Neopágina Diciembre 2018

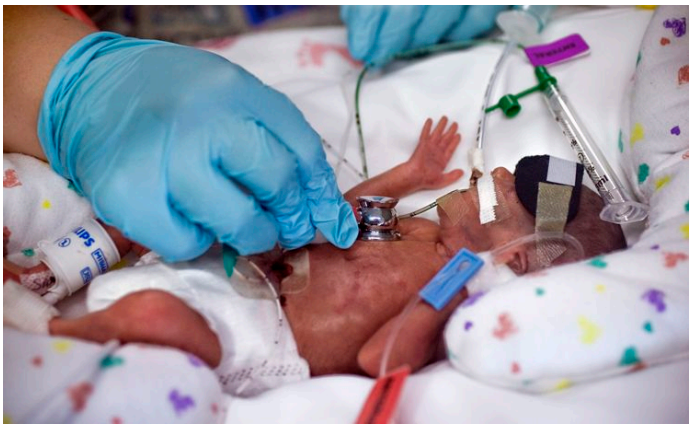


Tabla de contenido

Original Paper

Neonatology

Neonatology 2019;115:127–133
DOI: 10.1159/000493358

Received: May 5, 2018
Accepted after revision: August 27, 2018
Published online: November 12, 2018

Outcome of Infants with Therapeutic Hypothermia after Perinatal Asphyxia and Early-Onset Sepsis

Mariam Hakobyan[a] Koen P. Dijkman[b] Sabrina Laroche[c] Gunnar Naulaers[d] Monique Rijken[e] Katerina Steiner[f] Henrica L.M. van Straaten[g] Renate M.C. Swarte[h] Hendrik J. ter Horst[i] Alexandra Zecic[j] Inge A. Zonnenberg[k] Floris Groenendaal[a]

[a]Department of Neonatology, Wilhelmina Children's Hospital, University Medical Center Utrecht and Utrecht University, Utrecht, The Netherlands; [b]Department of Neonatology, Máxima Medical Centre, Veldhoven, The Netherlands; [c]Department of Neonatology, University Hospital, Antwerp, Belgium; [d]Department of Neonatology, University Hospital, Leuven, Belgium; [e]Department of Neonatology, Leiden University Medical Center, Leiden, The Netherlands; [f]Department of Neonatology, Radboud University Medical Center, Radboud Institute for Health Science, Amalia Children's Hospital, Nijmegen, The Netherlands; [g]Department of Neonatology, Isala Clinics, Zwolle, The Netherlands; [h]Department of Neonatology, Erasmus Medical Center Sophia, Rotterdam, The Netherlands; [i]Department of Neonatology, Beatrix Children's Hospital, University Medical Centre Groningen, Groningen, The Netherlands; [j]Department of Neonatology, University Hospital, Gent, Belgium; [k]Department of Neonatology, VU University Medical Center, Amsterdam, The Netherlands

Keywords
Early-onset sepsis · Perinatal asphyxia · Therapeutic hypothermia · Cerebral palsy · Neurodevelopmental impairment · Group B streptococcus

Abstract
Background: Animal models suggest that neuroprotective effects of therapeutic hypothermia (TH) after perinatal asphyxia are reduced in infants with early-onset sepsis. ***Objectives:*** To assess the outcome of infants with perinatal asphyxia, neonatal encephalopathy, and TH in the presence of early-onset sepsis. ***Methods:*** In a retrospective cohort of 1,084 infants with perinatal asphyxia and TH, the outcome of 42 infants (gestational age 36.1–42.6 weeks and birth weight 2,280–5,240 g) with proven sepsis ($n = 14$) and probable sepsis ($n = 28$) was analyzed. Death, cerebral palsy, or a delayed development at 2 years was considered an adverse outcome. ***Results:*** Sepsis was caused mostly by group B streptococci ($n = 17$), other Gram-positive bacteria ($n = 5$), and *Candida albicans* ($n = 1$). Of the 42 infants, 9 (21.4%) died, and 5 (11.9%) showed impairments on follow-up. The outcome is comparable to the previously reported outcome of infants with TH without early-onset sepsis. ***Conclusion:*** A good outcome was reported in the majority of infants with perinatal asphyxia, TH, and early-onset sepsis. Cooling should not be withheld from these infants.

© 2018 The Author(s)
Published by S. Karger AG, Basel

KARGER

E-Mail karger@karger.com
www.karger.com/neo

© 2018 The Author(s)
Published by S. Karger AG, Basel

Karger Open access

This article is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND) (http://www.karger.com/Services/OpenAccessLicense). Usage and distribution for commercial purposes as well as any distribution of modified material requires written permission.

Floris Groenendaal
Department of Neonatology, Room KE.04.123.1
Wilhelmina Children's Hospital, University Medical Center Utrecht, Lundlaan 6
NL–3584 EA Utrecht (The Netherlands)
E-Mail F.Groenendaal@umcutrecht.nl

Introduction

Neonatal encephalopathy (NE) following perinatal asphyxia in term neonates is still a common and serious condition. The prevalence of NE after perinatal asphyxia is approximately 1–6 per 1,000 full-term live births [1, 2]. It is well known that infants with moderate-to-severe NE carry a high risk of adverse outcome, such as cerebral palsy (CP), neurodevelopmental impairment, or mortality, even after therapeutic hypothermia (TH) [1, 3, 4]. In addition, early-onset sepsis which is mostly caused by group B streptococcus (GBS) or Gram-negative organisms, such as *Escherichia coli*, carries a high risk of an adverse outcome [5]. The outcome of infants with perinatal asphyxia, early-onset sepsis, and TH has not been reported in much detail. It has been suggested that encephalopathic newborns with early-onset sepsis may have a worse outcome compared to nonseptic neonates [6]. Studies in adults with sepsis did not show benefits of hypothermia [7]. In addition, in the study by Geurts et al. [8] an increased risk for pneumonia and sepsis was observed, although the overall infection risk was not significantly higher. At present, little is known about the interplay of hypothermia and sepsis. Several animal models have examined the neuroprotective effect of TH in the presence of bacterial infections and results are inconclusive [9–12].

In the present study, the outcome of infants with perinatal asphyxia, NE treated with hypothermia, and early-onset sepsis was assessed.

Subjects and Methods

Infants with a gestational age between 36 + 0 and 42 + 0 weeks with perinatal asphyxia, NE, and TH admitted to one of the level III participating Neonatal Intensive Care Units (NICU) in the Netherlands or Flanders, Belgium, between January 2008 and December 2016 were included. During this period, 1,084 infants were treated with TH in the participating hospitals. Retrospectively, data were collected from the medical files. Growth percentiles were calculated according to the Netherlands Perinatal Registry Birth Weight centiles (www.perined.nl) [13].

Infants with positive blood cultures within 48 h after birth and clinical signs of sepsis were considered to have a proven sepsis. Infants with clinical signs of early-onset sepsis and an elevated CRP (≥50 mg/L) or positive surface cultures, but no positive blood culture, were considered to have a probable sepsis. All infants were treated with antibiotics for at least 7 days, and all had signs of multi-organ failure. Most infants were too ill to undergo lumbar punctures.

The severity of encephalopathy was graded according to Sarnat. TH was used as described previously [3]. Although 3 infants appeared to have a mild encephalopathy, aEEG showed a suppressed background pattern and TH was applied. In 3 infants with a good aEEG background pattern on admission, TH was started because of a high Thompson score.

In all infants, aEEG was used routinely, and patterns were analyzed as described previously [14]. Clinical and/or aEEG-detected seizures were treated according to the Dutch/Flemish neonatal seizure protocol which includes phenobarbital with midazolam and/or lidocaine as add-on therapy [15]. Brain imaging (cranial ultrasound and MRI) was collected from the files. MRI abnormalities were reported as watershed lesions, lesions in the basal ganglia and thalamus (BGT), or near total injury [16].

Outcome

After discharge, follow-up assessments were performed in the participating hospitals at regular intervals up to at least 18 months in the routine follow-up program. Death, CP, neurodevelopmental impairment of >3 months, a Griffiths' developmental quotient <88 (–1 standard deviation, SD), or a score on the Bayley Scales of Infant and Toddler Development-III <85 (–1 SD) were all considered an adverse outcome. In addition, infants ($n = 4$) with a normal MRI at birth and having no neurological abnormalities at the age of 6 months, and 2 additional infants with a normal MRI and no follow-up data were categorized in the group with no adverse outcome.

Statistical Analysis

Mortality and adverse neurodevelopmental outcome data were compared to the data reported previously in our units [3] and a Cochrane review [4], using χ^2 tests, Fisher tests, or analysis of variance (ANOVA) where appropriate. Data were expressed as mean ± SD, median with interquartile range (IQR), or in percentages. With the number of 42 patients, it would be possible to compare neuroprotective effects of hypothermia in septic patients (both proven and probable sepsis combined) with all hypothermia patients presented in the studies mentioned above [3, 4] with an alpha of 0.05 and a power of 0.80. This retrospective study was approved by the local ethics committee, and the requirement to obtain informed consent for this study with anonymous data analysis was waived according to national regulations.

Results

Between January 2008 and December 2017, 42 infants with perinatal asphyxia and TH showed early-onset sepsis. Of these 42 infants, 14 infants had proven sepsis and 28 probable sepsis. Clinical data of our patients are presented in Table 1. Clinical data were not significantly different between the proven and probable sepsis groups. Gestational age and birth weight were lower in the neonates who died ($n = 9$) compared to the ones who survived ($n = 33$); however, the 5th and the 10th percentile birth weights were similar. Infants who died had a higher Thompson score and a more severe encephalopathy. Clinical data of the patients with sepsis, such as gestational age, birth weight, and severity of encephalopathy were comparable to those reported in other studies of

Table 1. Clinical data

Characteristics	All early-onset septic infants (n = 42)	Probable sepsis (n = 28)	Proven sepsis (n = 14)	p value*	Survived (n = 33)	Died (n = 9)	p value+
Gestational age, weeks	40.1±1.55	40.0±1.49	40.2±1.72	0.75	40.3±1.45	39.1±1.65	**0.04**
Female	21 (50.0)	14 (50.0)	7 (50.0)	1.00	16 (48.5)	5 (55.6)	0.71
Birthweight	3,659±662	3,660±726	3,658±534	0.99	3,814±619	3,094±502	**0.003**
P5 for SGA	4 (9.5)	3 (10.7)	1 (7.1)	1.00	2 (6.1)	2 (22.2)	0.20
P10 for SGA	6 (14.3)	4 (14.3)	2 (14.3)	1.00	3 (9.1)	3 (33.3)	0.10
Mode of birth (n = 39[a])				0.56			0.42
Section	16 (41.0)	10 (37.0)	6 (50.0)		12 (40.0)	4 (44.4)	
SVD	18 (46.2)	14 (51.9)	4 (33.3)		13 (43.3)	5 (55.6)	
Vacuum extraction	5 (12.8)	3 (11.1)	2 (16.7)		5 (16.7)	0 (0.0)	
Meconium (n = 34[a])	19 (55.9)	11 (50.0)	8 (66.7)	0.35	14 (51.9)	5 (71.4)	0.35
Grade of encephalopathy on admission (n = 21[b])				0.75			**<0.0001**
Mild	3 (14.3)	2 (16.7)	1 (11.1)		3 (18.8)	0 (0.0)	
Moderate	12 (57.1)	6 (50.0)	6 (66.7)		12 (75.0)	0 (0.0)	
Severe	6 (28.6)	4 (33.3)	2 (22.2)		1 (6.3)	5 (100)	
Apgar score (n = 41[a])							
1 min	1 [2]	1 [2]	1.5 [2]	0.57	1 [2]	0 [2]	0.14
5 min	3 [3]	3 [3]	3.5 [3]	0.39	3.5 [2]	2 [5]	0.4
pH[c] (n = 34[a])	6.98±0.20	7.03±0.20	6.91±0.17	0.07	6.98±0.21	7.0±0.13	0.93
Thompson score (n = 35[a])	10 [3]	10 [3]	10 [2]	0.89	10 [3]	13 [3]	**0.02**
Antiepileptic drugs ≥1 (n = 37[a])	26 (70.3)	15 (60.0)	11 (91.7)	0.06	20 (71.4)	6 (66.7)	0.79
Highest CRP during hypothermia (n = 39[a])	106±72.5	94±54.5	135±103	0.11	103±69.0	116±89.5	0.65
aEEG on admission (n = 33[a])				0.58			0.06
CNV	3 (9.1)	3 (13.6)	0 (0.0)		3 (12.0)	0 (0.0)	
DNV	6 (18.2)	4 (18.2)	2 (18.2)		6 (24.0)	0 (0.0)	
BS	13 (39.4)	7 (31.8)	6 (54.5)		11 (44.0)	2 (25.0)	
LV	5 (15.2)	4 (18.2)	1 (9.1)		2 (8.0)	3 (37.5)	
FT	6 (18.2)	4 (18.2)	2 (18.2)		3 (12.0)	3 (37.5)	
Mortality	9 (21.4)	6 (21.4)	3 (21.4)	1.00	–	–	–

Values are expressed as means ± SD, numbers with percentage in parentheses, or medians with IQR in square brackets. Bold values are $p < 0.05$. SGA, small for gestational age; P5, 5th percentile; P10, 10th percentile; SVD, spontaneous vaginal delivery; aEEG, amplitude-integrated electroencephalography; CNV, continuous normal voltage; DNV, discontinuous normal voltage; BS, burst suppression; LV, low voltage; FT, flat trace.

[a] Percentages are based on data that were available from the number of infants in the institutions. It means that some data were missing. [b] Data were missing for 21 infants. [c] The umbilical arterial pH was not reported in 8 infants. * p values were calculated between probable and proven sepsis. + p values were calculated between infants who survived and those who died.

TH in the Cochrane review by Jacobs et al. [4] and in Groenendaal et al. [3].

Microbiology

The children with proven sepsis showed a positive blood culture with Gram-positive bacteria, which included GBS (n = 10), *Actinomyces* (n = 1), coagulase-negative staphylococci (n = 1), *Streptococcus viridans* (n = 1), and *Streptococcus milleri* (n = 1, Table 2). The infant with *S. viridans*-proven sepsis died. The infants with proven sepsis who survived developed no neurological impairments. Some neonates in the probable sepsis group had no surface cultures taken (n = 19). These infants were diagnosed with probable sepsis based on their high CRP values and clinical symptoms, leaving 9 neonates with a positive surface with Gram-positive bacteria (GBS, n = 7, and *Enterococcus hirae*, n = 1) or fungus (*Candida*, n = 1). There was no significant difference in adverse outcome, considering the type of organism found in blood or surface culture (Table 2).

Table 2. Bacteria cultured in infants with a proven or probable sepsis

	Proven sepsis (n = 14)	Probable sepsis (n = 28)	Adverse outcome
Surface or blood culture[a]			
GBS	10 (71.4)	7 (77.8)	6
CNS	1 (7.1)	–	0
Actinomyces oris	1 (7.1)	–	0
Streptococcus milleri	1 (7.1)	–	0
Streptococcus viridans[b]	1 (7.1)	–	1
Enterococcus hirae	–	1 (11.1)	0
Candida	–	1 (11.1)	0
Unknown[c]	–	19 (45.2)	7

Values are expressed as numbers with percentages in parentheses. GBS, group B streptococcus; CNS, coagulase-negative staphylococcus.

[a] Surface culture was taken from the ear and/or umbilicus, with missing data for 19 infants. [b] This infant with *S. viridans* sepsis developed a coinfection with CNS at birth. [c] These infants were included in the probable sepsis group due to clinical signs of sepsis and a CRP >50 mg/L.

Table 3. MRI data and survival

Characteristics	Probable sepsis (n = 28)	Proven sepsis (n = 14)	Survived (n = 33)	Died (n = 9)
MRI (n = 35)[a]				
Normal	13 (46.4)	5 (35.7)	18 (54.5)	0 (0.0)
WS	5 (17.8)	2 (14.3)	7 (21.2)	0 (0.0)
BGT	4 (14.3)	2 (14.3)	5 (15.2)	1 (11.1)
NT	3 (10.7)	1 (7.1)	1 (3.0)	3 (33.3)
Not performed	3 (10.7)	4 (28.6)	2 (6.1)	5 (55.6)

Values are expressed as numbers with percentages in parentheses. MRI, magnetic resonance imaging; WS, watershed pattern of injury; BGT, basal ganglia and thalamus pattern of injury; NT, "near total" pattern of injury.

[a] MRI was not performed in 7 infants, of whom 5 died.

Outcome

Imaging

Findings of cranial MRI examinations at follow-up are presented in Table 3. The MRI showed no abnormalities in 51.4% of the infants with sepsis. Infants who died had more severe MRI abnormalities ($p < 0.0001$). Four infants (11.4%) had a near total pattern on the MRI. Of the 4 infants with a near total pattern, 3 died and 1 survived but developed neurological disabilities. The aEEG of these 4 neonates showed a flat trace or continuous low voltage and 2 had a Thompson score of >11. Furthermore, 6 neonates (17.1%) had BGT involvement on the MRI, and 7 neonates (20%) had a watershed-type injury. One infant with a BGT pattern died and 2 developed neurological disabilities. Finally, there was no difference in MRI results between proven and probable sepsis ($p = 0.992$).

Mortality

The overall mortality among septic infants with TH after asphyxia was 21.4%. Two infants died shortly after admission due to severe sepsis, 7 others died after redirection of care following severe brain injury which was demonstrated using MRI. Postmortem examination was performed in 2 infants, confirming the multi-organ involvement and MRI findings. No significant difference was found in mortality between the proven and probable sepsis groups. The mortality in the present study (21.4%) was comparable to the previous study (31.8%) and the Cochrane review (26.8%; Table 4).

Follow-Up

Outcome data on Neurodevelopmental disabilities or CP are presented in Table 4. Of the 42 neonates, 33 (78.6%) infants survived. Among the survivors, 5 (15.1%) had neurodevelopmental impairment including CP. Three infants were too young to be formally tested or had no follow-up. The remaining 25 infants with perinatal asphyxia and early-onset sepsis were normal (59.5%). Hypothermia-treated survivors with sepsis had no difference in the incidence of adverse outcome compared to the previous TH studies.

Discussion

In the present study, the outcome of septic neonates who underwent TH was reported. During the study period, 42 of the 1,084 infants (3.9%) had proven or probable early-onset sepsis. Whereas one-third had an adverse outcome, more than 60% was normal at 18 months or later. An additional 2 younger infants were too young to be formally tested but were normal at this younger age. These outcomes are comparable to the data reported in large RCTs and the results of previously reported patients in the Netherlands and Flanders, Belgium, without sepsis [4, 17–20].

Table 4. Outcome of septic neonates with TH after asphyxia compared to previous studies [3, 4]

Outcome	Early-onset sepsis (n = 42)	Groenendaal et al. [3] (n = 308)	p value*	Cochrane review [4] (n = 678)	p value^{+}
Normal outcome	26 (61.9)	168 (54.5)	0.37	366 (54.0)	0.32
Neurodevelopmental impairment or CP	5 (11.9)	42 (13.6)	0.76	130 (19.2)	0.24
Mortality	9 (21.4)	98 (31.8)	0.17	182 (26.8)	0.44
Adverse outcome	14 (33.3)	140 (45.5)	0.14	312 (46.0)	0.11
Too young to be tested	2 (4.8)	–	–	–	–

Values are expressed as numbers with percentages in parentheses. * Significant difference was calculated between the early-onset sepsis group and Groenendaal et al. [3]. $^{+}$ Significant difference was calculated between the early-onset sepsis group and the Cochrane review [4].

Infections with GBS are still an important cause of serious morbidity in neonates [21]. In the present study, the outcome of infants with infections caused by GBS was not different from infections caused by other organisms. Infections with Gram-negative organisms were not seen in the present study. In the Netherlands, early-onset sepsis with Gram-negative organisms in full-term infants is very rare (data from the Netherlands Perinatal Registry, www.perined.nl).

TH has a neuroprotective effect by influencing different pathways including metabolism, cerebral blood flow, the release of excitatory amino acids, and apoptosis. Furthermore, TH has an antioxidant effect, the ability to block the proinflammatory cascade and reduce ATP loss [6, 22]. During sepsis, metabolic demands in different organs are high due to the inflammation response, which may increase neuronal apoptosis and subsequent neurological damage. Based on this theory, TH could also be effective in infants with early-onset sepsis. In contrast, hypothermia may suppress the potentially protective inflammatory cascade and may result in functional immune compromise, leading to an adverse outcome in infants with sepsis [23]. Animal experiments have described conflicting results in models of perinatal asphyxia and infections. Neuroprotective effects have been described in neonatal models of Gram-positive sepsis and TH [11], whereas a lack of effects has been detected in neonatal models of Gram-negative sepsis and TH [9, 10]. In contrast, prolonged survival in Gram-negative sepsis was documented in adult models of Gram-negative and Gram-positive sepsis and TH [12, 24].

The large trials of TH in perinatal asphyxia and NE have not described the effects of TH in infants with early-onset sepsis in much detail. TH may increase the risk of infections [23]. A meta-analysis in adults strongly suggested an association between TH and the risk of pneumonia and sepsis [8]. In main randomized trails, solely 5–11.3% of infants developed sepsis [17, 18, 20, 25]. However, early-onset sepsis has not been defined in much detail in most trials, and in many studies no difference was reported between early and late-onset sepsis. In the present study, 14 of the infants developed late-onset sepsis which is higher number compared to the study of Jacobs et al. [25].

Our study has several limitations. First, it had a retrospective design, and some clinical data were not reported in much detail. Furthermore, some units did not perform routine surface cultures, thereby limiting the detection of the causative organism in infants with clinical sepsis. The effect of TH on CRP levels is controversial [26, 27]. Nevertheless, by using very high cutoff values for CRP (>50 mg/L), twice the upper level as those mentioned by others [28, 29], and a clinical picture of early-onset sepsis, we considered the risk of false positives to be low. Second, follow-up was not performed uniformly, which may have led to somewhat diverse outcome data. By using cutoff values of the separate tests, we were able to identify infants with an adverse outcome. Third, the numbers of sepsis cases were too small to provide detailed information on the outcome of Gram-positive versus Gram-negative neonatal sepsis, but the sample size was large enough to demonstrate that neuroprotection by TH was retained in infants with perinatal asphyxia and early-onset sepsis. Furthermore, no lumbar puncture was performed in most infants because of the severity of illness, and the presence of accompanying meningitis is unknown.

Conclusion

A good outcome was reported in more than 60% of infants with perinatal asphyxia, sepsis, and therapeutic hypothermia. Therapeutic hypothermia should not be withheld from infants with perinatal asphyxia, neonatal encephalopathy, and early-onset sepsis.

Acknowledgements

The authors, who are members of the Dutch-Flemish Working Group on Neonatal Neurology, thank the other members for the valuable comments and suggestions.

Statement of Ethics

For this observational study analyzing and reporting a large set of anonymized data a waiver of informed consent was obtained according to European legislation.

Funding Sources

Financial support for publication of the study was received from the Stichting Neonatale Neurologie Utrecht (www.snnu.nl).

References

1 Pin TW, Eldridge B, Galea MP. A review of developmental outcomes of term infants with post-asphyxia neonatal encephalopathy. Eur J Paediatr Neurol. 2009 May;13(3):224–34.
2 Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev. 2010 Jun;86(6):329–38.
3 Groenendaal F, Casaer A, Dijkman KP, Gavilanes AW, de Haan TR, ter Horst HJ, et al. Introduction of hypothermia for neonates with perinatal asphyxia in the Netherlands and Flanders. Neonatology. 2013;104(1):15–21.
4 Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013 Jan;1(1):CD003311.
5 Jenster M, Bonifacio SL, Ruel T, Rogers EE, Tam EW, Partridge JC, et al. Maternal or neonatal infection: association with neonatal encephalopathy outcomes. Pediatr Res. 2014 Jul;76(1):93–9.
6 Hassell KJ, Ezzati M, Alonso-Alconada D, Hausenloy DJ, Robertson NJ. New horizons for newborn brain protection: enhancing endogenous neuroprotection. Arch Dis Child Fetal Neonatal Ed. 2015 Nov;100(6):F541–52.
7 Fries M, Stoppe C, Brücken D, Rossaint R, Kuhlen R. Influence of mild therapeutic hypothermia on the inflammatory response after successful resuscitation from cardiac arrest. J Crit Care. 2009 Sep;24(3):453–7.
8 Geurts M, Macleod MR, Kollmar R, Kremer PH, van der Worp HB. Therapeutic hypothermia and the risk of infection: a systematic review and meta-analysis. Crit Care Med. 2014 Feb;42(2):231–42.
9 Osredkar D, Thoresen M, Maes E, Flatebø T, Elstad M, Sabir H. Hypothermia is not neuroprotective after infection-sensitized neonatal hypoxic-ischemic brain injury. Resuscitation. 2014 Apr;85(4):567–72.
10 Osredkar D, Sabir H, Falck M, Wood T, Maes E, Flatebø T, et al. Hypothermia Does Not Reverse Cellular Responses Caused by Lipopolysaccharide in Neonatal Hypoxic-Ischaemic Brain Injury. Dev Neurosci. 2015;37(4–5):390–7.
11 Falck M, Osredkar D, Maes E, Flatebø T, Wood TR, Sabir H, et al. Hypothermic Neuronal Rescue from Infection-Sensitised Hypoxic-Ischaemic Brain Injury Is Pathogen Dependent. Dev Neurosci. 2017;39(1–4):238–47.
12 Rim KP, Kim K, Jo YH, Lee JH, Rhee JE, Kang KW, et al. Effect of therapeutic hypothermia according to severity of sepsis in a septic rat model. Cytokine. 2012 Dec;60(3):755–61.
13 Hoftiezer L, Hukkelhoven CW, Hogeveen M, Straatman HM, van Lingen RA. Defining small-for-gestational-age: prescriptive versus descriptive birthweight standards. Eur J Pediatr. 2016 Aug;175(8):1047–57.
14 Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics. 2010 Jul;126(1):e131–9.
15 van Rooij LG, Toet MC, van Huffelen AC, Groenendaal F, Laan W, Zecic A, et al. Effect of treatment of subclinical neonatal seizures detected with aEEG: randomized, controlled trial. Pediatrics. 2010 Feb;125(2):e358–66.
16 de Vries LS, Groenendaal F. Patterns of neonatal hypoxic-ischaemic brain injury. Neuroradiology. 2010 Jun;52(6):555–66.
17 Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet. 2005 Feb;365(9460):663–70.
18 Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al.; National Institute of Child Health and Human Development Neonatal Research Network. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med. 2005 Oct;353(15):1574–84.
19 Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al.; TOBY Study Group. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med. 2009 Oct;361(14):1349–58.
20 Simbruner G, Mittal RA, Rohlmann F, Muche R; neo.nEURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. Pediatrics. 2010 Oct;126(4):e771–8.
21 Tann CJ, Martinello KA, Sadoo S, Lawn JE, Seale AC, Vega-Poblete M, et al.; GBS Neonatal Encephalopathy Investigator Group. Neonatal Encephalopathy With Group B Streptococcal Disease Worldwide: Systematic Review, Investigator Group Datasets, and Meta-analysis. Clin Infect Dis. 2017 Nov;65 suppl_2:S173–89.
22 Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AJ. The mechanisms and treatment of asphyxial encephalopathy. Front Neurosci. 2014 Feb;8:40.
23 Jenkins DD, Lee T, Chiuzan C, Perkel JK, Rollins LG, Wagner CL, et al. Altered circulating leukocytes and their chemokines in a clinical trial of therapeutic hypothermia for neonatal hypoxic ischemic encephalopathy*. Pediatr Crit Care Med. 2013 Oct;14(8):786–95.

24 Chang YT, Wann SR, Tsai JS, Kao CH, Lee PT, Huang NC, et al. The role of autonomic nervous system function in hypothermia-mediated sepsis protection. Am J Emerg Med. 2013 Feb;31(2):375–80.
25 Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al.; Infant Cooling Evaluation Collaboration. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. Arch Pediatr Adolesc Med. 2011 Aug;165(8):692–700.
26 Okumuş N, Beken S, Aydın B, Erol S, Dursun A, Fettah N, et al. Effect of therapeutic hypothermia on C-reactive protein levels in patients with perinatal asphyxia. Am J Perinatol. 2015 Jun;32(7):667–74.
27 Chakkarapani E, Davis J, Thoresen M. T herapeutic hypothermia delays the C-reactive protein response and suppresses white blood cell and platelet count in infants with neonatal encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2014 Nov; 99(6):F458–63.
28 Celik IH, Demirel FG, Uras N, Oguz SS, Erdeve O, Biyikli Z, et al. What are the cut-off levels for IL-6 and CRP in neonatal sepsis? J Clin Lab Anal. 2010;24(6):407–12.
29 Xu L, Li Q, Mo Z, You P. Diagnostic value of C-reactive protein in neonatal sepsis: A meta-analysis. Eur J Inflamm. 2016;14(2):100–8.

BREASTFEEDING MEDICINE
Volume 12, Number 7, 2017
© Mary Ann Liebert, Inc.
DOI: 10.1089/bfm.2017.29047.aje

ABM Clinical Protocol #8: Human Milk Storage Information for Home Use for Full-Term Infants, Revised 2017

Anne Eglash,[1] Liliana Simon,[2] and The Academy of Breastfeeding Medicine

A central goal of The Academy of Breastfeeding Medicine is the development of clinical protocols, free from commercial interest or influence, for managing common medical problems that may impact breastfeeding success. These protocols serve only as guidelines for the care of breastfeeding mothers and infants and do not delineate an exclusive course of treatment or serve as standards of medical care. Variations in treatment may be appropriate according to the needs of an individual patient.

Background

Breastfeeding mothers may encounter unforeseen reasons for separation from their infants, but more often women express and store milk for planned events, lifestyle flexibility, and returning to work. Knowledge of appropriate human milk handling and storage is essential for breastfeeding success in these situations. One study indicated that although most women store their milk as recommended, ~12% heated their milk in a microwave, and 17% rinsed bottle nipples/teats with only water before reuse,[1] which may reduce the milk's biological properties and increase risk of contamination, respectively. Another study showed that neonatal nurses' knowledge and practice of breast milk collection and storage were adequate, however, there was inadequacy related to discarding, storing, and thawing breast milk.[2]

Human milk is a fresh, living food with many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients. Although some of these nutrients and health properties change with storage, there is good evidence that human milk storage can be safe, allowing provision of optimal nutrition to the child when breastfeeding or immediately expressed milk is not available. When direct breastfeeding is not possible, stored human milk maintains unique qualities, such that it continues to be the gold standard for infant feeding.

Preparation for Human Milk Storage

1. Washing: Women should wash their hands with soap and water, or a waterless hand cleanser if their hands don't appear dirty, before milk expression. Unclean hands may transmit viruses and bacteria, some of which can cause illness. Studies show that human milk containing fewer bacteria at the time of expression develops less bacterial growth during storage and has higher protein levels compared to milk that has an abundance of bacteria.[3–5] Additional hand hygiene and cleaning of the breasts before expression are not necessary.[6] (IIB) (Quality of evidence [levels of evidence IA, IB, IIA, IIB, III, and IV] is based on levels of evidence used for the National Guidelines Clearing House[7] and is noted in parentheses.)
2. Hand or Pump: Milk expression can be achieved by hand or by a pump. As long as the appropriate steps are taken for hand cleansing and cleaning of pump parts as per the pump manufacturer's instructions, there does not seem to be a difference in milk contamination with pumping versus hand expression.[8,9] (IIB, IV) There is no need to discard the first few drops of milk with initiating milk expression. This milk is not more likely to be contaminated than milk that is subsequently expressed.[7] One study found that milk expressed at home appears to have more bacterial contamination than milk expressed at the hospital, possibly related to equipment at home or transport, not related to personal hygiene.[6] (IIB)
3. Storage Container Choice: Several studies have been done to evaluate a range of available storage containers. There is a significant reduction in percent of fat and an increase in total protein and carbohydrate concentrations with either glass or polyethylene, polypropylene, polycarbonate, or polyethersulfone bottles or bags.[10] Glass and polypropylene containers appear similar in their effects on adherence of lipid-soluble nutrients to the container surface,[11] the concentration of immunoglobulin

[1]Department of Family and Community Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.
[2]Department of Pediatrics, Pediatric Critical Care, University of Maryland School of Medicine, Baltimore, Maryland.

A (IgA), and the numbers of viable white blood cells in the stored milk.[12] Use of polyethylene containers was associated with a marked drop (60%) of IgA[12] and milk's bactericidal effect when compared to Pyrex, a type of tempered glass.[13] Steel containers were associated with a marked decline in cell count and cell viability when compared to polyethylene[14] and glass.[15] (IIB)

There has been concern about possible contamination of milk stored in polypropylene bags because of the risk of contamination by puncturing the plastic.[16] (IV) However, one study showed no difference between contamination and fat loss when comparing hard and soft polypropylene containers.[17] Therefore, plastic bags used for human milk storage should be sturdy, sealed well, and stored in an area of the freezer where damage to the bag would be minimized. (IIB) Containers made with bisphenol A, which is found in several plastic containers including baby bottles, should be avoided based on strong evidence of its adverse effects as an endocrine disruptor.[18] There should be caution about the use of bottles with bisphenol S, a bisphenol A alternative, as it may also have deleterious effects, although this is not well established in the literature.

Human milk should not be stored in hospital plastic specimen storage containers such as those used for urine or other bodily fluids because there is insufficient evidence regarding their chemical safety and effects on infants' health;[19] only food grade plastic containers should be used for human milk storage. (IV)

4. Care of Containers: Containers for human milk storage and breast pump milk collection kits must be completely dismantled, washed in hot soapy water and rinsed or washed in a dishwasher,[8] and should always be thoroughly air dried or dried with paper towels.[20] They do not need to be sterilized. If soap is not available, then boiling water is preferable. (IIB) Chemical disinfection is not ideal, as the disinfectant can be easily deactivated and could expose infant to unnecessary risk of both inadequately clean containers and residual chemical disinfectant.[20] (IV)

Storage of Human Milk

1. Freshly expressed human milk may be stored safely at room temperature (10–29°C, 50–85°F) for some period of time. Studies suggest different optimal times for room temperature storage because conditions vary greatly in the cleanliness of milk expression technique and the room temperature. Warmer ambient temperatures are associated with faster growing bacterial counts in stored milk. For room temperatures ranging from 27°C to 32°C (29°C = 85°F), 4 hours may be a reasonable limit.[5,21,22] For very clean expressed milk with very low bacterial counts, 6–8 hours at lower room temperatures may be reasonable, but it is best to chill or refrigerate as soon as possible if the milk will not be used during that time.[4,23–25] (IIB)
2. Ice packs: Very few studies have evaluated milk storage safety at 15°C (59°F), which would be equivalent to an ice pack in a small cooler. Hamosh et al.[21] suggested that human milk is safe at 15°C for 24 hours, based on minimal bacterial growth noted in the samples from their study. (IIB)
3. Refrigeration: Several studies have demonstrated the safety of refrigerating human milk (4°C, 39.2°F), either by evaluating the bactericidal capacity of stored milk as a marker for milk quality or by measuring bacterial growth in the stored milk samples. Bactericidal capacity of stored refrigerated human milk declines significantly by 48–72 hours.[26–28] However, studies of expressed human milk with little contamination at the time of expression demonstrate safe, low levels of bacteria growth in milk at 72 hours[24] and even after 4–8 days of refrigeration.[3,4,29]

   Few studies have been done on the change in milk composition during refrigerator storage. One study found that lipid composition and lipase activity remained stable up to 96 hours in the refrigerator.[30] Lactoferrin levels are stable in the refrigerator for 4–5 days.[31,32] Many immunologic factors in colostrum such as IgA, cytokines, and growth factors are not diminished with refrigeration for 48 hours.[33] (IIB)
4. Freezing expressed human milk (0°F, −18°C) has been demonstrated to be safe for at least 3 months. Evidence indicates that thawed human milk, previously frozen for at least 6 weeks at −20°C (−4°F), has the same bacterial viability and diversity as it did when it was freshly expressed.[34] The basic principles of freezing dictate that frozen foods at −18°C (0°F) are safe indefinitely from bacterial contamination, although enzymatic processes inherent in food could persist, with possible changes in milk quality.[35]

   Fat, protein, and calories decrease in human milk when frozen for 90 days compared to fresh human milk.[36] Frozen human milk has a significant increase in acidity by 3 months, likely due to ongoing lipase activity, that increases free fatty acids in the milk.[37] Based on a few studies with very small samples sizes, vitamin E appears stable in frozen milk over time, and vitamin C levels decrease significantly after 1–5 months of storage.[38,39] There is a paucity of research on how freezer storage affects nearly all vitamins and minerals in human milk.[38–40]

   Bioactive factors in human milk variably diminish with freezing. Lactoferrin levels and bioactivity are significantly lower in human milk frozen at −20°C for 3 months.[13,31,32] However, several cytokines, IgA and growth factors from colostrum are stable for at least 6 months at −20°C (−4°F).[10,33] One trial evaluating milk frozen for 9 months found a progressive decline in pH and in bacterial counts, and increases in nonesterified fatty acids. Other macronutrients, osmolality, and immunoactive proteins remained unchanged in this study after 9 months.[41] Frozen human milk should be stored in the back of the freezer to prevent intermittent rewarming due to freezer door opening, and should be kept away from the walls of self-defrosting freezers. All containers with human milk should be well sealed to prevent contamination. (IIB)
5. Smell of stored milk: Refrigerated and frozen human milk may have an odor different from fresh milk due to lipase-mediated triglyceride breakdown, releasing fatty acids. The odor likely comes from oxidation of these fatty acids.[42,43] This lipolysis process has antimicrobial effects preventing the growth of microorganisms in thawed refrigerated milk.[44] There is no evidence to

suggest that infants often reject human milk due to this odor. Many foods that humans eat, such as eggs, cheese, and fish, have an unpleasant odor that does not affect taste. One study demonstrated that freezing human milk to −80°C (−112°F) leads to less change in smell as compared to conventional freezing to −19°C.[43] Heating milk to above 40°C to deactivate lipase is not advised because this may destroy many of the immunologically active factors in human milk. (IIB)

6. Expansion while freezing: When filling a container with human milk, space should be left at the top to allow for expansion with freezing. All stored containers of human milk should be labeled with the date of milk expression and the name of the child if the milk will be used in a child-care setting. It is typical for infants in daycare to take 60–120 mL (2–4 ounces) of human milk at one feeding. Therefore, storing human milk in a variety of small increments such as 15–60 mL is a convenient way to prevent waste of thawed human milk.
7. Mixing milk: Freshly expressed warm milk should not be added to already cooled or frozen milk, to prevent rewarming of the already stored milk. It is best to cool down the newly expressed milk first before adding it to older stored milk.

A summary of milk storage guidelines is given in Table 1.

Using Stored Human Milk

1. Cleaning of feeding devices: Containers and feeding devices used to feed the infant should be cleaned with soap and water and air dried or dried with a paper towel before/after every use. They do not need to be sterilized for a healthy infant. (IIB)
2. Using fresh milk first: Fresh milk is of higher quality than frozen milk. Fresh milk contains current maternal secretory IgA antibodies that may be relevant to the dyad's recent infectious exposures.[45] Freshly expressed milk is highest in antioxidants, vitamins, protein, fat, and probiotic bacteria compared to refrigerated or frozen milk.[27,36,38,39] Fresh human milk also has the greatest immunologic activity compared to refrigerated or frozen milk.[10,31,46] (IB)
3. Thawing frozen milk: There are several ways to thaw frozen human milk: by either placing the container in the refrigerator overnight; by running it under warm water; by setting it in a container of warm water; or by using a waterless warmer. Slow thawing in the refrigerator causes less fat loss than thawing in warm water.[47] (IIB)
4. Warming human milk: Most infants drink milk cool, at room temperature, or warmed; infants may demonstrate a preference. Warming thawed human milk to body temperature is best done over a period of 20 minutes in lukewarm water (at most 40°C). Even warming the milk just to 37°C brings the fat to its melting point, promoting changes from solid fat, which is present at 4°C refrigerator temperature, to liquid or oil fat. Oil fat appears to adhere to the side of the container at 37°C more than it does at 4°C, therefore lowering the fat content of the milk. One study compared tepid water warming at 37°C and waterless warming and found there was no difference between them in regards to changes in fat, protein, lactoferrin, and secretory IgA.[44]

   Milk placed in hot water bath (80°C, which is not uncommon in the real setting) creates islets of high temperature milk due to lack of stirring.[48] Overheating during the warming process causes denaturation and inactivation of milk's bioactive proteins and decreased fat content. (IIB)
5. Microwaving: Studies done on defrosting human milk in a microwave demonstrate that controlling the temperature in a microwave is difficult, causing the milk to heat unevenly.[49] Although microwaving milk decreases bacteria in the milk much like pasteurization does, it also significantly decreases the activity of immunologic factors, which may reduce its overall health properties for the infant.[50,51] (IIB)
6. Using thawed milk: Once frozen milk is brought to room temperature, its ability to inhibit bacterial growth is lessened, especially by 24 hours after thawing.[52] Previously frozen human milk that has been thawed for 24 hours should not be left out at room temperature for more than 2 hours.[44] (IIB)
7. Refreezing: There is little information on refreezing thawed human milk. Bacterial growth and loss of antibacterial activity in thawed milk will vary depending on the technique of milk thawing, duration of the thaw, and the amount of bacteria in the milk at the time of expression. At this time no recommendations can be made on the refreezing of thawed human milk.
8. Using previously fed milk: Once an infant begins drinking expressed human milk, some bacterial contamination occurs in the milk from the infant's mouth. The length of time the milk can be kept at room temperature once the infant has partially fed from the cup or bottle would theoretically depend on the initial bacterial load in the milk, how long the milk has been thawed, and the ambient temperature. There has been insufficient research done to provide recommendations in this regard. However, based on related evidence thus far, it seems reasonable to discard the remaining

Table 1. Milk Storage Guidelines

Location of storage	*Temperature*	*Maximum recommended storage duration*
Room temperature	16–29°C (60–85°F)	4 hours optimal 6–8 hours acceptable under very clean conditions
Refrigerator	~4°C (39.2°F)	4 days optimal 5–8 days under very clean conditions
Freezer	0°F (−18°C)	6 months optimal 12 months acceptable

milk within 1–2 hours after the infant is finished feeding. (IV) To avoid wasting or discarding unfed milk, mothers may consider storing milk in a variety of increments such as 15, 30, or 60 mL.

9. Handling: Expressed human milk does not require special handling (such as universal precautions), as is required for other bodily fluids such as blood. It can be stored in a workplace refrigerator where other workers store food, although it should be labeled with name and date.[53] (IV) Mothers may prefer to store their milk in a personal freezer pack or cooler, separate from communal refrigerator areas.
10. Infections: Uncontaminated human milk naturally contains nonpathogenic bacteria[54,55] that are important in establishing the neonatal intestinal flora. These bacteria are probiotics—they create conditions in the intestine that are unfavorable to the growth of pathogenic organisms.[55] If a mother has breast or nipple pain from a bacterial or yeast infection, there is no evidence that her stored expressed milk needs to be discarded. Human milk that appears stringy, foul, or purulent should, however, be discarded and not be fed to the infant. (IV)

Areas for Future Research

The evidence for some aspects of human milk storage is lacking. Many studies are older, and because of differences in methodology, are difficult to compare. The studies vary in many respects, such as technique of milk collection, cleanliness and types of containers, duration of storage, method of thawing and warming milk, temperature and type of storage unit, and culture techniques of milk samples. Large high-quality studies evaluating human milk storage in a variety of circumstances over a longer duration of time are needed. Standards for evaluating milk quality, such as culture techniques, need to be established. Although it is ideal to have a universal international guideline for human milk storage, it may be impossible for one guideline to represent unusual or limited circumstances in some cultures.

Human milk naturally has both prebiotic and probiotic activity that is essential in establishing the infant gut microbiome. Human milk's prebiotic components are nondigestible factors such as oligosaccharides that promote the growth of beneficial microorganisms in the intestines. Human milk's probiotic components are commensal organisms. Because of the impact of refrigeration, freezing, thawing, and warming on the bactericidal activity of human milk, feeding an infant stored human milk may have different consequences on infant intestinal health compared to breastfeeding, and this should be investigated further. Along the same lines, stored human milk changes in quality over time, as demonstrated by many of the referenced articles included in this protocol. The effect of stored human milk versus fresh human milk on the health of a child should be studied.

There is also no agreed-upon definition of unsafe milk. Several studies describe the degree of milk contamination over a period of time under certain temperature and storage time conditions, typically described as the number of colony-forming units per milliliter. There is no accepted limit at which point milk should not be consumed, although 1×10^4 colony-forming units/mL has been suggested. Other studies have investigated the bactericidal capacity of stored human milk, which would reflect its immunologic effectiveness for the infant and the risk of the milk becoming contaminated over time during storage. The percentage loss of bactericidal activity that would render human milk unfit has not been determined. A definition for adequate milk quality should be established, with guidelines on what would constitute unsafe milk or lower-quality milk that would necessitate discarding of stored milk.

There is only one study investigating human milk quality after 6 months of freezing. This is particularly concerning, given that a few very small studies have demonstrated a decline in some vitamins after 3 months of freezing. Because some infants rely entirely on frozen human milk for nutrition, studies should be done to confirm that this is nutritionally safe.

References

1. Labiner-Wolfe J, Fein SB. How US mothers store and handle their expressed breast milk. *J Hum Lact* 2013;29:54–58.
2. Gharaibeh H, Al-Sheyab N, Malkawi S. Breast milk collection and storage in the neonatal intensive care unit: Nurses' knowledge, practice, and perceived barriers. *J Contin Educ Nurs* 2016;47:551–557.
3. Sosa R, Barness L. Bacterial growth in refrigerated human milk. *Am J Dis Child* 1987;141:111–112.
4. Pardou A, Serruys E, Mascart-Lemone F, et al. Human milk banking: Influence of storage processes and of bacterial contamination on some milk constituents. *Biol Neonate* 1994;65:302–309.
5. Eteng M, Ebong P, Eyong E, et al. Storage beyond three hours at ambient temperature alters the biochemical and nutritional qualities of breastmilk. *Afr J Reprod Health* 2001;5:130–134.
6. Haiden N, Pimpel B, Assadian O, et al. Comparison of bacterial counts in expressed breast milk following standard or strict infection control regimens in neonatal intensive care units: Compliance of mothers does matter. *J Hosp Infect* 2016;92:226–228.
7. Shekelle P, Woolf S, Eccles M, et al. Developing guidelines. *Br Med J* 1999;318:593–596.
8. Pittard WB 3rd, Geddes K, Brown S, et al. Bacterial contamination of human milk: Container type and method of expression. *Am J Perinatol* 1991;8:25–27.
9. Boo N, Nordiah A, Alfizah H, et al. Contamination of breast milk obtained by manual expression and breast pumps in mothers of very low birthweight infants. *J Hosp Infect* 2001;49:274–281.
10. Chang Y-C, Chen C-H, Lin M-C. The macronutrients in human milk change after storage in various containers. *Pediatr Neonatol* 2012;53:205–209.
11. Garza C, Johnson C, Harrist R, et al. Effects of methods of collection and storage on nutrients in human milk. *Early Hum Dev* 1982;6:295–303.
12. Goldblum R, Garza C, Johnson C, et al. Human milk banking I. Effects of container upon immunologic factors in human milk. *Nutr Res* 1981;1:449–459.
13. Takci S, Gulmez D, Yigit S, et al. Effects of freezing on the bactericidal activity of human milk. *J Pediatr Gastroenterol Nutr* 2012;55:146–149.
14. Manohar A, Williamson M, Koppikar G. Effect of storage of colostrum in various containers. *Indian Pediatr* 1997;34:293–295.

15. Williamson M, Murti P. Effect of storage, time, temperature, and composition of containers on biologic components of human milk. *J Hum Lact* 1996;12:31–35.
16. Hopkinson J, Garza C, Asquith M. Human milk storage in glass containers. *J Hum Lact* 1990;6:104–105.
17. Janjindamai W, Thatrimontrichai A, Maneenil G, et al. Soft plastic bag instead of hard plastic container for long-term storage of breast milk. *Indian J Pediatr* 2013;80: 809–813.
18. Vom Saal F, Hughes C. An extensive new literature concerning low dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* 2005;113: 926–933.
19. Blouin M, Coulombe M, Rhainds M. Specimen plastic containers used to store expressed breast milk in neonatal care units: A case of precautionary principle. *Can J Public Health* 2014;105:e218–e220.
20. Price E, Weaver G, Hoffman P, et al. Decontamination of breast pump milk collection kits and related items at home and in hospital: Guidance from a Joint Working Group of the Healthcare Infection Society and Infection Prevention Society. *J Hosp Infect* 2016;92:213–221.
21. Hamosh M, Ellis L, Pollock D, et al. Breastfeeding and the working mother: Effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. *Pediatrics* 1996;97:492–498.
22. Nwankwo M, Offor E, Okolo A, et al. Bacterial growth in expressed breast milk. *Ann Trop Paediatr* 1988;8: 92–95.
23. Pittard WB 3rd, Anderson D, Cerutti E, et al. Bacteriostatic qualities of human milk. *J Pediatr* 1985;107:240–243.
24. Igumbor E, Mukura R, Makandiramba B, et al. Storage of breast milk: Effect of temperature and storage duration on microbial growth. *Cent Afr J Med* 2000;46:247–251.
25. Ajusi J, Onyango F, Mutanda L, Wamola. Bacteriology of unheated expressed breastmilk stored at room temperature. *East Afr Med J* 1989;66:381–387.
26. Martínez-Costa C, Silvestre M, López M, et al. Effects of refrigeration on the bactericidal activity of human milk: A preliminary study. *J Pediatr Gastroenterol Nutr* 2007;45: 275–277.
27. Silvestre D, López M, March L, et al. Bactericidal activity of human milk: Stability during storage. *Br J Biomed Sci* 2006;63:59–62.
28. Ogundele M. Effects of storage on the physicochemical and antibacterial properties of human milk. *Br J Biomed Sci* 2002;59:205–211.
29. Slutzah M, Codipilly C, Potak D, et al. Refrigerator storage of expressed human milk in the neonatal intensive care unit. *J Pediatr* 2010;156:26–28.
30. Bertino E, Giribaldi M, Baro C, et al. Effect of prolonged refrigeration on the lipid profile, lipase activity, and oxidative status of human milk. *J Pediatr Gastroenterol Nutr* 2013;56:390–396.
31. Raoof NA, Adamkin DH, Radmacher PG, et al. Comparison of lactoferrin activity in fresh and stored human milk. *J Perinatol* 2016;36:207–209.
32. Rollo DE, Radmacher PG, Turcu RM, et al. Stability of lactoferrin in stored human milk. *J Perinatol* 2014;34: 284–286.
33. Ramírez-Santana C, Pérez-Cano FJ, Audí C, et al. Effects of cooling and freezing storage on the stability of bioactive factors in human colostrum. *J Dairy Sci* 2012;95: 2319–2325.
34. Marín ML, Arroyo R, Jiménez E, et al. Cold storage of human milk: Effect on its bacterial composition. *J Pediatr Gastroenterol Nutr* 2009;49:343–348.
35. USDA. Freezing and food storage. 2013. Available at https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/freezing-and-food-safety/ct_index/!ut/p/a1/jVFtT8IwEP417Nto55CgSWMWDCoiaFAZ-7IUetuajHa2h4K_3g4kEQNKm1zu5Xnae-5IQmKSKP4uc45SK17WcdJO6RNtBxdd2h9dBD16N3x9Gt13u7QzPneA6R-AYXgi_8iJ6H_8_gkfnJmH7kNOkopj4UuVaRLngD5X9gOMJXGmtfAtzwDXfsbn6NsCAF2hzvmbasGVKKXKHdgAfDrP0YX_g0niOaZSCViRCUn2u6KBu3fDcNy67Q9DOmr9BhwY2xZwfC5OeF7q2WZH00jNwo5TaCADA6a5NC5dIFb2skEb1AI386JZd7tttpnr91366o3tNHkzVDfNFaNbZ_3tsPE (Accessed April 2, 2017).
36. García-Lara NR, Escuder-Vieco D, García-Algar O, et al. Effect of freezing time on macronutrients and energy content of breastmilk. *Breastfeed Med* 2012;7:295–301.
37. Vázquez-Román S, Escuder-Vieco D, García-Lara NR, et al. Impact of freezing time on dornic acidity in three types of milk: Raw donor milk, mother's own milk, and pasteurized donor milk. *Breastfeed Med* 2016;11:91–93.
38. Romeu-Nadal M, Castellote A, Lopez-Sabater M. Effect of cold storage on vitamins C and E and fatty acids in human milk. *Food Chem* 2008;106:65–70.
39. Buss I, McGill F, Darlow B, et al. Vitamin C is reduced in human milk after storage. *Acta Paediatr* 2001;90:813–815.
40. Bank MR, Kirksey A, West K, et al. Effect of storage time and temperature on folacin and vitamin C levels in term and preterm human milk. *Am J Clin Nutr* 1985;41:235–242.
41. Ahrabi A, Handa D, Codipilly C, et al. Effects of extended freezer storage on the integrity of human milk. *J Pediatr* 2016;177:140–143.
42. Spitzer J, Klos K, Buettner A. Monitoring aroma changes during human milk storage at +4°C by sensory and quantification experiments. *Clin Nutr* 2013;32:1036–1042.
43. Sandgruber S, Much D, Amann-Gassner U, et al. Sensory and molecular characterisation of the protective effect of storage at −80°C on the odour profiles of human milk. *Food Chem* 2012;130:236–242.
44. Handa D, Ahrabi AF, Codipilly CN, et al. Do thawing and warming affect the integrity of human milk? *J Perinatol* 2014;34:863–866.
45. Lönnerdal B. Bioactive proteins in breast milk. *J Paediatr Child Health* 2013;49 Suppl 1:1–7.
46. Akinbi H, Meinzen-Derr J, Auer C, et al. Alterations in the host defense properties of human milk following prolonged storage or pasteurization. *J Pediatr Gastroenterol Nutr* 2010;51:347–352.
47. Thatrimontrichai A, Janjindamai W, Puwanant M. Fat loss in thawed breast milk: Comparison between refrigerator and warm water. *Indian Pediatr* 2012;49:877–880.
48. Bransburg-Zabary S, Virozub A, Mimouni FB. Human milk warming temperatures using a simulation of currently available storage and warming methods. *PLoS One* 2015; 10:e0128806.
49. Ovesen L, Jakobsen J, Leth T, et al. The effect of microwave heating on vitamins B1 and E, and linoleic and linolenic acids, and immunoglobulins in human milk. *Int J Food Sci Nutr* 1996;47:427–436.
50. Quan R, Yang C, Rubinstein S, et al. Effects of microwave radiation on anti-infective factors in human milk. *Pediatrics* 1992;89:667–669.

51. Sigman M, Burke K, Swarner O, et al. Effects of microwaving human milk: Changes in IgA content and bacterial count. *J Am Diet Assoc* 1989;89:690–692.
52. Hernandez J, Lemons P, Lemons J, et al. Effect of storage processes on the bacterial growth-inhibiting activity of human breast milk. *Pediatrics* 1979;63:597–601.
53. CDC. Are special precautions required for handling breast milk? 2015. Available at https://www.cdc.gov/breastfeeding/faq/#Precautions (accessed June 26, 2017).
54. Delgado S, Arroyo R, Jimenez E, et al. Mastitis infecciosas durante la lactancia: Un problema infravalorado. *Acta Pediatr Esp* 2009;67:564–571.
55. Heikkilä M, Saris P. Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. *J Appl Microbiol* 2003;95:471–478.

ABM protocols expire 5 years from the date of publication.

Content of this protocol is up-to-date at the time of publication. Evidence-based revisions are made within 5 years or sooner if there are significant changes in the evidence.

The 2004 and 2010 editions of this protocol were authored by Anne Eglash.

The Academy of Breastfeeding Medicine Protocol Committee:

Wendy Brodribb, MBBS, PhD, FABM, Chairperson
Sarah Reece-Stremtan, MD, Co-Chairperson
Larry Noble, MD, FABM, Translations Chairperson
Nancy Brent, MD
Maya Bunik, MD, MSPH, FABM,
Cadey Harrel, MD
Ruth A. Lawrence, MD, FABM
Yvonne LeFort, MD, FABM
Kathleen A. Marinelli, MD, FABM
Casey Rosen-Carole, MD, MPH, MSEd
Susan Rothenberg, MD
Tomoko Seo, MD, FABM
Rose St. Fleur, MD
Michal Young, MD

For correspondence: abm@bfmed.org

This article has been cited by:

1. Hernández-Aguilar Maria-Teresa, Bartick Melissa, Schreck Paula, Harrel Cadey, The Academy of Breastfeeding Medicine. 2018. ABM Clinical Protocol #7: Model Maternity Policy Supportive of Breastfeeding. *Breastfeeding Medicine* **13**:9, 559-574. [Abstract] [Full Text] [PDF] [PDF Plus]
2. Enrico Bertino, Chiara Peila, Francesco Cresi, Elena Maggiora, Stefano Sottemano, Diego Gazzolo, Sertac Arslanoglu, Alessandra Coscia. 2018. Donor Human Milk: Effects of Storage and Heat Treatment on Oxidative Stress Markers. *Frontiers in Pediatrics* **6**. . [Crossref]
3. Caroline Steele. 2018. Best Practices for Handling and Administration of Expressed Human Milk and Donor Human Milk for Hospitalized Preterm Infants. *Frontiers in Nutrition* **5**. . [Crossref]
4. Schlotterer Hannah R., Perrin Maryanne T.. 2018. Effects of Refrigerated and Frozen Storage on Holder-Pasteurized Donor Human Milk: A Systematic Review. *Breastfeeding Medicine* **13**:7, 465-472. [Abstract] [Full Text] [PDF] [PDF Plus]
5. 2018. Correction to: ABM Clinical Protocol #8: Human Milk Storage Information for Home Use for Full-Term Infants, Revised 2017, by Eglash A, Simon L, and The Academy of Breastfeeding Medicine Breastfeed Med 2017;12(7):390–395. DOI: 10.1089/dna.2017.29047.aje. *Breastfeeding Medicine* **13**:6, 459-459. [Citation] [Full Text] [PDF] [PDF Plus]
6. Sriraman Natasha K., Evans Amy E., Lawrence Robert, Noble Lawrence, the Academy of Breastfeeding Medicine's Board of Directors. 2018. Academy of Breastfeeding Medicine's 2017 Position Statement on Informal Breast Milk Sharing for the Term Healthy Infant. *Breastfeeding Medicine* **13**:1, 2-4. [Citation] [Full Text] [PDF] [PDF Plus]
7. FouziaAbdulaziz AlHreashy. 2018. Non-maternal nursing in the muslim community: A health perspective review. *Journal of Clinical Neonatology* **7**:4, 191. [Crossref]

Acta Ophthalmologica

Thesis

Retinal oximetry and systemic arterial oxygen levels

Thorunn Scheving Eliasdottir CRNA

Department of Ophthalmology, University of Iceland, Reykjavik, Iceland

Acta Ophthalmol. 2018: 96, thesis 113: 1–44

© 2018 Acta Ophthalmologica Scandinavica Foundation. Published by John Wiley & Sons Ltd

doi: 10.1111/aos.13932

Thesis for the degree of Philosophiae Doctor

Supervisor:
Professor Guðrún Kristjánsdóttir, DrPH., Ph.D.

Advisor:
Professor Einar Stefánsson, M.D., Ph.D.

Doctoral committee:
Professor Einar Stefánsson, M.D., Ph.D.

Professor Guðrún Kristjánsdóttir, DrPH., Ph.D.

Professor Charles Vacchiano, CRNA., Ph.D.

Professor Þórarinn Gíslason, M.D., Ph.D.

Professor Gísli Heimir Sigurðsson, M.D., Ph.D.

June 2017

Thesis for a doctoral degree at the University of Iceland. All right reserved. No part of this publication may be reproduced in any form without the prior permission of the copyright holder.
© Þórunn Scheving Elíasdóttir 2017

ISBN 978-9935-9313-4-4
Printing by Háskólaprent
Reykjavik, Iceland 2017

ABSTRACT.

Purpose: Continuous peripheral pulse oximetry for monitoring adequacy of oxygenation is probably the most important technological advance for patients' monitoring and safety in the last decades. Pulse oximetry has the disadvantage of measuring the peripheral circulation, and the only mean to measure oxygen content of the central circulation is by invasive technology. Determination of blood oxyhaemoglobin saturation in the retinal vessels of the eye can be achieved noninvasively through spectrophotometric retinal oximetry which provides access to the central nervous system circulation. The aim of the thesis was to determine whether retinal oximetry technique can be applied for estimation of the central nervous system circulation which until now has only been possible invasively. This was achieved by measuring oxyhaemoglobin saturation in three adult subject study groups: in people with central retinal vein occlusion (CRVO) to observe local tissue hypoxia, in patients with severe chronic obstructive pulmonary disease (COPD) on long-term oxygen therapy to observe systemic hypoxaemia and in healthy subjects during hyperoxic breathing to observe systemic hyperoxemia. In addition, the fourth study that is mentioned was performed to test whether retinal oximetry is feasible for neonates.

Methods: *Retinal oximetry in central retinal vein occlusion:* Sixteen subjects with central retinal vein occlusion participated in the study. The oxyhaemoglobin saturation of the central retinal vein occlusion affected eye was compared with the fellow unaffected eye. *Retinal oximetry in healthy people under hyperoxia:* Thirty healthy subjects participated in the study, and the oxyhaemoglobin saturation of retinal arterioles and venules was compared between normoxic and hyperoxic breathing. *Retinal oximetry in severe chronic obstructive pulmonary disease:* Eleven patients with severe chronic obstructive pulmonary disease participated in the study. Retinal oximetry measurements were made with and without their daily supplemental oxygen therapy. Retinal arteriolar oxyhaemoglobin saturation when inspiring ambient air was compared with blood samples from the radial artery and finger pulse oximetry and healthy controls. The healthy control group was assembled from our database for comparison of oxyhaemoglobin saturation of retinal arterioles and venules during the ambient air breathing. The retinal oximeter is based on a conventional fundus camera and a specialized software. A beam splitter coupled with two high-resolution digital cameras allows for simultaneous acquisition of retinal images at separative wavelengths for calculation of oxyhaemoglobin saturation. In addition, retinal images of 28 full-term healthy neonates were obtained with scanning laser ophthalmoscope combined with modified Oxymap analysis software for calculation of the optical density ratio and vessel diameter

Results: *Retinal oximetry in central retinal vein occlusion:* Mean retinal venous oxyhaemoglobin saturation was 31 ± 12% in CRVO eyes and 52 ± 11% in unaffected fellow eyes (mean ± SD, $n = 14$, $p < 0.0001$). The arteriovenous oxygen difference (AV-difference) was 63 ± 11% in CRVO eyes and 43 ± 7% in fellow eyes ($p < 0.0001$). The variability of retinal venous oxyhaemoglobin saturation was considerable within and between eyes affected by CRVO. There was no difference in oxyhaemoglobin saturation of retinal arterioles between the CRVO eyes and the unaffected eyes ($p = 0.49$). *Retinal oximetry in healthy people under hyperoxia:* During hyperoxic breathing, the oxyhaemoglobin saturation in retinal arterioles increased to 94.5 ± 3.8% as compared with 92.0 ± 3.7% at baseline ($n = 30$, $p < 0.0001$). In venules, the mean oxyhaemoglobin saturation increased to 76.2 ± 8.0% from 51.3 ± 5.6% ($p < 0.0001$) at baseline. The AV-difference was markedly lower during hyperoxic breathing as compared with the normoxic breathing (18.3 ± 9.0% versus 40.7 ± 5.7%, $p < 0.0001$). *Retinal oximetry in severe chronic obstructive pulmonary disease:* During ambient air breathing, chronic obstructive pulmonary disease subjects had significantly lower oxyhaemoglobin saturation than healthy controls in both retinal arterioles (87.2 ± 4.9% versus 93.4 ± 4.3%, $p = 0.02$, $n = 11$) and venules (45.0 ± 10.3% versus 55.2 ± 5.5%, $p = 0.01$) but the AV-difference was not markedly different ($p = 0.17$). Administration of their prescribed oxygen therapy significantly increased the oxyhaemoglobin saturation in retinal arterioles (87.2 ± 4.9% to 89.5 ± 6.0%, $p = 0.02$) but not in venules (45.0 ± 10.3% to 46.7 ± 12.8%, $p = 0.3$). Retinal oximetry values were slightly lower than finger pulse oximetry (mean percentage points difference = −3.1 ± 5.5) and radial artery blood values (−5.0 ± 5.4). *Retinal oximetry study in neonates:* The modified version of the retinal oximetry instrument estimated the optical density ratio in retinal arterioles to be 0.256 ± 0.041 that was significantly different from the 0.421 ± 0.089 in venules ($n = 28$, $p < 0.001$, paired *t*-test). The vascular diameter of retinal arterioles was markedly narrower than of venules (14.1 ± 2.7 and 19.7 ± 3.7 pixels, $p < 0.001$).

Conclusion: The results of this thesis indicate that spectrophotometric retinal oximetry is sensitive to both local and systemic changes in oxyhaemoglobin saturation. Retinal oxyhaemoglobin saturation values are slightly lower than radial artery blood sample and finger pulse oximetry values. The discrepancies between the different modalities are expected to derive from countercurrent exchange between central retinal artery and vein within the optic nerve but calibration issues cannot be excluded as contributing to this difference. Despite these differences, the findings indicate the potential of retinal oximetry for noninvasive real-time measurements of oxyhaemoglobin saturation in central nervous system vessels. Following calibration upgrade and technological improvement, verification retinal oximetry may potentially be applied to critically ill and anaesthesia care patients. The study on combined scanning laser ophthalmoscope and retinal oximetry supports the feasibility of the technique for oximetry analysis in newly born babies.

Key words: central retinal vein occlusion – chronic obstructive pulmonary disease – oximetry – retinal vessels – systemic circulation

ÁGRIP.

Tilgangur: Innleiðing púlsoximælinga til samfelldrar mælingar á súrefnismettun blóðs er ein mikilvægasta tækniþróun undanfarinna áratuga fyrir öryggi og vöktun sjúklinga. Þær hafa þó þann veikleika að mæla útæðar (peripheral circulation) og eina leiðin til að mæla súrefnisstyrk í miðlægum æðum er með ífarandi slagæðamælingum. Mæling á súrefnismettun í sjónhimnuæðum augans með sjónhimnu-súrefnismæli er hins vegar mæling á miðlægum æðum án ífarandi tækni. Sjónhimnan er hluti miðtaugakerfisins og eru sjónhimnuæðar því miðlægar æðar, sem samsvara súrefnisástandi miðtaugakerfisins að nokkru leyti.Meginmarkmið verkefnisins er að meta hvort hægt sé að nota sjónhimnu-súrefnismælingar til að mæla súrefnismettun í miðlægri blóðrás sem hingað til hefur ekki verið mögulegt nema með ífarandi inngripum. Sannreynd er geta tækisins til að meta súrefnismettun í miðlægum æðum með því að skoða þrjá hópa fullorðins fólks; Fólks með miðbláæðarlokun (central retinal vein occlusion, CRVO) sem veldur staðbundnum súrefnisskorti í innri sjónhimnunni, sjúklinga með alvarlega langvinna lungnateppu (chronic obstructive pulmonary disease, COPD) sem einkennist af kerfisbundnum súrefnisskorti og heilbrigða einstaklinga til að meta kerfisbundin áhrif innandaðs súrefnis. Fjórða rannsóknin sem komið er inná var framkvæmd til að meta hvort sjónhimnu-súrefnismælingar eru álitlegur kostur fyrir nýbura.

Aðferðir: *Sjónhimnu-súrefnismælingar í miðbláæðarlokun:* Sextán einstaklingar með miðbláæðarlokun tóku þátt í rannsókninni og var súrefnismettun augans með bláæðastífluna borin saman við súrefnismettun í gagnstæða auganu. *Sjónhimnu-súrefnismælingar hjá heilbrigðum við innöndun 100% súrefnis:* Þrjátíu heilbrigðir einstaklingar tóku þátt í rannsókninni og var súrefnismettun sjónhimnuæða við innöndun á andrúmslofti borin saman við innöndun 100% súrefnis. *Sjónhimnu-súrefnismælingar í alvarlegri langvinnri lungnateppu:* Ellefu einstaklingar með alvarlega langvinna lungnateppu með varanlega þörf fyrir súrefni tóku þátt í rannsókninni. Súrefnismettun sjónhimnuæða hægra augans var mæld bæði með og án súrefnismeðferðar. Niðurstöðurnar voru bornar saman og jafnframt gerður samanburður án súrefnismeðferðar við blóðsýni frá sveifarslagæð, við fingurmælingu (pulse oximeter) og heilbrigðan samanburðarhóp sem fengin var úr gagnagrunni sem rannsóknarhópurinn hafði áður safnað. Súrefnismælirinn samanstendur af hefðbundinni augnbotnamyndvél og sérstökum hugbúnaði sem les úr myndunum. Ljósdeilir sér til þess að tvær stafrænarmyndavélar taka samtímis myndir af sama svæðinu með sitthvorri bylgjulengdinni fyrir útreikninga á

súrefnismettun sjónhimnuæða. *Sjónhimnu-súrefnismælingar í nýburum:* Að auki voru teknar myndir af 28 fullbura nýburum með laser skanna augnbotnamyndavél og fyrrgreindum hugbúnaði sem búið var að aðlaga laser skanna tækninni, til útreikninga á æðavídd og ljósþéttnihlutfalli í slag- og bláæðlingum.
Niðurstöður: *Sjónhimnu-súrefnismælingar í miðbláæðarlokun:* Meðaltal súrefnismettunar í bláæðlingum augna með miðbláæðarlokun mældist 31 ± 12% og 52 ± 11% í gagnstæðum augum (meðaltal ± staðalfrávik, $n = 14$, $p < 0.0001$). Mismunur súrefnismettunar í slag- og bláæðlingum mældist 63 ± 11% í augum með miðbláæðarlokun og 43 ± 7% í gagnstæðum augum ($p < 0.0001$). Breytileiki bláæðamettunar reyndist umtalsverður bæði innan augna og milli augna með miðbláæðarlokun. Ekki reyndist munur á súrefnismettun í slagæðlingum augna með miðbláæðarlokun og í gagnstæðum augum ($p = 0.49$). *Sjónhimnu-súrefnismælingar hjá heilbrigðum við innöndun 100% súrefnis:* Innöndun 100% súrefnis jók súrefnismettun slagæðlinga í 94.5 ± 3.8% til samanburðar við 92.0 ± 3.7% áður en hún hófst ($n = 30$, $p < 0.0001$). Í bláæðlingum jókst súrefnismettunin í 76.2 ± 8.0% frá 51.3 ± 5.6% ($p < 0.0001$) áður en innöndunin hófst. Mismunur súrefnismettunar í slag- og bláæðlingum lækkaði marktækt á meðan á innöndun súrefnisins stóð (18.3 ± 9.0% versus 40.7 ± 5.7% áður, $p < 0.0001$). *Sjónhimnu-súrefnismælingar í alvarlegri langvinnri lungnateppu:* Án súrefnismeðferðar mældist sjónhimnu-súrefnismettunin marktækt lægri hjá fólki með alvarlega langvinna lungnateppu en hjá heilbrigða samanburðarhópnum bæði í slag- (87.2 ± 4.9% versus 93.4 ± 4.3%, $p = 0.02$, $n = 11$) og í bláæðlingum (45.0 ± 10.3% versus 55.2 ± 5.5%, $p = 0.01$). Ekki reyndist mærktækur munur á mismuni súrefnismettunar í slag- og bláæðlingum milli þessara hópa ($p = 0.17$). Innöndun súrefnismeðferðar jók marktækt súrefnismettunina í slagæðlingum (87.2 ± 4.9% versus 89.5 ± 6.0%, $p = 0.02$) en ekki í bláæðlingum (45.0 ± 10.3% versus 46.7 ± 12.8%, $p = 0.3$). Sjónhimnu-súrefnismælingarnar sýndu lítið eitt lægri gildi en fingurmælingar (mean percentage points difference = −3.1 ± 5.5) og ífarandi slagæðamælingar (−5.0 ± 5.4). *Sjónhimnu-súrefnismælingar í nýburum:* Ljósþéttnihlutfallið í slagæðlingum sjónhimnunnar mældist marktækt lægra en í bláæðlingum (0.256 ± 0.041 versus 421 ± 0.089, $n = 28$, $p < 0.001$, parað t-próf). Æðavídd slagæðlinga reyndist marktækt minni en í blæðlingum (14.1 ± 2.7 versus 19.7 ± 3.7 pixlar, $p < 0.001$).
Ályktanir: Niðurstöður rannsóknanna sýna að sjónhimnu-súrefnismælirinn er næmur fyrir staðbundnum og kerfisbundnum breytingum á súrefnismettun í miðlægum æðum. Sjónhimnu-súrefnismælingar sýna eilítið lægri gildi en slagæða- og fingurmælingar. Mismuninn má að öllum líkindum rekja til nálægrar legu miðslagæðarinnar við miðbláæðina innan sjóntaugarinnar (countercurrent exchange) og kvörðunar á sjónhimnu-súrefnismælinum. Þrátt fyrir þennan mun, gefa rannsóknirnar vísbendingar um að víkka megi notagildi tækisins yfir í mælingar á súrefnisbúskap í miðlægum æðum blóðrásarinnar. Endurskoða þarf kvörðunina á mælitækinu og með tilkomu tækniframfara er mögulega unnt að sannreyna gildi mæliaðferðarinnar á svæfingadeildum og hjá alvarlega veikum sjúklingum á gjörgæslu. Rannsóknin á ungabörnunum gefur vísbendingar um að sjónhimnu-súrefnismælingar séu álitlegur kostur til mats á súrefnismettun hjá nýburum.
Lykilorð: Sjónhimnuæðar, súrefnismælingar, miðlæg blóðrás, miðbláæðalokun í sjónhimnu, langvinn lungnateppa.

Introduction

In situations of caring for patients in the intensive care units (ICU), in acute care settings and under sedation and general anaesthesia continuous monitoring of oxyhaemoglobin saturation using noninvasive peripheral pulse oximetry has become a standard of care. The peripheral pulse oximetry, however, depends on pulsatile arterial blood volume, and its measurements are therefore limited by inadequate tissue perfusion accompanying peripheral vasoconstriction. Clinical experience yields it difficult to obtain measurements under such conditions and may leave no other options but invasive measures. Unlike the central nervous system which is protected and preferred in shock and severe illnesses, peripheral pulse oximeter measurements do not represent the central vasculature. The development of a noninvasive retinal oximeter (Hardarson et al. 2006) to measure oxyhaemoglobin saturation in retinal vessels provides a prospect for central vascular oximetry. The retinal arterioles are derived from the ophthalmic artery which is the first branch from the internal carotid artery, and represents the central vasculature in the central nervous system. Presuming the retinal arterial oxygen content is identical to the systemic circulation, retinal oximetry may provide relevant information on oxygen delivery to the central nervous system. Such a method may enhance the monitoring and treatment of critically ill patients in the ICU, in the field of emergency and anaesthesia care. Thus, the aim of the thesis was to determine whether retinal oximetry technique can be applied for estimation of the central nervous system circulation which until now has only been possible through invasive measures.

Oxyhaemoglobin saturation monitoring

For survival of human beings, oxygen delivery to tissues must be sufficient to meet minimal oxygen consumption for cellular metabolism. Insufficient capillary oxygen supply leads to impaired cellular respiration (oxidative phosphorylation) and energy production that may rapidly progress to hypoxic injury and eventually death (Scheufler 2004). Early recognition of inadequate oxygen delivery and prompt intervention is therefore crucial for survival and health outcome of patients who are critically ill and in unstable hemodynamic conditions (Perel 2015).

Numerous techniques have been applied to monitoring oxygen delivery and tissue oxygenation but few have proceeded into routine clinical practice. Direct measurements of oxygen partial pressure (PO_2) with polarographic microelectrodes require a needle to be

inserted into the preferred tissue, for example to assess oxygenation of the brain. Reflectance spectrometry is a noninvasive alternative technique for continuous monitoring of microvascular oxyhaemoglobin saturation and intracellular oxygen availability (Scheufler 2004; Carreau et al. 2011). In clinical practice, the oxygen delivery by the systemic circulation can either be accessed directly by arterial blood gas analysis of oxygen partial pressure (PaO_2) and oxyhaemoglobin saturation or determined indirectly by transcutaneous pulse oximetry.

Arterial blood gas monitoring

Invasive arterial blood gas monitoring necessitates intermittent direct arterial blood sampling for estimation of oxyhaemoglobin saturation, most commonly from a peripheral radial artery or femoral artery in the groin. The arterial blood sample is processed on arterial blood gas analyser which calculates the estimated oxyhaemoglobin saturation based on empirical equations, operating PO_2 and pH values. Invasive arterial blood gas analysis is considered the gold standard technique for estimation on oxyhaemoglobin saturation (Haymond 2006; Collins et al. 2015), especially in critically ill patients where precision and accurate values are necessary for treatment and health outcome.

Transcutaneous pulse oximetry

The global marketing of transcutaneous pulse oximetry in the mid-eighties and coinciding reduction (90%) in anaesthesia-related fatalities (Severinghaus 2007) marked a milestone in patient monitoring care. Since then, complimentary continuous pulse oximetry has become a routine standard of care whenever tissue oxygenation is jeopardized such as in acute care settings and anaesthesia practice. Concurrently, its establishment is considered the most important technological monitoring advances for patients' safety (Severinghaus 2011) and widely viewed as the fifth vital sign in aforementioned hospital and out-of-hospital settings.

Pulse oximetry is based on photoplethysmography which calculate light absorption amplification as transmitted light intensity lessens when peripheral arterial blood volume increases during systolic left ventricular ejection. The stroke volume permits arterial blood saturation to be distinguished from venous blood saturation and is responsible for the pulsatile nature of this technique. Pulse oximetry incorporates the optical technique of difference in dual light absorption spectra to distinguish the oxyhaemoglobin from deoxyhaemoglobin in arterial blood. Deoxyhaemoglobin absorbs greater amount of red light (660 nm) whereas oxyhaemoglobin absorbs greater amount of near-infrared light (940 nm). The greater absorbability of near-infrared light and the scattering of red light is the reason for the oxygen-rich arterial blood to be distinguished from the oxygen poor venous blood.

The oximeter probe is most commonly put on a finger (also earlobe, toe and nose) where two light-emitting diodes emit the two different wavelengths of light through the peripheral vascular bed. A photodiode on the opposite site of the tissue receives the transmitted red and near-infrared light for calculation of their relative amount of oxygenated haemoglobin. Eventually, the arterial oxyhaemoglobin saturation is illustrated as photoplethysmographic waveform and digital number display (Sinex 1999; Alian et al. 2011; Chan & Chan 2013; Nitzan e al. 2014).

Several studies (Shamir et al. 1999; Golparvar et al. 2002; Westphal et al. 2009) have suggested the possible role of pulse oximetry other than monitoring arterial oxyhaemoglobin saturation and pulse rate. In this respect, a pulse oximetry analysis of the plethysmographic waveform has been proposed to give useful information on hemodynamic changes, including fluid volume status (Cannesson et al. 2007; McGrath et al. 2011) and cardiac arrhythmias (Cripps et al. 1992; Marinskis et al. 2006) in critically ill patients.

Limitations of peripheral pulse oximetry

For noninvasive pulse oximeter, the empirical calibration route is carried out on healthy volunteers with simultaneous assessment of the standard deviation of difference between oxyhaemoglobin saturation values obtained by pulse oximetry and invasive arterial blood sample. In general, the proclaimed accuracy of a pulse oximeter measurement is 2%. Derived from clinical studies on critically ill patients and preterm neonates, this number translates into a probability of an intrinsic error of 3–4% (Nitzan et al. 2014). Such discrepancy may have enormous impact on patients, when accurate values are warrant for precision of their titrated supplemental oxygen therapy.

Studies have, in general, reported good correlations between oxyhaemoglobin saturation values obtained by pulse oximetry and invasive arterial blood gas measures over the range of 70% to 100% in healthy people and patients with adequate perfusion. This correlation is, however, lost and pulse oximetry readings become inaccurate in patients with inadequate tissue perfusion and under hypoxic condition (Trivedi et al. 1997; Van de Louw et al. 2001; Perkins et al. 2003; Wilson et al. 2010).

Peripheral vasoconstriction is one of the earliest response to compromised central blood volume (Dutton 2007; Scheeren et al. 2012), severe hypoxia (Heistad & Abboud 1980) and acute pain (Hoiseth et al. 2015), signalling the acute sympathetic nervous system response for redistribution of blood flow from lower priority organs to vital organs, including the central nervous system (Dutton 2007). Because peripheral pulse oximetry depends on pulsatile arterial blood volume, their usage can be limited by inadequate tissue perfusion (Chan & Chan 2013; Nitzan et al. 2014). Under such circumstances, it may become difficult or even impossible to obtain sufficient signal for a pulse oximetry reading from peripheral vascular bed. In case of hypoxaemia, the pulse oximeter measurement may also lag behind the real-time oxygen deterioration of arterial blood (Fouzas et al. 2011).

Ongoing efforts to improve noninvasive oximetry

Despite recent advances and ongoing efforts to improve the existing technology, noninvasive oximetry modalities are still inferior to invasive monitoring. The drawback of current technology underpins the need for ongoing endeavour to improve and develop noninvasive method for estimation of oxyhaemoglobin saturation. Concurrently, near-infrared spectroscopy has gained increased attention in the acute patient care. Unlike pulse oximetry, cerebral and tissue near-infrared spectroscopy oximetry is independent of the pulsatile blood flow. It uses fixed

and relative 70% venous and 30% arterial blood to estimate capillary saturation and therefore tissue oxygen-haemoglobin saturation. For that reason, transcutaneous cerebral near-infrared spectroscopy offers information on intracerebral tissue oxygen supply and metabolic demand rather than cerebral oxygen delivery itself (Ikeda et al. 2014; Steppan & Hogue 2014).

Retinal oximetry

Measurements of oxyhaemoglobin saturation in retinal vessels may be a more direct indicator of oxygen delivery to the brain than the peripheral pulse oximetry or a cerebral near-infrared spectroscopy. In numerous clinical situations counting critical care, operating rooms, emergency departments and out-of-hospital traumatic injury, patients may suffer circulatory shock where peripheral vasoconstriction limits the pulse oximetry readings but ocular and cerebral perfusion are preserved (Denninghoff et al. 2003; Riva et al. 2011). For that reason, measurement of central nervous system vessels with retinal oximetry may be more reliable mean for estimation of oxygen delivery to the brain and to guide resuscitation.

Spectrophotometric retinal oximetry captures images of the retinal circulation for the calculation of oxyhaemoglobin saturation and thus an estimation of oxygen delivery to the central nervous system by the central circulation. Such method might be highly valuable and mark a milestone in patient care, especially for those patients at risk for central nervous system hypoxia. In addition to arterial oxygen delivery, the retinal oximetry allows direct noninvasive assessment of venous oxyhaemoglobin saturation and hence has the potential for microcircular hemodynamic assessment as well. Therefore, the method could be an important step in the development of improved approach for measurement oxyhaemoglobin saturation of the human body, imaging the only place where arterial and venous blood can be directly imaged with visible light.

Retina

The transparent structure of the eye allows images as light waves to pass through the cornea, aqueous humour, lens and vitreous humour before hitting the retina that lines the back of the eye (Fig. 1). The retina is a part of the central nervous system and formed embryonically as an outgrowth of the forebrain. It is approximately 0.3 mm thick and composed of numerous cell and nerve fibre layers the light must enter before reaching the photoreceptors in the outer retina. Once the light gets to the photoreceptors, it is transformed into nerve signals and transmitted by the optic nerve to the cerebral cortex for recreation into a visual image (Levin et al. 2011; Kolb 2012).

The optic disc (optic nerve head) is a white circular spot in the central retina (Fig. 2). On the temporal side in the centre of the retina is the macula, an avascular oval-shaped area, approximately 5 mm in diameter. The macula constitutes the fovea, which is densely packed with cones, the photoreceptors that are essential for sharp and detailed colour vision. The rods are the photoreceptors for black and white vision of dim light and are predominantly found outside the macula, in the periphery of the retina.

Retinal oxygenation

Ocular blood flow

The human retina is supported by two vascular systems which differ both anatomically and physiologically: the retinal circulation and the choroidal circulation. The retinal circulation supports the inner two-thirds of the retina whereas the choroidal vasculature supplies the outer third of the retina with at least 85% of the total ocular blood flow (Alm & Bill 1973; Nickla & Wallman 2010). Both these circulations arise from the ophthalmic artery, which supplies the entire eye. The ophthalmic artery is the first branch emanating the internal carotid artery on its way carrying metabolic substrates and oxygenated blood (Riva et al. 2011) from the aorta to the brain. The ophthalmic artery divides into the central retinal artery and ciliary arteries to supply the inner and outer retina at the back of the eye, respectively (Riva et al. 2011).

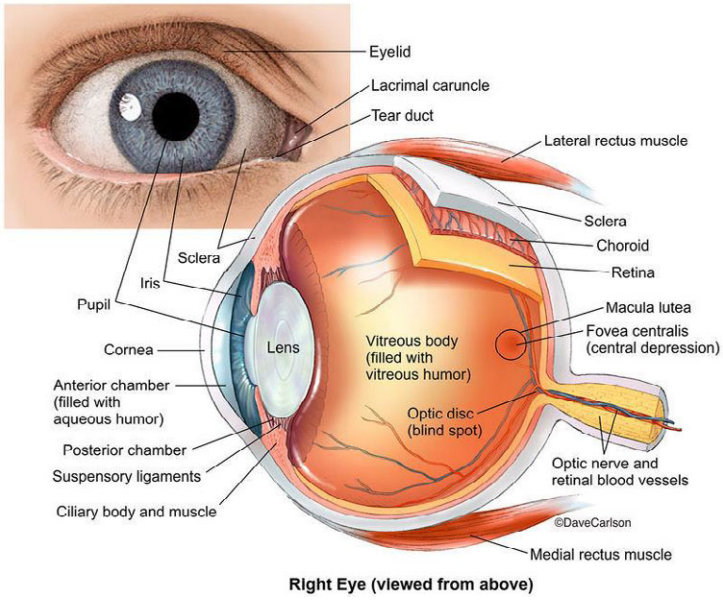


Fig. 1. A schematic view of the tissues of the eye. Illustration ©Dave Carlson/CarlsonStockArt.com.

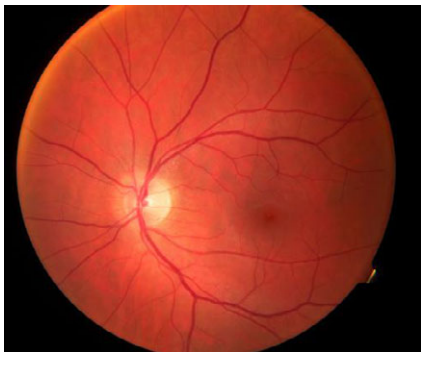


Fig. 2. Normal fundus photography of the left eye with retinal arterioles and venules piercing the optic disc. The dark spot in the centre is the macula consisting the fovea.

Retinal circulation. The central retinal artery (CRA) penetrates the optic nerve sheath about 10 mm behind the globe (Fig. 3). It runs centrally within the optic nerve to the optic disc where it bifurcates into superior and inferior retinal branches. These branches in turn divide into temporal and nasal arterioles where each supplies one quadrant of the inner retina (Pournaras et al. 2008; Hayreh 2011). The retinal circulation is an end-arterial circuit without anatomizes. Retinal arterioles arcade by dichotomous and side-arm branching towards the periphery, until terminating in two-layered capillary plexuses that connect with postcapillary venules. Larger arterioles and venules are found in the innermost layers of the retina at the inner limiting membrane and the outer plexiform layer. Capillary density is greatest in the centre of the retina with the more superficial capillary plexus situated in the ganglion cell and nerve fibre layer. The second capillary plexus lies deeper, at the border of the inner nuclear and outer plexiform layer with a single-layered capillary network proceeding until it finally vanishes, leaving an avascular zone in the farthest retinal periphery. In addition, a single-layered capillary network surrounds the area of the avascular fovea as well as the superficial peripapillary capillaries that enclose the optic disc, to chase the superior and inferior temporal retinal vessels.

The retinal venous system follows a similar pattern to the arteriolar structure. It runs independently in the periphery of the retina but in close proximity to the arterioles with occasionally crossing within the centre of the retina. The postcapillary venules drain into major branch venules that merge at the optic nerve head to form a central retinal vein (CRV). The CRV leaves the eye adjacent (temporal) to the CRA within the optic nerve before emptying either into the ophthalmic vein or directly into the cavernous sinus (Pournaras et al. 2008; Hayreh 2011; Riva et al. 2011).

Choroidal circulation. The choroidal circulation is located between the outer retina and the sclera that constitute the outermost membrane of the eye. It originates in 2–3 main ciliary arteries coming off the ophthalmic artery to supply the temporal and nasal portions of the choroidal sphere. Main ciliary arteries branches into 10–20 short posterior ciliary arteries and two long posterior ciliary arteries to form major choroidal arteries that support the posterior and anterior portion of the choroid, respectively. The shortest posterior ciliary arteries aim for a vascular structure near the macula to nourish the vicinity of fovea. Occasionally, a cilioretinal artery arises directly from a short posterior ciliary artery or the peripapillary choroid. It normally pierces the retina temporal to the optic disc to nourish the macular area but the location and contribution to the oxygen supply vary.

The choroid is made of three layers: the outermost Haller's layer of large vessels, the inner Sattler's layer of medium and small arteries and arterioles and the dense innermost network of single choriocapillary layer. Choriocapillary blood drains into the vortex veins before entering orbital veins (Hayreh 1975, 2011; Nickla & Wallman 2010; Riva et al. 2011) and eventually emptying into the cavernous sinus.

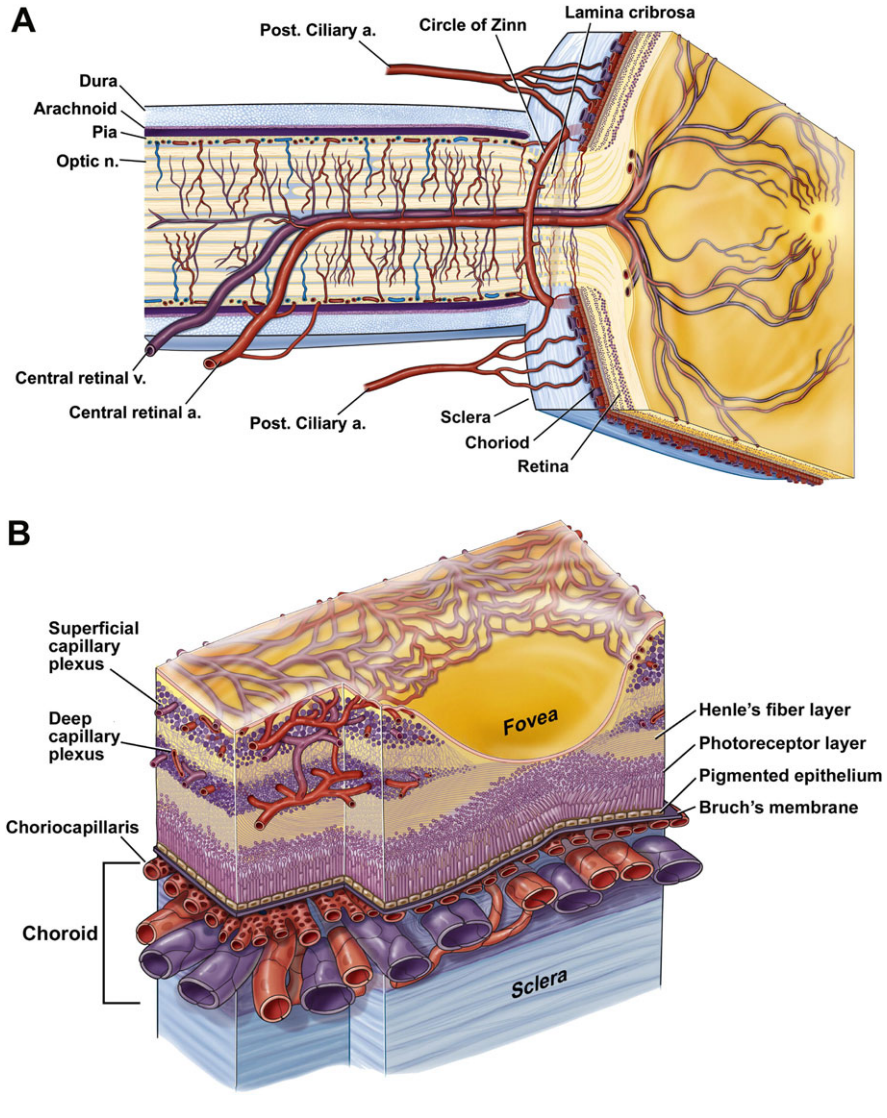


Fig. 3. Anatomy of ocular circulation (a – artery, b – vein, *n* – nerve). (A) Cut away drawing along the superior–inferior axis of the human eye through the optic nerve, showing the vascular supply to the retina and choroid. (B) Drawing showing vasculature of the retina and choroid. Drawings by Dave Schumick from Anand-Apte and Hollyfield (2009). Reprinted from Prog Retin Eye Res, 31(5), Kur et al. Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease, 377–406, © 2012, with permission of Elsevier Ltd.

Retinal metabolism

The retina is metabolically one of the most active tissues in the body, consuming oxygen faster than the brain

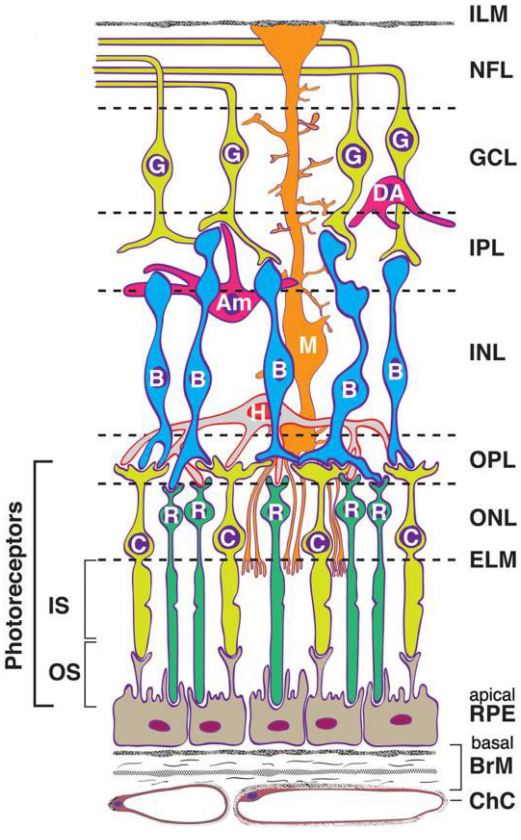


Fig. 4. Chorioretinal layers and major cell types. Modified from Zheng et al. (2012). The neurosensory retina has distinct layers (from top to bottom): inner limiting membrane (ILM), nerve fibre layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor inner segments (IS) and photoreceptor outer segments (OS). The retinal pigment epithelium (RPE) lies outside the neurosensory retina but is considered a part of the retina embryologically. The major retinal cell types are ganglion cells (G), diffuse amacrine cells (DA), amacrine cells (Am), Müller cells (M), bipolar cells (B), horizontal cells (H), rods (R) and cones (C). Reprinted from Prog Retin Eye Res, 41(2014), Pikuleva & Curcio., Cholesterol in the retina: The best is yet to come, 64–89, ©2014, with permission of Elsevier Ltd.

whilst lacking the capacity of oxygen reserve (Wangsa-Wirawan & Linsenmeier 2003). Oxygen delivery by the two separate vascular systems must therefore be highly efficient to meet the metabolic demand of the retinal tissue as reflected by their intrinsic properties in virtue. The retinal circulation is characterized by slow rate of blood flow (Alm & Bill 1973; Wang et al. 2009; Werkmeister et al. 2015), high oxygen extraction (Werkmeister et al. 2015) and about 35% arteriovenous oxygen difference (Schweitzer et al. 1999). In contrast, the choroidal circulation has exceedingly high blood flow (Riva et al. 2011) and very low oxygen extraction fraction or only 3% (Nickla & Wallman 2010). Choriocapillaries have large diameter (≥10 μm) which generate low resistance (compared with 5 μm of retinal capillaries, offering higher resistance) that explains the high blood flow and low rate of oxygen extraction of the choroidal circulation. This create oxygen abundance gradient across the Bruch′s membrane to the avascular zone of outer retinal layers for the energy consuming activity of the photoreceptors (Linsenmeier & Padnick-Silver 2000).

Because the choroidal circulation is located immediately behind the retina, a sufficient oxygen flux from the choriocapillaries through the Bruch′s membrane and retinal pigment epithelium (Fig. 4) is of vital importance for normal function of the photoreceptors (Delaey & Van De Voorde 2000; Linsenmeier & Padnick-Silver 2000; Jackobiec's et al. 2008). From choriocapillaries, the PO_2 declines sharply across the outer retina until it reaches a very low minimum in the inner segments, at the location of mitochondria in photoreceptors (Linsenmeier & Padnick-Silver 2000). Photoreceptors carry out the most energy demanding function of the retina (Buttery et al. 1991) that is influenced by illumination. The metabolic activity is higher in dark than in light when the oxygen consumption exceeds 90% of the blood supply. In darkness thus, approximately 90% of the oxygen supply comes from the choroidal circulation and the remainder 10% diffuses from the retinal circulation. In light however, the oxygen flux from the choroidal circulation fulfils the photoreceptors metabolic need and some oxygen may even reach the inner retina as well (Stefánsson et al. 1983; Linsenmeier & Braun 1992; Linsenmeier & Padnick-Silver 2000; Hardarson et al. 2009; Nickla & Wallman 2010).

Unlike choroidal blood flow that appears to be unaffected by increased metabolic activity of the retinal tissue, the blood flow amplifies in the retinal circulation (Garhofer et al. 2002) to supply photoreceptor oxygen consumption under increased metabolic demand (in a dim light). This is manifested by sharp decline in oxygen diffusion across the outer nuclear layer to the inner segments (Linsenmeier & Padnick-Silver 2000) at the location of the photoreceptors at the outer retina.

The spatial differences of oxygen diffusion between the retinal and choroidal circulations depend on the location and local metabolic activity within the retinal tissue (Braun et al. 1992; Linsenmeier & Braun 1992). Studies have shown that the superior and inferior temporal quadrants of the retina receive higher blood flow (Feke et al. 1989) and consume more oxygen (Schweitzer et al. 1999) than the nasal hemisphere that most likely reflects the metabolic activity as previously discussed.

The photoreceptors include rods and cones and are responsible for light absorption (Fig. 4). Horizontal and bipolar cells transmit signals from the photoreceptors to the ganglion cells which carry them via optic nerve to the brain. The photoreceptors are situated in the outer retina and receive oxygen from the choriocapillaries (ChC) located outside the retinal pigment epithelium, adjacent to the Bruch′s membrane (BrM). The retinal

circulation supplies the inner part of the retina with capillaries reaching down to the plane of photoreceptors.

Autoregulation

Vascular beds in organs have an intrinsic capacity to some extent to regulate the perfusion pressure locally. This property is referred to as autoregulation and is produced by an intrinsic capacity for a stretch response of the arteriolar vascular smooth muscle. This ability for a local regulation maintains the blood flow relatively constant despite of variations in the perfusion pressure (Arjamaa & Nikinmaa 2006) and keeps the PO_2 of the inner retina relatively unaffected by either systemic hypoxaemia or hyperoxia. This is an unique characteristic trait of the retinal tissue. Dissimilar to most other tissues of the body, the retinal vessels and the optic nerve head lack autonomic innervations and subsequently relay on the autoregulation for regulation of blood flow. The mechanism behind the retinal autoregulation is not fully understood but is balanced by the effects of myogenic and metabolic factors on the vascular resistance in adjustment of local blood flow to changes in perfusion pressure and the metabolic need of the tissue.

Retinal arteries have remarkably well-developed smooth muscle layer that differ them from arteries of the same size in other organs. Changes in arteriolar diameter are considered the main regulatory component of the retinal vasculature and are triggered either by systemic or by local factors (Delaey & Van De Voorde 2000; Pournaras et al. 2008; Hayreh 2011). Vascular resistance is generated along the entirely length of the arteriolar wall but terminal arterioles between 20 μm and 50 μm in diameter offer the main resistance, thereby playing a central role in the autoregulatory response either by vessel dilation or by vasoconstriction (Jeppesen et al. 2007; Hayreh 2011; Schmidl et al. 2011a). Contractile properties of intramural pericytes in retinal capillaries also play a role in the autoregulatory response (Anderson 1996; Anderson & Davis 1996). Pericytes possess similar properties as smooth muscle cells in that they can dilate and constrict the capillary lumen to some minor extent in response to vasoactive substances or to provocation by local blood gases (Pournaras et al. 2008; Kur & Newman 2014).

Systemic and local factors

Autoregulation of the retinal blood flow pertains to metabolic and myogenic mechanisms in reaction to activation of systemic and local factors as mentioned above. The autoregulatory mechanism is evoked by vasoactive substances that are released from the endothelium in arterioles and the adherent retinal tissue. Metabolic autoregulation strives for retinal tissue local blood flow regulation in unity with its metabolic requirements. For instance, in the case of accumulation of metabolic wastes in the tissue, the rate of blood flow increases. The myogenic autoregulation, however, is activated by alterations in the transmural pressure, secondary to constriction or stretching on the endothelium in the vessel wall. In return, vasoactive factors are released causing either a dilatation or constriction of the vessel (Delaey & Van De Voorde 2000; Pournaras et al. 2008).

Systemic factors. Systemic blood pressure, circulating hormones, arterial blood gases and pH are among the systemic factors that activate the autonomic local vascular reaction. Local factors incorporate the PO_2 and partial pressure of carbon dioxide (PCO_2), pH, endothelial factors (endothelium-derived relaxing factors and constricting factors) and retinal factors (Delaey & Van De Voorde 2000). Increase in arteriolar mural pressure and mechanical stretch trigger release of the endothelium-derived constricting factor, endothelin-1, resulting in vasoconstriction (Polak et al. 2003). Nitric oxide (NO) is one of the major endothelium-derived relaxing factor (EDRF), maintaining the basal vascular tone and mediating vasodilatation by several agonists (Schmetterer & Polak 2001). Acetylcholine, histamine and bradykinin are all examples of neurotransmitters that modulate the vascular tone by activation of EDRF (Yu et al. 2003). The role of retinal relaxing factor (RRF) is yet relatively unknown. It seems to play considerable role as an indirect mediator of the hypoxic response by the retinal tissue itself, independent of other vasoactive mediators (Maenhaut et al. 2007). Other local compounds that contribute to retinal blood flow regulation include adenosine and prostacyclin by increasing the arteriolar diameter. Angiotensin II, prostaglandin and cyclooxygenase narrow the arteriolar diameter whereas lactate modulates the vascular tone parallel to the local metabolic need either by constricting or by widening the vessel (Gidday & Park 1993; Yamanishi et al. 2006; Pournaras et al. 2008).

Circulating hormones like endothelin-1 and angiotensin II have negligible effects on the retinal circulation (Flammer & Mozaffarieh 2008) because the nonfenestrated endothelium of retinal capillaries and the complex network of tight junctions, that resemble the blood–brain barrier (Patton et al. 2005), prevent large molecules penetrating the inner blood–retinal barrier. Conversely, the fenestrated endothelium of choriocapillaries is highly permeable to molecules their size, allowing for their direct effects on smooth muscle cells (Flammer & Mozaffarieh 2008; Riva et al. 2011).

Hypoxaemia and hyperoxia. Hypoxaemia provokes reactivity in retinal vessels mainly through release of tissue metabolites in response to the abnormally low PO_2 in the arterial blood. Hypoxaemia frequently results in hypoxia, the inadequate level of oxygenation for retinal tissue metabolism. Reports on the impact of adenosine on retinal vascular relaxation are conflicting (Gidday & Park 1993; Delaey et al. 2000) but prostacyclin, lactate and the RRF are all released under hypoxic condition and are found to increase the retinal blood flow secondary to vasodilatory response (Delaey et al. 2000; Hata et al. 2000; Yamanishi et al. 2006; Maenhaut et al. 2007; Pournaras et al. 2008). Supposedly, RRF is one of the main modulators for hypoxic vascular response. It is independent of endothelial involvement in the arteriolar wall which may explain the slower onset of retinal vascular reaction as compared with that of the cerebral circulation.

In hyperoxia, retinal vasoconstriction has faster onset than the vasodilatation during hypoxaemia probably due to faster release of vasoconstrictive substances from the endothelial cells in retinal arterioles (Delaey et al. 2000; Maenhaut et al. 2007; Cheng 2014).

Gas challenges on retinal circulation. Similar to cerebral circulation (Pournaras et al. 2008), the retinal circulation adjusts the local retinal blood to changes in arteriolar PO_2 and PCO_2 by widening

or narrowing the vascular lumen. Hyperoxia-induced vasoconstriction is greater in retinal vessels than in cerebral vessels, in contrast with the hypercapnic-induced vasodilatation that is greater in the brain (Kisilevsky et al. 2008; Cheng et al. 2016).

Hyperoxic and hypocapnic gas challenges provoke vasoconstrictions in both retinal arterioles (Gilmore et al. 2004) and venules (Jean-Louis et al. 2005; Palkovits et al. 2014a; Palkovits et al. 2014b; Werkmeister et al. 2015; Cheng et al. 2016; Rose et al. 2016) whereas hypercapnic (Venkataraman et al. 2008) and hypoxic gas mixtures induce vasodilatation of those vessels (Brinchmann-Hansen et al. 1989; Choudhary et al. 2013; Palkovits et al. 2014c; Cheng et al. 2016; Rose et al. 2016). The blood flow regulation is found to be relatively stable when PaO_2 is above 32–37 mmHg but beneath these limits the autoregulatory response is found to be lost (Cheng et al. 2016). According to electroretinography studies, the inner retina show more sensitivity to transient hypoxic stress (at the level of the retinal ganglion cells) than the outer retina which is more resistant to the hypoxic challenges (Tinjust et al. 2002; Janaky et al. 2007; Caprara & Grimm 2012).

Perfusion pressure. The ability for local autoregulation in vascular beds maintains the retinal blood flow relatively constant and independent of fluctuations in the perfusion pressure, as long as the ocular perfusion pressure (OPP) is within a range of upper and lower limits of the autoregulatory plateau. Beyond these limits, the vascular reserve is lost and ocular blood flow becomes directly dependent on the pressure (Arjamaa & Nikinmaa 2006). By most studies, the upper limits of retinal blood flow regulation are attained when the mean arterial blood pressure (MAP) reaches approximately 40% above baseline (Schmidl et al. 2011a). Other studies have found the autoregulatory limits of the mean OPP in the range of 34–60% over baseline (Pournaras et al. 2008), more than 36% below baseline (Riva et al. 1981) or when the intraocular pressure (IOP) either reaches about 30 mmHg (Schulte et al. 1996) or drops below 10 mmHg (Williamson & Harris 1994)

Calculation of retinal blood flow. Retinal blood flow (BF) through the optic nerve head is directly proportional to the perfusion pressure and inversely proportional to the vascular resistance:

$$\mathrm{BF} = \frac{\mathrm{PP}}{R}, \tag{1}$$

where perfusion pressure (PP) is the force that drives blood through the vessel, determined by the difference between the arterial and venous pressure (ΔP). The resistance (R) of a vessel wall against the PP is a function of the vascular calibre and the vessel tone (Caprioli & Coleman 2010). Blood flow resistance can be calculated according to the Poiseulle's law:

$$R = \frac{8\eta L}{r^4}, \tag{2}$$

where vascular resistance (R) is directly related to the fluid viscosity (η) and the length of a vessel (L) and inversely related to the radius of the vessel in the fourth power (r). For that reason, even small changes in the vascular lumen have considerable effects on blood flow resistance. For instance, lessen the retinal arteriolar lumen by half will 16-fold the increase in OPP.

Ocular perfusion pressure. As already brought up, the mean OPP is the pressure driving the blood through the optic nerve. It is determined by the mean ophthalmic arterial pressure (MOAP) entering the eye, minus the mean ophthalmic venous pressure (MOVP), leaving the eye:

$$\mathrm{OPP} = \mathrm{MOAP} - \mathrm{MOVP}. \tag{3}$$

The MOVP is close to the intraocular pressure (IOP), and therefore, the OPP can be estimated as:

$$\begin{aligned}\mathrm{OPP} &= \mathrm{MOAP} - \mathrm{IOP} \\ &= 2/3\mathrm{MAP} - \mathrm{IOP}.\end{aligned} \tag{4}$$

The IOP is determined by the rate of aqueous humour production and the drainage of aqueous humour through the trabecular meshwork. The normal lower and upper limits of IOP are 10 and 22 mmHg, respectively, and the mean around 16 mmHg (Williamson & Harris 1994; Thariq Bhatti 2008).

Mean arterial pressure. Mean OPP is estimated to be two third of the mean brachial blood pressure. The mean arterial pressure is the time-weighted average calculation on arterial pressures during one pulse cycle (Butterworth et al. 2013), determined by the following equation:

$$\mathrm{MAP} = \frac{\mathrm{SBP} + 2(\mathrm{DBP})}{3}. \tag{5}$$

The MAP calculation is based on the fact that during a pulse cycle, one third of the time is spent near the systolic blood pressure (SBP) and two third near diastolic blood pressure (DBP; Butterworth et al. 2013)

The OPP may be affected by one or more of the variables that are used to calculate its value. For example, a low systemic arterial blood pressure or a high IOP (≥30 to 34 mmHg) will reduce the OPP whereas a high systemic blood pressure or a low IOP (<10 mmHg) will elevate the OPP (Williamson & Harris 1994). Consequently, the vessel lumen will distend or constrict to the magnitude of fall or rise in IOP, respectively, until reaching the point the compensatory ability is lost and retinal blood flow becomes a direct function of OPP.

Choroidal blood flow regulation

Regulation of retinal blood flow is believed to be more efficient than of the choroidal flow by the autoregulatory mechanism. Unlike the retinal circulation, the choroidal vascular bed and the central retinal artery up to the lamina cribrosa are innervated by the autonomic nervous system (Nickla & Wallman 2010). The choroidal circulation demonstrates limited autoregulatory capacity to changes in IOP (Schmidl et al. 2011b; Schmidl et al. 2012) but emerging evidence has verified some autoregulatory efforts to changes in MAP and OPP (Fuchsjager-Mayrl et al. 2003; Luksch et al. 2003; Schmidl et al. 2012).

It is also likely that the choroidal circulation is only autoregulated by changes in PCO_2 but not PO_2 (Thylefors et al. 2009). Under system hypoxaemic conditions, the level of PO_2 drops linearly from the choriocapillaries across the outer retina (Linsenmeier & Braun 1992) so the oxygen flux becomes insufficient for the outer retina oxygen requirements. In case the systemic PaO_2 becomes less than 60 mmHg, the retinal circulation contributes (10%) by oxygen diffusion to the outer

retinal segments (Linsenmeier & Braun 1992).

Hypercapnia on the other hand exerts strong vasodilatory NO-mediated effects on the choroidal vasculature, just like on the retinal circulation (Schmetterer & Polak 2001). Carbon monoxide seems to affect the rate of choroidal blood flow to some degree whilst hyperoxia has negligible effects on the blood flow (Schmidl et al. 2011a). Due to the lack of choroidal autoregulatory response to system hyperoxia, the abundant oxygen flux for the duration of supplemental oxygen breathing seems beneficiary for the inner retinal tissue by attenuating the effects of hyperoxic vasoconstriction on the retinal circulation (Palkovits et al. 2014a).

Oxygen transportation to tissues

Physiology of oxygenation

Oxygen is transported by the arterial blood to tissues in two forms: dissolved and combined with haemoglobin. Under normal condition, about 97% of the oxygen is bound with haemoglobin and the residual oxygen (3%) is dissolved in plasma (Guyton and Hall 2000). Oxygen combines reversibly with the haem portion of the haemoglobin molecule to form oxyhaemoglobin. This chemical reaction is reliant on the concentration of PO_2 dissolved in plasma. Each haemoglobin molecule has the capacity to combine with four oxygen molecules. Accordingly, the percentage of haemoglobin molecules occupied by oxygen molecules to the total oxygen binding capacity is expressed as oxyhaemoglobin saturation (West 2005).

Oxyhaemoglobin dissociation curve. The relationship between oxyhaemoglobin saturation and the PO_2 can be described by the oxyhaemoglobin dissociation curve (Fig. 5).

The oxyhaemoglobin dissociation curve has a sigmoidal shape representing the dynamic interaction between the oxyhaemoglobin saturation and the PO_2 in plasma. The binding of the first oxygen molecule is more difficult than subsequent oxygen-haemoglobin attachments. After the first oxygen combines with one of the four heme portions of the haemoglobin, configural changes of the haemoglobin protein facilitates additional binding to the other heme groups. Subsequently, The affinity for the fourth oxygen molecule is nearly 300-fold greater than

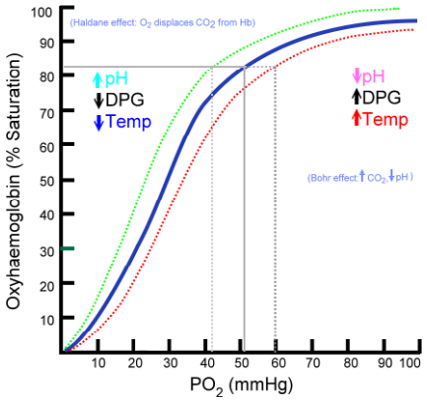


Fig. 5. The oxyhaemoglobin dissociation curve (blue) illustrates how oxyhaemoglobin saturation arises quickly up till approximately PO_2 of 50 mmHg, under normal physiological conditions. From PO_2 of 60 mmHg which represents about 90% oxyhaemoglobin saturation, the curve becomes flatter because of fewer available oxygen-haemoglobin binding sites, until complete saturation is reached at 100%. The red dotted line represents a condition of rightward shift of the curve and the green line leftward shift of the oxyhaemoglobin dissociation curve. Public domain figure. https://upload.wikimedia.org/wikipedia/commons/8/8a/Oxyhaemoglobin_dissociation_curve.png.

for the first one. The opposite is just true for oxygen release in tissues in that the dissociation of the first oxygen molecule facilitates subsequent oxygen release from the haemoglobin (Guyton and Hall 2000; Butterworth et al. 2013).

The chemical reaction of oxygen binding is illustrated by the steep sigmoidal upward shift in the region of 20–30 mmHg (Harvey and Ferrier 2011). Normally, 50% oxyhaemoglobin saturation (P50) is reached at approximately 26.6 mmHg. When the haemoglobin affinity for oxygen is increased, the 50% saturation is reached at a lower PO_2 with subsequent shifting of the oxyhaemoglobin dissociation curve to the left and less availability of oxygen for tissues. The exact opposite is true when the haemoglobin affinity for oxygen is decreased. Then, the oxyhaemoglobin dissociation curve is shifted to the right and a higher PO_2 is needed to reach the 50% oxyhaemoglobin saturation, and more oxygen becomes available for tissues. This condition is typical for a normal oxygen delivery in tissues where hydrogen ion (H^+) concentration accompanying elevated CO_2 production secondary to cellular metabolism shifts the oxyhaemoglobin dissociation curve to the right. Hence, the haemoglobin affinity is decreased and more oxygen is released at lower PO_2. This condition is represented at the steepest part on the curve where only small changes in PO_2 dislodge oxygen molecules from the haemoglobin. Other known factors that shift the curve to the right include increased 2,3 diphosphoglycerate (DPG) and elevated blood temperature (Guyton and Hall 2000; Butterworth et al. 2013).

Oxygen content of the blood. As previously stated, the oxyhaemoglobin saturation (SO_2) is the percentage of available haemoglobin binding sites that are combined with oxygen given by the following equation:

$$SO_2(\%) = \frac{[HbO_2]}{[Hb] + [HbO_2]} \times 100, \qquad (6)$$

where HbO_2 is the oxygen combined with haemoglobin and Hb is the deoxygenated haemoglobin. The SO_2 is multiplied by 100 to obtain the percentage. The SO_2 of arterial blood (SaO_2) at 100 mmHg is approximately 97,5% and 75% for the mixed venous blood (SvO_2) at PaO_2 of 40 mmHg (Guyton & Hall 2000; West 2005).

According to the Henry's law, the dissolved concentration of oxygen in the blood is proportional to the partial pressure. For each mmHg of PO_2, there is 0.003 ml oxygen (O_2) dissolved in 100 ml of blood. Therefore, a normal arterial blood (at sea level) with PO_2 of 100 mmHg contains 0.3 ml O_2 per 100 ml blood. The oxygen capacity is about 20.8 ml O_2 • 100 ml^{-1} of blood because one gram of pure Hb can combine with 1.39 ml O_2 and normal blood has about 15 gram of Hb • 100 ml^{-1}. However, under normal physiological conditions of the body, other species of haemoglobin, namely dyshaemoglobins, may exist in the blood as well (West 2005).

Because in reality the oxyhaemoglobin saturation never reaches the theoretical maximum of 1.39 ml O_2 and some measurements give 1.34 ml, the total oxygen content of blood is given by the following equation:

$$\text{Oxygen concentration} = (1.34 \times Hb \times SaO_2) + 0.003 \times PO_2, \qquad (7)$$

where 1.34 is the oxygen carrying capacity (ml/g) of haemoglobin (at sea level), Hb is the amount of

haemoglobin in the blood (g/dl), SaO_2 is the oxyhaemoglobin saturation of arterial blood at given PO_2 and 0.003 ml O_2 dissolved in 100 ml of blood per mmHg PO_2.

Dyshaemoglobin. Haemoglobin can be classified as a normal haemoglobin that is capable of carrying oxygen (Hb and HbO_2) and dyshaemoglobin that are haemoglobin derivatives and incapable of an oxygen holding. Dyshaemoglobin are further classified as methaemoglobin (MetHb), carboxyhaemoglobin (COHb) and sulfhemoglobin (SHb). Sulfhemoglobin is an uncommon form that is caused by reaction of sulpha-containing compounds, usually from excessive use of sulpha-based drugs (Haymond 2006). Carboxyhaemoglobin carries carbon monoxide that is formed during the metabolic pathway of haem into bilirubin and constitutes normally less than 1–3% of the total haemoglobin in the body (McClatchey 2002). It can, however, be as much as fivefold in a heavy smoker (Whincup et al. 2006). The greater affinity (200- to 300-fold) of carbon monoxide for haemoglobin than oxygen allows COHb to easily displace the HbO_2, shifting the oxyhaemoglobin dissociation curve to the left. Methaemoglobin is the oxidative deoxy form of normal haemoglobin where the iron of the haem group is in a ferric (Fe^{3+}) form instead of the ferrous (Fe^{2+}) state. The MetHb has no oxygen carrying capacity and similar to the COHb can shift the oxyhaemoglobin dissociation curve to the left (McClatchey 2002; Butterworth et al. 2013).

Some contemporary peripheral cooximeters differentiate aforementioned abnormal dyshaemoglobin structures from normal haemoglobin. However, at 660 nm, the COHb has an absorbance parallel with that of HbO_2 (Miller 2000) thereby necessitating information on whether the person is an active smoker before spectroscopic retinal oximetry is performed. The half-life of COHb is 4–6 hr on a room air so smoking should preferentially be abstained for 12–24 hr prior to retinal oximetry. Breathing 100% oxygen shortens COHb half-life to 40–80 min and hyperbaric oxygen breathing shortens it still further, or to 15–30 min (Nagelhout & Zaglaniczny 2001).

Fetal haemoglobin. Fetal haemoglobin (HgbF) constitutes about 75–84% of the total haemoglobin in new born babies (Chestnut 2004) and 1% of the normal adult haemoglobin (Schechter 2008), or haemoglobin A (HgbA). Functionally, HgbF diverges from HgbA in that it has somewhat higher affinity for oxygen because of decreased interaction with 2,3–DPG (Mosca et al. 2009) with P50 around 19–21 mmHg (Chestnut 2004) instead of 26.6 mmHg in adults. During the first month of life, the HgbF amount is progressively substituted by HgbA until dwindling off by 6 months of life (Edoh et al. 2006). As HgbA starts to substitute the HgbF, the level of 2,3DPG raise and the affinity becomes analogous to that of adults within the first few months, although the concentration of HgbF still remains 25% (Chestnut 2004). HgbF has nearly the same absorption spectrum for oxyhaemoglobin and deoxyhaemoglobin as HgbA and hence should not affect the oximetry outcome measures from of adults (Miller 2000; Chestnut 2004).

Matching oxygen supply to demand

Oxygen transport to tissues depends on adequate respiratory and circulatory function. Dry air at sea level (barometric pressure of 760 mmHg) has a PO_2 of 20.93% (~21%). The fully saturated vapour pressure at a sea level is 47 mmHg at normal body temperature of 37°C. Thus, the PO_2 of an inhaled air at sea level is expressed as follows:

$$\frac{(20.93)}{100}\times(760-47)=149\text{mmHg}. \quad (8)$$

When the inspired air reaches the alveoli of the lungs, the PO_2 has dropped to about 100 mmHg. The reason is that the alveolar PO_2 is a product of the balance between alveolar ventilation and the alveolar gas diffusion across the capillary blood gas interface (West 2005).

Global oxygen delivery. Global oxygen delivery (DO_2) to tissues is a product of cardiac output (CO), or the blood flow, and the total oxygen content of the arterial blood (CaO_2; McLellan and Walsh 2004). The DO_2 is calculated by the following formula:

$$DO_2 = CO \times CaO_2, \quad (9)$$

where cardiac output is a product of the heart rate (HR) and stroke volume (SV) and the stroke volume is the amount of blood ejected by the left ventricle during one heart beat. The oxygen concentration depends on both oxyhaemoglobin saturation and PO_2 dissolved in plasma, as previously discussed. Therefore, an inadequate oxygen delivery may either originate from impaired cardiac output, insufficient PaO_2 or a low haemoglobin count (Butterworth et al. 2013).

Global oxygen delivery to tissues depends on two processes: convection of the blood down the arterial tree and diffusion from capillaries to adjacent tissues (Leach & Treacher 2002). Elastic properties and calibre of the vessel wall determine the vascular resistance against the cardiac output. Subsequently, the compliance in the arterial system serves the function of damping the pulsatile output from the left ventricle. Hence, the pulsatile pressure is minimized and a continuous blood flow facilitated down to the microvascular level (Guyton and Hall 2000).

The rate of oxygen uptake by pulmonary capillaries is governed by oxygen consumption in tissues that is fairly constant under normal resting conditions (West 2005). The cardiac output is constantly adapted to the overall metabolic need of the body. It is controlled by the sum of various factors (as previously discussed) that regulate local blood flow according to metabolic need of tissues. These factors add together to make the venous return which in turn is delivered by the pumping activity of the heart and convective flow down the arterial tree to capillaries (Guyton and Hall 2000). From the capillary blood, the oxygen diffuses down its concentration gradient to the much lower PO_2 of the mitochondria of individual cells in tissues (Leach & Treacher 2002).

Presuming the retinal arterial oxygen content is identical to the systemic circulation, the oxygen delivery to a retinal tissue can be derived from equation 9, by substituting total retinal blood flow (TRBF) for the cardiac output:

$$DO_2 = TRBF \times CaO_2. \quad (10)$$

Oxygen consumption. Tissue oxygen consumption (VO_2) is equivalent to the tissue metabolic rate under aerobic conditions per minute. The Fick's equation describes the relationship between oxygen consumption, cardiac

output or retinal blood flow and the oxygen content in both retinal arterioles (CaO_2) and venules (CvO_2):

$$VO_2 = CO \times (CaO_2 - CvO_2) \quad (11)$$

or

$$VO_2 = TRBF \times (CaO_2 - CvO_2), \quad (11)$$

where CaO2 – CvO2 is the difference between the arterial and venous oxygen content (arteriovenous oxygen difference, AV-difference). The global arterial oxygen content is about 20 ml/100 ml blood and the venous oxygen content about 15 ml/100 ml blood and thus producing arteriovenous oxygen difference of approximately 5 ml/100 ml blood (Butterworth et al. 2013).

Arteriovenous oxygen difference. By rearranging equation 11, it is apparent the oxygen consumption and the local blood flow determine the arteriovenous oxygen difference in the retinal tissue:

$$\text{AV-difference} = \frac{VO_2}{BF}. \quad (12)$$

The AV-difference is directly related to the local tissue oxygen consumption and inversely to the local blood flow. The relationship is clearly manifested in both retinal and choroidal circulations. In this context, the retinal circulation is characterized by low VO_2/BF ratio and a large AV-difference, resulting in relatively low oxygen content on the venous side of the circulation. In contrast, the choroidal circulation is characterized by high VO_2/BF ratio and low AV-difference with high oxygen content on the venous side of the circulation.

Oxygen extraction fraction. The amount of oxygen consumption is reflected by the fraction of oxygen extraction (OEF) across the perfused capillary network. The oxygen extraction fraction can be calculated as follows:

$$OEF = \frac{CaO_2 - CvO_2}{CaO_2}. \quad (13)$$

Normal oxygen extraction fraction for the majority of tissues is 25% (5 ml/20 ml). In other words, under normal conditions, most tissues consume only one-fourth of the oxygen delivered to the capillary bed. When oxygen supply exceeds the oxygen demand for the metabolic activity, the extraction fraction becomes less than 25%. However, when the oxygen supply is less than the metabolic demand, the extraction fraction becomes greater than 25% (Butterworth et al. 2013).

Blood flow distribution varies enormously among tissues, depending on their momentary functional requirements. Some tissues, including the retina (Wangsa-Wirawan & Linsenmeier 2003) and the brain, have steady energy requirements whilst perfusion and energy utilization of others (e.g. the liver) are predominated by their activity level (Scheufler 2004). Consequently, tissues with greater energy expenditure, like the inner retina, myocardium and the brain, extract higher oxygen fraction than less active tissues. The oxygen extraction fraction of the cerebral tissue is close to 40% and the arteriovenous oxygen difference is about 34% (Hatazawa et al. 1995; Qin et al. 2011). Likewise, the inner retina oxygen extraction fraction is 37% and arteriovenous oxygen difference approximately 35% (Schweitzer et al. 1999; Felder et al. 2015). Consequently, a duration of reduced oxygen supply (hypoxaemia) will directly affect metabolic processes in retinal and cerebral tissue cells (Kergoat et al. 2006; Ozcimen et al. 2016).

The application of noninvasive fundus reflectometry in human studies has made calculation of the inner retinal oxygen extraction achievable. Studies in young healthy individuals indicate no changes in oxygen extraction under acute mild systemic hypoxia (Palkovits et al. 2014c). During 100% oxygen breathing however, the oxygen extraction is lessened by more than 60% from the normoxic breathing (Palkovits et al. 2014a). For those reasons, the retina autoregulatory response seems to be efficient under both acute system hypoxaemic and hyperoxic conditions.

Inadequate tissue oxygenation

Oxygen diffusion from a capillary lumen to tissues is directly related to the capillary area and the PO_2 difference across the vessel wall and is inversely related to the distance between the two sites (Leach & Treacher 1998, 2002; West 2005). Normally above 1–3 mmHg of oxygen pressure is required to maintain cellular oxidative metabolism which allows for a large safety margin in animals (Leach & Treacher 1998; Guyton & Hall 2000; Scheufler 2004). This is true for the inner retina as well which has remarkably higher PO_2 that measures around 20 mmHg in animals with circulatory structure that is analogous to humans (Wangsa-Wirawan & Linsenmeier 2003).

During the final step of a cellular respiration, oxygen-dependent adenosine triphosphate (ATP) is made as an energy basis for the tissue metabolism. Oxygen deficiency depletes the ATP stores and the speed of cellular injury will be determined by the tissue oxygen consumption and the ability to attain ATP under anaerobic conditions. Anaerobic metabolism can only serve as a temporarily reserve because of the inability to match tissue oxygen consumption. Consequently, the rapidity of cellular damage differs remarkably among tissues where neurological cells can only survive for few minutes (Leach & Treacher 1998; Guyton & Hall 2000). This holds true for the photoreceptors and other retinal neurological cells that require high oxygen consumption for the energy demanding process of transmitting light waves into neurological signs for interpretation by the brain (Caprara & Grimm 2012). The physiological response of prioritization blood flow to the retina and brain is thus of uttermost importance during hemodynamic stress and makes the retina indeed the most advantageous source for noninvasive measures of oxygen delivery and viability of the central nervous system.

Similarities between retina and the brain. Embryologically, the retina is a direct outgrowth of the diencephalon, sharing similarities in microvascular structure with the cerebral circulation, blood flow and regulatory mechanisms (Delaey & Van De Voorde 2000; Patton et al. 2005). Based on the homogeneity, emerging evidence suggests that the retinal vasculature may be a surrogate for pathological changes on the cerebrovasculature and even the cardiovascular system as well (Patton et al. 2005; McClintic et al. 2010; Flammer et al. 2013). Like in retina, the rate of oxygen consumption by the cerebral tissue is quite constant (3.5 ml/100 gm tissue, or nearly 49 ml O_2/min the entire brain) except under intense brain activity (Clarke & Sokoloff 1999). Consequently, the high oxygen consumption and the inability to store oxygen and any metabolic

products such as glucose render the cerebral tissue extremely vulnerable to an ischaemic insult (Pittman 2011). When arterial blood PO_2 level goes below about 60 mmHg (Ainslie & Poulin 2004), the oxyhaemoglobin saturation becomes less than 90% (Gupta et al. 1997) and the cerebral tissue PO_2 becomes less than 30 mmHg (normal 35–40 mmHg), a prompt provocation of compensatory response with increased cerebral blood flow (Guyton & Hall 2000; Carreau et al. 2011) is initiated secondary to amplification of cardiac output (Zhou et al. 2007).

As previously discussed, the retinal blood is dependent on the blood flow delivered by the carotid arteries to the brain. Impaired global oxygen delivery in case of severe hypoxaemia (Heistad & Abboud 1980) or haemorrhagic shock initiates compensatory response of intense peripheral vasoconstriction to redirect blood flow from lower priority organs to the vital ones (Dutton 2007) to preserve cerebral and ocular perfusion (Denninghoff et al. 2003; Riva et al. 2011). The initial compensatory response is aimed at increasing oxygen extraction to match the oxygen demand for cellular metabolism as evident by decreased oxyhaemoglobin saturation on the venous site of the circulation. In contrast, a reduced oxygen consumption for example during hypothermia or with tissue necrosis lessens the fraction of oxygen extraction as shown by increased venous oxyhaemoglobin saturation (Schober & Schwarte 2012). Eventually, whenever inadequate oxygenation jeopardizes the normal cellular function, vigilant oxygen monitoring with timely restorative and preventive measures is crucial for viability and functionality of individual tissues and body organs.

Retinal oximetry

The retina possesses highly sophisticated architecture of neurological layers and vascular structure that is analogous to the cerebral circulation. The transparent structure at the front of the eye offers an unique opportunity for a direct noninvasive observation of the retinal circulation. Dual-wavelength spectrophotometric retinal oximetry allows for a direct noninvasive assessment of oxyhaemoglobin saturation in retinal vessels which serve as a window to the central nervous system. The technique is based on different light absorption of oxyhaemoglobin and deoxyhaemoglobin and gives information on the oxyhaemoglobin saturation.

Principles of retinal oximetry

Spectrophotometric oximetry measures the attenuation of light travelling through a blood column as a function of wavelength, based on the fact that oxy- and deoxyhaemoglobin have different light absorption spectra (Fig. 6). Oxyhaemoglobin is light red in colour whilst deoxyhaemoglobin is darker red in colour. These differences of shades permit calculation for oxyhaemoglobin saturation in retinal arterioles and venules.

Like conventional oxyhaemoglobin saturation measurements in a whole blood sample, the retinal oximetry exploits the Beer–Lambert law to its system. Supposedly, the light absorption is dependent on the extinction coefficient (molar absorptivity) of the blood (ε); the distance of light has to travel through the sample (d) and the concentration (c):

$$I = I_0 \times 10^{-\varepsilon c d}, \qquad (14)$$

where I_0 is the original light intensity not interacting with the blood, and I is the intensity of light transmitted through the blood.

Dual-wavelength spectrophotometric oximetry simultaneously acquires two automatic monochromatic retinal images at two different wavelengths. One is at the isosbestic point and one at a nonisosbestic wavelength. Vessel points are automatically selected (Fig. 7) for measurement of light intensity inside (I) and outside the vessel (I_O) for calculation of optical density (OD). Because of light absorbance by the erythrocytes, the intensity of reflected light from the vessel is less than from the immediate surrounding retina.

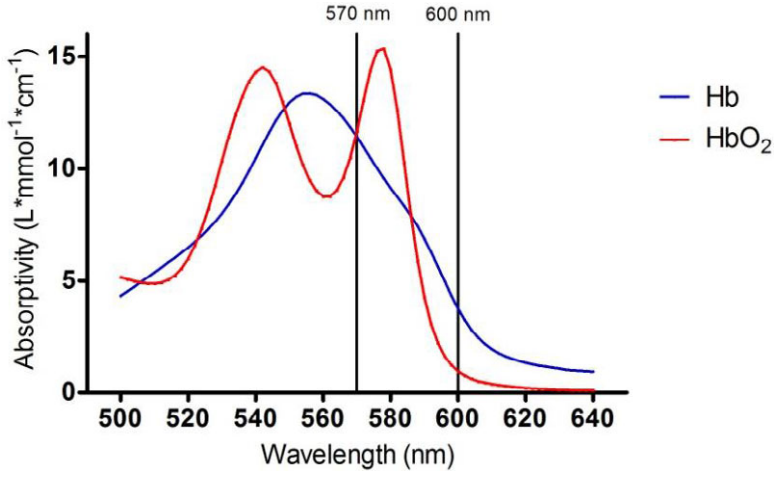


Fig. 6. Light absorptivity at isosbestic (570 nm) and nonisosbestic (600 nm) wavelengths. Isosbestic wavelengths are insensitive to oxyhaemoglobin saturation whereas nonisosbestic wavelengths are sensitive to distinctive oxyhaemoglobin saturation. For the Oxymap T1 retinal oximeter, wavelength of 570 nm is at the reference isosbestic point whilst 600 nm is sensitive to the oxyhaemoglobin. The figure was created by Jona Valgerdur Kristjansdottir based on data from Zijlstra et al. (2000).

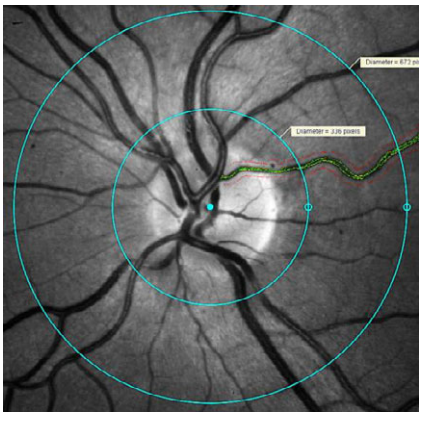


Fig. 7. Monochrome retinal oximetry image with a pseudocolour overlay of selected measure points along the vessel. Green overlay (600 nm) shows the light intensity of the measure inside the vessel (I). Red measure-points illustrate the light intensity of the background, to the sides of the vessel (I_o).

The OD describes the light absorbance and can be calculated as:

$$OD = \log(I_0/I). \quad (15)$$

The higher the OD, the higher is the absorbance. The OD inside a vessel at the isosbestic point depends on the vascular diameter in addition to the distance that the light travels (d) and the erythrocyte concentration (c) but is independent of oxyhaemoglobin saturation. In addition to the vascular diameter and the $c{\cdot}d$, the OD at the nonisosbestic wavelength depends on the oxyhaemoglobin saturation. Consequently, the OD ratio at these two wavelengths is sensitive to oxyhaemoglobin saturation whilst the vascular width and $c{\cdot}d$ tend to cancel out.

Optical density at the two different wavelengths allows for calculation of an optical density ratio (ODR):

$$ODR = \frac{\text{OD nonisosbestic}}{\text{OD isosbestic}}. \quad (16)$$

The ODR has nearly linear relationship to the oxyhaemoglobin saturation (Beach et al. 1999; Harris et al. 2003; Hardarson et al. 2006) that permits calculation of oxyhaemoglobin saturation (SO_2):

$$SO_2 = a + b \times ODR. \quad (17)$$

In the equation, a and b are calibration constants based. The calibration constant of the retinal oximeter used for the purpose of this thesis will be discussed in Chapter 3.2.3.2.

Development of retinal oximetry

Noninvasive spectrophotometric analysis dates back to 1959, when Hickam and associates used broadband light filters, two wavelengths of light and standard photographic films to measure oxyhaemoglobin saturation in retinal vessels (Hickam et al. 1960). Subsequently, photoelectric technique (Delori 1988), imaging spectroscopy (Schweitzer et al. 1999), multispectral confocal imaging (Smith et al. 2000) and digital camera systems (Beach et al. 1999) evolved from other research groups. For review, see Harris et al. (2003), Geirsdottir et al. (2012) and Beach (2014).

Currently, two different systems are commercially available for retinal oximetry that allots noninvasive direct measurement of arterioles and venules: the Imedos system (RVA; Imedos, Jena, Germany) and the Oxymap retinal oximeter (Oxymap; Oxymap ehf. Reykjavik, Iceland).

Retinal oximetry with scanning laser ophthalmoscope (SLO). Recently, a scanning laser ophthalmoscope (SLO) was combined with modified Oxymap analysis software for development of SLO retinal oximetry with a dual-wavelength algorithm. SLO uses low power laser wavelengths (instead of white light) to scan through individual separate retinal layers at once from the inner sensory layers down to the choroid. A green laser (532 nm) scans from the inner retina to the pigment epithelium whereas red laser (366 nm) gives a choroidal view by scanning from the retinal pigment epithelium down to the choroid.

The SLO brings some theoretical advances to retinal oximetry. First, it minimizes any unnecessary light exposure to the fundus by using laser (instead of white light imaging in regular fundus cameras) to obtain the monochromatic images at the dual different wavelengths. Second, it acquires wide-field scanning of nearly the entire fundus but conventional fundus cameras are limited to quite narrow images of the posterior pole. Third, pupil dilation using mydriatic eye drops is unnecessary. Finally, it pierces other optical opacities in the eye such as cataracts better than conventional spectrophotometric fundus camera (Kristjansdottir et al. 2014). In addition, SLO has a potential for application of retinal oximetry to neonates and infants. This could mark a milestone in management of preterm neonates (and other paediatric groups) who are treated in incubators at neonatal intensive care units.

The risk of retinopathy of prematurity (ROP) is inversely related to gestational age and weight at birth. Extremely premature infants and those with low birthweight are therefore at highest risk for severe ROP and consequently a poor visual outcome due to the immaturity of retina at birth (Cavallaro et al. 2014). The interruption of normal retinal vascular development following transition from the relatively hypoxic intrauterine environment to the oxygen-rich ambient air after birth which is further aggravated by administration of supplemental oxygen therapy is twofold: the first phase is characterized by suppression of insulin-like growth factor I and normal vascular endothelial growth factor (VEGF). The second phase is characterized by hypoxic-induced neovascularization that is analogous to other proliferative retinopathies, as the retina becomes more metabolically demanding (Smith 2004). Careful titration of supplemental oxygen to preterm neonates to reduce the risk of visual impact (i.e. blindness) from hyperoxia is therefore of vital importance for retinal vascular development. Currently, oxygen supplementation to those babies is monitored by continuous peripheral pulse oximetry and intermittent invasive arterial blood gas measurements.

The noninvasive dual-wavelength confocal SLO combined with oximetry analysis for retinal oximetry may improve monitoring and management of these babies. Prior to allowing oxyhaemoglobin saturation measurements of retinal arterioles and venules, it is necessary to determine the ODR in full-term healthy neonates, shortly after birth.

Retinal oximetry in healthy individuals

Retinal oximetry has shown sensitivity and reliability with high reproducibility and repeatability of retinal oxyhaemoglobin saturation measurements during normoxia in healthy humans (Hardarson et al. 2006; Hardarson et al. 2009; Blondal, Sturludottir, Hardarson, Halldorsson, and Stefansson 2011; Geirsdottir et al. 2012; Palsson et al. 2012; Heitmar & Cubbidge 2013; Man et al. 2013; Man et al. 2014; Yip et al. 2014; Werkmeister et al. 2015), rhesus monkeys (Li et al. 2016) and pigs (Traustason et al. 2013) and during induced hypoxaemia (Choudhary et al. 2013; Palkovits et al. 2014c; Rose et al. 2016) and hyperoxia (Palkovits et al. 2014a; Werkmeister et al. 2015; Rose et al. 2016) in humans. Most of these studies have been carried out to test the reproducibility and sensitivity of the technique but also to study normal physiological reactions to oxygen challenges and retinal metabolism.

Report on the groundwork of SLO with dual-wavelength retinal oximetry revealed sensitivity and repeatability in healthy humans and that the emplacement is technically feasible and promising for advancement of retinal

oximetry in the future (Kristjansdottir et al. 2014).

Normoxia. Recently, some research reports were published on normal retinal vessel oxyhaemoglobin saturation in human subjects of multiethnicities (Table 1). These studies show similar results although some small deviations exist between different versions of retinal oximeters and between different research groups. The differences are attributable to the retinal oximeter hardware, the calibration of the oximeter, the analysis software and the analytical approaches. When comparing oxyhaemoglobin saturation measurements between different studies, the importance of identical calibration between retinal oximeters devices (Geirsdottir et al. 2012) must be kept in mind and whether methodological procedures for oximetry analysis were according to standardized protocol. None of the studies have found difference in oxyhaemoglobin saturation between the left and right eyes but the effects of age on oxyhaemoglobin saturation are conflicting.

Geirsdottir et al. (2012) found the oxyhaemoglobin saturation in retinal venules to decrease significantly with advanced age, or 1.9% for every 10 years in males and 0.7% for females. The oxyhaemoglobin saturation in retinal arterioles was not affected by age. Consequently, the AV-difference increased by 1.5 ± 0.5% for every 10 years in males and 1.0 ± 0.4% in females. The vessel diameter was inversely affected by the age whereas the OPP rose with age which the authors suggested might partly compensate for the increase in vascular resistance caused by the decreased vessel lumen. The oxyhaemoglobin saturation measurements of both retinal arterioles and venules were significantly lower in the inferotemporal quadrants than other retinal quadrants (Geirsdottir et al. 2012). Jani et al. (2014) found no difference across ethnicity, genders nor iris/fundus pigmentations. They found both the arteriolar and venous oxyhaemoglobin saturation to decrease with age and an increase in AV-difference. The oxyhaemoglobin saturation values were lowest in the inferotemporal quadrant and highest in the superonasal quadrant. Conversely, the AV-difference was greatest in the inferotemporal quadrant and lowest in the superotemporal quadrant (Jani et al. 2014). Yip et al. (2014) reported the age to act as a negative factor for oxyhaemoglobin saturation in venules, similar to the finding of Geirsdottir et al. and Jani et al. (Yip et al. 2014). In contrast to the above studies, Mohan et al. (2015) found the arteriolar and venous oxyhaemoglobin saturation to increase with age but the AV-difference to remain unaffected. There was no statistical correlation between the retinal oximeter measurements and finger pulse oximetry readings. They speculated that heavily pigmented fundus like in Indians has more melanin exposed for light absorption which could hypothetically alter the ODR and hence the oximetry measures (Mohan et al. 2015). Yang et al. (2016) obtained difference in oxyhaemoglobin saturation measurements between retinal quadrants. The arteriolar measures from inferotemporal quadrant gave the lowest value followed by the superotemporal, inferonasal and superonasal quadrants, respectively. The retinal venules followed a similar pattern (Yang et al. 2016). Nakano et al. (2016) measured increased oxyhaemoglobin saturation in major retinal arteries (0.67% per 10 years) with advanced age but not in venules. They found the mean oxyhaemoglobin saturation to be markedly lower in both retinal arterioles and venules in the temporal hemispheres, particularly in the inferotemporal vessels that are similar to the findings of Geirsdottir et al. Palsson et al. Mohan et al. and Yang et al. (Nakano et al. 2016). Recently, Liu et al.(2017) published the first study on retinal oximetry

Table 1. Published data on retinal oxyhaemoglobin saturation (%) in healthy subjects. All values are mean ± standard deviation, except*.

Author (year)	Ethnicity (number of subjects)	Age (years)	Device (software)	Arterioles	Venules	AV-difference
Geirsdottir et al. (2012)	Caucasians (120)	18 to 80 median 47	Oxymap T1 (2.2.1)	92.2 ± 3.7	55.6 ± 6.3	36.7 ± 5.4
Jani et al. (2014)	Caucasian (18) Hispanic (13) African-American (17) Asian (13)	19 to 74 mean 44.1 ± 14.7	Oxymap T1 (2.3.1)	90.4 ± 4.3	55.3 ± 7.1	35.4 ± 4.0
Yip et al. (2014)	Asian (118)	≥40	Oxymap T1 (2.3.1)	93.64 ± 6.9	54.22 ± 6.9	39.43 ± 8.9
Mohan et al. (2015)	Asian Indian (98)	18 to 63 Mean 33	Oxymap T1 (2.4.2)	90.3 ± 6.6	56.9 ± 6.3	33.2 ± 5.2
Yang et al. (2016)	Chinese	19 to 30	Oxymap T1(2)	93.2 ± 6.3	60.4 ± 5.3	32.9 ± 6.4
Nakano et al. (2016)	Japanese (252)	20 to 93 Mean 61.1 ± 18.8	Oxymap T1 (2.4.2)	97.0 ± 6.9	52.8 ± 8.3	44.2 ± 9.2
Liu et al. (2017)	Chinese (122)	5 to 13 Mean 13.0 ± 2.9	Oxymap T1 (2.4.2 and 2.5.0)	85.5 ± 7.1	48.2 ± 5.5	37.3 ± 6.5
Man et al. (2013)	Caucasian (20)	19 to 45 Mean 30.1 ± 7.56	Imedos	94.0 ± 4.9	61.8 ± 4.99	
Man et al. (2014)	Caucasian (50)	18 to 58 Median 26	Imedos	*95.94 range: 91.53–98.49	*62.35 range 57.65–64.17	33.79 ± 3.37
Kristjansdottir et al. (2014)	Caucasian (11)	Mean: 34 ± 10	SLO (modified Oxymap analyser Software)	92 ± 13	57 ± 12	

SLO = scanning laser ophthalmoscope.
* Median of the right eye.

in children. According to their results, the oxyhaemoglobin saturation of retinal arterioles and venules is lower in children than in adults. In arterioles, the oxyhaemoglobin saturation increased considerable with age and showed a similar trend in venules. They observed a statistical difference between measurements in all retinal quadrants, being lowest in the inferotemporal region, followed by superotemporal, inferonasal and superonasal quadrants, respectively. The AV-difference, however, was similar between retinal hemispheres. The authors hypothesized the reason for lower oxyhaemoglobin saturation in children is due to their yet not fully developed retina, resulting in a higher oxygen consumption and is inversely related to the retinal nerve fibre layer thickness (Liu et al. 2017).

Man et al. (2014) reported similar findings, using the Imedos system, to Mohan et al. (2015) on increased oxyhaemoglobin saturation in both retinal arterioles and venules by age and unchanged AV-difference. They did not observe any correlation of oxyhaemoglobin saturation in retinal vessels with OPP. They reported, however, a strong correlation of the finger pulse oximetry readings with oxyhaemoglobin saturation in retinal arterioles and the AV-difference which is in contrast with the findings of Geirsdottir et al. (2012) and Mohan et al. (2015).

The results on measured oxyhaemoglobin saturation by Kristjansdottir et al. (2014) on the employed SLO retinal oximetry with a dual-wavelength algorithm of modified Oxymap analysis software (Kristjansdottir et al. 2014) are in agreement with other publications of retinal oximetry normative data.

Induced hypoxaemia. Retinal oximetry studies on induced acute systemic hypoxaemia in healthy young adults show sensitivity to changes of oxygen concentration in both retinal arterioles and venules. In other words, these studies show the subnormal oxygen level delivered by the systemic circulation is being manifested in the central circulation by retinal oximetry.

Choudhary et al. (2013) used multispectral image-replicating imaging spectrometer to measure the effects of acute mild hypoxaemia on retinal vessel oxyhaemoglobin saturation in 10 healthy subjects with the mean age of 25 ± 5 years. During normoxic breathing, the mean oxyhaemoglobin saturation of retinal arterioles was 98.5 ± 1.6%, 70.7 ± 2.7% in venules and the AV-difference was 27.8 ± 2.9%. During hypoxic breathing of 15% oxygen, the oxyhaemoglobin saturation in retinal arterioles decreased to 90.3 ± 2.0% and 62.4 ± 2.2% in venules. The AV-difference, however, remained unchanged whilst the vessel diameter increased by 3% and 4%, respectively. The oxyhaemoglobin saturation in retinal arterioles was similar to their finger pulse oximetry readings or 98.5 ± 1.6% under normoxic breathing and 89.6 ± 0.5% with hypoxic breathing (Choudhary et al. 2013).

Palkovits et al. (2014c) included 27 healthy Caucasians in a statically analysis of retinal oximeter measurements and blood flow velocity during normoxia and different breathing regimens of hypoxic mixture. The participants age ranged from 18 to 35 years (mean 25.2 ± 3.9 years). The Imedos retinal oximetry system was used to measure oxyhaemoglobin saturation and vessel diameter in one major temporal retinal artery and venule. Blood velocity was measured by bidirectional laser Doppler velocimetry at the same location as oxyhaemoglobin saturation and diameter were quantified. The study was performed in randomized two-way crossover design and parameters were obtained under normoxia and under isocapnic hypoxia when participants inhaled oxygen 12% + nitrogen 88% or oxygen 15% + nitrogen 85%. Oxyhaemoglobin saturation in both retinal arterioles and venules, the AV-difference and finger pulse oximetry readings all decreased during the hypoxic gas mixtures breathing. The PO_2 of a capillary earlobe blood measured 45.5 ± 7.0 mmHg and 56.9 ± 4.3 mmHg during 12% and 15% oxygen breathing, respectively. The hypoxic breathing regimen induced vasodilatation in both retinal arteries and venules as compared with normoxia that was more prominent during 12% oxygen breathing. The blood flow increased during both breathing regimens but was more augmented during 12% oxygen breathing (Palkovits et al. 2014c). It appears that the retinal circulation was well autoregulated during the hypoxic challenges which is in agreement with earlier statements in that above a PaO_2 of 32–37 mmHg the autoregulatory response of the retinal circulation is well preserved. The authors concluded that the retinal oxygen extraction fraction was unaffected by the graded systemic hypoxia. Rose et al. (2016) used metabolic hyperspectral retinal camera to measure oxyhaemoglobin saturation of retinal arterioles and venules of one eye in 11 healthy people, under conditions of normoxia, isocapnic hyperoxia and isocapnic hypoxia. Total retinal blood flow was quantified with Doppler spectral-domain optical coherence tomography (SD-OCT). Participants mean age was 33.36 ± 6.03 years. When the end-tidal partial pressure of oxygen (E_tO_2), which is an indicator of arterial PO_2, was reduced from baseline (100 mmHg) to 80, 60 and 50 mmHg, the retinal arterial and venous oxyhaemoglobin saturation decreased from 99.3 ± 5.8% and 56.3 ± 4.2% to 95.6 ± 5.1% and 52.5 ± 4.1%, 89.6 ± 2.8% and 49.5 ± 2.9%, 83.3 ± 3.9% and 45.0 ± 6.1%, respectively. The retinal blood flow increased markedly during the hypoxic gas mixture breathing as compared with the normoxic baseline breathing (Rose et al. 2016).

Induced hyperoxia. Like studies on induced hypoxaemia, retinal oximetry demonstrates sensitivity to changes of oxyhaemoglobin saturation for the period of acute systemic hyperoxia.

Palkovits et al. (2014a) used the Imedos system to measure oxyhemoglobin saturation of one major temporal retinal artery and vein in 41 healthy humans during isocapnic 100% oxygen breathing. The age of the participants ranged from 18 to 35 years. Retinal venous blood velocity was measured using bidirectional laser Doppler velocimetry, and the blood flow was calculated. At baseline during normoxic breathing, the oxyhaemoglobin saturation in retinal arterioles measured 92.3 ± 3.9% and 61.8 ± 4.4% in venules and the AV-difference 30.5 ± 7.9%. During 100% oxygen breathing, these values increased to 96.4 ± 3.1%, 73.9 ± 10.0% and 22.4 ± 10.4%, respectively. The increase in oxyhaemoglobin saturation was greater in retinal venules than arterioles and the calculated blood flow was markedly reduced during the hyperoxic breathing (Palkovits et al. 2014a). Werkmeister et al. (2015) used the Imedos system to

measure the oxyhaemoglobin saturation and vessel diameter in retinal arterioles and venules and dual-beam Doppler FD-OCT system for retinal blood velocities. Measurements were performed in one eye during both normoxic and 100% oxygen breathing. Participants included eight healthy persons between the age of 18 and 35 years. During normoxic breathing, the mean oxygen saturation of retinal arterioles was 95.3 ± 1.9% and 68.0 ± 3.9% of venules. During hyperoxic breathing, these values raise to 99.4 ± 0.3% and 76.6 ± 5.2%, respectively, with a drop in the AV-difference (Werkmeister et al. 2015).

Rose et al (2016) reported marked increase in oxyhaemoglobin saturation of retinal venules during baseline level versus 200 mmHg and baseline level versus 300 mmHg (above-mentioned method in 1.8.3.2). The oxyhaemoglobin saturation in retinal arterioles at 200 and 300 mmHg remained unchanged as compared with 100 mmHg at baseline. Yet, the total retinal blood flow did significantly decrease under the hyperoxic breathing (Rose et al. 2016). These results of increased oxyhaemoglobin saturation on the venous site of the circulation and reduced blood flow under system hyperoxia indicate the effective autoregulatory response and reduced oxygen extraction of the inner retina with enhanced global oxygen delivery as already stated in former chapters.

Retinal oximetry in eye diseases

Retinal oximetry has been widely used for retinal imaging and appraisal of retinal vessel oxyhaemoglobin saturation in patients with variety of eye diseases. Those include glaucoma (Michelson & Scibor 2006; Olafsdottir et al. 2011; Vandewalle et al. 2012; Vandewalle et al. 2013), diabetic retinopathy (Hardarson & Stefánsson 2012b; Khoobehi et al. 2013; Jørgensen & Bek 2014; Jørgensen et al. 2014; Kashani et al. 2014; Klefter et al. 2015; Dong et al. 2016; Klefter et al. 2016; Man et al. 2015; Sin et al. 2016), retinitis pigmentosa (Eysteinsson et al. 2014; Battu et al. 2015; Ueda-Consolvo et al. 2015; Zong et al. 2016) age related macular degeneration (AMD; Geirsdottir et al. 2014), retinal vein occlusions (Hardarson & Stefánsson 2010, 2012a; Lin et al. 2016) and retinal arterial occlusions (Hardarson et al. 2013). All these studies reveal notable changes in retinal vessel oxyhaemoglobin saturation and retinal tissue metabolism. For instance, in severe glaucomatous eyes, increased venous oxyhaemoglobin saturation and reduced AV-difference indicate reduced oxygen extraction secondary to retinal tissue atrophy (Olafsdottir et al. 2011; Vandewalle et al. 2013). In patients with diabetic retinopathy increased oxyhaemoglobin saturation in both retinal arterioles and venules in addition to unaltered AV-difference (Hardarson & Stefánsson 2012b; Jørgensen et al. 2014; Man et al. 2015), are indicative of disease progression to more advanced stages. In retinitis pigmentosa, changes in retinal oximetry values and reduced vascular calibre most likely mirror retinal tissue atrophy and decrease in oxygen consumption (Eysteinsson et al. 2014; Battu et al. 2015). In patients with exudative AMD, an increase in both retinal venous oxyhaemoglobin saturation and AV-difference indicate abnormally low oxygen consumption of the inner retina (Geirsdottir et al. 2014). In both retinal artery occlusions and retinal vein occlusions, retinal oximeter measurements give valuable information on the oxygen changes and the effect on retinal tissue metabolic function by comparing the affected eye with the fellow unaffected eye. In central retinal vein occlusion (CRVO), the mean oxyhaemoglobin saturation has shown to be markedly lower and the inter eye variability greater than of the fellow unaffected eye (Hardarson & Stefánsson 2010; Traustason et al. 2014). In branch retinal vein occlusion (BRVO), the oxyhaemoglobin saturation of retinal venules is decreased to a variable degree and in some cases the arteriolar saturation is higher in the affected eye than in the opposite unaffected eye (Hardarson & Stefánsson 2012a; Lin et al. 2016).

Up to date, only a few studies have been published on retinal oximetry in people with branch and central retinal artery occlusion. These reports give novel insight of the consequences of obstructed arterial blood flow on the retinal tissue oxygen supply. Initially, after the clinical onset of symptoms the oxyhaemoglobin saturation of retinal arterioles is subnormal but gets better with time and treatment (Hammer et al. 2009; Hardarson et al. 2013) signalling facilitation of the local blood flow and hence improved oxygen delivery to the inner retina.

Central retinal vein occlusion (CRVO)

Central retinal vein occlusion is a sight threatening disease that is a frequent cause for visual loss in humans. It usually affects only one eye (McAllister 2012) although the incidence of CRVO in the contralateral eye may reach 7% within 5 years from the development in the first eye (Hahn et al. 2013). The clinical existence of the disease has been known since 1878 (Hayreh 2014) but its pathophysiology is yet to be fully elucidated (Glueck et al. 2005; Kang et al. 2011; Hayreh et al. 2012). The risk factors have been associated with variety of systemic factors, such as hypertension and diabetic mellitus, haematologic and local factors, including ocular hypertension and glaucoma (Elman et al. 1990; Hayreh et al. 2001, 2004). In 2008, CRVO was estimated to affect 2.5 million people worldwide, based on a prevalence ratio of 0,8 per 1000 individuals from population-derived studies around the world (Rogers et al. 2010). As an advanced age has been identified as an important risk factor (McAllister 2012) for CRVO, the prevalence and disease burden may be expected to rise with the growth rate of the ageing population during the 21st century.

Pathophysiology. CRVO is caused by thrombotic occlusion of the central retinal vein most commonly within the optic nerve at a variable location posterior to the lamina cribrosa. Colour Doppler imaging and fluorescein angiography demonstrate central retinal venous outflow obstruction and retinal capillary nonperfusion to various degrees (Hayreh 2005), along with reduced venous blood flow velocity and prolonged arteriovenous transit time (Arsene et al. 2002). Clinical entities of the disease consists as well of visual impairment (Martinet et al. 2012), intraretinal haemorrhage, venous tortuosity, vascular congestion and macular oedema (London & Brown 2011). The impediment to the venous blood flow amplifies the upstream intraluminal pressure (venous dilatation) which elevates the hydrostatic pressure causing extravasations of fluid into the extracellular space (Kaur et al. 2008) resulting in retinal tissue oedema. Retinal hypoxia stimulates VEGF

production, which increases vascular permeability and leakage of osmotically active plasma proteins into tissue. Increased hydrostatic difference between vasculature and tissue and reduced osmotic pressure difference combine to cause retinal oedema according to Starling's law. Eventually, the extent of the venous stasis retinopathy together with or without tissue hypoxia depends on the severity (Hayreh et al. 2011) and location of the obstruction against blood flow within the central retinal vein.

Depending on the implicated retinal tissue hypoxic injury, the temporary and long-term visual morbidities are primarily caused by vitreous haemorrhage, the magnitude of macular oedema, concomitant development of neovascularization and the progression to neovascular glaucoma (McIntosh et al. 2010).

Retinal blood flow in CRVO. Fluorescein angiographies always manifest some evidence of blood flow within the vascular bed in CRVO. The obstruction to venous outflow is determined by the number of tributaries anterior to the occlusion in the central retinal vein for collateral flow. The farther away from lamina cribrosa, the greater the amount of tributaries situated anterior to the occlusion to create anastomosis with nearby veins for rerouting the blood around the thrombus. On the contrary, the closer the thrombus is situated to the lamina cribrosa, fewer tributaries are available for this collateral flow (Hayreh et al. 2011). In addition, histopathological studies on enucleated eyes due to neovascular glaucoma after CRVO has shown evidence of recanalization of the thrombus that most likely have an onset early on and proceeds over time (Green et al. 1981).

Classification of CRVO. CRVO is classified as either nonischaemic or ischaemic type of CRVO. In the former, the thrombi is located more distal in the optic nerve whereas the occlusion is closer to the lamina cribrosa in the ischaemic type. Subsequently, the clinical entity and visual outcome of the nonischaemic and ischaemic CRVO are very divergent based on the number of tributaries anterior to the occlusion for venous blood shunting (Hayreh et al. 2011).

Nonischaemic CRVO is relatively benign with better prognosis and visual outcome than the ischaemic type which has poor prognosis (Hayreh 2014). Macular oedema is seen in both types but is markedly less and not always present in the nonischaemic form. Unlike the ischaemic type, neovascularization never develops in nonischaemic CRVO eyes. Transient visual deterioration (central scotoma) secondary to macular oedema is the major complication in nonischaemic CRVO that tends to resolve over time. Ischaemic CRVO on the other hand is characterized by severe hypoxia and capillary closure with the risk of neovascular glaucoma and permanent blindness due to an irreversible ischaemic damage of macular ganglion cells (Hayreh et al. 1983; Hayreh 2014).

Tissue hypoxia. Similar to chronic systemic hypoxic condition in cardio- and pulmonary diseases (Arjamaa & Nikinmaa 2006), vascular disorders like CRVO may result in local hypoxia of the inner retina tissue (Williamson et al. 2009). The local hypoxia involves only the retinal circulation secondary to the obstruction of the central retinal vein whereas the oxygen delivery by the systemic circulation is normal.

Hypoxia sets off cascade of hypoxia signals, primarily by the hypoxia-inducible factor (HIF) protein. HIF is a key factor and a common denominator for all hypoxia-dependent events in cells including angiogenesis regulation and transcription of several others genes. HIF comprises two subunits: a stable HIF-β unit which is continuously expressed and a labile HIF-α unit which is regulated and stabilized by normal cellular oxygen tension. Under hypoxic conditions, HIF-1α evades its degrading and starts to build up before moving into the nucleus of the cell where it proceeds as a transcription factor for several gene arrays (Arjamaa & Nikinmaa 2006). These target genes direct glucose transport to tissue cells, glycolysis, a modulation of the vascular tone and an erythropoiesis for homeostasis and survival of retinal cells (Carreau et al. 2011). In oxygen-depleted tissues, the HIF-1α is a chief stimulant for upregulation of VEGF for neovascular sprouting (Pages & Pouyssegur 2005) and angiogenesis throughout the body. The VEGF expression and inflammatory induction disrupt the blood–retinal barrier (Arjamaa & Nikinmaa 2006; Kaur et al. 2008; Flammer et al. 2013) that is analogous to the breakdown of the blood–brain barrier (Schoch et al. 2002; Yeh et al. 2007) in cerebral ischaemia (Croll et al. 2004). Consequently, the inflammatory mediators (Kaur et al. 2008) and VEGF expression jointly add to the vascular hyperpermeability, retinal oedema, intraretinal haemorrhage and the retinal capillary nonperfusion manifested in CRVO (Boyd et al. 2002; Campochiaro et al. 2008; Noma et al. 2009; Campochiaro 2012).

In ischaemic CRVO, the angiogenic factors diffuse across the disrupted blood–retinal barrier to establish neovascularization at remote locations throughout the intraocular tissues (Kaur et al. 2008), on the optic nerve head and the iris which carries the risk for neovascular glaucoma. For instance, the concentration of VEGF in the aqueous humour was found to be directly correlated with the onset and progression of neovascularization of the iris (Boyd et al. 2002) and to be inversely related with the patient's visual outcome (Campochiaro 2012). Because the VEGF upregulation is a chief stimulant for blood–retinal barrier breakdown, a treatment for anti-VEGF formation is considered an effective means for improving visual outcome in CRVO-affected eyes (Campochiaro et al. 2011; Epstein et al. 2012) either as a monotherapy or in conjunction with other forms of treatment such as vitrectomy or a panretinal photocoagulation.

Oxygen measurements in CRVO. Four studies have published their findings on oxygen content in humans with CRVO. All of them quantified lower oxygenation of the inner retina in CRVO-affected eyes than in the opposite unaffected eyes. In 2002, Yoneya and associates used spectral imaging to measure oxyhaemoglobin saturation of retinal vessel in eyes affected by ischaemic CRVO. They reported semi-quantitative correlation between fluorescein angiography and decreased oxyhaemoglobin saturation of retinal venules (less than 40%) in patients with ischaemic CRVO. They also noted decreased oxyhaemoglobin saturation in adjacent capillary areas that seemed to be circulatory intact. In addition, unaffected retinal hemispheres in CRVO eyes appeared to be influenced by the circulatory disruption (Yoneya et al. 2002). In 2009, Williamson and associates used oxygen sensitive electrode probes to measure intravitreal PO_2 during vitrectomy. Patients with

ischaemic CRVO had lower preretinal PO_2 than patients undergoing vitrectomy for either epiretinal membrane removal or macular hole (Williamson et al. 2009). In 2010, Hardarson and Stefánsson used the Oxymap, noninvasive spectrophotometric retinal oximeter (2nd version), with a 45-degree view of the fundus to measure retinal vessel oxyhaemoglobin saturation in eight patients with unilateral CRVO. The mean oxyhaemoglobin saturation in venules was 49 ± 12% in CRVO eyes and 65 ± 6% in the unaffected eyes ($p = 0.003$). There was no difference, however, in arteriolar oxyhaemoglobin saturation (99%) of CRVO-affected and unaffected fellow eyes (Hardarson & Stefánsson 2010). Most recently, Traustason et al. (2014) used a similar noninvasive spectrophotometric technique (Oxymap Retinal Oximeter P3) for retinal oximetry in 11 patients with unilateral CRVO. At baseline, before intravitreal VEGF inhibitor treatment (Ranibizumab) was initiated, the mean oxyhaemoglobin saturation of retinal venules was 32 ± 12% and 59 ± 10% in the unaffected eyes ($p = 0.001$). Concurrently, the oxyhaemoglobin saturation of retinal arterioles was significantly higher in CRVO eyes than in fellow eyes, or 95 ± 8% and 91 ± 3%, respectively ($p = 0.04$). The oxyhaemoglobin saturation in CRVO eyes improved with time and intravitreal anti-VEGF treatment but still remained subnormal or roughly halfway normalized at 3 and 6 months follow-up point in time (Traustason et al. 2014).

Hayreh and associates have studied the effectiveness of different parameters for delineating ischaemic CRVO from nonischaemic CRVO in the early stages of the disease. They found fluorescein angiography to provide optimal reliable information about retinal capillary nonperfusion in only 50–60% of patients whereas combining information from electroretinography and relative afferent papillary defect captured 97% of the cases and hence to be the best tests (Hayreh 2014). Information on retinal oxyhaemoglobin saturation may be helpful in classification of CRVO in the early stages of the disease although the value of this is beyond the scope of this thesis. Retinal oximetry may also be valuable in the management and observation of CRVO patients over time. Timely intervention on retinal tissue hypoxia is supposedly essential for preventing and interrupting any detrimental effects of the hypoxia signalling cascades in CRVO where retinal oximetry may possibly play a vital role in the future.

Earlier oximetry studies on CRVO have been confined to imaging a small portion of the fundus. In this thesis, we use the Oxymap T1 oximeter to acquire wider images of the fundus, than previous studies, to analyse oxyhaemoglobin saturation in retinal vessels in patients with CRVO and to observe the disease process over time.

Retinal oximetry in systemic diseases

Retinal oximetry has been shown to be reliable and valid in patients suffering from chronic systemic hypoxia secondary to Eisenmenger syndrome and in patients with severe chronic obstructive pulmonary disease (COPD).

Traustason et al. (2011) utilized the Oxymap device to detect hypoxaemia of both retinal arterioles and venules in clinically stable patients with Eisenmenger syndrome, a congenital cyanotic cardiac defect. The oxyhaemoglobin saturation in retinal arterioles was 81 ± 9% and 44 ± 12% in venules as compared with 93 ± 3% ($p < 0.001$) and 59 ± 5% ($p < 0.001$), respectively, in healthy controls. The AV-difference was not markedly different between the groups (37% ± 6% and 34% ± 5%, respectively). The oxyhaemoglobin saturation of retinal arterioles in the Eisenmenger group correlated with both intrafemoral artery oxyhaemoglobin saturation (83 ± 5%, $p = 0.82$; $p < 0.001$) and finger pulse oximetry (88 ± 5%). Also, the decrease in retinal venous oxyhaemoglobin saturation was decreased in proportion to the decrease in femoral artery saturation. Palkovits et al. (2013) reported retinal vessel hypoxia in patients with severe COPD during cessation of their supplemental oxygen therapy, using the Imedos device. The AV-difference remained unchanged during both breathing regimens of the ambient air and oxygen supplementation. According to their findings, the retinal oxyhaemoglobin saturation correlated with both capillary earlobe blood gas sample and finger pulse oximeter measurements (Palkovits et al. 2013).

In people with Giant cell arteritis, retinal oximetry has revealed reduced oxyhaemoglobin saturation in retinal arterioles and elevation in venules despite no ophthalmological manifestation of the disease (Turksever et al. 2014). Mild-to-moderate Alzheimer disease also appears to be expressed by elevated oxyhaemoglobin saturation of both retinal arterioles and venules as compared with healthy control group (Einarsdottir et al. 2015).

Although the literature is sparse on retinal oximetry in systemic diseases, those results suggest the retinal circulation represents the systemic and central nervous system involvement. Retinal oximetry imaging opens the opportunity to examine the central nervous oxyhaemoglobin saturation in systemic diseases and thus gain new insight into oxygen delivery and retinal metabolism in systemic disorders.

Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease is characterized by progressive airflow limitation (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016), chronic inflammation of the airways and systemic inflammatory response (van Eeden & Sin 2008). The airflow limitation eventually creates ventilation–perfusion mismatching that unavoidable leads to systemic hypoxaemia, either with or without CO_2 retention (West 2003). The severity of airflow limitation is classified by spirometry measurement of a forced expiratory volume in one second (FEV_1) to the forced vital capacity (FVC), after maximum inspiration. Patients with less than 50% and 30% of the predicted forced expiratory volume in one second (FEV_1/FVC) are classified with severe (stage 3) and very severe (stage 4) COPD, respectively. Long-term oxygen therapy (>15 hr/day) has shown to increase survival in patients with severe resting hypoxaemia and is recommended for patients with a resting PO_2 at or below (≤) 55 mmHg or arterial oxyhaemoglobin saturation ≤88%, with or without hypercapnia (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016). The aim of the supplemental oxygen therapy is to target the pulmonary oxygen concentration between 60–80 mmHg and the oxygen flow titrated according to arterial blood gas and peripheral pulse oximeter

measurements (Hines & Marschall 2012).

The magnitude of systemic inflammation in COPD has been affiliated with the severity of the airflow obstruction and is believed to underlie the extrapulmonary pathogenesis of the disease (Clarenbach et al. 2012). Many organ systems are negatively affected by the systemic inflammatory response including the cardiovascular and autonomic nervous system (van Gestel & Steier 2010). Cardiovascular events are considered a frequent reason for mortality in patients classified with mild-to-moderate COPD (Sin et al. 2006; van Eeden & Sin 2008). Presumably, the systemic inflammatory response in addition to the systemic hypoxia, oxidative stress and sympathetic activation leads to vascular dysfunction and hence a cardiovascular disease (Clarenbach et al. 2012). Hypoxia seems to provoke vascular dysfunction by disrupting the physiological equilibrium between vascular constriction and vasodilatation by means of upregulation of vasoconstrictive mediators, such as endothelin-1 and inhibition of nitrous oxide activity (McQuillan et al. 1994).

It has been speculated that hypoxaemia is a key modulator for development of polyneuropathy in COPD patients (Ozge et al. 2005; Oncel et al. 2010) and thus possibly playing role in the optic nerve and retinal involvement (Demir et al. 2012) in the disease. As already mentioned, Palkovits et al. (2013) reported markedly reduced oxyhaemoglobin saturation of both retinal arterioles and venules in patients with severe COPD. According to their findings, there was a significant positive correlation of oxyhaemoglobin saturation in retinal arterioles with capillary earlobe blood measures and finger pulse oximetry (Palkovits et al. 2013). Based on their findings, retinal oximetry has the ability to identify systemic hypoxaemia of the retinal circulation which supports its potential for applicability to acute patients' care settings in the future.

Aims

The overall aims of this thesis are to test whether the retinal oximetry can be applied to measuring systemic oxygen levels in healthy subjects as well as subjects with compromised oxygenation in order to improve non-invasive monitoring of critically ill and patients in anaesthesia care in the future.

The specific objective is to test whether oxyhaemoglobin saturation of the systemic circulation can be measured through the retinal circulation in health and disease. The research questions and hypothesis are the following:

Research question 1: Is the retinal oximeter sensitive to changes in oxyhaemoglobin saturation (Papers I-V)?
(a) Is the retinal oximeter sensitive to local hypoxia in retina, that is CRVO (Paper I)?
(b) Is the retinal oximeter sensitive to systemic changes in oxygen levels? (Papers II and III)?

(i) Is the oxyhaemoglobin saturation in retinal vessels affected by the system hypoxaemia and supplemental oxygen breathing in people with severe chronic obstructive pulmonary disease (Paper III)?
(ii) Is oxyhaemoglobin saturation in retinal vessels affected by hyperoxic breathing in healthy individuals (Paper II)

(c) Is a retinal oximetry applicable to infants (Paper IV)?

Hypothesis 1:
(a) The retinal oximeter is sensitive to the various extent of retinal tissue hypoxia in people with CRVO (Paper I).
(b) The retinal oximeter is sensitive to changes in systemic arterial oxygen content.
(i) Oxyhaemoglobin saturation in retinal vessels is affected by systemic hypoxaemia in people with severe COPD, and the oxyhaemoglobin saturation improves with supplemental oxygen breathing (Paper III).
(ii) Oxyhaemoglobin saturation of retinal vessels is increased from normal baseline level during systemic hyperoxia (Paper II).
(c) Combined scanning laser ophthalmoscope and retinal oximetry is sensitive to different oxygen content in retinal arterioles and venules. The modified version of retinal oximetry is feasible for neonates (Paper IV).

Research question 2: Is retinal oximetry comparable to radial artery blood measures and finger pulse oximetry in people with chronic hypoxia secondary to severe COPD (Paper III)?

Hypothesis 2: Spectrophotometric retinal oximetry is at least as good indicator of the systemic oxyhaemoglobin saturation as invasive radial artery blood sample measurements and peripheral finger plethysmography in people with severe COPD (Paper III).

Materials and Methods

Protection of human subjects and ethical standards

The studies were approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority. Protocols were in compliance with the tenets of Declaration of Helsinki. A signed informed consent was obtained from all adult participants (Papers I-III) and parents of the neonates (Paper IV) prior to the study enrolment.

Retinal oximetry studies

Study population

CRVO patients (Paper I). Nineteen consecutive Caucasian patients who presented with symptoms of unilateral CRVO at the Department of Ophthalmology at the Landspitali University Hospital in Iceland enrolled in the study. All patients were recruited using convenience sampling of referral by their ophthalmologist for retinal oximetry. The study design is a prospective observational case series.

Three study subjects were excluded from the analysis due to either insufficient oximetry image quality or lack of images from the first visit. Of the 16 patients included in the analysis, eleven were males and five females. The age ranged from 48 to 81 years with the mean of 64 ± 9 years (mean ± SD). Five patients were referred by their ophthalmologist for repeated oximetry imaging over time.

Four patients were treated for glaucoma, two patients had COPD, two had diabetes mellitus, five had ischaemic heart disease, four were on a treatment for arterial hypertension, and single patients had aortic valve replacement, atrial fibrillation, ipsilateral carotid endarterectomy, chronic renal failure, renal cancer and migraine. Oxyhaemoglobin saturation measurements of retinal vessels of both eyes were made before initiating any

treatment (except for seven days of latanoprost eye drops in one case).

Healthy people under hyperoxia (Paper II). In total, 33 healthy individuals with healthy eyes were recruited through advertisement for the study. Thirty subjects were included in the analysis. Eleven were males and 19 females with the mean age of 44 ± 18 years.

Exclusion criteria included any eye disease, stroke, epilepsy and seizure disorders, smoking and systemic diseases. Systemic diseases pertained to disorders that could have pathological effects on the eye such as diabetes mellitus or affecting the systemic oxygen content like cardiopulmonary diseases including coronary artery disease and COPD.

It was required that expiratory end-tidal oxygen plateau was reached during the hyperoxic breathing (100% oxygen) period. Three participants were precluded from the study: two of them did not reach end-tidal oxygen plateau and one was considered a glaucoma suspect. All study subjects underwent eye examination by ophthalmologist within seven months before participation in the study.

COPD patients (Paper III). Eleven Caucasian (7 female, 4 male) people with severe COPD [GOLD (Global Initiative in Obstructive Lung Disease) stage 3 or 4] as classified by a forced expiratory volume of less than 50% of predicted in one second ($FEV_1 < 50\%$) were recruited in the study. The mean age was 70.4 ± 5.4 years (ranging 66 to 82 years). All COPD participants were on long-term oxygen therapy and had lightweight portable concentrator devices that supplied their prescribed oxygen (Luxfer, Salford M50 3XE, UK). The long-term oxygen therapy was based on meeting the international criteria for sustained hypoxia, generally defined as an arterial oxyhaemoglobin saturation of <90%. All COPD participants received their ambulatory pulmonary care at the National University Hospital in Iceland and were clinically evaluated to have sufficient respiratory reserve capacity to endure ceasing their supplemental oxygen for 15 min. They were all in a stable condition and had a baseline finger pulse oximetry of greater than 89% on their prescribed oxygen.

Study exclusion criteria included signs or symptoms of a coronary arterial disease; history of atrial fibrillation, congestive heart failure, carotid stenosis, brain tumour, stroke, anticoagulation treatment with blood test coagulation factors outside the normal range; diabetes mellitus, mental illness or any eye disease. Before participating in the study, all the COPD subjects underwent a complete eye examination.

Eleven age and gender matched healthy subjects served as a control group data. The control group was selected from a set of 120 healthy subjects in a database who had undergone retinal oximetry analysis of oxyhaemoglobin saturation whilst breathing ambient air prior to the current investigation.

Neonates (Paper IV). Fifty-nine full-term healthy neonates were recruited at the paediatric department of the University Hospital in Iceland during a routine fifth day postpartum infant examination. The mean gestational age was 40 weeks. Thirty-four were female and 25 male gender. The mean age on the day of the study was 16 ± 4.8 days. Inclusion criteria included healthy neonates with gestational age of 37 to 42 weeks and normal weight at birth. Exclusion criteria included any complications of the mother or the foetus during pregnancy or in the perinatal period.

Successively retinal images were obtained from 55 babies. Three babies were excluded from the study because they did not collaborate in opening their eyes for image acquisition. One was precluded due to prematurity at birth.

Study protocol (Papers I-IV)

All studies were conducted according to a standard protocol. Medical histories including smoking were obtained from self-reported questioners (Papers I-III) and patient records (Papers I & III). Prior to the study, participants sat comfortable on a chair for measurement of vital signs at baseline. Pupil dilation (mydriasis) was achieved using 1% tropicamide (Mydriacyl, S.A. Alcon-Couvreur N.V., Puurs, Belgium) followed by retinal oximetry. In neonates, a detail on the maternal and fetal health was obtained prior to the study (Paper IV).

CRVO (Paper I). A finger pulse oximetry reading (Ohmeda Biox 3700; Ohmeda, Boulder, CO), blood pressure and pulse rate measurements (Omron M6 Comfort [HEM-7000-E]; Omron Healthcare Europe, Hoofddorp, the Netherlands) were obtained prior to and following retinal oximetry and recorded on the patient chart.

Hyperoxia (Paper II). Oxyhaemoglobin saturation of retinal arterioles and venules by retinal oximetry imaging were compared between three inspired gas conditions: (1) ambient air (baseline), (2) hyperoxic breathing for 10 min, and (3) ambient breathing for 10 min (recovery). Under each circumstances; finger pulse oximetry, pulse rate, blood pressure, fraction of inspired oxygen (FiO_2), end fraction of expired oxygen (EtO_2), end-tidal carbon dioxide ($EtCO_2$), fraction of inspired carbon dioxide ($FiCO_2$) and respiratory rate (RR) were measured.

First, both pupils were dilated for measurement of IOP (iCare TAO1 Tonometer; Tiolat Oy, Helsinki, Finland). Next, with the study participant sitting comfortable in a chair, a finger pulse oximetry, pulse rate (Datex-Ohmeda, Oxytip+ Healthcare, Finland) and blood pressure readings ([HEM-7221-E]; Omron Healthcare Europe, Hoofddorp, the Netherlands) were obtained followed by retinal oximetry imaging for baseline.

Then, an appropriately sized inflatable soft cushion face mask (Flexicare, Flexicare Medical Ltd., Mountain Ash., UK) was placed over the mouth and nose of the subject′s face. The face mask was connected to a circle system with carbon dioxide absorber of an anaesthesia machine (Dameca: Siesta 10770, Rodovre, Denmark). Airtight seal was created by fasten a head strap to retaining hooks surrounding the facial mask orifice which was further supported by the participant′s hand to prevent leakage. The adequacy of the face mask fitting was tested by checking the tidal volume and capnography waveform with oxygen flowing through the anaesthesia machine (Dameca: Siesta 10770, Rodovre, Denmark). Subsequently, the oxygen flow was set to 6 l/min and 100% concentration inhaled for 10 min that was immediately followed by a second session of retinal oximetry imaging. Because the face mask did not fit in front of the fundus camera, it was removed and the study subjects held their breath whilst the retinal oximetry images were captured (about 30 seconds).

The face mask was also attached to a respiratory gas analyser (Datex-

Ohmeda D-LCC15.03, Planar Systems Inc., Beaverton Oregon, USA) with the ability to continuously monitor the subjects' respiratory parameters (FiO_2, EtO_2, $FiCO_2$, $EtCO_2$, RR) throughout the study. Blood pressure cuff was applied over the brachial artery and the blood pressure measured under the three breathing regimens: (1) on ambient air at baseline, (2) 9 min into hyperoxic breathing and (3) recovery ambient air breathing for 10 min. Systolic and diastolic blood pressures were used to calculate mean arterial pressure (MAP) using the formula: (1/3 systolic blood pressure) + (2/3 diastolic blood pressure). The ocular perfusion pressure (OPP) was calculated as 2/3 MAP – IOP.

Two male study subjects, 24 and 33 years old, underwent retinal oximetry imaging of the left eye every 5 seconds for a total of 120 seconds. The procedure started at once when the supplemental oxygen was halted and lasted into the first 2 min of the recovery ambient air breathing.

COPD patients (Paper III). Oxyhaemoglobin saturation of retinal arterioles and venules in subjects with severe COPD was compared to healthy controls whilst breathing ambient air. Additionally, the COPD subjects were exposed to three inspired gas conditions: 1) prescribed supplemental oxygen, 2) ambient air and 3) prescribed supplemental oxygen. Under each breathing regimen, finger pulse oximetry, pulse rate, blood pressure, fraction of inspired and expired oxygen (FiO_2/EtO_2), inspired and end-tidal carbon dioxide ($FiCO_2$/$EtCO_2$), respiratory rate and retinal oximetry images of both eyes were obtained (Fig. 8). Radial arterial blood samples for blood gas analysis were drawn under the ambient air breathing period only.

Subjects were seated comfortably in a chair whilst breathing their prescribed supplemental oxygen from a lightweight portable oxygen concentrator. Finger oximetry sensor was applied to a finger for which a stable reading could be obtained and the first peripheral arterial oxyhaemoglobin saturation value and pulse rate were measured (Datex-Ohmeda OxyTip+ Healthcare, Finland). The patient's portable oxygen concentrator device and nasal cannula were then substituted with a dual nasal cannula system (Flexicare Medical Limited, UK) connected to an oxygen cylinder with the flow meter set at the subjects prescribed rate of flow. In addition, the cannula was connected to a respiratory gas analyser (Datex-Ohmeda D-LCC15.03, Planar Systems Inc., Beaverton Oregon, USA) with the ability to continuously monitor the respiratory rate and measure the respiratory parameters. Finger pulse oximetry, pulse rate, respiratory rate, $EtCO_2$ and FiO_2 were continuously measured during the study. Noninvasive blood pressure cuff was applied over the brachial artery (Omron M6 Comfort [HEM-7000-E]; Omron Healthcare Europe, Hoofddorp, the Netherlands) on the opposite arm to the finger pulse oximeter. Blood pressure measurements were obtained at three different time-points in the study. Mean arterial pressure was calculated from systolic and diastolic blood pressure values, using the formula: (1/3 systolic blood pressure) + (2/3 diastolic blood pressure).

After pupil dilation, baseline retinal oximetry images were obtained with subjects inhaling their prescribed supplemental oxygen (first baseline period). Then, the supplemental oxygen was discontinued and the subject inhaled ambient air (ambient air period) followed by acquisition of the second session of retinal oximetry images. The prescribed supplemental oxygen was then reinstituted for a period of 20 min (second baseline period) followed by acquisition of the final session of retinal oximetry images.

Prior to the second retinal oximetry session, whilst on ambient air for 10 min, a modified Allen's test (Habib et al. 2012) was made on the nondominant hand to verify the arterial competency. Instantaneously, after the oximetry imaging, an radial artery blood sample was drawn (BD Preset with needle, Becton, Dickinson and Company, UK) and sent for immediate blood gas analysis using co-oximetric blood gas analysis (ABL 800, Radiometer A/S, Husum, Denmark).

Neonates (Paper IV). The neonate lay in a prone position on the lower arm of the parent. The parent stabilized the back by supporting the chin and chest with the other hand (modified flying baby position). The researcher assisted the parent by aligning the head of the baby towards the SLO device with the adequacy of alignment confirmed on the Optomap monitor. The researcher spread the infants' eyelid by hand using a rubber glove or a cotton tip. A different researcher obtained images of this one eye.

Oxymap retinal oximeter (Papers I-III)
The noninvasive dual-wavelength Oxymap T1 retinal oximeter (Oxymap ehf., Reykjavik, Iceland) is based on a conventional fundus camera (Topcon TRC-50DX; Topcon Corporation, Tokyo, Japan) connected with a costume-made optical adapter (Fig. 9). Two narrow band-pass filters (5 nm) and a beam splitter are coupled with two high-resolution digital cameras (Insight IN 1800, 1600 × 1200 square pixels; Diagnostic Instruments Inc., Sterling Heights, MI) that generate 50° view of the fundus.

First, the fundus camera briefly illuminates the ocular fundus with white light for retinal oximetry. Subsequently, the two narrow band filters allow the retinal oximeter to simultaneously acquire two monochromatic retinal images at 570 nm and 600 nm for spectrophotometric analysis. A broader 80 nm band-pass filter is

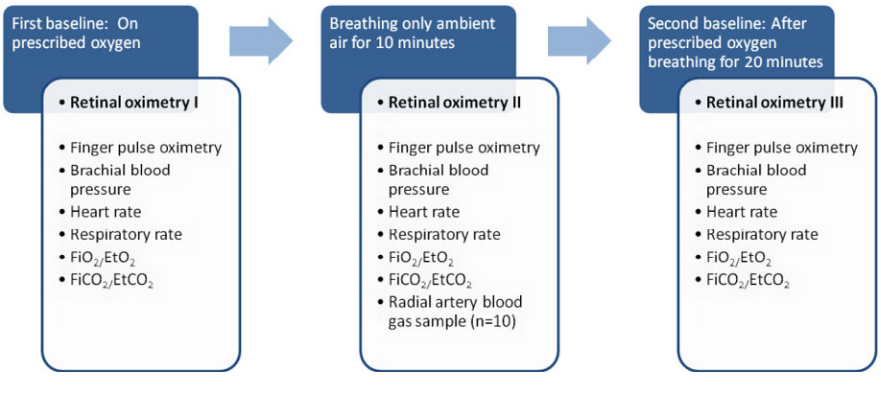


Fig. 8. Order of experimental protocol.

Fig. 9. The retinal Oxymap T1 oximeter.

situated in the light path of the fundus camera (585 nm centre wavelength) in order to restrict redundant light exposure to the subjects' eyes by permitting only 545 to 625 nm light to exit the camera lens.

Spectrophotometric oximetry is based on the fact that oxyhaemoglobin and deoxyhaemoglobin have different light absorption spectra. The monochrome image at 570 nm is at isosbestic wavelength that is insensitive to oxyhaemoglobin. The absorbance of arterioles and venules is similar at this point so they come into view equally dark on the image. The monochromatic image at 600 nm wavelength, however, is at nonisosbestic point that is sensitive to oxyhaemoglobin (Fig. 10). The absorbance of the arterioles at this wavelength is less than of venules so the arterioles appear brighter than venules on the image.

Oximetry imaging. The room was dimmed with window blinds by the only light source coming from the fundus camera and a computer screen that was configured at the dimmest setting. Image acquisition was performed according to a standard protocol with the study subjects sitting at the fundus camera with their chin on a chin rest and forehead against the head bar in front of them. The same experienced researcher took all the images with participants sitting in front of the fundus camera. Five images of the right eye were taken first and then of the left eye in Papers II and III. In Paper I, these five images were first taken of the CRVO-affected eye and then the opposite unaffected eye. The first image was centred on the macula. Second image was centred on the optic disc. On the third image, the subject gazed up and on the fourth image down. The fifth image was replication of the second image with the optic disc in the centre. The average time for obtaining these five images of each eye was approximately 30 seconds. The first good quality image with the optic disc in centre was selected manually for analysis.

Image processing. Specialized software (Oxymap Analyzer software 2.2.1, Papers I and II, and 2.4 Paper III, version 3847) automatically selects vessel points on the monochromatic images for measurement of light intensity inside (I) and outside the vessel (I_O) for calculation of optical density *(OD)* and optical density ratio (ODR) for estimation of oxyhaemoglobin saturation. The calibration of the retinal oximeter is based on calibration constants where a = −1.28 and b = 1,24. These calibration factors are derived from the work of Schweitzer et al. (1999), who measured the average oxyhaemoglobin saturation of whole blood in vivo and vitro for healthy people using calibrated imaging spectroscopy. Their findings of 92.2% for retinal arterioles and 57.9% for venules are used to extrapolate the oxyhaemoglobin saturation in subjects' vessel by comparison of the ODR to that of the calibration settings. The calculated oxyhaemoglobin saturation is then automatically presented as a pseudocolour overlay (Fig. 11) on the fundus image.

The software also quantifies the vessel width at particular point by counting the pixels on an orthogonal cross section of the vessel. These numbers of pixels are then averaged for over 100 cross sections along the measured vessel to obtain the mean.

Data analysis. First- and second-degree arterioles and venules in each quadrant on the fundus images were selected for analysis and calculation of mean oxyhaemoglobin saturation values. Vessels were matched so that measured vessels in the fellow eye were parallel to the CRVO eye (Paper I), within the eye between oximetry sessions (Papers II and III) or between the eye of the COPD subject and of a healthy subject from the control group (Paper III). To get this matching, two vessel segments

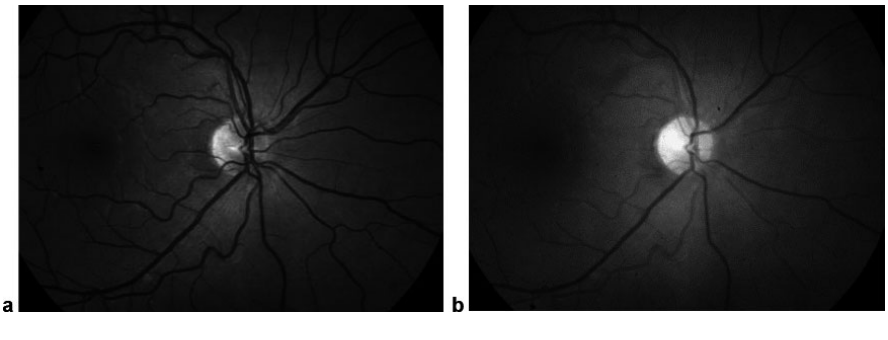


Fig. 10. Oxymap T1 acquisition of two monochromatic fundus images at different wavelengths with the optic disc in the centre. (A) Isosbestic wavelength (570 nm) that is not sensitive to oxygen. (B) Oxyhaemoglobin-sensitive wavelength (600 nm). The arterioles and venules are of similar density on the 570 nm image (left), but on the 600 nm image (right), the arterioles have a much lower optical density and appear brighter, due to lower absorption of oxyhaemoglobin at 600 nm.

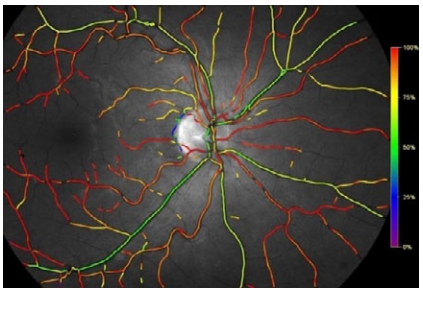


Fig. 11. Pseudocolour image of the right fundus in a healthy subject. The colours indicate oxyhaemoglobin saturation as seen on the scale on the right. In general, arterioles are orange to red, indicating oxygen saturation about 90–100%. Venules may vary from bluish to yellow but are normally green, indicating normal oxyhaemoglobin saturation around 50–60%.

were sometimes averaged and inserted as one for calculation of the mean for the eye. For example, in CRVO, if a selected vessel segment in the inferior temporal venule in the fellow eye was too short (<100 pixels) before branching, both daughter vessels on the other side of the bifurcation were measured. The calculated mean average was then inserted as one and compared with the mean value of the matching parent vessel before branching (if ≥100 pixels) in the CRVO-affected eye.

The AV-difference was represented as the calculated difference between arterial and venous oxyhaemoglobin saturation.

CRVO (Paper I)—Vessel segments close to haemorrhages were excluded to bypass artefacts. Vessels were matched so that measured vessels in the fellow eye were parallel to the CRVO eye.

Of the sixteen patients included in the analysis, single measurement was made in 11 cases and repeated measurements in five cases over a time period ranging from two weeks to 20 months. Data from two patients were not included in the comparative statistical analysis because images of the unaffected eye were lacking. Hence, the values of their oxyhaemoglobin saturation and numbers of measured vessels are given in the result Table 4 (Chapter 4.1) for information only.

Hyperoxia (Paper II)—Oxyhaemoglobin saturation was calculated for all first- or second-degree arterioles and venules in each quadrant of the right eye. Vessels were matched between retinal images of the three breathing regimens.

COPD (Paper III)—First- and second-degree arterioles and venules in each quadrant of the right eye were matched between the retinal images of the three breathing conditions and with a retinal image of a healthy control subject. Identical image acquisition and oximetric analyses were performed for the healthy subjects.

Of the 11 COPD participants, one was not breathing the prescribed oxygen on arrival so there was no measurement at first baseline for this subject and we were unable to draw arterial blood sample from another. Hence, data from 10 subjects within the COPD group underwent statistical analysis at each study time period for comparison of mean and standard deviation. Furthermore, one COPD patient was 'a mouth breather', and therefore, it was not possible to get adequate $EtCO_2$ and FiO_2 data from the dual nasal cannula at any of the three study period. All 11 COPD subjects were included in the comparison with healthy controls under ambient air breathing (Table 2).

Table 2. Selection criteria for retinal vessel segment measurement of oxyhaemoglobin saturation with the Oxymap T1 retinal oximeter. One pixel is approximately 9.3 μm.

Begin vessel selection	Papers I and II: a) at least 15 pixels around the optic disc excluded b) at least 15 pixels on a border of any bright area around the optic disc excluded c) start as close to the optic disc as possible Paper III: An area that was demarcated 1.5 disc diameters (344 pixels)
End vessel selection	Papers I and II: Never closer than 30 pixels to the rim of the image Papers III: 3 disc diameters (690 pixels)
Minimum vessel diameter	Papers I and III : 8.0 pixels (~75 μm) Paper II: 6.0 pixels (~56 μm)
Vessel length	Paper I: 100–300 pixels (as close to 300 pixels as possible) Paper II: 50–200 pixels (as close to 200 pixels as possible) Paper III: 50 pixels at minimum but as close to the total length of the segment between the two circles (1.5 and 3 disc diameters) as possible.
Vessel segment exclusion criteria	a) Vessel branching with 15 pixels b) Vessel crossing c) Any extremes in background brightness such as haemorrhage to avoid artifacts

Neonates (Paper IV)

The Optomap 200Tx scanning laser ophthalmoscope (Optos plc., Dunfermline, Scotland, UK) for the oximetry imaging in neonates, uses 532 nm and 633 nm laser wavelengths of light to obtain two monochrome spectral images of the fundus. Although the reference wavelength at 532 nm nearer an isosbestic point, it is not completely isosbestic. The great distinction between oxy- and deoxyhaemoglobin light absorption at 532 nm and 633 nm (oxygen sensitive wavelength) allows for application of SLO for retinal oximetry. The ODR is calculated according to the following equation:

$$\mathrm{ODR} = \frac{\mathrm{OD}\,633\,\mathrm{nm}}{\mathrm{OD}\,532\,\mathrm{nm}}. \qquad (18)$$

The calculated oxyhaemoglobin saturation is then inversely and near linearly related to the ODR.

Oximetry imaging of neonates. The room was dimmed with window blinds and the only light source coming from the SLO and a computer screen that was configured at the dimmest setting. The same researcher took all the images with the babies aligned in front of the SLO in a modified baby flying position. Both Ultra-Widefield (200°) and ResMax (100°) retinal images were obtained of a unilateral eye. For the purpose of the study, 250 ResMax images were acquired in total with the median of four images (range 0–8) per neonate. Ultra-Widefield images were obtained for further research purpose only.

Image processing. A modified version of the dual-wavelength Oxymap Analyzer software 2.3 (version 5206) algorithms was used for the spectral imaging and to process the monochromatic ResMax images for calculation of ODR and vessel diameter. The calibration of the modified version of Oxymap Analyzer software is based on calibration constants where $a = -2.4733$ and $b = 1.4388$. These calibration factors are derived from the work of Kristjansdottir et al. (2014) which based on the work of Schweitzer et al. (1999) for mean oxyhaemoglobin saturation of 92.2% for retinal arterioles and of 57.9% for venules for healthy people as has previously been discussed (Chapter 3.2.3.2). The oxyhaemoglobin saturation can then be calculated according to the $(a \times \mathrm{ODR} + b) \times 100$. The calculated oxyhaemoglobin saturation was automatically presented as a pseudocolour map on a 100° oximetry fundus image (Fig. 12).

Image analysis. In total, 28 fundus images of 28 neonates were manually selected for analysis by the modified Oxymap Analyzer software for calculation of ODR and vessel diameter. The image analysis was standardized prior to the study. The image of the

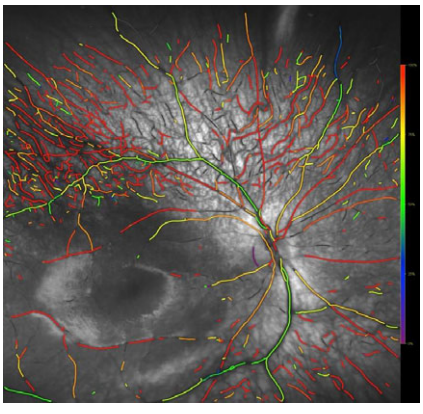


Fig. 12. Pseudocolour overlay, using Oxymap algorithm, over monochrome spectral SLO image of the fundus.

best contrast and focus quality of the main superotemporal arteriole and venule with the optic disc in the centre was manually selected for oximetry analysis (Table 3).

If the superotemporal vessel segment from the optic disc rim to the first branching was shorter than 60 pixels, the segment was excluded. The vessels segment (≥60 pixels) posterior to the branching until the second branching was selected instead for oximetry analysis.

Statistical analysis (Papers I-IV)

Statistical data analysis for Papers I-III was carried out with Prism version 5 (GraphPad Software Inc, LaJolla, California, USA) for comparison of means by using 2-tailed paired *t*-tests. Resulting data are presented as mean ± SD. p value of <0.05 was considered to be statistically significant.

For Paper III, the resultant data were in addition to the 2-tailed paired *t*-tests, presented as repeated-measures one–way ANOVA for comparison of means. Dunnett's and Tukey's multiple comparison post-tests were performed to compare the means of group pairs. A p value of < 0.05 was considered to be statistically significant and the data presented as mean ± SD (95% confidence interval (CI)). Bland–Altman plots were used to determine the level of agreement between different measurement devices, that is retinal oximetry, pulse oximetry and radial artery blood samples. The degree of error, defined as the difference between the measured values, was reviewed in terms of bias and variability, where bias was calculated as the average difference between the measurements and the variability as the mean bias ±1.96 standard deviations. A power analysis indicated that seven subjects were necessary for Paper III to identify a difference of three percentage points (%) in oxyhaemoglobin saturation between retinal oximetry measurements of subjects inspiring supplemental oxygen versus ambient air with 90% power.

For Paper IV, statistical data analysis was carried out using SPSS version 22 (Release 22.0.0.0, IBM). A 2-tailed paired *t*-test was used for comparison of the mean oxyhaemoglobin saturation in superotemporal arterioles and venules. Resulting data are presented as mean ± SD. p value of <0.05 was determined to be statistically significant.

Results

Three studies on retinal oximetry (Papers I-III) were carried out to test whether the Oxymap T1 retinal oximeter is sensitive to changes in oxyhaemoglobin saturation. The first study was on retinal vessel hypoxia in people affected by CRVO (Paper I). The second study was on changes of oxyhaemoglobin saturation in retinal arterioles and venules in healthy people during hyperoxic breathing (Paper II). The third study was on retinal vessel oxyhaemoglobin saturation in people with systemic hypoxaemia with and without their supplemental oxygen therapy. In that study, a comparison was made with a healthy cohort and invasive radial artery blood gas and finger pulse oximetry values during ambient air breathing (Paper III). The fourth study was adjunctive to the former studies in order to learn whether a retinal oximetry is applicable to infants (Paper IV).

Retinal oximetry in CRVO patients (Paper I)

For the 14 eyes affected by CRVO, the oxyhaemoglobin saturation of retinal arterioles was 95 ± 5% and 31 ± 12% in venules (mean ± SD). In unaffected fellow eyes of the same patients, the oxyhaemoglobin saturation of retinal arterioles was 94 ± 6% and 52 ± 11% in venules. The venous oxyhaemoglobin saturation in the CRVO-affected eyes was significantly lower than in the fellow unaffected eyes (31 ± 12% versus 52 ± 11%, $p < 0.0001$, paired *t*-test; Fig. 13). There was no statistical difference between oxyhaemoglobin saturation of retinal arterioles in eyes affected by CRVO and the fellow unaffected eyes ($p = 0.49$).

Greater variability of retinal venous oxyhaemoglobin saturation was observed in CRVO eyes as compared with unaffected fellow eyes (Table 4). The AV-difference in eyes with CRVO

Table 3. Selection criteria for retinal vessel segments for measurement of oxyhaemoglobin saturation with the combined SLO and modified Oxymap analysis software.

Begin vessel selection	a) at least 15 pixels around the optic disc excluded b) at least 15 pixels on a border of any bright area around the optic disc excluded c) start as close to the optic disc as possible
End vessel selection	Never closer than 30 pixels to the rim of the image
Minimum vessel diameter	6.0 pixels
Vessel length	60–350 pixels (as close to 360 pixels as possible)
Vessel segment exclusion criteria	a) Vessel branching with 15 pixels b) Vessel crossing c) Any extremes in background brightness

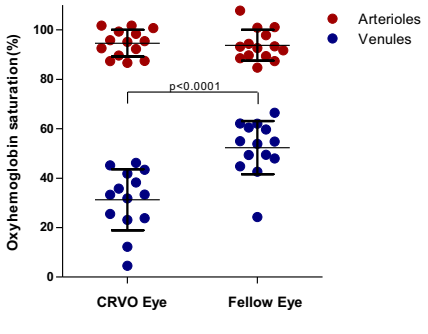


Fig. 13. Oxyhaemoglobin saturation in retinal arterioles (red dots) and venules (blue dots) in CRVO eyes and unaffected fellow eyes. Each dot stands for mean oxyhaemoglobin saturation (%) of individual eye in first- and second-degree retinal vessels. Bars designate mean ± SD ($n = 14$, $p < 0.0001$).

Table 4. Oxyhaemoglobin saturation (%) in first- and second-degree retinal arterioles and venules in 16 patients with central retinal vein occlusion. The values show mean ± standard deviation and number of measured retinal vessels (parentheses). SpO_2 is finger pulse oximetry. The diameter of retinal venules is shown in pixels. Patients' number 15 and number 16 were not included in statistical analysis. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653–1661, ©2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology).

	CRVO eye		Fellow unaffected eye			CRVO eye	Fellow eye
Patient no.	Arterioles	Venules	Arterioles	Venules	SpO_2	Retinal venule diameter	Retinal venule diameter
1	87 ± 5 (2)	23 ± 26 (4)	87 ± 6 (2)	49 ± 6 (4)	94	16	17
2	87 ± 3 (6)	5 ± 32 (3)	85 ± 8 (5)	24 ± 6 (3)	93	14	14
3	101 ± 4 (6)	24 ± 12 (4)	101 ± 8 (6)	62 ± 4 (4)	98	20	16
4	96 ± 16 (4)	33 ± 22 (3)	101 ± 6 (4)	54 ± 5 (3)	95	18	15
5	95 ± 5 (5)	42 ± 13 (5)	93 ± 11 (5)	61 ± 4 (4)	97	16	16
6	98 ± 7 (3)	38 ± 17 (5)	94 ± 1 (3)	55 ± 6 (5)	97	13	13
7	95 ± 3 (7)	45 ± 12 (4)	98 ± 7 (7)	60 ± 7 (4)	95	18	20
8	92 ± 12 (5)	46 ± 24 (2)	90 ± 4 (6)	55 ± 2 (2)	97	19	17
9	102 ± 8 (3)	33 ± 24 (5)	108 ± 2 (3)	62 ± 13 (5)	97	17	17
10	87 ± 10 (6)	12 ± 15 (6)	89 ± 5 (6)	43 ± 6 (6)	95	18	14
11	102 ± 7 (7)	32 ± 22 (6)	92 ± 11(7)	48 ± 9 (6)	94	19	17
12	93 ± 7 (4)	25 ± 14 (3)	89 ± 3 (4)	49 ± 7 (3)	96	14	16
13	99 ± 6 (5)	36 ± 6 (5)	93 ± 7 (5)	45 ± 5 (4)		20	16
14	90 ± 6 (5)	43 ± 6 (6)	93 ± 4 (5)	66 ± 4 (6)		16	15
15	95 ± 5 (7)	34 ± 3 (4)					
16	102 ± 9 (4)	50 ± 8 (4)			96		
Patients 1–14 Mean ± SD	95 ± 5	31 ± 12	94 ± 6	52 ± 11		17 ± 2	16 ± 2
Patients 1–16 Mean ± SD	95 ± 5	33 ± 12					

The standard deviation reflects the oxyhaemoglobin saturation variation within individual eyes.

was 63 ± 11% compared with 43 ± 7% in unaffected fellow eyes ($p < 0.0001$). The mean arteriolar vessel diameter in pixels (each pixel is about 9.3 μm) in CRVO eyes was 10 ± 1 and 12 ± 1 in fellow eyes ($p < 0.0001$). The diameter of retinal venules in eyes with CRVO was 17 ± 2 and 16 ± 2 in unaffected fellow eyes ($p = 0.02$).

There were no statistical differences between finger pulse oximetry (96% ± 2%, $n = 13$) and oxyhaemoglobin saturation of retinal arterioles either in the CRVO eye ($p = 0.28$) or in the fellow eye ($p = 0.24$).

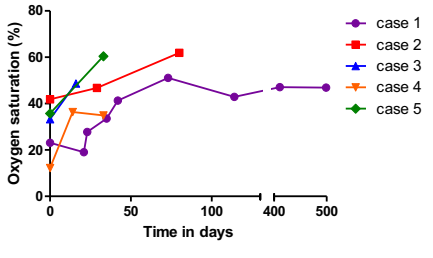


Fig. 14. Mean oxyhaemoglobin saturation of retinal venules during the follow-up period of five patients with CRVO. Each dot indicates the oxyhaemoglobin saturation in first- and second-degree venules at a point in time. Case 1. The patient developed iris neovascularization, glaucoma (Fig. 15) and poor visual outcome. Case 2. The patient received dorzolamide eye drops. Clinical signs of CRVO resolved (Fig. 16). Case 3. The patient presented with ischaemic CRVO, macular oedema and elevated intraocular pressure. Treatment consisted of dorzolamide eye drops, intravitreal bevacizumab and panretinal photocoagulation. At week two, the mean retinal venous oxyhaemoglobin saturation had improved. Case 4. The patient was treated with dorzolamide eye drops for 4 weeks. During the 8-week follow-up period, clinical signs and symptoms resolved. Case 5. The patient presented with mild CRVO on arrival with no macular oedema. The patient was treated with dorzolamide eye drops and established full recovery.

Patients follow-up

All five patients who were followed with repeated retinal oximetry images received treatment; dorzolamide eye drops, intravitreal anti-VEGF bevacizumab and/or laser photocoagulation. In all five CRVO cases, the venular oxyhaemoglobin saturation improved over time (Fig. 14). In two patients, the clinical situation improved and the venous oxyhaemoglobin saturation recovered. In two eyes, the clinical signs and symptoms of CRVO completely resolved but the venular oxyhaemoglobin saturation did not return to normal. One eye developed iris neovascularization, glaucoma and poor visual outcome where the venous oxyhaemoglobin saturation remained slightly subnormal. Measured values at the first and last visit are listed in Table 5.

Table 5. Retinal venous oxyhaemoglobin saturation values (%) in the five patients that were followed with repeated oximetry imaging.

Case	Follow-up period	On arrival	Last image
1	20 months	23 ± 26	40 ± 23
2	80 days	42 ± 13	62 ± 8
3	16 days	33 ± 23	49 ± 12
4	76 days	12 ± 15	35 ± 10
5	33 days	36 ± 6	60 ± 7

The values show mean ± standard deviation.

Retinal oximetry images on the patient who developed iris neovascularization, glaucoma and poor visual outcome are presented in Fig. 15. Images on the patient who resolved from the clinical signs and symptoms are presented in Fig. 16.

Retinal oximetry in healthy under hyperoxemia (Paper II)

After 10 min of hyperoxic facemask breathing, the mean FiO_2 was 96 ± 2% and the EtO_2 91 ± 4% (mean ± SD, $n = 30$). The $EtCO_2$ measured 36 ± 2 mmHg ($n = 29$). The physiological parameters are presented in Table 6.

Both the brachial blood pressure and the heart rate were unaffected by hyperoxic breathing as compared with baseline normoxic breathing ($p = 0.86$,

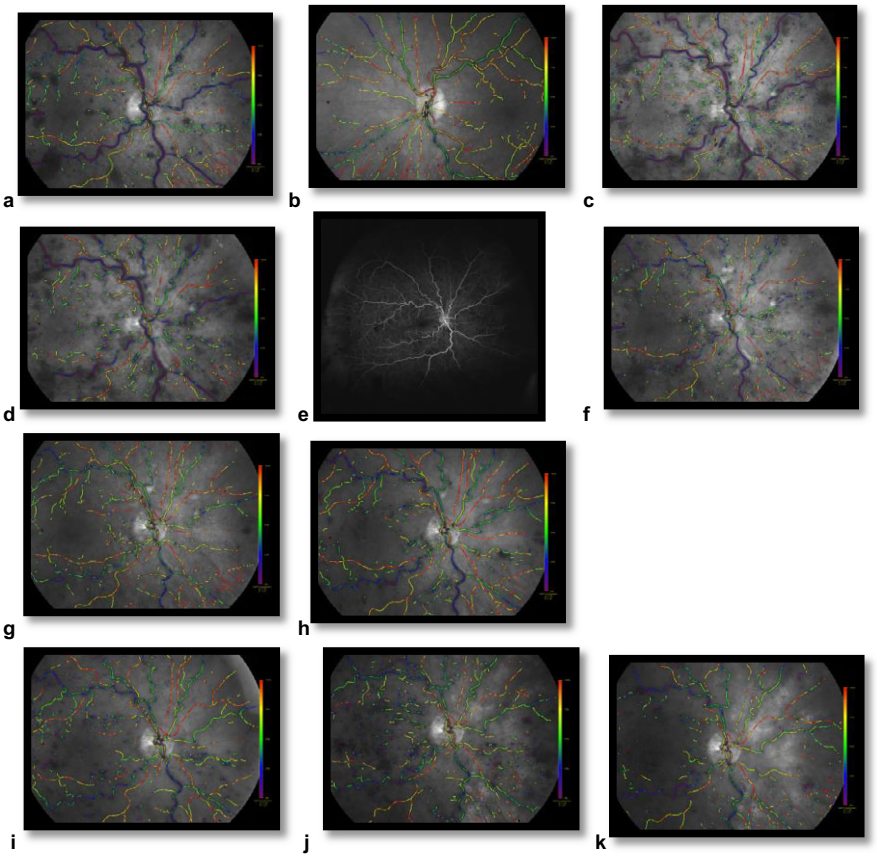


Fig. 15. The patient was followed with repeated oximetry images (Fig. 14, case 1) for 20 months. In the first oximetry image (A), oxygen saturation of retinal venules ranged from minus 4% to 49% as indicated by blue and green colours, respectively, on the image. Three weeks later (C) mean oxygen saturation of retinal venules had decreased. Clinical evaluation revealed cystoid macular oedema and worsened visual acuity. Three days later (D), after the first dosage of intravitreal bevacizumab, retinal venous oxygen saturation and macular oedema improved. At week five (F) and fifteen (G) retinal venous oxygen saturation had improved but regional variability remained high. From week twenty-one (H) clinical symptoms worsened. At 20 months (K), after twelve intravitreal bevacizumab injections and three sessions of panretinal photocoagulation, the CRVO eye had developed neovascular glaucoma. (A) First retinal oximetry image of the CRVO eye, 2 weeks after onset of symptoms. Retinal venous oxyhaemoglobin saturation is 23 ± 26% (mean ± SD) and retinal arteriolar oxyhaemoglobin saturation 87 ± 5%. Visual acuity (VA) 0.7. No macular oedema. Central macular thickness 160 μm. (B) Unaffected left fellow eye. Oxyhaemoglobin saturation in retinal venules is 49 ± 6% and 87 ± 6% in retinal arterioles. VA 0.7. (C) Three weeks after the first retinal oximetry image. Retinal venous oxyhaemoglobin saturation is 19 ± 22%. VA has deteriorated to 0.1. Macular oedema. Central macular thickness 626 μm. (D and E) At three and a half weeks, three days after first bevacizumab injection. Retinal venous oxyhaemoglobin saturation is 28 ± 21%. Macular oedema has resolved. Central macular thickness 190 μm. Fluorescein angiography at 72 seconds after injection shows poorly perfused areas of the retina. (F) At 5 weeks. Clinical signs improving. Retinal venous oxyhaemoglobin saturation is 34 ± 20%. VA 0.3. No macular oedema. Central macular thickness 170 μm. (G) At fifteen weeks, after three bevacizumab injections. Retinal venous oxyhaemoglobin saturation is 51 ± 5%. VA 0.2. No macular oedema. Central macular thickness 132 μm. (H) At week twenty-one. Worsening clinical signs. Retinal venous oxyhaemoglobin saturation is 43 ± 21%. VA 0.1. Recurrence of macular oedema. (I) At thirteen months, three months after ninth bevacizumab injection. Retinal venous oxyhaemoglobin saturation is 47 ± 18%. VA 0.3. Central macular thickness 494 μm. (J) At sixteen months, after ten bevacizumab injections and panretinal photocoagulation. Retinal venous oxyhaemoglobin saturation is 47 ± 20%. VA 0.17. Central macular thickness 316 μm. (K) At twenty months. Neovascular glaucoma. Retinal venous oxyhaemoglobin saturation is 40 ± 23% and retinal arteriolar oxyhaemoglobin saturation 88 ± 5%. VA 0.3. Central macular thickness 140 μm. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653–1661,© 2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology).

n = 30 and p = 0.17, n = 26, respectively).

The oxyhaemoglobin saturation of retinal arterioles was 92.0 ± 3.7% at baseline ambient air breathing and increased to 94.5 ± 3.8% during the hyperoxic breathing (n = 30, p < 0.0001). The oxyhaemoglobin saturation of retinal venules was 51.3 ± 5.6% at the baseline and increased to 76.2 ± 8.0% during the hyperoxic breathing (p < 0.0001). Concurrently, the AV-difference measured 40.7 ± 5.7% at baseline versus 18.3 ± 9.0% during the hyperoxic breathing (p < 0.0001). There were no statistical differences between oxyhaemoglobin saturation measurements and the AV-difference during ambient air breathing at baseline and 10 min of recovery breathing (p = 0.2 and 0.8; Fig. 17).

Under hyperoxic condition, the vessel diameter narrowed in both retinal arterioles and venules. In arterioles, the diameter decreased from 10.3 ± 1.3 pixels at baseline to 9.7 ± 1.4 pixels (p < 0.0001) during hyperoxic breathing. In venules, the diameter of the vessel wall decreased from 13.3 ± 1.5 pixels at baseline to 11.4 ± 1.2 pixels (p < 0.0001) with hyperoxic breathing. The retinal venules′ diameter was slightly narrower at recovery as compared with baseline breathing (13.1 ± 1.4 versus 13.3 ± 1.5, p = 0.007). There was no difference in arteriolar diameter between baseline and recovery breathing (p = 0.3).

During hyperoxic breathing (Fig. 18), the mean oxyhaemoglobin saturation in retinal arterioles was markedly different from finger pulse oximeter measurements (n = 30, 94.5 ± 3.8% and 99.1 ± 0.3, respectively, p < 0.0001, paired t-test).

During ambient air breathing, the mean oxyhaemoglobin saturation in retinal arterioles showed also difference from finger pulse oximeter measurements (n = 27, 92.0 ± 3.7 versus 97.5 ± 0.7, respectively, p < 0.0001).

The finger pulse oximetry increased during hyperoxic breathing as compared with baseline ambient air breathing (n = 27, p < 0.0001).

Figure 19 shows retinal oximetry analysis of the images that were captured every five seconds of two healthy male subjects. The oximetry image session started immediately after cessa-

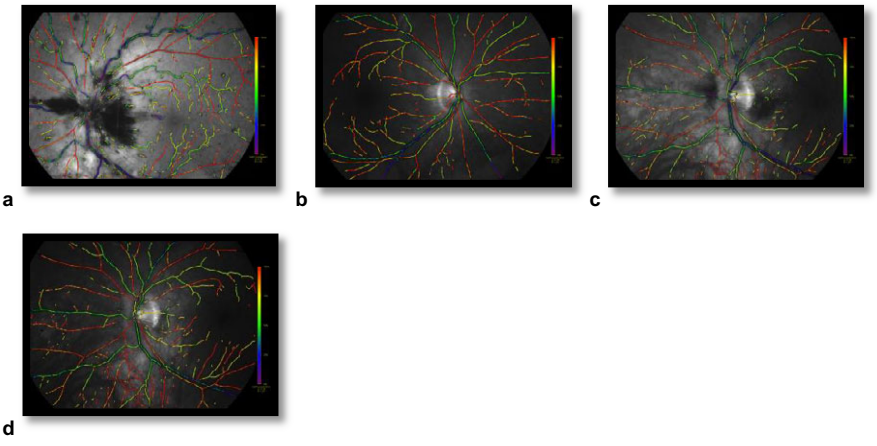


Fig. 16. The patient suffered vision loss six weeks before presenting to the University Hospital. The person was followed with repeated oximetry images for a period of 11 weeks (Fig. 14, case 2). Clinical evaluation confirmed left eye CRVO (A) and haemorrhagic fundus without much oedema. Dorzolamide eye drop treatment was implemented. At week 11 (D) clinical signs and symptoms had resolved. (A) CRVO eye at presentation, six weeks after onset of symptoms. Retinal venous oxyhaemoglobin saturation is 42 ± 13% (mean ± SD). Retinal arteriolar oxyhaemoglobin saturation is 95 ± 5%. VA 0.2 to 0.4. (B) Fellow eye. Retinal venous oxyhaemoglobin saturation is 61 ± 4% and retinal arteriolar oxyhaemoglobin saturation 93 ± 11%. VA 1.0. (C) At four weeks. Less retinal haemorrhage. Retinal venous oxyhaemoglobin saturation is 47 ± 13%. VA 0.6. (D) At eleven weeks. Clinical signs and symptoms resolved. Retinal venous oxyhaemoglobin saturation is 62 ± 8% and retinal arteriolar oxyhaemoglobin saturation 100 ± 3%. VA 0.9–1.0. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653–1661,© 2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology)

Table 6. Physiological parameters (mean ± SD) in 30 healthy subjects at baseline, under experimental condition of hyperoxic breathing for 10 min and after recovery period on ambient air for 10 min.

Physiological parameters	Baseline	Hyperoxia	Recovery
FiO_2 / EtO_2 (%)	–	96 ± 2 / 91 ± 4	–
$EtCO_2$ (mmHg)	–	36 ± 2*	–
Heart rate (bpm)	72 ± 11	70 ± 11	–
SBP (mmHg)	132 ± 20	127 ± 22	128 ± 19
DBP (mmHg)	84 ± 12	86 ± 14	84 ± 14
MAP (mmHg)	100 ± 14	100 ± 15	99 ± 15
SpO2 (%)	97.5 ± 0.7	99.1 ± 0.3	–
IOP (mmHg)	15 ± 4	–	–
OPP (mmHg)	52 ± 10	–	–

DBP = diastolic blood pressure; $EtCO_2$ = end-tidal carbon dioxide; EtO_2 = concentration of exhaled oxygen; FiO_2 = concentration of inhaled oxygen; HR = heart rate; IOP = intraocular pressure; MAP = mean arterial blood pressure; OPP = ocular perfusion pressure; SBP = systolic blood pressure; SpO_2 = finger pulse oximetry.
* $EtCO_2$ is missing from one of the study participants.

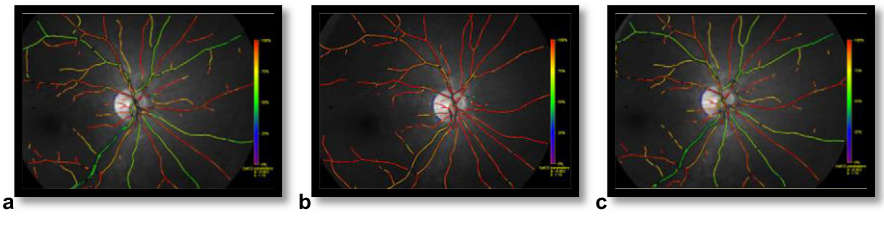


Fig. 17. Retinal oxyhaemoglobins saturation in retinal arterioles and venules under the different breathing regimens. (A) Baseline ambient air breathing. (B) After 10 min of hyperoxic breathing. (C) Recovery ambient air breathing for 10 min.

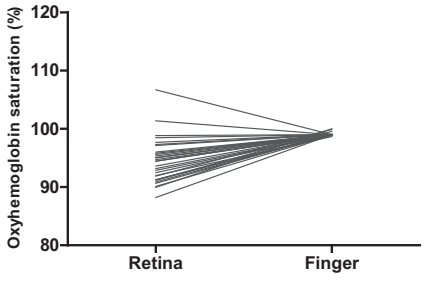


Fig. 18. The graph show individual oxyhaemoglobin saturation measurements by retinal and peripheral pulse oximetry in hyperoxic breathing.

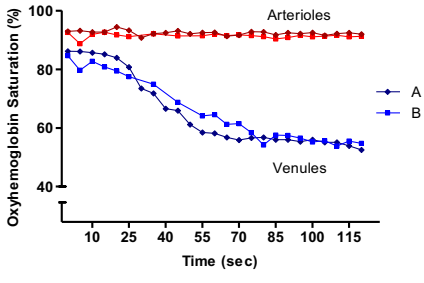


Fig. 19. The slope illustrates the return of oxyhaemoglobin saturation of retinal vessels down to baseline values in two study subjects after cessation of face mask oxygen breathing.

tion of hyperoxic breathing and lasted into the first 2 min of recovery breathing. The oxyhaemoglobin saturation of both retinal arterioles and venules returned to baseline values within these 2 min.

Retinal oximetry in COPD patients (Paper III)

All enrolled COPD subjects finished the study, and no adverse reactions were observed. Physiological parameters are presented in Table 7.

COPD subjects compared to healthy control subjects

COPD patients ($n = 11$) breathing ambient air had significantly lower mean oxyhaemoglobin saturation as compared with healthy controls in both retinal arterioles (87.2 ± 4.9% versus control = 93.4 ± 4.3%, 95% CI: −11.31 to −1.02, p = 0.02, paired *t*-test) and venules (45.0 ± 10.3% versus control = 55.2 ± 5.5%, 95% CI: −17.95 to −2.37, p = 0.01; Fig. 20). The AV-difference was not markedly different between the COPD group and the healthy subject group (42.2 ± 8.0%

Table 7. Basic physiological parameters (mean ± SD) at first baseline (on prescribed oxygen prior to the first retinal oximetry image), then after 10 min of only ambient air breathing and at second baseline after a recovery period of 20 min with oxygen breathing.

	Oxygen therapy First baseline $n = 10^*$	Ambient air Breathing $n = 11$	Oxygen therapy Second baseline $n = 11$
SBP (mmHg)	133 ± 21	127 ± 19	129 ± 13
DBP (mmHg)	82 ± 15	78 ± 10	82 ± 11
MAP (mmHg)	99 ± 15	94 ± 11	97 ± 11
SpO_2 (%)	94 ± 4	90 ± 3	95 ± 2
Heart rate (bpm)	82 ± 10	77 ± 13	76 ± 12
RR (min^{-1})	18 ± 4	15 ± 3	15 ± 3
	$n = 9^\dagger$	$n = 10$	$n = 10$
FiO_2 (%)	39 ± 20	21 ± 3	43 ± 15
$EtCO_2$ (%)	34 ± 8	33 ± 7	33 ± 8

DBP = diastolic blood pressure; $EtCO_2$ = end-tidal carbon dioxide; FiO_2 = concentration of inhaled oxygen; MAP = mean arterial blood pressure; RR = respiratory rate; SBP = systolic blood pressure; SpO_2 = finger pulse oximetry.

* One participant was not breathing the prescribed supplemental oxygen on arrival. Therefore, no basic physiological parameters at first baseline from that subject are presented on the table.

† Reliable measures of FiO_2 and $EtCO_2$ from one of the participants were not possible to acquire due to the subject being a mouth breather.

versus control = 38.2 ± 4.0%, 95% CI: −1.98 to 9.98, p = 0.17).

Administration of the prescribed supplemental oxygen increased the oxyhaemoglobin saturation in retinal arterioles (87.2% ± 4.9% to 89.5% ± 6.0%, 95% CI: −4.13 to −0.31, p = 0.02) but not in venules (45.0% ± 10.3% to 46.7% ± 12.8%, 95% CI: −5.15 to 1.76, p = 0.3). When COPD subjects were on their supplemental oxygen, the difference in oxyhaemoglobin saturation showed a non-significance as compared with the healthy group in retinal arterioles (95% CI: −9.37 to 1.49, p = 0.14).

No differences were observed between COPD subjects and healthy controls, respectively, in vessel diameter of arterioles (106.6 ± 10.6 μm versus 114.2 ± 10.9 μm, 95% CI: −17.06 to 2.00, p = 0.11) or venules (147.7 ± 14.1 μm versus 153.4 ± 15.1 μm, 95% CI: −24.28 to 12.85, p = 0.51).

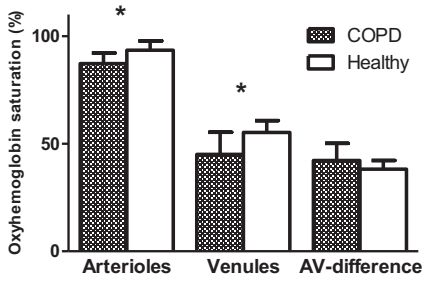


Fig. 20. COPD subjects ($n = 11$) breathing ambient air had significantly lower mean oxyhaemoglobin saturation in both retinal arterioles and venules as compared with healthy controls. Oxyhaemoglobin values are presented as mean ± standard deviation. *Significant difference between COPD patients and healthy control subjects.

COPD subjects under experimental protocol

After termination of inspiring supplemental oxygen and 10 min of ambient air breathing, the oxyhaemoglobin saturation in retinal arterioles and finger oximetry reading decreased considerably (Table 8). Inhalation of supplemental oxygen for 20 min (second baseline period) returned the oxyhaemoglobin saturation in retinal arterioles and finger nearly to the values at first baseline. Cessation or reapplication of supplemental oxygen breathing neither significantly affected the retinal venule oxyhaemoglobin saturation, AV-difference, nor retinal vessel diameter. No significant differences were found between oximetric measurements at first and second baseline.

The comparison of retinal arteriolar oximetry with finger pulse oximetry and radial artery co-oximetry in the COPD subjects during the ambient air breathing is shown in Fig. 21.

Individual radial artery blood gas measurements are presented in Table 9. For the COPD group the mean PaO_2 was 61.3 ± 10.5 mmHg and 42.2% ± 5.9 mmHg for PCO_2. The mean value for bicarbonate was 26.4 ± 3.4 mEq/l, and the pH 7.4 ± 0.0.

Bland–Altman plots compared retinal arteriolar oximetric oxyhaemoglobin saturation values with radial artery blood gas and finger pulse oximetry under ambient air breathing. Radial artery blood gas measurement and finger pulse oximetry revealed a bias and limit of agreement of

Table 8. Comparison of oxyhaemoglobin saturation (%) between retinal vessels, finger pulse oximetry and radial artery blood with and without oxygen therapy in 10 patients with severe COPD. Arteriole and venule oxyhaemoglobin saturation difference and diameters are also shown.

$n = 10$	Oxygen therapy First baseline	Ambient air 10 min	Oxygen therapy recovery Second baseline	95% CI of difference
Retinal arterioles	91.0 ± 4.5	87.5 ± 5.1	90.0 ± 5.9	*1.03 to 6.05† *−5.09 to −0.07‡
Finger pulse oximetry	93.7 ± 3.6	90.6 ± 2.8	94.7 ± 2.5	*0.14 to 6.05† *−7.05 to −1.14‡
Radial artery	–	92.5 ± 3.6	–	
Retinal venules	47.6 ± 12.7	45.6 ± 10.6	46.5 ± 13.5	ns −2.13 to 6.11† ns −4.96 to 3.28‡
AV-difference	43.4 ± 10.6	41.8 ± 8.3	43.5 ± 9.6	ns −2.68 to 5.79† ns −5.98 to 2.49‡
Arteriolar diameter	107.8 ± 18.4	104.7 ± 8.7	102.6 ± 10.1	ns −4.26 to 10.63† ns −5.42 to 9.47‡
Venular diameter	142.7 ± 19.2	147.3 ± 14.8	143.5 ± 17.5	ns −13.10 to 3.85† ns −4.66 to 12.29‡

ns = nonsignificant.

Values are mean ± SD. Repeated-measures one-way ANOVA and Tukey's multiple comparison post-test. (CI: 95% confidence interval of the difference).

† Oxygen therapy at first baseline versus ambient air breathing for 10 min.

‡ Ambient air breathing versus oxygen therapy at second baseline.

* $p < 0.05$.

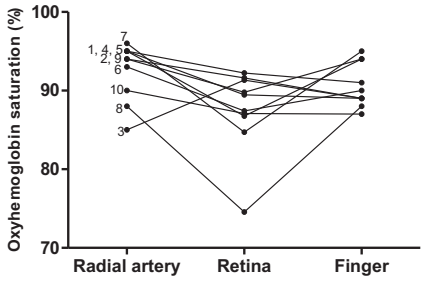


Fig. 21. Comparison of 10 individual COPD subjects' oxyhaemoglobin saturation in retinal arterioles, radial artery blood sample and finger after 10 min of ambient air breathing. The mean oxyhaemoglobin saturation in retinal arterioles was 87.5 ± 5.1% compared to 92.5 ± 3.6% in the radial artery (95% CI: −8.65 to −1.35, $p < 0.05$) and 90.6 ± 2.8% in the finger (95% CI: −6.75 to 0.54, $p > 0.05$). Each number indicates a COPD subject with reference to Table 9. Each data point represents mean oxyhaemoglobin saturation (%) in a single COPD subject. Repeated-measures one-way ANOVA and Dunnett's multiple comparison post-test.

−3.1 ± 5.5; 95% CI: −14.05 to 7.84 and −5.0 ± 5.4; 95% CI: −15.68 to 5.67, respectively (Fig. 22a,b).

Retinal oximetry in neonates with SLO (Paper IV)

The mean ODR of 0.256 ± 0.041 for arterioles and 0.421 ± 0.089 for venules is considerably different ($n = 28$, $p < 0.001$, paired t-test). The median values were 0.255 (range 0.150–0.337) and 0.409 (range 0.268–0.626), respectively. The average vessel diameter measured 14.1 ± 2.7 pixels for arterioles and 19.7 ± 3.7 pixels for venules ($n = 28$, $p < 0.001$; Fig. 23).

Discussion

The outcome of this thesis suggests that oxyhaemoglobin saturation of the systemic circulation can be measured through the retinal circulation. The Oxymap T1 retinal oximeter is shown to be sensitive to the changes in oxyhaemoglobin saturation with hypoxaemia and systemic hyperoxia. In the following discussion, the main focus is on the clinical studies in the adult subjects (Papers I-III) followed by a brief deliberation on the results of the neonatal study (Paper IV). The neonatal study gives indications that the retinal oximetry may also be applicable to newborns when combined with scanning laser ophthalmoscope.

The major findings of each study will be discussed independently before concluding on the results.

Retinal oximetry in CRVO patients (Paper I)

The retinal oximetry study on CRVO patients was carried out to test whether the retinal oximeter is sensitive to retinal tissue hypoxia. Central retinal vein occlusion creates obstruction to the venous outflow with disturbances against blood flow and retinal tissue hypoxia to a variable degree. Earlier oximetry studies on CRVO were restricted to 20-degree field of view images on the fundus. The Oxymap T1 oximeter that was used in this thesis yield 50-degree field of view and thereby wider fundus images than previous studies for estimation of oxyhaemoglobin saturation of retinal vessels in CRVO. Following up on patients with repeated oximetry imaging provides a novel insight on the changes of oxyhaemoglobin saturation with the course of the CRVO disease over time.

Earlier studies on CRVO patients with the older versions of the retinal oximeter had revealed markedly lower oxyhaemoglobin saturation of retinal venules in CRVO-affected eyes than in fellow unaffected eyes (Hardarson & Stefánsson 2010; Traustason et al. 2014). Our results of lower mean oxyhaemoglobin saturation of 31 ± 12% in CRVO eyes as compared with 52 ± 11% in the fellow eyes verify these earlier findings. The results are also in agreement with the semiquantitative findings reported by Yoneya et al. (2002) of retinal tissue hypoxia in CRVO-affected human eyes.

The retinal oximeter captured local hypoxic areas to a variable degree in CRVO-affected eyes. The oxyhaemoglobin saturation in some of the venules was 6% which is equivalent to the average PO_2 of 8 mmHg previously measured with invasive oxygen probe at the retinal surface in patients

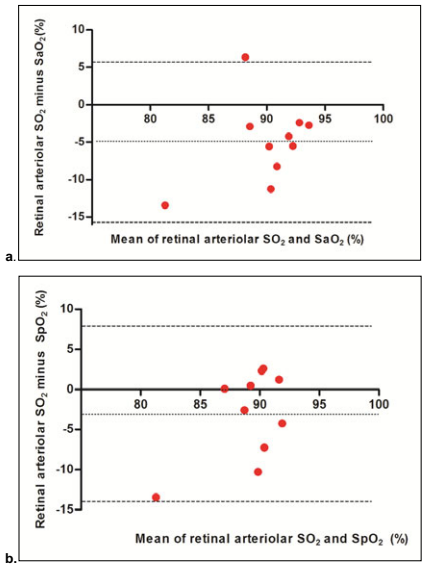


Fig. 22. Bland–Altman plots comparing arteriole oxyhaemoglobin saturation values of retinal oximetry with (A) peripheral pulse oximetry from finger and (B) radial artery blood during ambient air breathing in 10 patients with systemic hypoxaemia secondary to severe COPD. Dotted lines indicate mean difference between measurements, and dash lines indicate 95% limits of agreement. SO_2, retinal arteriole oxyhaemoglobin saturation; SpO_2, finger pulse oximetry; SaO_2, radial artery oxyhaemoglobin saturation.

Table 9. Selected characteristics of the COPD patients: arterial blood gas analysis, retinal arteriolar oxyhaemoglobin saturation and finger pulse oximetry.

Subject	Age	SaO_2	Retina	SpO_2	PaO_2	PCO_2	pH	bicarb	O_2oxyhgl
1	68	95.0	92.2	91.0	64	43	7.43	28	92
2	64	94.0	89.8	94.0	68	43	7.39	25	92
3	71	85.0	91.3	89.0	42	52	7.44	33	84
4	67	95.0	86.7	94.0	78	38	7.37	22	94
5	77	95.0	89.5	89.0	60	40	7.45	27	94
6	76	93.0	87.4	90.0	69	49	7.4	29	92
7	68	96.0	84.7	95.0	66	42	7.39	25	95
8	66	88.0	74.5	88.0	55	43	7. 43	27	86
9	82	94.0	91.6	89.0	63	42	7.43	27	92
10	68	90.0	87.0	87.0	53	30	7.41	21	90

SaO_2, arterial oxyhaemoglobin saturation; retina, arterial oxyhaemoglobin saturation; SpO_2, finger pulse oximetry; PaO_2, mean partial pressure of oxygen; $PaCO_2$, mean partial pressure of carbon dioxide; Bicarb, bicarbonate; O_2oxyhgl, oxyhaemoglobin.

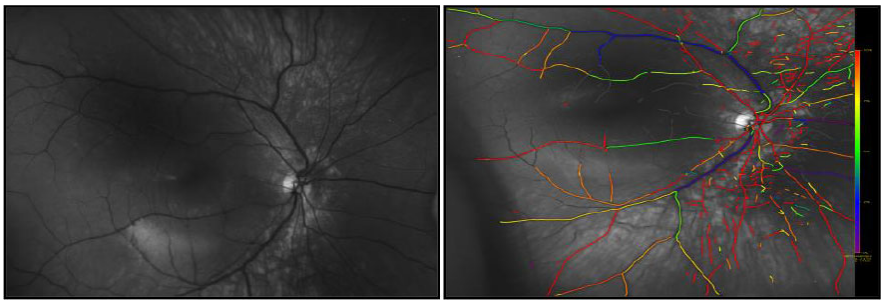


Fig. 23. Retinal oximetry image of an infant (left) without and (right) with a pseudocolour overlay.

with CRVO (Williamson et al. 2009). Animal studies have shown that the preretinal PO_2 is overall a good indicator of the intraretinal PO_2 because the retinal circulation is the main oxygen source for the vitreous (Wangsa-Wirawan & Linsenmeier 2003). The difference between mean retinal oximetry values and preretinal findings can at least be partially explained by different methods used in calculating their values; at one hand, invasive PO_2 values are determined by closeness of the oxygen probe to blood vessels across the retinal surface. On the other hand, retinal venous oxyhaemoglobin saturation is the weighted average of all venular segments that are analysed in the pertaining eye.

Although calculations of PO_2 from the haemoglobin dissociation curve holds for retinal vessels only, the mid-vitreous cavity PO_2 of 19.8 mmHg that had previously been measured in CRVO eyes (Williamson et al. 2009) matches our findings of mean oxyhaemoglobin saturation of 31% in CRVO-affected eyes. In healthy cats, an uniformity has been found between retinal venous PO_2 and mid-vitreous PO_2 (Alder & Cringle 1985) that becomes disrupted under pathological condition like diabetic retinopathy (Linsenmeier et al. 1998). This is probably the case in human CRVO-affected eyes because the direction of oxygen gradient is reversed and the vitreous may indeed provide oxygen support for the hypoxic retina instead (Williamson et al. 2009).

Our hypoxic measures in obstructed venules are also in agreement with invasive measures on preretinal PO_2 of BRVO areas in pigs, miniature pigs (Pournaras et al. 1990; Pournaras et al. 2004; Noergaard et al. 2008) and cats (Stefánsson et al. 1990). In addition, our mean oxyhaemoglobin saturation findings of 52% in the fellow unaffected eyes and 55.6% in healthy subjects (Geirsdottir et al. 2012) do convert into 28 and 29 mmHg PO_2, respectively, that fit nicely to the preretinal PO_2 of pigmented Long Evans rats (Lau & Linsenmeier 2012).

Effects of the venous occlusion on the oxygen extraction

The retinal tissue has higher oxygen extraction under normal condition (8 ml per 100 ml blood) with lower retinal venous and tissue PO_2 than most other tissues in the body (Wangsa-Wirawan & Linsenmeier 2003). The subnormal venous oxyhaemoglobin saturation in CRVO eyes indicates the effects of the venous obstruction on the retinal circulatory closed circuit, where the venous pressure is increased and the perfusion pressure decreased. Consequently, more oxygen is extracted per volume as the sluggish blood flow moves through the microcirculatory network. The PO_2 differences create a higher gradient for oxygen to diffuse from the retinal capillaries into the hypoxic tissue, to be consumed in the oxidative synthesis of ATP for cellular metabolism. Moreover, the obstruction of the retinal circulation will reduce the clearance rate of H^+ and most likely induce H^+ accumulation from the lactate production in the anaerobic glycolysis as well (Nielsen et al. 1998; Birol et al. 2005). Consequently, the elevated H^+ concentration and acidification of the inner retina shifts the oxyhaemoglobin dissociation curve further to the right. As the oxygen′s affinity for haemoglobin decreases, more oxygen is unloaded from retinal capillaries to the hypoxic tissue that adding to the subnormal venous oxyhaemoglobin saturation as shown by the retinal oximetry results. The increased AV-difference in CRVO is ultimately a biomarker of acute retinal tissue hypoxia that might become reversible with timely restoration of oxygen supply for the retinal tissue oxygen demand.

Increased AV-difference in CRVO

The enlarged AV-difference in CRVO eyes as compared with opposite unaffected eyes is in line with previous findings of Hardarson & Stefánsson (2010) and Traustason et al. (2014). The mean AV-difference is bigger in fellow unaffected eyes ($43 \pm 7\%$) of CRVO patients than reported on healthy people ($36.7 \pm 5.4\%$) with healthy eyes (Geirsdottir et al. 2012). The reason is unclear but may partially be explained by lack of control for confounding variables, whereas the healthy group is younger (median age 47 years) and systemic risk factors in the CRVO subjects are not controlled for in this study. Some of the risk factors may indeed lower the oxyhaemoglobin saturation of retinal venules. A possible explanation could also be if the blood flow is slower in the fellow unaffected eyes of people with CRVO than of healthy subjects with healthy eyes. We did not quantify the retinal blood flow so this speculation remains to be elucidated.

Intra- and interindividual variability

The retinal venous oxyhaemoglobin saturation is variable within each eye and between individual eyes as shown by the standard deviation. The inconsistency is markedly greater in eyes with CRVO than in unaffected fellow eyes. The variability in the oxyhaemoglobin saturation is shown by the colour-coded map on the oximetry images, changing from normoxic green to bluish and purple, determined by the hypoxic state of the inner retina. This wide range of intra- and interindividual oxyhaemoglobin saturation of retinal venules is similar to those previously published by Hardarson & Stefánsson (2010). The variability most certainly signifies the individual distinction of the magnitude in retinal venous obstruction that is determined by the location (Hayreh 2005) and the various reparatory mechanisms engaged against the occlusion. Collateral pathways for establishment of venous drainage (Takahashi et al. 1998; Paques &

Gaudric 2002; Hayreh et al. 2011) are undertaken to variable degree and progressive channelizing of the thrombus may start within days or weeks from the initial onset of the occlusion (Green et al. 1981), potentially contributing to the retinal oximetry and clinical improvement over course of the disease.

Vessel diameter

Retinal venules in the CRVO-affected eyes are characterized by vessel dilation and venous tortuosity most likely due to elevated intraluminal pressure (Kristinsson et al. 1997), resulting from backup pressure because of the obstruction to the central venous outflow. The mean difference of venular diameter by only one pixel is probably a matter of accuracy from resolution of measurements. In some CRVO patients, the mean vessel diameter is wider in the unaffected fellow eye than the CRVO eye. Some of the patients have comorbidities that are known to influence the retinal vessel calibre and may contribute to increased venous diameter and tortuosity of the fellow unaffected eye.

Retinal arterioles

The oxyhaemoglobin saturation in retinal arterioles is unaffected by the CRVO, as compared with the fellow control eye. The finding is in agreement with the previous finding by Hardarson & Stefánsson (2010) but Traustason et al. (2014) found the oxyhaemoglobin saturation to be slightly elevated in CRVO-affected eyes. They speculated the elevation may in part be explained by technique error since the numbers of very high values were more frequently observed in CRVO eyes than in contralateral eyes. In this thesis, the extremes of intra- and inter individual variability are similar between CRVO eyes and unaffected control eyes.

In arterioles, the mean vessel diameter was narrower in CRVO eyes than in unaffected eyes. The reason for the unchanged arteriolar oxyhaemoglobin saturation in CRVO eyes and narrowness of the vessel lumen is unclear. Whether it is of technological origin or perhaps a natural course of the disease remains to be answered. This latest version of the Oxymap T1 software automatically correct for any artifactual changes in the oxyhaemoglobin saturation derived from widening or narrowing of the vascular diameter. As such, the calculation of oxyhaemoglobin saturation of the inner retina should be unaffected by the vascular width of both retinal arterioles and venules. Ultimately, reduced retinal arteriolar calibre in CRVO eyes points towards diminished oxygen delivery of the inner retina secondary to reduced blood flow, winding-up with higher oxygen extraction and increased arteriovenous oxygen difference than in the fellow unaffected eyes.

Follow-up on patients over time

The retinal oximeter detects temporal and topographical variance of oxyhaemoglobin saturation of the retinal circulation over time. By following up on patients, it becomes evident that venous oxyhaemoglobin saturation is lowest at first and improves with time and treatment as had previously been reported by Traustason et al. (2014). It also appears that even though clinical signs and symptoms resolve, the venous oxyhaemoglobin saturation remains lower than normal. As was previously discussed, different compensatory mechanisms against the vein occlusion are expected to be underlying the improved oxyhaemoglobin saturation. Increased oxyhaemoglobin saturation is a sign of better balance between oxygen supply and demand of the retinal tissue despite of the impending hypoxic injury secondary to CRVO.

Limitations of the retinal oximetry study on CRVO

The study has several limitations some of which have already been discussed. Confounding variables were not controlled for and therefore systemic coexisting diseases may have influenced the resultant differences between the CRVO eye and fellow control eye. The subject group is small and control group for the unaffected eye is lacking. Affiliated blood flow measures were not performed and hence its correlation with variability of venous oxyhaemoglobin saturations within and between eyes is based on assumptions. However, one patient underwent fluorescein angiography that revealed capillary nonperfusion that was consistent with hypoxic areas on retinal oximetry images. Although a certain measures were taken to avoid technical limitations, they could not be entirely avoided and some of the images were of poor quality. Increased age and some systemic (and ocular) diseases are known to alter the media transparency. Cataract is known to alter lens morphology and thus creating more artificial scattering of light which affects the oxyhaemoglobin saturation (Patel et al. 2013) and lower measurements of venules (Hardarson et al. 2015). Despite the above-mentioned limitations, spectrophotometric retinal oximetry enables analysis of oxyhaemoglobin saturation in the inner retinal vasculature and our estimation of oxygen levels in CRVO seems realistically accurate. In the future, study on larger patient group with CRVO is needed in order to investigate the impact of the disease on retinal vessel oxyhaemoglobin saturation and to observe the natural course of the disease and the effectiveness of treatment over time.

Retinal oximetry in healthy under hyperoxemia (Paper II)

The retinal oximetry study on healthy subjects was performed to test whether the retinal oximeter is sensitive to hyperoxic changes of the retinal circulation. Inhalation of high oxygen concentration elevates the systemic arterial oxygen content and optimizes the oxyhaemoglobin saturation. The increased oxygen delivery is supposedly reflected in increased oxyhaemoglobin saturation of retinal vessels by retinal oximeter measurements.

The retinal oximeter calculates stable oxyhaemoglobin saturation level at baseline. System hyperoxia elevates the oxyhaemoglobin saturation and narrows the vascular lumen of both retinal arterioles and venules. These changes are more pronounced on the venous site than the arteriolar site of the retinal circulation. Systemic hyperoxia markedly lessen the AV-difference. Retinal oximeter measurements demonstrate lower oxyhaemoglobin saturation values than finger oximetry during both ambient air and supplemental oxygen breathing.

Choroidal interaction on the inner retina during hyperoxia

Increased oxyhaemoglobin saturation of retinal arterioles and venules is in agreement with recent reports

(Palkovits et al. 2014a; Werkmeister et al. 2015) on the ability of retinal oximetry to recognize those systemic changes of oxyhaemoglobin saturation. Continual retinal oximetry imaging reveals prompt retinal circulatory recovery when supplemental oxygen is halted. Utilizing rebreathing circuit made possible to induce isocapnic hyperoxia and thus the vasoconstriction of both retinal arterioles and venules is a marker of the effective autoregulatory response from the hyperoxic provocation. The pronounced elevation of venous oxyhaemoglobin saturation supports the former belief which is based on invasive PO_2 animal studies (Linsenmeier & Yancey 1989; Pournaras et al. 1989) that the high systemic oxygen concentration causes oxygen to flux from the poorly regulated choroidal circulation to the innermost layers of the retinal tissue. It is likely that the influx contributes to the metabolic need of the inner retinal tissue and some oxygen molecules end up in the retinal veins as well. Because the retinal vessel diameter is reduced and the blood flow is known to diminish under hyperoxic condition (Palkovits et al. 2014a; Werkmeister et al. 2015), the concomitant decrease of oxygen extraction from the retinal circulation is the proposed mechanism behind the reduced AV-difference seen in our results.

Retinal oximetry readings during acute hyperoxia

Both retinal oximetry and finger pulse oximetry measured considerable elevated oxyhaemoglobin saturation from baseline with supplemental oxygen breathing. Retinal oximeter measurements display relatively lower average oxyhaemoglobin saturation values than peripheral pulse oximeter measurements, both at a baseline and during system hyperoxia. It would be expected to see the oxyhaemoglobin binding sites of retinal arterioles nearly fully saturated during system hyperoxia so the results are somewhat perplexing and the reason for the disparity between the two methods is unclear. The causation can be twofold, either technical or physiological, and both. The former reason is based on the fact that the calibration factor of the instrument is derived from laboratory values that were calibrated in vivo and are somewhat lower than given values in the systemic circulation (Guyton and Hall 2000). Palkovits et al. (2014) got similar results on induced systemic hyperoxia, using the Imedos system that utilizes the same calibration factor as ours. The latter reason is probable, due to countercurrent exchange between the central retinal artery and the central retinal vein as they run adjacent each other within the optic nerve.

Limitations of oximetry study on healthy subjects

Measurements on arteriolar oxyhaemoglobin saturation with the retinal oximeter give lower values than expected during systemic hyperoxia. This is most likely due to the abovementioned biological countercurrent exchange mechanism but some calibration issues of the instrument cannot be ruled out.

The Oxymap T1 retinal oximeter quantifies retinal oxyhaemoglobin saturation based on calibration factors presuming that average oxyhaemoglobin saturation in healthy individuals is 92.2% for arterioles and 57.9% for venules. These reference values were initially attained with a laboratory oximeter, which was calibrated in vitro and are somewhat lower than quoted normal values in the systemic circulation (Guyton and Hall 2000). Inhalation of 100% oxygen by facemask (FiO_2 of approximately 96%) in healthy subjects in this thesis increases the retinal arteriolar oxyhaemoglobin saturation to 94.5 ± 3.8 and $76.2 \pm 8.0\%$ in venules which is closer to normal mixed venous oxyhaemoglobin saturation during ambient air breathing at sea level. Hence, the calibration factor may be accountable for the lower retinal arteriolar oxyhaemoglobin saturation than expected. The notion of lower estimated values during systemic hyperoxia than expected is further supported by the findings of Palkovits et al. (2014) with their arteriolar oxyhaemoglobin saturation results of $96.4 \pm 3.1\%$. Of interest, their retinal oximeter (Imedos) is based on the same calibration factor as the Oxymap T1 instrument. They speculated the reason for not reaching oxyhaemoglobin saurian of 100% would most likely be due to the calibration or the countercurrent exchange of oxygen within the optic nerve.

Although it may be speculated, the third reason for lower values than expected during hyperoxic breathing, stem from study subjects removing the oxygen facemask whilst the oximetry images were obtained, it is highly unlikely; first, the inspired and expired oxygen concentration had reach equilibrium (EtO_2 $91 \pm 4\%$) before oximetry images were obtained. Second, the average time for oximetry imaging was fast, only about 30 seconds. Third, study subjects did not inhale during the imaging but exhaled slowly if needed.

Ultimately, the lower oxyhaemoglobin saturation values obtained by the Oxymap T1 retinal oximeter than expected, give reason for its calibration to be reconsidered and to contemplate the countercurrent effects of the retinal circulation.

Retinal oximetry in COPD patients (Paper III)

The retinal oximetry study on patients with severe COPD was conducted to test whether the retinal oximeter is sensitive to systemic hypoxaemia. COPD is a systemic disease, characterized by hypoxia and inflammatory response that is known to negatively affect tissues and organ systems of the body. In patients with severe COPD, the systemic hypoxaemia is expected to be captured with retinal oximeter measurements. The daily supplemental oxygen therapy improves oxygenation and thus should be reflected in increased oxyhaemoglobin saturation of the retinal vessels.

The retinal circulation offers direct noninvasive assessment of the central nervous circulation and thus the systemic circulation. Spectrophotometric retinal oximetry is hypothetically at least as good indicator of the systemic oxyhaemoglobin saturation as invasive radial artery blood sample measurements and peripheral finger plethysmography.

COPD subjects compared to healthy controls

Retinal oximetry captures systemic hypoxaemia in both retinal arterioles and venules in people with severe COPD. During ambient air breathing, the oxyhaemoglobin is substantially lower in both retinal arterioles and venules than of healthy controls. When

COPD subjects inspire their prescribed oxygen therapy, the arterial oxyhaemoglobin saturation shows trend towards that of the healthy controls. The tendency must, however, be interpreted with caution since the number of the study group is small ($n = 11$). The AV-difference in COPD subjects is greater than in the control group and no considerable changes are observed when they are exposed to different breathing regimens during the experimental protocol. The mean vessel diameter of both retinal arterioles and venules is slightly narrower in COPD patients but statistically nonsignificant. The variance of vascular width between vessels segments (standard deviation) both within eyes and between eyes is similar to that healthy of controls.

Comparison with other studies

The improved arteriolar oxyhaemoglobin saturation and unaltered AV-difference are in agreement with recent publication of similar patient group by Palkovits et al. (2013) using the Imedos retinal device ($n = 15$). In their study, the arteriolar oxyhaemoglobin saturation during supplemental oxygen breathing was $92.2 \pm 4.6\%$ and decreased by 2.1 per cent points during ambient air breathing. The AV-difference was close to $25 \pm 5\%$ under both breathing conditions. In contrast to our findings, they reported the venous oxyhaemoglobin saturation of $67.6 \pm 7.8\%$ during the supplemental oxygen breathing to be higher than in their healthy subject group. They also found a tendency ($p = 0.05$) of decreased venous oxyhaemoglobin saturation with cessation of oxygen breathing whereas we did not acquire statistical significance. It is important, however, to keep in mind that both studies represent small groups of COPD subjects and therefore future studies should include more patients in order to enhance the capability to detect any latent changes therein.

Results on oxyhaemoglobin saturation of retinal arterioles and venules in our COPD subjects match to the findings on patients with congenital system hypoxaemia secondary to Eisenmenger syndrome (Traustason et al. 2011), using the same instrument but an older version. Those patients are shown to have abnormally low oxyhaemoglobin saturation in retinal arterioles or $81 \pm 9\%$ and in venules, or $44 \pm 12\%$ when breathing ambient air. These values are similar to our findings but information on measurements during supplemental oxygen breathing is not available.

COPD subjects under the experimental protocol

All COPD patients in this thesis were hemodynamically stable throughout the study period. In consonance with retinal oximeter measurements, all COPD participants suffered from moderate-to-severe hypoxaemia during the ambient air breathing as manifested by the subnormal PO_2 arterial blood gas analysis. Two COPD patients were CO_2 retainers and all pH values were within normal limits. The bicarbonate level was mildly elevated in few of the cases, confirming the chronic hypoxia is compensated for. The unchanged AV-difference obtained by the retinal oximeter is indicative of the retinal tissue metabolic compensatory response to the chronic hypoxia.

Effects of chronic systemic hypoxaemia on the inner retina

Systemic arterial hypoxaemia is a limiting factor in the cellular metabolism of the inner retina as evident by the reduced venous oxyhaemoglobin saturation. The reduction in mean arteriole and venules oxyhaemoglobin saturation in patients with severe COPD inspiring ambient air indicates that they experience inadequate oxygen delivery to the inner retina and thus, hypoxaemic conditions of the tissue.

The retina has a high oxygen demand, and local blood vessel autoregulatory mechanisms maintain the oxygen concentration of the inner retinal tissue at relatively stable levels with a hypoxic vasodilatory threshold to decreased PaO_2 similar in both the retinal (Cheng et al. 2016) and cerebral circulations (Kety & Schmidt 1948; Gupta et al. 1997). According to the Fick′s principle, the circulatory response to arterial hypoxaemia is double; additional capillaries are recruited to enlarge the interface for oxygen exchange and shortening the diffusion distance. Secondly, the vascular resistance is reduced by arteriolar vasodilatation for enhanced oxygen delivery and tissue perfusion (Pittman 2011). The increase in vascular calibre is evident in both retinal arterioles and venules under induced acute systemic hypoxaemia in healthy people (Palkovits et al. 2014a). In our COPD study subjects however, the vasodilatory response is seemingly lost. In fact, the vessel lumen of both retinal arterioles and venules is slightly narrower as compared with the control group, both under the ambient air and supplemental oxygen breathing conditions.

Retinal adaption to chronic systemic hypoxaemia

Absence of the inner retina vasodilatory response is most likely caused by long-term retinal adaption to the system hypoxaemic condition. Animal studies have shown the initial cerebral blood flow augmentation is attenuated by increased capillary density and oxygen carrying capacity of the blood (Boero et al. 1999; Dunn et al. 2004) over time. Although studies on cerebral blood flow in COPD patients are lacking, most human studies on acclimatization to high-altitude point towards unchanged blood flow over time, hypothetically by offset of the initially hypoxic vasodilatory response. Progressive ventilatory adjustment counteracts the initial rise in blood flow along with increased release of local factors and endothelium-derived vasoconstrictors and elevated sympathetic nervous system activity. These factors are likely to pertain to the unchanged cerebral blood flow that has been found in COPD patients (Ainslie & Ogoh 2010) and hypothetically to the long-term retinal circulatory adjustment as well.

It is known that the systemic inflammatory response of COPD involves aberration of the systemic vascular function although the role of the endothelial-dependent and endothelium-independent mechanism for the impaired vasodilatation is of controversy (Eickhoff et al. 2008; Maclay et al. 2009). This vascular dysfunction including arterial stiffness (Maclay et al. 2009) is expected to be the cause for retinal vasoconstriction in people with severe COPD. However, retinal blood flow measurements and clarification on the mechanism behind the retinal circulatory hypoxic adjustment remain to be elucidated for future studies.

Some studies have supported evidence of both functional and structural changes of the retina in stable COPD

patients. As already mentioned, the system inflammatory response and chronic hypoxaemia are believed to provoke pathological changes on the ocular vasculature underlying those retinal changes. The average subfoveal choroidal thickness and peripapillary retinal nerve fibre layer (RNFL) thickness are found to be thinner than in healthy controls (Ozcimen et al. 2016; Ugurlu et al. 2016). Such degenerative tissue changes are indicative of the detrimental effects of the hypoxaemia (Kergoat et al. 2006) on RNFL and consequent ganglion cells death. Seemingly, the retinal circulation is unable to compensate for the metabolic changes that underlie these structural damages (Kergoat et al. 2006; Ozcimen et al. 2016). Latencies of visual evoked potentials and amplitude anomalies have also been reported (Ozge et al. 2005; Demir et al. 2012) along with defect on the visual field, which implies the neuropathological aspect of mild-to-moderate hypoxaemia on the retina and the optic nerve itself (Demir et al. 2012).

Subnormal retinal venous oxyhaemoglobin saturation

A hypoxic effect on the retinal tissue metabolism is demonstrated by the reduced average retinal venous oxyhaemoglobin saturation which indicates increased oxygen extraction by the retinal tissue. The intra- and inter eye variability of those hypoxic effects are demonstrated by the standard deviation. Increased CO_2 production coupled with elevated H^+ production and reduced pH in the retinal tissue shifts the oxyhaemoglobin dissociation curve to the right which facilitates dissociation of the oxygen molecule from the haemoglobin binding site into the cell. Subsequently, a higher oxygen fraction is removed from retinal capillaries and the oxyhaemoglobin saturation on the venous site becomes abnormally low in patients with severe COPD.

The unchanged AV-difference during cessation and reapplication of the supplemental oxygen therapy suggests an unaltered mitochondrial consumption for ATP production of the hypoxic inner retinal tissue in patients with severe COPD. Since the autoregulatory response of the choroidal circulation to changes in PaO_2 is minimal, the oxygen influx from choriocapillaries declines linearly across the outer retinal tissue under systemic hypoxaemia (Pournaras et al. 2008). Under normal conditions, increased photoreceptors metabolic activity already demands some additional oxygen flux from the retinal circulation (Linsenmeier & Braun 1992) to the retinal outer segments. In people with severe COPD, the chronic systemic hypoxaemia of the outer segments most likely constantly demands the inner retinal circulation to contribute oxygen for the energy consuming photoreceptor activity. This will exceed the capacity of the already hypoxaemic retinal circulation for oxygen contribution as manifested by low venous oxyhaemoglobin saturation and increased AV-difference from normal, eventually leading to functional and structural changes of the retina in stable COPD patients that was mentioned above.

Effects of prescribed oxygen on retina in COPD

Supplemental oxygen therapy improves global oxygen delivery and consequently the oxyhaemoglobin saturation of retinal arterioles and venules as shown in the COPD subjects group. A high FiO_2 augments the oxygen influx from choriocapillaries, not only to the outer retina but to the inner retina as well as is shown in the hyperoxia study of this thesis (Olafsdottir et al. 2015). Although the effect of supplemental oxygen on retinal venous oxyhaemoglobin saturation is not statistically significant, it shows improvement. Inference of posteriori power analysis implicates that the lack of significance may be owed to inadequate power to detect a difference of 5.5% in oxyhaemoglobin saturation (power 90%, $p = 0.05$). Nonetheless, retinal oximetry implicates local metabolic changes associated with systemic hypoxaemia in people with COPD. Those metabolic changes are probably causative for the retinal neuropathological changes that have started to evolve around the systemic effects on the retina in COPD.

Retinal oximetry compared with radial artery blood and finger pulse oximetry

Despite of some intra-individual variations, retinal arteriole oxyhaemoglobin saturation values were in general lower than those measured from radial artery blood samples and with finger pulse oximetry. The Bland–Altman plots illustrate the tendency of retinal oximetry to produce lower oxyhaemoglobin saturation measures but show a fair agreement with the other two modalities. Bland–Altman plot of retinal oximetry and arterial blood sample oxyhaemoglobin values indicate a degree of bias with three retinal oximetry outliers contributing substantially to the width of the limits of agreement. Nevertheless, the calculated variability implicates fair agreement between the radial artery blood sample and retinal oximetry measurements of oxyhaemoglobin saturation. It should be kept in mind that estimated oxyhaemoglobin saturation in arterial blood is quantified from normal values of PaO_2 and pH and standard oxyhaemoglobin dissociation curve based on probable oxygen-haemoglobin affinity and 2,3-DPG concentration. Therefore, the radial artery oxyhaemoglobin saturation should be interpreted with care in the presence of pathology (Haymond 2006).

A Bland–Altman plot of the finger and the retinal oximetry oxyhaemoglobin saturation values also show some difference between the two modalities. The plot illustrates the tendency of retinal oximetry to measure lower oxyhaemoglobin saturation values but, like with radial artery blood sample, demonstrated fair agreement between those two techniques.

The lower retinal arteriole values acquired by retinal oximetry could simply be due to calibration of the device. If the difference is real however, a likely reason for lower retinal arterial oxyhaemoglobin saturation is that the central retinal artery and vein lie adjacent to each other (about 1 cm) within the optic nerve where the utmost oxygen countercurrent exchange may occur between the artery and vein. This could result in somewhat lower oxyhaemoglobin saturation in retinal arterioles compared with the larger arteries. In addition, one possible reason is that the fairly small retinal arterioles that are measured loose more oxygen through their vessel walls by diffusion than arteries measured in the finger or the wrist. Retinal measurements are made on vessel segments that stretch for some length into the retina whereas measurements in the finger and the wrist are confined. Moreover, the retinal oximetry calculation is based on average measurements of

multiple vessel segments of very metabolically active tissue of the eye. Peripheral radial artery and finger calculation, however, represent single measure of oxygen delivery to a tissue that is not that metabolically active.

Validity of retinal oximetry in systemic hypoxaemia

In humans, noninvasive spectrophotometric retinal oximetry has shown sensitivity to alteration in oxyhaemoglobin saturation in people with systemic hypoxaemia secondary to Eisenmenger syndrome (Traustason et al. 2011) and severe COPD (Palkovits et al. 2013). These retinal measures significantly correlated with earlobe capillary blood (Palkovits et al. 2013), femoral artery (Traustason et al. 2011) and finger pulse oximeter measurements (Traustason et al. 2011; Palkovits et al. 2013). Significant correlation has also been revealed between retinal vessel oxyhaemoglobin saturation and finger pulse oximetry during induced hypoxaemia in healthy people (Palkovits et al. 2014c). Moreover, the sensitivity of the Oxymap retinal oximeter has been confirmed in pigs exposed to acute hypoxaemia with a good correlation with the intravitreal and femoral artery oxygen content (Traustason et al. 2013). In addition, Denninghoff et al. (1998) used noninvasive low power scanning laser eye oximeter to estimate the sensitivity of retinal venous oxyhaemoglobin saturation for early haemorrhage and resuscitations in swine. They reported a good correlation of retinal venous oxyhaemoglobin saturation with mixed venous saturation, cardiac output, blood volume (Denninghoff et al. 2003) and the rate of blood loss in which retina demonstrated higher sensitivity for blood loss than conventional vital signs, that is blood pressure and heart rate (Denninghoff et al. 1998). These animal studies of exsanguinations and resuscitation demonstrate the potential of retinal oximetry monitoring during acute haemorrhage whilst the compensatory hemodynamic response is still intact.

Limitations on retinal oximetry in COPD patients

The study on hypoxaemia in patients with COPD has some limitation and some have already been discussed previously such as the small number of subjects, which may have resulted in the wide confidence interval. Invasive radial artery blood gas sample was only obtained after cessation of the prescribed oxygen breathing. Preferably, a radial artery blood sample would also have been drawn for oxyhaemoglobin saturation measurement at baseline, when COPD patients were still on their prescribed oxygen therapy. It was, however, deemed unjustifiable due to its invasive nature including complication risks and local discomfort. As already argued, the calibration factor for the oximeter is based on laboratory values for healthy subject which are lower than the reference normal values in the systemic circulation and call for reassessment on calibration constants of the device. The subjects of this study are older than this reference group and suffer from systemic hypoxaemia that probable cause bias on calculated values based on those calibrational constants.

A number of experimental spectrophotometric measurements of retinal arteriolar and venous oxyhaemoglobin saturation in human subjects have been carried out. Despite of that, no 'normal' gold standard values exist because of the invasive nature of the procedure needed to acquire the essential parameters to establish such normal values *in vivo*. This obstacle is also held accountable for the lack of an absolute margin for retinal vessel hypoxaemia. Nevertheless, retinal oximeter measurements give relative values (not absolute) and can give important information with respect to relative and trend oxyhaemoglobin saturation in retinal arterioles and venules.

At the present, there is little known about the effects of chronic systemic hypoxaemia on the central circulation in patients with severe COPD. Most case–control studies are conducted on subjects that have less severe health conditions. Additional studies are needed to investigate the impact of acute and chronic systemic hypoxaemia on the central circulation in real-life situations and retinal oximetry might be a valuable tool in these investigations.

Retinal oximetry in neonates with SLO (Paper IV)

The retinal oximetry study on neonates was performed to test whether retinal oximetry can be applied to infants by combining SLO with retinal oximetry. At the present, retinal oximetry is only performed in adult persons. Extending the technique to paediatrics could be a vital step for managing neonates and preterm babies at the neonates' intensive care units in the future.

The software algorithm allows for assessment of oxyhaemoglobin saturation in retinal vessels by evaluating the relationship between oxyhaemoglobin and deoxyhaemoglobin in retinal arterioles and venules. Since this is a new technology in children, the initial step is to test whether the method can be used in neonates and to estimate the optical density ratio for retinal oximetry analysis and future estimation of normal oxyhaemoglobin saturation.

Normative data were obtained on the ODR for arterioles and venules in healthy full-term neonates. The combined SLO and retinal oximetry technique are sensitivity to oxyhaemoglobin and deoxyhaemoglobin content of the retinal circulation, as indicated by the statistically significant difference between the ODR of arterioles and venules. This is a novel technique on children and the first study to conduct retinal oximetry analysis on neonates. Only one study has been published on the combined SLO and retinal oximetry method. In that study, Kristjansdottir and associates measured the ODR of arterioles (0.210) and venules (0.351) in healthy adults. They fitted the results with calibrated oxyhaemoglobin saturation values obtained in vitro by Schweitzer et al. (1999) and was previously described in this thesis. By using the mean values of 92.2% for arterioles and 57.9% they defined the calibration constants as: $a = -2.4733$ and $b = 1.4388$ for the combined SLO and retinal oximetry method. Subsequently, they calculated the mean oxyhaemoglobin saturation of the study subjects to be $92 \pm 13\%$ for arterioles and $57 \pm 12\%$ for venules. The repeatability of the measures as demonstrated by standard deviation of 3.5% for arterioles and 4.4% for venules was considered adequate bearing in mind the early development of this technique. The sensitivity of the instrument was confirmed under induced hyperoxic condition (oxygen 100%) and local hypoxia secondary to retinal vein occlusion in the eyes (Kristjansdottir et al. 2014).

In neonates, the ODR was quite variable with the optical density

ranging from 0.150 to 0.337 for the arterioles and more pronounced in venules or 0.268 to 0.626. Up to date, there is no background work on appropriate calibration factors for infants and adult calibration constants are considered inapplicable to neonates given the disparity between the ODR of these age groups. More work on calibration is needed before a step can be taken to estimate the oxyhaemoglobin saturation of central nervous system circulation in full-term neonates in the future.

Limitation on the oximetry study in neonates with SLO

Given the fact that retinal oximetry is a novel technique, the study on neonates has several limits some of which have already been discussed above. First, additional studies are needed for determination of precise calibrational constants for calculation of oxyhaemoglobin saturation values to base on. Secondly, technological advantages are warrant to reduce the inconsistency in measurements of the optical density. That could possibly be achieved by modification of the software and improving the laser imaging as the existing system is optimized for colour images. Currently, the bulk size of the instrument is also a limiting factor for its use whereby the infant is needed to be held in a flying baby position in order to adjust the eye next to the device.

Conclusions

This thesis demonstrates the spectrophotometric retinal oximetry is sensitive to local and systemic changes in oxyhaemoglobin saturation. The Oxymap T1 retinal oximeter identifies retinal tissue hypoxia and variability of the local hypoxia in people with central retinal vein occlusion. The method is able to detect systemic hyperoxia in healthy subjects and confines the recovery process back to the pre-experimental baseline values. The instrument captures system hypoxaemia in patients with severe COPD and is sensitive to changes of their inspired oxygen concentration. Measured retinal oxyhaemoglobin saturation values are, however, shown to be slightly lower than finger pulse oximetry and radial artery blood values. These discrepancies are expected to originate in a countercurrent exchange between the central retinal artery and vein within the optic nerve, where they lie closely together for a centimetre. The countercurrent exchange would lower oxyhaemoglobin saturation in retinal arterioles compared with aorta and other central arteries. Calibration issues however, cannot be excluded as a contributing factor to this difference. The reason for the differences in oxyhaemoglobin saturation of retinal arterioles and peripheral vascular beds needs to be clarified. Reconsideration of the instrument calibration and further studies on larger groups of healthy subjects and patients suffering from systemic hypoxic condition are necessary to address the matter of absolute arterial oxyhaemoglobin saturation and retinal arteriolar relative values.

The thesis indicates the ability of retinal oximetry to detect hypoxic metabolic changes of the inner retinal tissue. Retinal oximetry is the only system that allows for noninvasive venous oxyhaemoglobin saturation measures. Up to date, such measures stipulate invasive catheterization for central or mixed venous oxygen monitoring in patients who are critically ill.

The study on combined scanning laser ophthalmoscope and retinal oximetry demonstrates the feasibility of that technique in newly born babies. This is the first study on retinal oximetry in neonates and validates its sensitivity to oxyhaemoglobin and deoxyhaemoglobin in full-term neonates. The primary task with this novel technique was to obtain optical density ratio on full-term healthy neonates for the purpose of further oximetry analysis. Next steps need to aim at technological advantages to improve consistency in optical density measurements. More studies are required for furthering the precision and determination of calibration factors for the verification of the calculations of oxyhaemoglobin saturation values. Extending the retinal oximetry technique to the neonatal population is feasible and relevant for the refinement of the method and future application studies.

Future perspectives

This thesis suggests the oxyhaemoglobin saturation of the systemic circulation can be measured through the retinal circulation. Following calibration upgrade and technological improvement, experimental verification retinal oximetry applied to critically ill and anaesthesia care patients may be considered in order to test the feasibility of the technique for noninvasive monitoring in the future.

One of the significant barriers to the application of this technology in clinical settings is the sheer size and bulk of the oximeter equipment. Currently, study subjects are required to sit in front of the fundus camera after mydriasis for on time retinal imaging. Handheld version without pupil dilation is a consequent plan for furthering this technique. The instrument has already been miniaturized, and the prototype is currently being tested on healthy subjects. The next step is to re-evaluate the calibration constants before bringing the miniaturized version of the device to the bedside of patients for retinal oximetry analysis studies of their central nervous system vessels and hence the systemic circulation.

Acknowledgements

This project has become a reality through collaboration of many people. I want to emphasize my deepest gratitude to the people that were willing to devote their effort and time to participate in the studies. Their contribution is the foundation for this thesis and future studies to build on. It is impossible to mention every person who contributed to the project but I acknowledge you all.

To my instructors: First of all I express my sincere gratitude to my advisor, Dr. Einar Stefánsson, M.D., Ph.D., Professor at the Ophthalmology at the University of Iceland for inviting me to the fascinating world of retinal oximetry. His vast expertise on retinal oxygenation and over thirty years of pioneering work in retinal oximetry is an endless inspiration and origin of intelligence. His exceptional mentoring and leadership within the retinal oximetry team has always been encouraging and a source to rise above obstacles. Einar is the key person behind the conceptualization of this thesis. He played a major role in planning for the studies, interpretation of the data and in manuscript preparations.

My sincere gratitude to my supervisor Dr. Guðrún Kristjánsdóttir, RN,

DrPH, Ph.D., Professor of at the faculty of Nursing at the University of Iceland for welcoming my studies to the field of advanced nursing. Her great expertise and experience in the methodological field has been a great source for challenging questions and intellectual discussions. Her encouragement and support has been invaluable throughout my journey.

My sincere gratitude to my doctoral committee for their contribution to the project; Þórarinn Gíslason, M.D., Ph.D., Charles Vacchiano, CRNA, Ph.D. and Gísli H. Sigurðsson, M.D., Ph.D. Þórarinn Gíslason was involved with a study design, interpretation of results and a manuscript preparation. Charles Vacchiano helped with a manuscript preparation and shared his extensive experience as a colleague and his expertise on physiology. Gísli H. Sigurðsson made invaluable input to my thesis and has been supportive of this project during the whole time.

To my research colleagues: My deepest thanks to Ólöf Birna Ólafsdóttir, Ph.D., one of the major scientists on retinal oximetry in glaucoma worldwide. Her deep knowledge in the field of retinal oximetry has been a priceless resource along my studies. She is the first author of the study on induced hyperoxia in healthy people and without her conceptualization the study would not be a part of my thesis. Her generous motivation and contribution to my project is very much appreciated. I am extremely thankful to Sveinn Hákon Harðarson, Ph.D., which is one of the leading scientists in the field of retinal oximetry. He is a co-author of all manuscripts of this thesis. His experience on retinal oximetry and the tremendous help on data analysis, interpretation of the data, manuscript preparation along with general motivation for my project is highly valued. My sincere gratitude to Davíð Þór Bragason, M.D., for his collaboration on my research projects. He examined the study subjects when needed and helped with conducting my studies. He is also a co-author of the manuscripts where I am the first author. My sincere gratitude to Wouter B. Vehmeijer, M.D., Ph.D student, for his collaboration on retinal oximetry. He is the first author for the novice study on retinal oximetry in neonates. It has been intellectually stimulating and a pleasure to be involved in his projects. I am grateful to Jóna Valgerður Kristjánsdóttir, M.Sc., for gathering the data on oximetry imaging and collaborating to the oximetry project in so many ways. Also, many thanks to the other members of the retinal oximetry group in Reykjavik; including Þór Eysteinsson, Ph.D., Ásbjörg Geirsdóttir, M.D., Ph.D., and Ólafur Pálsson, M.D., for enhancing my understanding of retinal oximetry and the contribution to my work. Special thanks to the engineers and other personnel at Oxymap Ehf., who made the Oxymap T1 into a reality and for the continuous, strive for improvement of the software. Those include but are not limited to Róbert Karlsson, Árni Þór Árnason, Svava Ágústsóttir and Gísli Hreinn Halldórsson. In addition I like to thank Inga Lís Östrup Hauksdóttir for her helpfulness and delightful conversations during my course of work.

To the people at Landspítali – the National University Hospital of Iceland: All the studies which the data in this thesis are based on were conducted at the Department of Ophthalmology at Landspítali - the National University Hospital of Iceland. I want to thank Landspítali for supporting the project including the housing. Also thanks to all the good people that work there and contributed to my work on this project.

Stella S. Hrafnkelsdóttir, RN.,B.S., and Rósa Karlsdóttir, RN.,B.S., at the Department of ambulatory pulmonary care are thanked for their invaluable assistance with recruiting people for the study of people with COPD and with implementation of the study protocol. Sincere thanks to Margrét Jónasdóttir, RNA., for her kindness and support when I was beginning my research studies and to Aðalbjörn Þorsteinsson, M.D., Ph.D., for his support and expert counseling. I also want to thank Kristín Einarsdóttir, RN., at the pediatric department for her invaluable assistance with recruiting participants for the study on neonates.

I wish to express my deepest gratitude to Rikka Mýrdal, RNA., and Alma D. Möller, M.D., Ph.D., for their support and approval for a leave of absence from my clinical practice during my study and preparation of the thesis.

To others: I thank Ólafur Pétur Pálsson for his advice on a statistical analysis and Jakob Torfi Jörundsson for his technical computer support.

To my funding providers: I want to express my special gratitude to all the research funds that financially supported my project: Icelandic Fund for Prevention of Blindness, The Icelandic Center for Research (Rannís), Landspítali University Hospital Research Fund, Memorial Fund of Helga Jónsdóttir and Sigurliði Kristjánsson, Ingibjörg R. Magnúsdottir Research Fund, University of Iceland Research Fund, the Icelandic Nurses′ Association Science Fund and the Kristín Thoroddsen Memorial Fund.

To my relatives and friends: I want to express a great appreciation to my family and friends who backed me up and bore with me during the years of this journey; Especially thanks to my mother, Sigrún Scheving, and her husband Sigurgrímur Jónsson, for being such cherishing grandparents to my son and taking care of him whenever needed. To my sister Guðný Ósk Scheving for embracing my son (and her sister) and making her home an adventurous place for him to stay during the summer vocations. To my stepmother, Ólöf Eyjólfsdóttir and father Elías Einarsson, for their willingness to help out and for their caring. To my friends and neighbors, Ingibjörg Ólafsdóttir and Valdimar Hjaltason for keeping their home and family life open for my son. To Robbie Makroczy and Colin Wolf for providing me sanctuary asylum at their Yyoga studio in the heart of Brussels. This kept me focused and gave me the endurance to reach the final goal while staying sane.

Last but not least, my deepest gratitude to my husband Ragnar G. Kristjánsson for his unconditional support and encouragement throughout the years; for being my best friend and for always keeping faith in me. He has been an exceptional father to our son, Matthias Tumi, and periodically taken the sole responsibility for him when I have been busy working on this thesis. And finally to Matthías Tumi who truly is a blessing in my life. Thanks for your patience and for being just who you are.

Conflict of interest

None.

References

Ainslie PN & Ogoh S (2010): Regulation of cerebral blood flow in mammals during chronic hypoxia: a matter of balance. Exp Physiol **95**: 251–262.

Ainslie PN & Poulin MJ (2004): Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. J Appl Physiol (1985) **97**: 149–159.

Alder VA & Cringle SJ (1985): The effect of retinal circulation on vitreal oxygen tension. Curr Eye Res **4**: 121–129.

Alian AA, Galante NJ, Stachenfeld NS, Silverman DG & Shelley KH (2011): Impact of central hypovolemia on photoplethysmographic waveform parameters in healthy volunteers part 2: frequency domain analysis. J Clin Monit Comput, **25**: 387–396.

Alm A & Bill A (1973): Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys (*Macaca irus*): a study with radioactively labelled microspheres including flow determinations in brain and some other tissues. Exp Eye Res, **15**: 15–29.

Anand-Apte B & Hollyfield JG (2009): Developmental anatomy of the retinal and choroidal vasculature. In: Besharse J, Bok D, editors. Encyclopedia of the Eye. Academic Press, Elsevier Books; London: 9–15.

Anderson DR (1996): Glaucoma, capillaries and pericytes. 1. Blood flow regulation. Ophthalmologica, **210**: 257–262.

Anderson DR & Davis EB (1996): Glaucoma, capillaries and pericytes. 2. Identification and characterization of retinal pericytes in culture. Ophthalmologica, **210**: 263–268.

Arjamaa O & Nikinmaa M (2006): Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. Exp Eye Res, **83**: 473–483.

Arsene S, Giraudeau B, Le Lez ML, Pisella PJ, Pourcelot L & Tranquart F (2002): Follow up by colour Doppler imaging of 102 patients with retinal vein occlusion over 1 year. Br J Ophthalmol, **86**: 1243–1247.

Battu R, Mohan A, Khanna A, Kumar A & Shetty R (2015): Retinal oxygen saturation in retinitis pigmentosa and macular dystrophies in asian-Indian eyes. Invest Ophthalmol Vis Sci **56**: 2798–2802.

Beach JM (2014): Pathway to retinal oximetry. Transl Vis Sci Technol **3**: 2.

Beach JM, Schwenzer KJ, Srinivas S, Kim D & Tiedeman JS (1999): Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. J Appl Physiol **86**: 748–758.

Birol G, Budzynski E, Wangsa-Wirawan ND & Linsenmeier RA (2005): Retinal arterial occlusion leads to acidosis in the cat. Exp Eye Res **80**: 527–533.

Blondal R, Sturludottir MK, Hardarson SH, Halldorsson GH & Stefansson E (2011): Reliability of vessel diameter measurements with a retinal oximeter. Graefes Arch Clin Exp Ophthalmol **249**: 1311–1317.

Boero JA, Ascher J, Arregui A, Rovainen C & Woolsey TA (1999): Increased brain capillaries in chronic hypoxia. J Appl Physiol **86**: 1211.

Boyd SR, Zachary I, Chakravarthy U, Allen GJ, Wisdom GB, Cree IA, Martin JF & Hykin PG (2002): Correlation of increased vascular endothelial growth factor with neovascularization and permeability in ischemic central vein occlusion. Arch Ophthalmol **120**: 1644–1650.

Braun RD, Linsenmeier RA & Yancey CM (1992): Spontaneous fluctuations in oxygen tension in the cat retina. Microvasc Res **44**: 73–84.

Brinchmann-Hansen O, Myhre K & Sandvik L (1989): Retinal vessel responses to exercise and hypoxia before and after high altitude acclimatisation. Eye (Lond) **3**: 768–776.

Butterworth JF, Mackey DC & Wasnick JD (2013): Morgan & Mikhail′s clinical anesthesiology, 5th edn. New York, NY: The McGraw-Hill Companies, Inc.

Buttery RG, Hinrichsen CF, Weller WL & Haight JR (1991): How thick should a retina be? A comparative study of mammalian species with and without intraretinal vasculature Vision Res **31**: 169–187.

Campochiaro PA (2012): Anti-vascular endothelial growth factor treatment for retinal vein occlusions. Ophthalmologica **227**: 30–35.

Campochiaro PA, Hafiz G, Shah SM et al. (2008): Ranibizumab for macular edema due to retinal vein occlusions: implication of VEGF as a critical stimulator. Mol Ther **16**: 791–799.

Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, Murahashi WY & Rubio RG (2011): Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. Ophthalmology **118**: 2041–2049.

Cannesson M, Attof Y, Rosamel P, Desebbe O, Joseph P, Metton O, Bastien O & Lehot JJ (2007): Respiratory variations in pulse oximetry plethysmographic waveform amplitude to predict fluid responsiveness in the operating room. Anesthesiology **106**: 1105–1111.

Caprara C & Grimm C (2012): From oxygen to erythropoietin: relevance of hypoxia for retinal development, health and disease. Prog Retin Eye Res **31**: 89–119.

Caprioli J & Coleman AL (2010): Blood pressure, perfusion pressure, and glaucoma. Am J Ophthalmol **149**: 704–712.

Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C & Kieda C (2011): Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia J Cell Mol Med **15**: 1239–1253.

Cavallaro G, Filippi L, Bagnoli P et al. (2014): The pathophysiology of retinopathy of prematurity: an update of previous and recent knowledge. Acta Ophthalmol **92**: 2–20.

Chan ED & Chan MM (2013): Pulse oximetry: understanding its basic principles facilitates appreciation of its limitations. Respir Med **107**: 789–799.

Cheng R (2014): Vascular reactivity response characteristics to hypoxia. (Thesis) University of Toronto. Available at: http://hdl.handle.net/1807/44005. (Accessed date May 20, 2015).

Cheng RW, Yusof F, Tsui E, Jong M, Duffin J, Flanagan JG, Fisher JA & Hudson C (2016): Relationship between retinal blood flow and arterial oxygen. J Physiol **594**: 625–640.

Chestnut DH (2004): Obstetric anesthesia: principles and practice, 3rd edn. Philadelphia, PA: Elsevier Mosby.

Choudhary TR, Ball D, Fernandez Ramos J, McNaught AI & Harvey AR (2013): Assessment of acute mild hypoxia on retinal oxygen saturation using snapshot retinal oximetry. Invest Ophthalmol Vis Sci **54**: 7538–7543.

Clarenbach CF, Thurnheer R & Kohler M (2012): Vascular dysfunction in chronic obstructive pulmonary disease: current evidence and perspectives. Expert Rev Respir Med **6**: 37–43.

Clarke DD, Sokoloff L (1999): Regulation of cerebral metabolic rate. In: Siegel GJ, Agranoff BW & Albers RW et al. (Ed.), Basic neurochemistry: molecular, cellular and medical aspects, 6th edn. Philadelphia, PA: Lippincott-Raven, Chapter 31. Available at: https://www.ncbi.nlm.nih.gov/books/NBK28194/. (Accessed date March 18, 2015).

Collins JA, Rudenski A, Gibson J, Howard L & O'Driscoll R (2015): Relating oxygen partial pressure, saturation and content: the haemoglobin-oxygen dissociation curve. Breathe (Sheff) **11**: 194–201.

Cripps T, Rocker G & Stradling J (1992): Nocturnal hypoxia and arrhythmias in patients with impaired left ventricular function. Br Heart J **68**: 382–386.

Croll SD, Ransohoff RM, Cai N et al. (2004): VEGF-mediated inflammation precedes angiogenesis in adult brain. Exp Neurol **187**: 388–402.

Delaey C & Van De Voorde J (2000): Regulatory mechanisms in the retinal and choroidal circulation. Ophthalmic Res **32**: 249–256.

Delaey C, Boussery K & Van de Voorde J (2000): A retinal-derived relaxing factor mediates the hypoxic vasodilation of retinal arteries. Invest Ophthalmol Vis Sci **41**: 3555–3560.

Delori FC (1988): Noninvasive technique for oximetry of blood in retinal vessels. Appl Opt **27**: 1113–1125.

Demir HD, Inonu H, Kurt S, Doruk S, Aydin E & Etikan I (2012): Evaluation of visual field parameters in patients with chronic obstructive pulmonary disease. Acta Ophthalmol **90**: e349–e354.

Denninghoff KR, Smith MH, Hillman LW, Redden D & Rue LW (1998): Retinal venous oxygen saturation correlates with blood volume. Acad Emerg Med **5**: 577–582.

Denninghoff KR, Smith MH, Lompado A & Hillman LW (2003): Retinal venous oxygen saturation and cardiac output during controlled hemorrhage and resuscitation. J Appl Physiol **94**: 891–896.

Dong Y, Lin L, Yan H et al. (2016): Shifts in retinal vessel diameter and oxygen saturation in Chinese type 2 diabetes mellitus patients. BMC Ophthalmol **16**: 43.

Dunn JF, Roche MA, Springett R, Abajian M, Merlis J, Daghlian CP, Lu SY & Makki M (2004): Monitoring angiogenesis in brain using steady-state quantification of DeltaR2 with MION infusion. Magn Reson Med **51**: 55–61.

Dutton RP (2007): Current concepts in hemorrhagic shock. Anesthesiol Clin **25**: 23–34.

Edoh D, Antwi-Bosaiko C & Amuzu D (2006): Fetal hemoglobin during infancy and in sickle cell adults. Afr Health Sci **6**: 51–54.

van Eeden SF & Sin DD (2008): Chronic obstructive pulmonary disease: a chronic systemic inflammatory disease. Respiration **75**: 224–238.

Eickhoff P, Valipour A, Kiss D, Schreder M, Cekici L, Geyer K, Kohansal R & Burghuber OC (2008): Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med **178**: 1211–1218.

Einarsdottir AB, Hardarson SH, Kristjansdottir JV, Bragason DT, Snaedal J & Stefánsson E (2015): Retinal oximetry imaging in Alzheimer's disease. J Alzheimers Dis **49**: 79–83.

Elman MJ, Bhatt AK, Quinlan PM & Enger C (1990): The risk for systemic vascular diseases and mortality in patients with central retinal vein occlusion. Ophthalmology **97**: 1543–1548.

Epstein DL, Algvere PV, von Wendt G, Seregard S & Kvanta A (2012): Bevacizumab for macular edema in central retinal vein occlusion: a prospective, randomized, double-masked clinical study. Ophthalmology **119**: 1184–1189.

Eysteinsson T, Hardarson SH, Bragason D & Stefánsson E (2014): Retinal vessel oxygen saturation and vessel diameter in retinitis pigmentosa. Acta Ophthalmol **92**: 449–453.

Feke GT, Tagawa H, Deupree DM, Goger DG, Sebag J & Weiter JJ (1989): Blood flow in the normal human retina. Invest Ophthalmol Vis Sci **30**: 58–65.

Felder AE, Wanek J, Blair NP & Shahidi M (2015): Inner retinal oxygen extraction fraction in response to light flicker stimulation in humans. Invest Ophthalmol Vis Sci **56**: 6633–6637.

Flammer J & Mozaffarieh M (2008): Autoregulation, a balancing act between supply and demand. Can J Ophthalmol **43**: 317–321.

Flammer J, Konieczka K, Bruno RM, Virdis A, Flammer AJ & Taddei S (2013): The eye and the heart. Eur Heart J **34**: 1270–1278.

Fouzas S, Priftis KN & Anthracopoulos MB (2011): Pulse oximetry in pediatric practice. Pediatrics **128**: 740–752.

Fuchsjager-Mayrl G, Luksch A, Malec M, Polska E, Wolzt M & Schmetterer L (2003): Role of endothelin-1 in choroidal blood flow regulation during isometric exercise in healthy humans. Invest Ophthalmol Vis Sci **44**: 728–733.

Garhofer G, Huemer KH, Zawinka C, Schmetterer L & Dorner GT (2002): Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow. Curr Eye Res **24**: 109–113.

Geirsdottir A, Palsson O, Hardarson SH, Olafsdottir OB, Kristjansdottir JV & Stefánsson E (2012): Retinal vessel oxygen saturation in healthy individuals. Invest Ophthalmol Vis Sci **53**: 5433–5442.

Geirsdottir A, Hardarson SH, Olafsdottir OB & Stefánsson E (2014): Retinal oxygen metabolism in exudative age-related macular degeneration. Acta Ophthalmol **92**: 27–33.

van Gestel AJ & Steier J (2010): Autonomic dysfunction in patients with chronic obstructive pulmonary disease (COPD). J Thorac Dis **2**: 215–222.

Gidday JM & Park TS (1993): Adenosine-mediated autoregulation of retinal arteriolar tone in the piglet. Invest Ophthalmol Vis Sci **34**: 2713–2719.

Gilmore ED, Hudson C, Venkataraman ST, Preiss D & Fisher J (2004): Comparison of different hyperoxic paradigms to induce vasoconstriction: implications for the investigation of retinal vascular reactivity. Invest Ophthalmol Vis Sci **45**: 3207–3212.

Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2016): From the Global Strategy for the Diagnosis, Management and Prevention of COPD.

Glueck CJ, Wang P, Bell H, Rangaraj V & Goldenberg N (2005): Associations of thrombophilia, hypofibrinolysis, and retinal vein occlusion. Clin Appl Thromb Hemost **11**: 375–389.

Golparvar M, Naddafnia H & Saghaei M (2002): Evaluating the relationship between arterial blood pressure changes and indices of pulse oximetric plethysmography. Anesth Analg **95**: 1686–1690.

Green WR, Chan CC, Hutchins GM & Terry JM (1981): Central retinal vein occlusion: a prospective histopathologic study of 29 eyes in 28 cases. Retina **1**: 27–55.

Gupta AK, Menon DK, Czosnyka M, Smielewski P & Jones JG (1997): Thresholds for hypoxic cerebral vasodilation in volunteers. Anesth Analg **85**: 817–820.

Guyton AC & Hall JE (2000): Textbook of medical physiology, 10th edn. Philadelphia, PA: W.B. Saunders Company.

Habib J, Baetz L & Satiani B (2012): Assessment of collateral circulation to the hand prior to radial artery harvest. Vasc Med **17**: 352–361.

Hahn P, Mruthyunajaya P & Fekrat S (2013). Central retinal vein occlusion. In: Ryan SJ (ed.). Retina, 5th edn, vol. 2. Philadelphia, PA: Elsevier Health Science 1039–1049.

Hammer M, Riemer T, Vilser W, Gehlert S & Schweitzer D (2009). A new imaging technique for retinal vessel oximetry: principles and first clinical results in patients with retinal arterial occlusion and diabetic retinopathy. Ophthal Technol **XIX**: 7163 71630P-1.

Hardarson SH & Stefánsson E (2010): Oxygen saturation in central retinal vein occlusion. Am J Ophthalmol **150**: 871–875.

Hardarson SH & Stefánsson E (2012a): Retinal oxygen saturation is altered in diabetic retinopathy. Br J Ophthalmol **96**: 560–563.

Hardarson SH & Stefánsson E (2012b): Oxygen saturation in branch retinal vein occlusion. Acta Ophthalmol **90**: 466–470.

Hardarson SH, Harris A, Karlsson RA et al. (2006): Automatic retinal oximetry. Invest Ophthalmol Vis Sci **47**: 5011–5016.

Hardarson SH, Basit S, Jonsdottir TE et al. (2009): Oxygen saturation in human retinal vessels is higher in dark than in light. Invest Ophthalmol Vis Sci **50**: 2308–2311.

Hardarson SH, Elfarsson A, Agnarsson BA & Stefánsson E (2013): Retinal oximetry in central retinal artery occlusion. Acta Ophthalmol **91**: 189–190.

Hardarson SH, Jonsson BS, Karlsson RA et al. (2015): Image quality affects measurements of retinal vessel oxygen saturation. Invest Ophthalmol Vis Sci **56**: 3316.

Harris A, Dinn RB, Kagemann L & Rechtman E (2003): A review of methods for human retinal oximetry. Ophthalmic Surg Lasers Imaging **34**: 152–164.

Harvey RA & Ferrier DR (2011): Biochemistry, 5th edn. Philadelphia, PA: Wolters Kluwer.

Hata Y, Clermont A, Yamauchi T et al. (2000): Retinal expression, regulation, and functional bioactivity of prostacyclin-stimulating factor. J Clin Invest **106**: 541–550.

Hatazawa J, Fujita H, Kanno I et al. (1995): Regional cerebral blood flow, blood volume, oxygen extraction fraction, and oxygen utilization rate in normal volunteers measured by the autoradiographic technique and the single breath inhalation method. Ann Nucl Med **9**: 15–21.

Haymond S (2006): Oxygen saturation: a guide to laboratory assessment. 10–12. Available at: http://www.optimedical.com/pdf/articles/oxygen-saturation-laboratory-assessment.pdf

Hayreh SS (1975): Segmental nature of the choroidal vasculature. Br J Ophthalmol **59**: 631–648.

Hayreh SS (2005): Prevalent misconceptions about acute retinal vascular occlusive disorders. Prog Retin Eye Res **24**: 493–519.

Hayreh SS (2011): Acute retinal arterial occlusive disorders. Prog Retin Eye Res **30**: 359–394.

Hayreh SS (2014): Ocular vascular occlusive disorders: natural history of visual outcome. Prog Retin Eye Res **41**: 1–25.

Hayreh SS, Rojas P, Podhajsky P, Montague P & Woolson RF (1983): Ocular neovascularization with retinal vascular occlusion-III Incidence of ocular neovascularization with retinal vein occlusion. Ophthalmology **90**: 488–506.

Hayreh SS, Zimmerman B, McCarthy MJ & Podhajsky P (2001): Systemic diseases associated with various types of retinal vein occlusion. Am J Ophthalmol **131**: 61–77.

Hayreh SS, Zimmerman MB, Beri M & Podhajsky P (2004): Intraocular pressure abnormalities associated with central and hemicentral retinal vein occlusion. Ophthalmology **111**: 133–141.

Hayreh SS, Podhajsky PA & Zimmerman MB (2011): Natural history of visual outcome in central retinal vein occlusion. Ophthalmology **118**: 119–133 e111–e112.

Hayreh SS, Zimmerman MB & Podhajsky PA (2012): Retinal vein occlusion and the optic disk. Retina **32**: 2108–2118.

Heistad DD & Abboud FM (1980): Dickinson W Richards Lecture: circulatory adjustments to hypoxia. Circulation **61**: 463–470.

Heitmar RC & Cubbidge RP (2013): The impact of flash intensity on retinal vessel oxygen measurements using dual wavelength oximetry. Invest Ophthalmol Vis Sci **3**: 2807–2811.

Hickam JB, Sieker HO & Frayser R (1960): Studies of retinal circulation and A-V difference in man. Trans Am Clin Climatol Assoc **71**: 34–44.

Hines RL & Marschall KE (2012): Stoelting's anesthesia and co-existing diseases, 6th edn. Philadelphia, PA: Saunders.

Hoiseth LO, Hisdal J, Hoff IE, Hagen OA, Landsverk SA & Kirkeboen KA (2015): Tissue oxygen saturation and finger perfusion index in central hypovolemia: influence of pain. Crit Care Med **43**: 747–756.

Ikeda K, MacLeod DB, Grocott HP, Moretti EW, Ames W & Vacchiano C (2014): The accuracy of a near-infrared spectroscopy cerebral oximetry device and its potential value for estimating jugular venous oxygen saturation. Anesth Analg **119**: 1381–1392.

Jackobiec′s A, Miller JW, Azar B & & Blodi BA (2008): Principles and practice of ophthalmology, 3rd edn. Philadelphia, PA: Saunders.

Janaky M, Grosz A, Toth E, Benedek K & Benedek G (2007): Hypobaric hypoxia reduces the amplitude of oscillatory potentials in the human ERG. Doc Ophthalmol **114**: 45–51.

Jani PD, Mwanza J, Billow KB, Waters AM, Moyer S & Garg S (2014): Normative values and predictors of retinal oxygen saturation. Retina **34**: 394–401.

Jean-Louis S, Lovasik JV & Kergoat H (2005): Systemic hyperoxia and retinal vasomotor responses. Invest Ophthalmol Vis Sci **46**: 1714–1720.

Jeppesen P, Sanye-Hajari J & Bek T (2007): Increased blood pressure induces a diameter response of retinal arterioles that increases with decreasing arteriolar diameter. Invest Ophthalmol Vis Sci **48**: 328–331.

Jørgensen C & Bek T (2014): Increasing oxygen saturation in larger retinal vessels after photocoagulation for diabetic retinopathy. Invest Ophthalmol Vis Sci **55**: 5365–5369.

Jørgensen CM, Hardarson SH & Bek T (2014): The oxygen saturation in retinal vessels from diabetic patients depends on the severity and type of vision-threatening retinopathy. Acta Ophthalmol **92**: 34–39.

Kang MH, Balaratnasingam C, Yu PK, Morgan WH, McAllister IL, Cringle SJ & Yu DY (2011): Morphometric characteristics of central retinal artery and vein endothelium in the normal human optic nerve head. Invest Ophthalmol Vis Sci **52**: 1359–1367.

Kashani AH, Lopez Jaime GR, Saati S, Martin G, Varma R & Humayun MS (2014): Noninvasive assessment of retinal vascular oxygen content among normal and diabetic human subjects: a study using hyperspectral computed tomographic imaging spectroscopy. Retina **34**: 1854–1860.

Kaur C, Foulds WS & Ling EA (2008): Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. Prog Retin Eye Res **27**: 622–647.

Kergoat H, Hérard ME & Lemay M (2006): RGC sensitivity to mild systemic hypoxia. Invest Ophthalmol Vis Sci **46**: 5423–5427.

Kety SS & Schmidt CF (1948): The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. J Clin Invest **27**: 484–492.

Khoobehi B, Firn K, Thompson H, Reinoso M & Beach J (2013): Retinal arterial and venous oxygen saturation is altered in diabetic patients. Invest Ophthalmol Vis Sci **54**: 7103–7106.

Kisilevsky M, Hudson C, Mardimae A, Wong T & Fisher J (2008): Concentration-dependent vasoconstrictive effect of hyperoxia on hypercarbia-dilated retinal arterioles. Microvasc Res **75**: 263–268.

Klefter ON, Vilsboll T, Knop FK & Larsen M (2015): Retinal vascular and structural dynamics during acute hyperglycaemia. Acta Ophthalmol **93**: 697–705.

Klefter ON, Hommel E, Munch IC, Norgaard K, Madsbad S & Larsen M (2016): Retinal characteristics during 1 year of insulin pump therapy in type 1 diabetes: a prospective, controlled, observational study. Acta Ophthalmol **94**: 540–547.

Kolb H (2012): Simple anatomy of the retina by Helga Kolb. In Kolb H, Nelson R, Fernandez E & Jones B (eds). Webvision: The Organization of the Retina and Visual System. Available at: http://webvision.med.utah.edu/book/part-i-foundations/simple-anatomy-of-the-retina/

Kristinsson J, Gottfredsdottir MS & Stefánsson E (1997): Retinal vessel dilatation and elongation precedes diabetic macular oedema. Br J Ophthalmol **81**: 274–278.

Kristjansdottir JV, Hardarson SH, Halldorsson GH, Karlsson RA, Eliasdottir TS & Stefánsson E (2014): Retinal oximetry with a scanning laser ophthalmoscope. Invest Ophthalmol Vis Sci **55**: 3120–3126.

Kur J & Newman EA (2014): Purinergic control of vascular tone in the retina. J Physiol **592**: 491–504.

Lau JCM & Linsenmeier RA (2012): Oxygen consumption and distribution in the Long-Evans rat retina. Exp Eye Res **102**: 50–58.

Leach RM & Treacher DF (1998): Oxygen transport 2: tissue hypoxia. BMJ **317**: 1370–1373.

Leach RM & Treacher DF (2002): The pulmonary physician in critical care 2: oxygen delivery and consumption in the critically ill. Thorax **57**: 170–177.

Levin LA, Nilsson SFE, Ver Hoeve J, Wu SM, Kaufman PL & Alm A (2011): Adler's physiology of the eye. New York, NY: Elsevier Inc. Saunders.

Li J, Yang Y, Yang D, Liu X, Sun Y, Wei S & Wang N (2016): Normative values of retinal oxygen saturation in rhesus monkeys: the Beijing intracranial and intraocular pressure (iCOP) study. PLoS ONE **11**: e0150072.

Lin LL, Dong YM, Zong Y et al. (2016): Study of retinal vessel oxygen saturation in ischemic and non-ischemic branch retinal vein occlusion. Int J Ophthalmol **9**: 99–107.

Linsenmeier RA & Braun RD (1992): Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. J Gen Physiol **99**: 177–197.

Linsenmeier RA & Padnick-Silver L (2000): Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. Invest Ophthalmol Vis Sci **41**: 3117–3123.

Linsenmeier RA & Yancey CM (1989): Effects of hyperoxia on the oxygen distribution in the intact cat retina. Invest Ophthalmol Vis Sci **30**: 612–618.

Linsenmeier RA, Braun RD, McRipley MA, Padnick LB, Ahmed J, Hatchell DL, McLeod DS & Lutty GA (1998): Retinal hypoxia in long-term diabetic cats. Invest Ophthalmol Vis Sci **39**: 1647–1657.

Liu X, Wang S, Liu Y, Liu LJ, Lv YY, Tang P, Jonas JB & Xu L (2017): Retinal oxygen saturation in Chinese adolescents. Acta Ophthalmol **95**: 54–61.

London NJ & Brown G (2011): Update and review of central retinal vein occlusion. Curr Opin Ophthalmol **22**: 159–165.

Luksch A, Polska E, Imhof A, Schering J, Fuchsjager-Mayrl G, Wolzt M & Schmetterer L (2003): Role of NO in choroidal blood flow regulation during isometric exercise in healthy humans. Invest Ophthalmol Vis Sci **44**: 734–739.

Maclay JD, McAllister DA, Mills NL et al. (2009): Vascular dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med **180**: 513–520.

Maenhaut N, Boussery K, Delaey C & Van de Voorde J (2007): Control of retinal arterial tone by a paracrine retinal relaxing factor. Microcirculation **14**: 39–48.

Man RE, Kawasaki R, Wu Z, Luu CD, Wang JJ, Wong TY & Lamoureux EL (2013): Reliability and reproducibility of retinal oxygen saturation measurements using a predefined peri-papillary annulus.. Acta Ophthalmol **91**: e590–e594.

Man RE, Sasongko MB, Kawasaki R, Noonan JE, Lo TC, Luu CD, Lamoureux EL & Wang JJ (2014): Associations of retinal oximetry in healthy young adults. Invest Ophthalmol Vis Sci **55**: 1763–1769.

Man RE, Sasongko MB, Xie J et al. (2015): Associations of retinal oximetry in persons with diabetes. Clin Experiment Ophthalmol **43**: 124–131.

Marinskis G, Lip GY, Aidietis A et al. (2006): Blood oxygen saturation during atrio-ventricular dissociation with wide-QRS complex tachycardias. Int J Cardiol **107**: 134–135.

Martinet V, Guigui B, Glacet-Bernard A, Zourdani A, Coscas G, Soubrane G & Souied EH (2012): Macular edema in central retinal vein occlusion: correlation between optical coherence tomography, angiography and visual acuity. Int Ophthalmol **32**: 369–377.

McAllister IL (2012): Central retinal vein occlusion: a review. Clin Experiment Ophthalmol **40**: 48–58.

McClatchey KD (2002). Blood cell counting Clinical Laboratory Medicine, 2nd edn. Philadelphia, PA: Wolters Kluwer 806.

McClintic BR, McClintic JI, Bisognano JD & Block RC (2010): The relationship between retinal microvascular abnormalities and coronary heart disease: a review. Am J Med **123**: 374 e371-377.

McGrath SP, Ryan KL, Wendelken SM, Rickards CA & Convertino VA (2011): Pulse oximeter plethysmographic waveform changes in awake, spontaneously breathing, hypovolemic volunteers. Anesth Analg **112**: 368–374.

McIntosh RL, Rogers SL, Lim L et al. (2010): Natural history of central retinal vein occlusion: an evidence-based systematic review. Ophthalmology **117**: 1113–1123 e1115.

McLellan SA & Walsh TS (2004): Oxygen delivery and haemoglobin. Cont Educ Anaesth Crit Care Pain **4**: 123–126.

McQuillan LP, Leung GK, Marsden PA, Kostyk SK & Kourembanas S (1994): Hypoxia inhibits expression of eNOS via transcriptional and posttranscriptional mechanisms. Am J Physiol Heart Circ Physiol **267**: 1921–1927.

Michelson G & Scibor M (2006): Intravascular oxygen saturation in retinal vessels in normal subjects and open-angle glaucoma subjects. Acta Ophthalmol Scand **84**: 289–295.

Miller RD (2000): Anesthesia, 5th edn. New York, NY: Churchill Livingstone.

Mohan A, Dabir S, Yadav NK, Kummelil M, Kumar RS & Shetty R (2015): Normative database of retinal oximetry in Asian Indian eyes. PLoS ONE **10**: e0126179.

Mosca A, Paleari R, Leone D & Ivaldi G (2009): The relevance of hemoglobin F measurement in the diagnosis of thalassemias and related hemoglobinopathies. Clin Biochem **42**: 1797–1801.

Nagelhout J & Zaglaniczny K (2001): Nurse anesthesia, 2nd edn. New York: W.B. Saunders Co.

Nakano Y, Shimazaki T, Kobayashi N et al. (2016): Retinal oximetry in a healthy Japanese population. PLoS ONE **11**: e0159650.

Nickla DL & Wallman J (2010): The multifunctional choroid. Prog Retin Eye Res **29**: 144–168.

Nielsen HB, Madsen P, Svendsen LB, Roach RC & Secher NH (1998): The influence of PaO_2, pH and SaO_2 on maximal oxygen uptake. Acta Physiol Scand **164**: 89–97.

Nitzan M, Romem A & Koppel R (2014): Pulse oximetry: fundamentals and technology update. Med Devices (Auckl) **7**: 231–239.

Noergaard MH, Bach-Holm D, Scherfig E, Bang K, Jensen PK, Kiilgaard JF, Stefánsson E & la Cour M (2008): Dorzolamide increases retinal oxygen tension after branch retinal vein occlusion. IOVS **49**: 1136–1141.

Noma H, Funatsu H, Mimura T, Harino S & Hori S (2009): Vitreous levels of interleukin-6 and vascular endothelial growth factor in macular edema with central retinal vein occlusion. Ophthalmology **116**: 87–93.

Olafsdottir OB, Hardarson SH, Gottfredsdottir MS, Harris A & Stefánsson E (2011): Retinal oximetry in primary open-angle glaucoma. Glaucoma **52**: 6409–6413.

Olafsdottir OB, Eliasdottir TS, Kristjansdottir JV, Hardarson SH & Stefánsson E (2015): Retinal vessel oxygen saturation vessel oxygen saturation during 100% oxygen breathing in healthy individuals. PLoS ONE **10**: e0128780.

Oncel C, Baser S, Cam M, Akdağ B, Taspinar B & Evyapan F (2010): Peripheral neuropathy in chronic obstructive pulmonary disease. COPD **7**: 11–16.

Ozcimen M, Sakarya Y, Kurtipek E et al. (2016): Peripapillary choroidal thickness in patients with chronic obstructive pulmonary disease. Cutan Ocul Toxicol **35**: 26–30.

Ozge C, Ozge A, Yilmaz A, Yalçinkaya DE & Calikoğlu M (2005): Cranial optic nerve involvements in patients with severe COPD. Respirology **10**: 666–672.

Pages G & Pouyssegur J (2005): Transcriptional regulation of the Vascular endothelial growth factor gene-a concert of activating factors. Cardiovasc Res **65**: 564–573.

Palkovits S, Lasta M, Boltz A et al. (2013): Measurement of retinal oxygen saturation in patients with chronic obstructive pulmonary disease. Invest Ophthalmol Vis Sci **54**: 1008–1013.

Palkovits S, Lasta M, Told R et al. (2014a): Retinal oxygen metabolism during normoxia and hyperoxia in healthy subjects. Invest Ophthalmol Vis Sci **55**: 4707–4713.

Palkovits S, Told R, Boltz A, Schmidl D, Popa Cherecheanu A, Schmetterer L & Garhofer G (2014b): Effect of increased oxygen tension on flicker-induced vasodilatation in the human retina. J Cereb Blood Flow Metab **34**: 1914–1918.

Palkovits S, Told R, Schmidl D et al. (2014c): Regulation of retinal oxygen metabolism in humans during graded hypoxia. Am J Physiol Heart Circ Physiol **307**: H1412–H1418.

Palsson O, Geirsdottir A, Hardarson SH, Olafsdottir OB, Kristjansdottir JV & Stefánsson E (2012): Retinal oximetry images must be standardized: a methodological analysis. Invest Ophthalmol Vis Sci **53**: 1729–1733.

Paques M & Gaudric A (2002): Peripheral retinochoroidal anastomosis after central retinal vein occlusion. Br J Ophthalmol **86**: 1446–1447.

Patel SR, Hudson C, Flanagan JG & Heitmar R (2013): The effect of simulated cataract light scatter on retinal vessel oximetry. Exp Eye Res **116**: 185–189.

Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ & Dhillon B (2005): Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat **206**: 319–348.

Perel A (2015): Non-invasive monitoring of oxygen delivery in acutely ill patients: new frontiers. Ann Intens Care **5**: 24.

Perkins GD, McAuley DF, Giles S, Routledge H & Gao F (2003): Do changes in pulse oximeter oxygen saturation predict equivalent changes in arterial oxygen saturation? Crit Care **7**: R67.

Pittman RN (2011): *Regulation of tissue oxygenation*. Morgan & Claypool Life Sciences.

Polak K, Luksch A, Frank B, Jandrasits K, Polska E & Schmetterer L (2003): Regulation of human retinal blood flow by endothelin-1. Exp Eye Res **76**: 633–640.

Pournaras CJ, Riva CE, Tsacopoulos M & Strommer K (1989): Diffusion of O2 in the retina of anesthetized miniature pigs in normoxia and hyperoxia. Exp Eye Res **49**: 347–360.

Pournaras CJ, Tsacopoulos M, Strommer K, Gilodi N & Leuenberger PM (1990): Experimental retinal branch vein occlusion in miniature pigs induces local tissue hypoxia and vasoproliferative microangiopathy. Ophthalmology **97**: 1321–1328.

Pournaras JA, Petropoulos IK, Munoz JL & Pournaras CJ (2004): Experimental retinal vein occlusion: effect of acetazolamide and carbogen (95% O_2/5% CO_2) on preretinal PO_2. Invest Ophthalmol Vis Sci **45**: 3669–3677.

Pournaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH & Stefánsson E (2008): Regulation of retinal blood flow in health and disease. Prog Retin Eye Res **27**: 284–330.

Qin Q, Grgac K & van Zijl PC (2011): Determination of whole-brain oxygen extraction fractions by fast measurement of blood T(2) in the jugular vein. Magn Reson Med **65**: 471–479.

Riva CE, Sinclair SH & Grunwald JE (1981): Autoregulation of retinal circulation in response to decrease of perfusion pressure. Invest Ophthalmol Vis Sci **21**: 34–38.

Riva CE, Alm A & Pournaras CJ (2011): Ocular circulation. In Levin LA, Nilsson SFE, Ver Hoeve J, Wu SM, Kaufman PL & Alm A (eds). Adler's physiology of the eye, 11th edn. New York, NY: Elsevier Inc. Saunders 243–273.

Rogers S, McIntosh RL, Cheung N et al. (2010): The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and

Australia. Ophthalmology **117**: 313–319 e311.
Rose K, Kulasekara SI & Hudson C (2016): Intervisit repeatability of retinal blood oximetry and total retinal blood flow under varying systemic blood gas oxygen saturations. IOVS **57**: 188–197.
Schechter AN (2008): Hemoglobin research and the origins of molecular medicine. Blood **112**: 3927–3938.
Scheeren TWL, Schober P & Schwarte LA (2012): Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications. J Clin Monit Comput **26**: 279–287.
Scheufler KM (2004): Tissue oxygenation and capacity to deliver O2 do the two go together? Transfus Apher Sci **31**: 45–54.
Schmetterer L & Polak K (2001): Role of nitric oxide in the control of ocular blood flow. Prog Retin Eye Res **20**: 823–847.
Schmidl D, Garhofer G & Schmetterer L (2011a): The complex interaction between ocular perfusion pressure and ocular blood flow – relevance for glaucoma. Exp Eye Res **93**: 141–155.
Schmidl D, Weigert G, Dorner GT, Resch H, Kolodjaschna J, Wolzt M, Garhofer G & Schmetterer L (2011b): Role of adenosine in the control of choroidal blood flow during changes in ocular perfusion pressure. Invest Ophthalmol Vis Sci **52**: 6035–6039.
Schmidl D, Boltz A, Kaya S et al. (2012): Comparison of choroidal and optic nerve head blood flow regulation during changes in ocular perfusion pressure. Invest Ophthalmol Vis Sci **53**: 4337–4346.
Schober P & Schwarte LA (2012): From system to organ to cell: oxygenation and perfusion measurement in anesthesia and critical care. J Clin Monit Comput **26**: 255–265.
Schoch HJ, Fischer S & Marti HH (2002): Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain. Brain **125**: 2549–2557.
Schulte K, Wolf S, Arend O, Harris A, Henle C & Reim M (1996): Retinal hemodynamics during increased intraocular pressure. Ger J Ophthalmol **5**: 1–5.
Schweitzer D, Hammer M, Kraft J, Thamm J, Königsdörffer E & Strobel J (1999): In vivo measurement of the oxygen saturation of retinal vessels in healthy volunteers. IEEE Trans Biomed Eng **46**: 1454–1465.
Severinghaus JW (2007): Takuo Aoyagi: discovery of pulse oximetry. Anesth Analg **105** (6 Suppl): S1–S4, tables of contents.
Severinghaus JW (2011): Monitoring oxygenation. J Clin Monit Comput **25**: 155–161.
Shamir M, Eidelman LA, Floman Y, Kaplan L & Pizov R (1999): Pulse oximetry plethysmographic waveform during changes in blood volume. Br J Anaesth **82**: 178–181.
Sin DD, Anthonisen NR, Soriano JB & Agusti AG (2006): Mortality in COPD: role of comorbidities. Eur Respir J **28**: 1245–1257.
Sin M, Chrapek O, Karhanova M, Sinova I, Spackova K, Langova K & Rehak J (2016): The effect of pars plana vitrectomy and nuclear cataract on oxygen saturation in retinal vessels, diabetic and non-diabetic patients compared. Acta Ophthalmol **94**: 41–47.
Sinex JE (1999): Pulse oximetry: principles and limitations. Am J Emerg Med **17**: 59–67.
Smith LE (2004): Pathogenesis of retinopathy of prematurity. Growth Horm IGF Res **14** (Suppl A): S140–S144.
Smith MH, Denninghoff KR, Lompado A & Hillman LW (2000): Retinal vessel oximetry: toward absolute calibration. Proceedings of SPIE – The International Society for Optical Engineering, Vol. 3908. Society of Photo-Optical Instrumentation Engineers 217–226.
Stefánsson E, Wolbarsht ML & Landers MB 3rd (1983): In vivo O2 consumption in rhesus monkeys in light and dark. Exp Eye Res **37**: 251–256.
Stefánsson E, Novack RL & Hatchell DL (1990): Vitrectomy prevents retinal hypoxia in branch retinal vein occlusion. Invest Ophthalmol Vis Sci **31**: 284–289.
Steppan J & Hogue CW Jr (2014): Cerebral and tissue oximetry. Best Pract Res Clin Anaesthesiol **28**: 429–439.
Takahashi K, Muraoka K, Kishi S & Shimizu K (1998): Formation of retinochoroidal collaterals in crvo. Am J Ophthalmol **126**: 91–99.
Thariq Bhatti M (2008): Ophthalmologic complications. In Lobato EB, Gravenstein N & Kirby RR (eds), Complications in anesthesiology. Philadelphia, PA: Lippincott Williams & Wilkins 399–428.
Thylefors J, Piitulainen E & Havelius U (2009): Dark adaptation during systemic hypoxia induced by chronic respiratory insufficiency. Invest Ophthalmol Vis Sci **50**: 1307–1312.
Tinjust D, Kergoat H & Lovasik JV (2002): Neuroretinal function during mild systemic hypoxia. Aviat Space Environ Med **73**: 1189–1194.
Traustason S, Jensen AS, Arvidsson HS, Munch IC, Søndergaard L & Larsen M (2011): Retinal oxygen saturation in patients with systemic hypoxemia. Invest Ophthalmol Vis Sci **52**: 5064–5067.
Traustason S, Kiilgaard JF, Karlsson RA, Hardarson SH, Stefánsson E & la Cour M (2013): Spectrophotometric retinal oximetry in pigs. Invest Ophthalmol Vis Sci **54**: 2746–2751.
Traustason S, la Cour M & Larsen M (2014): Retinal vascular oximetry during ranibizumab treatment of central retinal vein occlusion. Br J Ophthalmol **98**: 1208–1211.
Trivedi NS, Ghouri AF, Lai E, Shah NK & Barker SJ (1997): Pulse oximeter performance during desaturation and resaturation: a comparison of seven models. J Clin Anesth **9**: 184–188.
Turksever C, Daikeler T, Konieczka K & Todorova MG (2014): Retinal vessel oxygen saturation in giant cell arteritis patients without ocular symptoms. Klin Monbl Augenheilkd **231**: 442–446.
Ueda-Consolvo T, Fuchizawa C, Otsuka M, Nakagawa T & Hayashi A (2015): Analysis of retinal vessels in eyes with retinitis pigmentosa by retinal oximeter. Acta Ophthalmol **93**: e446–e450.
Ugurlu E, Pekel G, Altinisik G, Bozkurt K, Can I & Evyapan F (2016): New aspect for systemic effects of COPD: eye findings. Clin Respir J **12**: 247–252.
Van de Louw A, Cracco C, Cerf C, Harf A, Duvaldestin P, Lemaire F & Brochard L (2001): Accuracy of pulse oximetry in the intensive care unit. Intensive Care Med **27**: 1606–1613.
Vandewalle E, Abegao Pinto L, Olafsdottir OB & Stalmans I (2012): Phenylephrine 5% added to Tropicamide 0.5% eye drops does not influence retinal oxygen saturation values or retinal vessel diameter in glaucoma patients. Acta Ophthalmol **91**: 733–737.
Vandewalle E, Abegão Pinto L, Olafsdottir OB et al. (2013): Oximetry in glaucoma: correlation of metabolic change with structural and functional damage. Acta Ophthalmol **92**: 105–110.
Venkataraman ST, Hudson C, Fisher JA, Rodrigues L, Mardimae A & Flanagan JG (2008): Retinal arteriolar and capillary vascular reactivity in response to isoxic hypercapnia. Exp Eye Res **87**: 535–542.
Wang Y, Lu A, Gil-Flamer J, Tan O, Izatt JA & Huang D (2009): Measurement of total blood flow in the normal human retina using Doppler Fourier-domain optical coherence tomography. Br J Ophthalmol **93**: 634–637.
Wangsa-Wirawan ND & Linsenmeier R (2003): Retinal Oxygen fundamental and clinical aspects. Arch Ophthalmol **121**: 547–557.
Werkmeister RM, Schmidl D, Aschinger G et al. (2015): Retinal oxygen extraction in humans. Sci Rep **5**: 15763.
West JB (2003): Pulmonary pathophysiology: the essentials, 6th edn. Philadelphia, PA: Lippincott Williams & Wilkins.
West JB (2005): Respiratory physiology: the essentials, 7th edn. Philadelphia, PA: Lippincott Williams & Wilkins.
Westphal GA, Silva E, Gonçalves AR, Caldeira Filho M & Poli-de-Figueiredo LF (2009): Pulse oximetry wave variation as a noninvasive tool to assess volume status in cardiac surgery. Clinics **64**: 337–343.
Whincup P, Papacosta O, Lennon L & Haines A (2006): Carboxyhaemoglobin levels and their determinants in older British men. BMC Public Health **6**: 189.
Williamson TH & Harris A (1994): Ocular blood flow measurement. Br J Ophthalmol **78**: 939–945.
Williamson TH, Grewal J, Gupta B, Mokete B, Lim M & Fry CH (2009): Measurement of PO_2 during vitrectomy for central retinal vein occlusion, a pilot study. Graefes Arch Clin Exp Ophthalmol **247**: 1019–1023.
Wilson BJ, Cowan HJ, Lord JA, Zuege DJ & Zygun DA (2010): The accuracy of pulse oximetry in emergency department patients with severe sepsis and septic shock: a retrospective cohort study. BMC Emerg Med **10**: 9.

Yamanishi S, Katsumura K, Kobayashi T & Puro DG (2006): Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. Am J Physiol Heart Circ Physiol **290**: H925–H934.

Yang W, Fu Y, Dong Y, Lin L, Huang X, Li Y, Lin X & Gao Q (2016): Retinal vessel oxygen saturation in a healthy young Chinese population. Acta Ophthalmol **94**: 373–379.

Yeh WL, Lu DY, Lin CJ, Liou HC & Fu WM (2007): Inhibition of hypoxia-induced increase of blood-brain barrier permeability by YC-1 through the antagonism of HIF-1alpha accumulation and VEGF expression. Mol Pharmacol **72**: 440–449.

Yip W, Siantar R, Perera SA, Milastuti N, Ho KK, Tan B, Wong TY & Cheung CY (2014): Reliability and determinants of retinal vessel oximetry measurements in healthy eyes. Invest Ophthalmol Vis Sci **55**: 7104–7110.

Yoneya S, Saito T, Nishiyama Y, Deguchi T, Takasu M, Gil T & Horn E (2002): Retinal oxygen saturation levels in patients with central retinal vein occlusion. Ophthalmology **109**: 1521–1526.

Yu DY, Su EN, Cringle SJ & Yu PK (2003): Isolated preparations of ocular vasculature and their applications in ophthalmic research. Prog Retin Eye Res **22**: 135–169.

Zheng W, Reem RE, Omarova S, Huang S, DiPatre PL, Charvet CD, Curcio CA & Pikuleva IA (2012): Spatial distribution of the pathways of cholesterol homeostasis in human retina. PLoS ONE **7**: e37926.

Zhou H, Saidel GM & Cabrera ME (2007): Multi-organ system model of O2 and CO2 transport during isocapnic and poikilocapnic hypoxia. Physiol Neurobiol **156**: 320–330.

Zijlstra WG, Buursma A & Van Assendelft OW (2000): Visible and near infrared absorption spectra of human and animal haemoglobin – determination and application. Utrecht: VSP.

Zong Y, Lin L, Yi C et al. (2016): Retinal vessel oxygen saturation and vessel diameter in retinitis pigmentosa at various ages. Graefes Arch Clin Exp Ophthalmol **254**: 243–252.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE, 2017
https://doi.org/10.1080/14767058.2017.1378337

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Check for updates

Effect of caffeine on superior mesenteric artery blood flow velocities in preterm neonates

Mohamed A. Abdel Wahed[a], Hanan M. Issa[b], Soha M. Khafagy[a] and Shaimaa M. Abdel Raouf[a]

[a]Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [b]Department of Radiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Objective: To investigate the effect of caffeine infusion on superior mesenteric artery (SMA) blood flow velocities (BFV) in preterm infants.

Methods: Prospective observational study on 38 preterm neonates 28–33^{+6} weeks gestation, who developed apnea on their first day of life, and caffeine citrate infusion was initiated at a loading dose of 20 mg/kg, followed by a maintenance dose of 5–10 mg/kg/day. Duplex ultrasound measurements of SMA BFV were recorded: peak systolic velocity (PSV), end diastolic velocity (EDV) and resistive index (RI), at 15 min before, 1-, 2- and 6-h after caffeine loading dose, and 2 h after two maintenance doses.

Results: There was a significant reduction in PSV 1-h ($p = .008$), a significant decrease in EDV 1- and 2-h ($p = .000$ and $p = .005$, respectively) and a significant increase in RI 1- and 2-h ($p = .003$ and $p = .005$, respectively) following caffeine loading dose, as compared to values before caffeine infusion. No significant effect of caffeine maintenance doses on SMA BFV was observed ($p > .05$).

Conclusion: Blood flow in SMA is significantly reduced after caffeine citrate infusion at a loading dose of 20 mg/kg. This effect continues for at least 2 h. Meanwhile, SMA BFV seems not affected by maintenance doses.

ARTICLE HISTORY
Received 2 August 2017
Revised 29 August 2017
Accepted 7 September 2017

KEYWORDS
Apnea of prematurity; duplex ultrasound; intestinal blood flow; necrotizing enterocolitis; respiratory center

Introduction

Caffeine is one of the most commonly prescribed drugs in the Neonatal Intensive Care Unit (NICU), and it has been described as a "silver bullet" in neonatology [1]. Its effect has been well established in the treatment and prevention of apnea of prematurity [2]. Caffeine reduces the frequency of apnea, intermittent hypoxemia, and extubation failure in mechanically ventilated preterm infants [3]. Evidence had also been suggested for additional short-term benefits on decreasing the incidence of bronchopulmonary dysplasia and patent ductus arteriosus (PDA) [4].

When the Food and Drug Administration approved caffeine citrate for the treatment of apnea of prematurity, a warning was included on the label about the possible association between the use of methyl xanthines and the development of necrotizing enterocolitis (NEC) [5]. Although the precise etiology of NEC is unknown, it is generally accepted that the most important etiologic factor in its development is intestinal ischemia or hypoperfusion leading to altered mucosal integrity [6,7].

Few studies had evaluated the effects of caffeine loading dose infusion on intestinal blood flow in preterm infants, but to the best of our knowledge, no one had assessed the effect of caffeine maintenance doses on the intestinal blood flow and whether there is a cumulative effect for caffeine on the splanchnic blood flow velocities (BFV). In this study, we aimed at investigating the effect of intravenous infusion of caffeine citrate at a loading dose of 20 mg/kg followed by a maintenance dose of 5–10 mg/kg on the superior mesenteric artery (SMA) BFV in preterm infants.

Subjects and methods

This was a prospective study, carried out at the NICU of Obstetrics & Gynecology Hospital, Ain Shams University over a period of 10 months from September 2012 to June 2013. The local ethical committee of Pediatric Department approved the study and an informed consent was obtained from one of the parents.

CONTACT Soha M. Khafagy soha_khafagy@yahoo.com Department of Pediatrics, Faculty of Medicine, Ain Shams University, Abbassayia, Cairo, Egypt

© 2017 Informa UK Limited, trading as Taylor & Francis Group

Subjects

This study was performed on 38 preterm neonates with gestational ages between 28 and 33^{+6} weeks, who developed apnea on their first day of life. Caffeine citrate therapy was initiated for each neonate by an intravenous infusion of a bolus and maintenance doses of caffeine, and was continued until the babies completed 34 weeks of postmenstrual age, and did not suffer apnea attacks for at least 1 week.

Preterm neonates were excluded from the study if they had history of perinatal asphyxia, congenital anomalies, early onset sepsis (diagnosed based on Rodwell hematologic sepsis score [8]), suspected or proven NEC, or hemodynamically significant PDA (PDA diameter >1.4 mm, left atrium to aorta ratio >1.4, and diastolic reverse flow in the aorta [9]). Also, preterm infants with abnormal umbilical artery Doppler and preterm infants with birth weights below third centiles were excluded.

Methods

Clinical data

A full history was taken for all neonates including maternal, obstetric, and perinatal history. Gestational age was calculated based on the date of last menstrual period and confirmed by neonatal examination using the modified Ballard score [10]. Birth weights, sex, Apgar score at 1 and 5 min were recorded. Complete physical examination was done including heart rate and blood pressure monitoring throughout the study.

Laboratory investigations done on admission included quantitative C-reactive protein (CRP) assay by latex agglutination test, and complete blood count using Max M. Counter (Coulter Corporation, Miami, FL).

A loading dose of 20 mg/kg caffeine citrate (equivalent to 10 mg/kg caffeine base) was given over 30 min by intravenous infusion (Caffeinospire 60 mg/ml, manufactured by Memphis for Pharmaceuticals & Chemical Industries, for Inspire Pharmaceutical Co., Cairo, Egypt) followed by maintenance doses of 5–10 mg/kg caffeine citrate (equivalent to 2.5–5 mg/kg of caffeine base) every 24 h [11].

Assessment of BFV measurements in the SMA using Duplex-pulsed Doppler ultrasound

A LOGIQ 400 PRO Series (General Electric, Boston, MA) Duplex-pulsed Doppler ultrasound machine with a 5 MHz short focus probe and high pass filter at 50 Hz was used for imaging the SMA at its origin from the abdominal aorta. When the clinician decided to start caffeine citrate, SMA peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured using Doppler ultrasound. The resistive index (RI) was calculated as the difference between PSV and EDV divided by the PSV: $RI = (PSV - EDV)/(PSV)$.

The first measurements were taken 15 min before caffeine administration. These measurements were repeated, at 1-, 2- and 6-h after caffeine loading dose administration, and were repeated 2 h after the caffeine maintenance dose administration, for 2 successive days. Each baby served as his or her own control. In all preterms, enteral feeding was started on the first day at 10 cc/kg divided every 3 h with a daily increment of 10 cc/kg. Feds were given after the caffeine citrate loading or maintenance doses, just after recording the Doppler SMA BFV measurements.

All infants were also observed for signs of NEC for at least 72 h after completion of SMA BFV measurements.

Statistical analysis

Sample size justification

The sample size was calculated using Epi Info version 7.0, setting the power at 0.8, and the two-sided confidence level at 95%. Data from a previous study [12] showed that caffeine administration was associated with an 18% reduction in the PSV of SMA. Calculation according to these values produced a sample size of 38. Therefore, 38 neonates were recruited in the current study.

Statistical analysis was done using SPSS software package, version 15.0, 2006, Eco Soft Corporation. Data was expressed descriptively as mean ± SD for quantitative parametric data, and number (percentage) for qualitative data. Comparison between measurements before and after caffeine administration was done using the paired *t*-test for parametric data and Wilcoxon's rank-sum test for skewed data. *p* value was considered significant if $< .05$.

Results

Thirty-eight patients were included in the study (Figure 1). The demographic and clinical characteristics and laboratory data of studied neonates are listed in Table 1.

None of the studied patients needed inotropes administration during the period of the study and none of them had a high umbilical catheter. Twenty-one of the patients were on nasal continuous positive

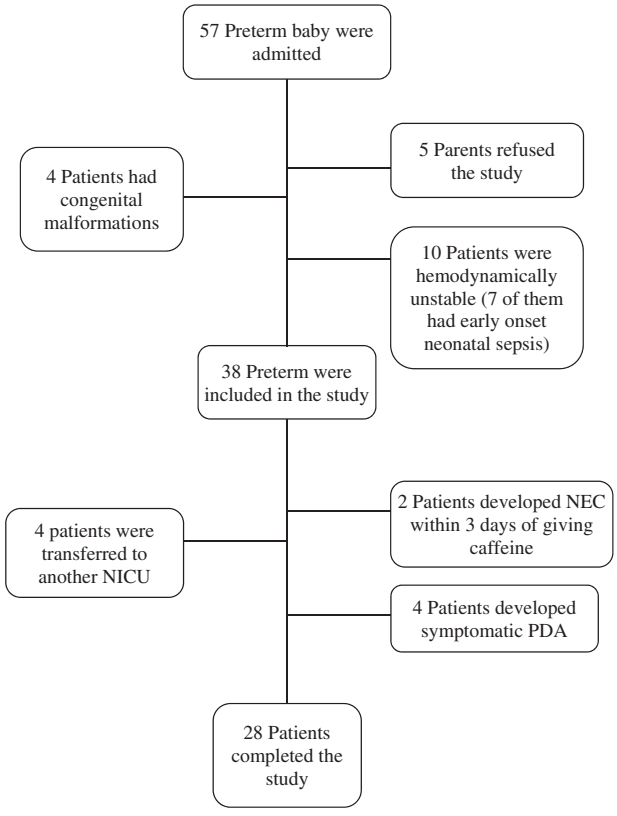


Figure 1. Algorithm of patients included in the study.

Table 1. Characteristics of the studied patients.

$N = 38$	Mean (SD)	Min	Max
GA (wks)	30.29 (2.25)	27	34
Wt. (kg)	1.37 (0.395)	0.6	2
Maintenance dose (mg/kg)	2.48 (0.825)	2.5	5
Duration of maintenance dose (days)	9.71 (7.47)	2	30
CBC TLC	16.17 (7.27)	4.8	42
Neutrophil	5.74 (3.26)	1.9	13.4
Platelet	218.10 (71.73)	42	381
HB	16.60 (2.33)	11.2	21.5
Apgar 1 min*	7 (6–7)	2	8
Apgar 5 min*	8 (7–9)	6	9
Sex#			
Male	16 (42.1%)		
Female	22 (57.9%)		
MOD#			
Vaginal	4 (10.5%)		
C.S	34 (89.5%)		
Wt for GA#			
AGA	31 (81.6%)		
LGA	3 (7.9%)		
SGA (3^{rd}–10^{th} centile)	4 (10.5%)		
CRP# (mg/dl)			
Positive (>6)	4 (10.5%)		
Negative (<six)	34 (89.5%)		
NEC within 3 d of loading dose#			
Yes	2 (5.3%)		
No	36 (94.75%)		

GA: gestational age; wks: weeks; Wt: weight; kg: kilogram; C.S: caesarean section; AGA: appropriate for gestational age; LGA: large for gestational age; SGA: small for gestational age; CBC: complete blood count; TLC: total leucocyte count; HB: haemoglobin; CRP: C-reactive protein; NEC: necrotizing enterocolitis.
*: Median (interquartile range); #: Number (percentage).

airway pressure, five patients were mechanically ventilated, while 12 patients did not need any respiratory support. Two of the studied patients (5.3%) developed NEC within 3 d of the loading dose of caffeine.

There was no statistically significant increase in values of mean blood pressure of studied patients at 1, 2 or 6 h after giving the loading dose of caffeine or after giving the maintenance doses. Also, no significant variations in the heart rates were found along the study. None of the studied patients received prophylactic indomethacin.

There was a statistically significant decrease in values of PSV of SMA 1-h ($p = .008$) after caffeine loading dose infusion as compared to values before the loading dose. There was also a statistically significant decrease in values of EDV of SMA 1-h ($p = .000$) and 2-h ($p = .005$) after caffeine loading dose as compared to values before loading dose infusion. Meanwhile, there was a statistically significant increase in calculated values of SMA RI after 1-h ($p = .003$) and 2-h ($p = .005$) of infusion of the caffeine-loading dose as compared to values before infusion. However, there were no statistically significant differences in the SMA BFV measurements 2 h after the maintenance doses of caffeine, recorded on 2 successive days as compared to the corresponding initial SMA BFV values before starting the caffeine citrate infusion therapy (Table 2).

Discussion

In this study, we used the usual standard dose for caffeine citrate; 20 mg/kg (10 mg/kg caffeine base) loading dose followed by 5–10 mg/kg per day (2.5–5 mg/kg caffeine base) as maintenance dose [13]. Our results demonstrated that an intravenous loading dose of 20 mg/kg of caffeine citrate given over a 30-minute period causes significant changes in SMA blood flow measurements reflecting a significant reduction in splanchnic perfusion. These changes in SMA BFV measurements persisted for 2 h after the caffeine loading dose infusion and returned to preinfusion levels after 6 h. This observed decrease in SMA blood flow might possibly lead to an ischemic injury to the gastrointestinal tract.

Caffeine is known to inhibit adenosine-induced vasodilatation [14], and it had been reported that endothelium dependent and independent vasoconstrictions occur in canine SMA following caffeine infusion [15]. Thus, the reduction of BFV following caffeine administration may result in increased incidence of NEC due to this vasoconstrictor effect.

Caffeine citrate administration in preterm infants at a loading dose of 25–50 mg/kg was reported to be associated with a reduction of mesenteric BFV [16–18]. However, when the high oral loading dose of

Table 2. Comparison between measurements of superior mesenteric artery blood flow velocity before caffeine loading dose and after caffeine administration.

	PSV		EDV		RI	
$N = 38$	Mean (SD)	p value	Mean (SD)	p value	Mean (SD)	p value
Before loading dose	42.46 (19.03)	–	13.12 (6.96)	–	0.70 (0.08)	–
1 h after loading dose	38.85 (16.15)	.008*	9.35 (3.12)	.000*	0.74 (0.08)	.003*
2 h after loading dose	41.71 (16.07)	.153	10.60 (3.91)	.005*	0.73 (0.08)	.005*
6 h after loading dose	42.56 (19.48)	.444	12.42 (5.94)	.316	0.70 (0.07)	.621
D2 maintenance ($n = 37$)	42.71 (14.68)	.055	11.58 (4.79)	.051	0.72 (0.08)	.399
D3 maintenance ($n = 30$)	43.68 (15.87)	.068	10.67 (3.32)	.015	0.74 (0.07)	.099
After DC of caffeine ($n = 28$)	54.13 (17.46)	.278	13.35 (4.85)	.407	0.74 (0.09)	.056

D2: day two of caffeine administration; D3: day three of caffeine administration; DC: discontinuation of caffeine; PSV: peak systolic velocity; EDV: end diastolic velocity; RI: resistive index; *significant.

25 mg/kg was divided into two equal doses of 12.5 mg/kg that were administered 4 h apart, no significant changes in BFV were reported in either the celiac or the superior mesenteric arteries [19]. These observations suggested that the effect of caffeine on intestinal BFV might be dose-dependent.

Caffeine pharmacokinetic data are limited in very low birth weight (VLBW) infants [11]. Clearance in infants born preterm is markedly lower and the volume of distribution is higher than at term-equivalent age and beyond [20]. It has a longer serum half-life of 101 h in neonates [20], whereas its half-life ranges from 3 to 6 h in adults [21]. This longer half-life did not result in a cumulative effect on the intestinal blood flow in our study as demonstrated by the non-significant effect of caffeine maintenance doses at 5–10 mg/kg/day on SMA BFV 2 h following its intravenous infusion.

Two of our patients developed NEC within 3 d of starting caffeine therapy. Our study was not powered to test the association of caffeine administration and development of NEC but several studies did. A study found that in preterm infants who received caffeine citrate, no significant difference in serum caffeine concentrations existed between patients who developed NEC and those who did not develop NEC [22]. A recent meta-analysis suggests that early caffeine use did not increase the risk of NEC, and at the same time has beneficial effects on neonatal outcomes [23], another randomized controlled trial (RCT) reported no increment in the rates of NEC in preterm infants after receiving a loading dose of caffeine citrate of 20 mg/kg per day followed by maintenance doses of 10 mg/kg per day [24].

In summary, this study demonstrated a significant decrease in SMA BFV after IV infusion of caffeine loading dose of 20 mg/kg caffeine citrate. This effect lasted for at least 2 h after receiving the loading dose and improved after 6 h. Maintenance doses of caffeine were not followed by significant changes in SMA BFV. So it seems better to withhold feeding for at least 2 h following administration of caffeine loading dose. Studies on a larger scale are needed to determine whether or not caffeine administration is associated with the development of NEC. These studies should concentrate on preterm neonates with smaller gestational ages (<28 weeks).

Acknowledgements

Thanks to all of the team in the Neonatal Intensive Care, Faculty of Medicine, Ain Shams University, who cared for our preterm infants till their discharge.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Aranda JV, Beharry K, Valencia GB, et al. Caffeine impact on neonatal morbidities. J Matern Fetal Neonatal Med. 2010;23(3):20–23.
[2] Larsen PB, Brendstrup L, Skov L, et al. Aminophylline versus caffeine citrate for apnea and bradycardia prophylaxis in premature neonates. Acta Paediatr. 1995;84(4):360–364.
[3] Steer PA, Flenady VJ, Shearman A, et al. Periextubation caffeine in preterm neonates: a randomized dose response trial. J Paediatr Child Health. 2003;39(7):511–515.
[4] Abu Jawdeh EG, O'Riordan M, Limrungsikul A, et al. Methylxanthine use for apnea of prematurity among an international cohort of neonatologists. J Neonatal Perinatal Med. 2013;6(3):251–256.
[5] New drug application: CAFCIT (NDA). p. 020793. Washington, DC: Food and Drug Administration; 2000.
[6] Noerr B. Current controversies in the understanding of necrotizing enterocolitis. Part 1. Adv Neonatal Care. 2003;3(3):107–120.
[7] Franco A, Ramji FG. Utility of abdominal sonography to diagnose necrotizing enterocolitis. Eur J Radiol Extra. 2008;65(1):13–16.

[8] Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988;112(5):761–767.
[9] Sehgal A, McNamara PJ. Does echocardiography facilitate determination of hemodynamic significance attributable to the ductus arteriosus? Eur J Pediatr. 2009;168(8):907–914.
[10] Ballard JL, Khoury JC, Wedig K, et al. New Ballard score, expanded to include extremely premature infants. J Pediatr. 1991;119(3):417–423.
[11] Leon AE, Michienzi K, Ma CX, et al. Serum caffeine concentrations in preterm neonates. Am J Perinatol. 2007;24(1):39–47.
[12] Soraisham AS, Elliott D, Amin H. Effect of single loading dose of intravenous caffeine infusion on superior mesenteric artery blood flow velocities in preterm infants. J Paediatr Child Health. 2008;44(3): 119–121.
[13] Dobson NR, Hunt CE. Pharmacology review: caffeine use in neonates: indications, pharmacokinetics, clinical effects, outcomes. NeoReviews. 2013;14(11): e540–e550.
[14] Smits P, Lenders JW, Thien T. Caffeine and theophylline attenuate adenosine-induced vasodilation in humans. Clin Pharmacol Ther. 1990;48(4):410–418.
[15] Jino H, Kurahashi K, Usui H, et al. Pharmacological natures of caffeine-induced endothelium- dependent and -independent contraction in canine mesenteric artery. Life Sci. 1995;57(12):1155–1162.
[16] Hassanein SM, Gad GI, Ismail RI, et al. Effect of caffeine on preterm infants' cerebral cortical activity: an observational study. J Matern Fetal Neonatal Med. 2015;28(17):2090–2095.
[17] Lane AJP, Coombs RC, Evans DH, et al. Effect of caffeine on neonatal splanchnic blood flow. Arch Dis Child Fetal Neonatal Ed. 1999;80(2):F128–F129.
[18] Hoecker C, Nelle M, Poeschl J, et al. Caffeine impairs cerebral and intestinal blood flow velocity in preterm infants. Pediatrics. 2002;109(5):784–787.
[19] Hoecker C, Nelle M, Beedgen B, et al. Effects of a divided high loading dose of caffeine on circulatory variables in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2006;91(1):F61–F64.
[20] Charles BG, Townsend SR, Steer PA, et al. Caffeine citrate treatment for extremely premature infants with apnea: population pharmacokinetics, absolute bioavailability, and implications for therapeutic drug monitoring. Ther Drug Monit. 2008;30(6):709–716.
[21] Carrillo JA, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clin Pharmacokinet. 2000;39(2):127–153.
[22] Lampkin SJ, Turner AM, Lakshminrusimha S, et al. Association between caffeine citrate exposure and necrotizing enterocolitis in preterm infants. Am J Health Syst Pharm. 2013;70(7):603–608.
[23] Park HW, Lim G, Chung SH, et al. Early caffeine use in very low birth weight infants and neonatal outcomes: a systematic review and meta-analysis. J Korean Med Sci. 2015;30(12):1828–1835.
[24] Mohammed S, Nour I, Shabaan AE, et al. High versus low-dose caffeine for apnea of prematurity: a randomized controlled trial. Eur J Pediatr. 2015;174(7):949–956.

Systematic Review and Meta-Analysis

Neonatology

Neonatology 2019;115:116–126
DOI: 10.1159/000492859

Received: July 24, 2018
Accepted after revision: August 12, 2018
Published online: November 8, 2018

Neonatal Glycaemia and Neurodevelopmental Outcomes: A Systematic Review and Meta-Analysis

Rajesh Shah[a] Jane Harding[a] Julie Brown[a] Christopher McKinlay[a–c]

[a]Liggins Institute, University of Auckland, Auckland, New Zealand; [b]Department of Paediatrics: Child and Youth Health, University of Auckland, Auckland, New Zealand; [c]Kidz First Neonatal Care, Counties Manukau Health, Auckland, New Zealand

Keywords
Hypoglycaemia · Infant · Newborn · Neurodevelopmental disorders · Child development

Abstract
Background: Hypoglycaemia is the most common metabolic problem in neonates but there is no universally accepted threshold for safe blood glucose concentrations due to uncertainty regarding effects on neurodevelopment. ***Objective:*** To systematically assess the association between neonatal hypoglycaemia on neurodevelopment outcomes in childhood and adolescence. ***Methods:*** We searched MEDLINE, EMBASE, CINAHL, and PsycINFO from inception until February 2018. We included studies that reported one or more prespecified outcomes and compared children exposed to neonatal hypoglycaemia with children not exposed. Studies of neonates with congenital malformations, inherited metabolic disorders and congenital hyperinsulinism were excluded. Two authors independently extracted data using a customized form. We used ROBINS-I to assess risk of bias, GRADE for quality of evidence, and REVMAN for meta-analysis (inverse variance, fixed effects). ***Results:*** 1,665 studies were screened, 61 reviewed in full, and 11 included (12 publications). In early childhood, exposure to neonatal hypoglycaemia was not associated with neurodevelopmental impairment (n = 1,657 infants; OR = 1.16, 95% CI = 0.86–1.57) but was associated with visual-motor impairment (n = 508; OR = 3.46, 95% CI = 1.13–10.57) and executive dysfunction (n = 463; OR = 2.50, 95% CI = 1.20–5.22). In mid-childhood, neonatal hypoglycaemia was associated with neurodevelopmental impairment (n = 54; OR = 3.62, 95% CI = 1.05–12.42) and low literacy (n = 1,395; OR = 2.04, 95% CI = 1.20–3.47) and numeracy (n = 1,395; OR = 2.04, 95% CI = 1.21–3.44). No data were available for adolescents. ***Conclusions:*** Neonatal hypoglycaemia may have important long-lasting adverse effects on neurodevelopment that may become apparent at later ages. Carefully designed randomized trials are required to determine the optimal management of neonates at risk of hypoglycaemia with long-term follow-up at least to school age.
© 2018 S. Karger AG, Basel

Introduction

Neonatal hypoglycaemia is the most common metabolic problem in newborn infants and a readily preventable cause of brain injury in infancy. However, clinical thresholds for diagnosis and treatment of neonatal hypoglycaemia are widely debated, with no universally accepted safe blood glucose concentration for newborns [1, 2]. This uncertainty is largely due to a lack of evidence regarding the effect of low neonatal glucose concentra-

KARGER
© 2018 S. Karger AG, Basel

E-Mail karger@karger.com
www.karger.com/neo

Christopher McKinlay
Liggins Institute, University of Auckland, Private Bag 92019
Victoria Street West
Auckland 1142 (New Zealand)
E-Mail c.mckinlay@auckland.ac.nz

tions on neurodevelopmental outcomes. Further, recent studies have suggested that higher glucose concentrations after hypoglycaemia may also contribute to brain injury [3], thus adding complexity to this common clinical problem.

Key risk factors for neonatal hypoglycaemia include being born preterm, large for gestational age or high birth weight, small for gestational age or low birth weight, and being an infant of a diabetic mother. Approximately 30% of all neonates are considered at risk, of whom approximately 50% develop hypoglycaemia [4]. The most common definition of neonatal hypoglycaemia is a blood glucose concentration <47 mg/dL (2.6 mmol/L), but lower and higher thresholds have been recommended. For example, the American Academy of Pediatrics advises that intravenous treatment is not needed until glucose concentrations are <25 mg/dL (1.4 mmol/L) within the first 4 h after birth, or <35 mg/dL (2.0 mmol/L) from 4 to 24 h [5]. However, the Pediatric Endocrine Society recommends that in babies at risk of hypoglycaemia, glucose concentrations should be maintained >50 mg/dL (2.8 mmol/L), or >60 mg/dL (3.3 mmol/L) if interventions beyond normal feeds are required [6]. This lack of consensus reflects the paucity of evidence about long-term outcomes after neonatal hypoglycaemia.

In 2006, Boluyt et al. [7] carried out a systematic review of the available studies on prognosis after neonatal hypoglycaemia. The review concluded that the extent of neurodevelopment impairment after neonatal hypoglycaemia in the first week of life was unclear, and thus the authors proposed an optimal study design to establish the relationship between neonatal hypoglycaemia and subsequent neurodevelopment. In 2008, the Eunice Kennedy Shriver National Institute of Child Health and Human Development Workshop on Neonatal Hypoglycaemia also identified major gaps in knowledge about neonatal hypoglycaemia and its clinical implications and prioritized it as a key area for research. Since then, although several review articles on the topic have appeared [8–10], no new systematic review has emerged.

This aim of this systematic review was to assess the association between neonatal hypoglycaemia on neurodevelopment outcomes at early childhood (2–5 years), mid-childhood (6–11 years), and adolescence (12–18 years).

Methods

This systematic review was conducted in accordance with the PRISMA statement, and was registered in PROSPERO (CRD42017073430, http://www.crd.york.ac.uk/PROSPERO/).

Search Strategy

We searched MEDLINE, EMBASE, CINAHL, and PsycINFO databases using the search terms infant, newborn, hypoglycaemia, neurodevelopmental disorders, neurological sequelae, neuroimaging, brain imaging, computed tomography scan, ultrasonography, and magnetic resonance imaging, including spelling variants (online suppl. material for full search strategy; see www.karger.com/doi/10.1159/000492859 for all online suppl. material). The search was restricted to studies involving humans and published in English. There was no limit on the year of publication. The search was last updated on February 12, 2018. We also hand-searched bibliographies of included studies, review papers and conference abstracts to identify additional items. One author conducted the search and initial title and abstract screening. Records identified for full-text screening were reviewed by two authors. Screening and eligibility assessments were performed using COVIDENCE (http://www.covidence.org/). Conflicts were resolved by consensus or after consultation with a third author.

Inclusion Criteria

We included all studies (trials, cohort, and case-control) that reported one or more of the primary or secondary outcomes and compared children or adolescents who were screened and found to be hypoglycaemic to those who were screened but were not hypoglycaemic. Studies were limited to neonates born at ≥32 weeks' gestation and who were screened for hypoglycaemia in the first week after birth. We excluded case series, conference abstracts, and studies that reported outcomes in neonates with congenital malformations, inherited metabolic disorders or congenital hyperinsulinism.

Primary outcomes were neurodevelopmental impairment, visual-motor impairment, and executive dysfunction, as defined by authors. Secondary outcomes were cognitive impairment (as defined by authors), mild cognitive impairment (developmental or intelligence quotient from 2 to 1 standard deviation below the mean), moderate-severe cognitive impairment (developmental/intelligence quotient more than 2 standard deviations below the mean), epilepsy (afebrile seizures or as defined by authors), highest educational level (adolescence), death, measures of general health and health care utilization, emotional-behavioural difficulty, abnormal brain imaging findings, visual impairment, hearing impairment, motor impairment, low literacy and low numeracy (mid-childhood and early adolescence), all as defined by authors.

Data Extraction and Analysis

Data for primary and secondary outcomes were extracted independently by two authors using a customized data form. Conflicts were resolved by consensus or following consultation with a third author.

We planned meta-analysis using the inverse variance, fixed effects method in REVMAN (version 5.3), with the inclusion of adjusted analyses where possible. If there were data for more than one age within an age band, then the most recent data were used. We assessed statistical heterogeneity using the I^2 statistic; values >30% were regarded as evidence of substantial heterogeneity. Forest plots are provided in the online supplementary material. We planned sensitivity analysis of the primary outcomes including only studies at low risk of bias and only those that used accurate methods for measuring glucose concentrations.

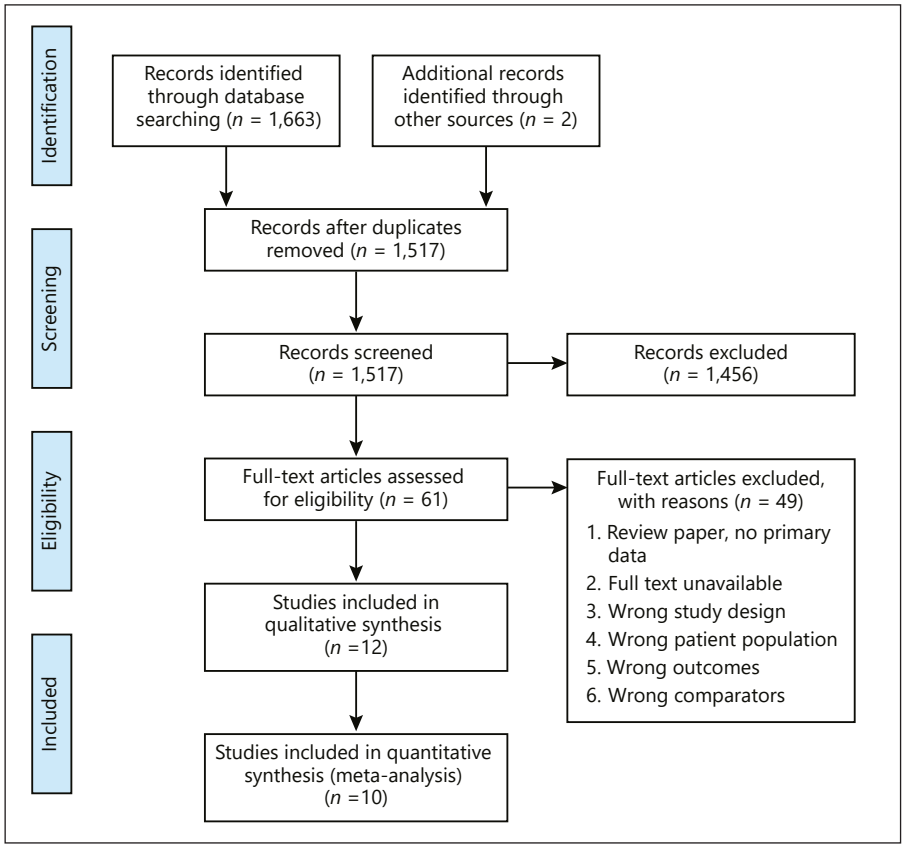


Fig. 1. Flow diagram of study identification and selection.

Quality of Evidence

We assessed the risk of bias for each study using a modified version of the ROBINS-I tool for non-randomized studies of interventions, as previously described [11]. This included assessment of the following domains for bias: recruitment and selection of participants, confounding, ascertainment of exposures, measurement of outcomes, missing data, and reporting of results. Two authors independently performed risk of bias assessments. Conflicts were resolved by consensus or by consultation with a third author.

We evaluated the overall quality of evidence for each research question using the GRADE approach [12]. Seven outcomes were selected for GRADE assessment: neurodevelopmental impairment, cognitive impairment, visual-motor impairment, low language/literacy, low numeracy, epilepsy, and executive dysfunction. Two authors independently assessed the quality of evidence. Conflicts were resolved by consensus or by consultation with a third author.

Results

Search Results

Of 1,665 records identified through databases and hand searching, 148 were duplicates and were removed. Of the remaining 1,517 studies, 1,456 were excluded following title and abstract screening, and a further 49 were excluded following full-text review (Fig. 1). One cohort study reported outcomes separately at 2 and 4.5 years of age [3, 13]. Thus, a total of 11 studies (12 publications), comprising 4,041 infants were included, of which 9 (10 publications) provided data suitable for meta-analysis in early and mid-childhood. No studies reported outcomes in adolescence.

Characteristics of the Selected Studies

All of the included studies were cohort studies; 3 were prospective [13–15], 6 were retrospective [16–21], and for 2 it was unclear whether all data were collected prospectively [22, 23] (Table 1). All studies were conducted in developed countries, including Europe, the USA, Canada, and New Zealand. Four studies were conducted in the 1970s [14, 15, 20, 22], 2 in the 1990s [17, 21], 1 in the 2000s [16] and 4 in the 2010s [13, 18, 19, 23]. In 10 studies the study population comprised infants at risk of hypoglycaemia; 1 study included all the infants born at the hospital. Only 4 studies (5 publications) each had uncertain or low risk of bias in one or more domains, and each adjusted results for potential confounding [3, 13, 18, 19,

Table 1. Characteristics of included studies

First author and date	Study type	Study population	Sample size	Definition of hypoglycaemia	Glucose screening protocol	Test method	Target range for treatment	Treatments	Age at follow-up	Neurodevelopmental tests	Included in meta-analysis	Adjustment for potential confounding
Griffiths [22], 1971	Cohort	Neonates admitted to special care unit	41 exposed, 41 unexposed	<1.11 mmol/L (<20 mg/dL)	Single blood glucose measurement within 24 h of admission; further blood glucose measurement only if hypoglycaemic or if symptomatic	Capillary whole blood; modified Watson method	Not specified	Not specified	4.2 years (mean)	Cognition: Stanford-Binet or Griffiths Behavior: Stott Systemic Interview Motor: Griffiths Locomotor Scale Vision: Stycar	Yes	No
Koivisto [20], 1972	Retrospective cohort	At-risk or symptomatic neonates screened for hypoglycaemia	151 exposed, 56 unexposed	<1.7 mmol/L (<30 mg/dL)	Blood glucose measured 3 times daily for 2–4 days in at-risk and symptomatic infants, and continued for 24 h after euglycaemic or discontinuation of treatment	Capillary whole blood; laboratory-modified Hultman or glucose oxidase method	Not specified	10–20% intravenous glucose; hydrocortisone	1–4 years	Cognitive, language and motor tests not specified Behavioural assessment not specified Ophthalmologic examination	Yes	No
Pildes [15], 1974	Prospective cohort	At-risk or symptomatic neonates screened for hypoglycaemia (mostly preterm or SGA)	39 exposed, 41 unexposed	<1.11 mmol/L (<20 mg/dL)	Daily blood glucose measurement for 1–4 days	Capillary whole blood; laboratory glucose oxidase method	Not specified	Oral dextrose; 10–15% intravenous dextrose; hydrocortisone or ACTH	1–7 years	Cognitive: Cattell Infant Scale, Stanford-Binet or Wechsler Intelligence Scale for Children Social: Vineland Social Maturity Scale Electroencephalogram	Yes	No
Haworth [14], 1976	Prospective cohort	Infants of diabetic mothers	25 exposed, 12 unexposed	≤1.11 mmol/L (≤20 mg/dL) in low birth weight babies (<2.5 kg) and ≤1.65 mmol/L (≤30 mg/dL) in normal weight babies	0.5, 1, 2, 3, 6,12, 24, 48 and 72 h, and more frequently if hypoglycaemic	Capillary whole blood; laboratory Huggett and Nixon method	Not specified	Intravenous glucose, long-acting adrenalin or both	4.5 years (mean)	Yale Developmental Schedule	Yes	No
Stenninger [21], 1998	Retrospective cohort	Infants of diabetic mothers	13 exposed 15 unexposed	<1.5 mmol/L (<27 mg/dL)	Glucose measurements for the first 24 h, frequency not specified	Capillary whole blood; laboratory glucose oxidase method	Not specified	Not specified	7–8 years	Cognitive: Griffiths' Developmental Scales Motor: Movement Assessment Battery for Children Behaviour: questionnaires (not specified) Validated neurological screening test for evaluation of minimal brain dysfunction Electroencephalogram	Yes	No
Duvanel [17], 1999	Retrospective cohort	Preterm (≤34 weeks) and SGA	62 exposed, 23 unexposed	<2.6 mmol/L (47 mg/dL)	Blood glucose measured approximately every 4–5 h for the first 24 h	Dextrostix for screening; if <2 mmol/L confirmed by laboratory venous sample using glucose oxidase or hexokinase method	Not specified	10% intravenous dextrose	6, 12, and 18 months, and 3.5 and 5 years	Cognitive: Griffith's Developmental Scales, McCarthy Scales of Aptitude	No	No
Brand [16], 2005	Retrospective cohort	Term and large for gestational age	60 exposed, 15 unexposed	<2.2 mmol/L (<40 mg/dL)	1, 3, and 5 h after birth and more frequently if hypoglycaemic	Capillary whole blood; laboratory glucose oxidase method	Not specified	Additional feeding or intravenous dextrose	4 years	Cognitive: Denver Developmental Scale, Snijders-Oomen non-verbal intelligence test Behaviour: Dutch version of the Child Behaviour Check List	Yes	No
Kerstjens [23], 2012	Cohort	Moderate preterm (32–35 weeks' gestation)	67 exposed, 765 unexposed	<1.7 mmol/L (<30 mg/dL)	Blood glucose measured several times during the first 24 h	Bedside glucometer; laboratory confirmation if <3.0 mmol/L (54 mg/dL) or <2.5 mmol/L (45 mg/dL), depending on site protocol	Not specified	Not specified	3.5–4 years	Ages and Stages Questionnaire	Yes	Yes

Neonatology 2019;115:116–126
DOI: 10.1159/000492859

Neonatology 2019;115:116–126
DOI: 10.1159/000492859

Table 1 (continued)

First author and date	Study type	Study population	Sample size	Definition of hypoglycaemia	Glucose screening protocol	Test method	Target range for treatment	Treatments	Age at follow-up	Neurodevelopmental tests	Included in meta-analysis	Adjustment for potential confounding
McKinlay 2015 [3]	Prospective cohort	Term and late-preterm neonates born at risk for hypoglycaemia	216 exposed, 188 unexposed	<2.6 mmol/L (<47 mg/dL)	1 h, then before feeds 3–4 h for first 24 h, then 6–8 h for next 24 h and until hypoglycaemia no longer a clinical concern. More frequent if hypoglycaemic or receiving intravenous dextrose	Capillary whole blood; laboratory glucose oxidase method; masked continuous interstitial glucose monitoring	≥2.6 mmol/L (47 mg/dL)	Additional feeding, buccal dextrose gel, intravenous dextrose	2 years	Cognitive: Bayley Scales of Infant Development III. Executive: a battery of four tasks, and Behavior Rating Inventory of Executive Function (Preschool version) Vision: visual screening using four assessment categories and random-dot kinematograms of varying coherence Hearing: audiologic screening	Yes	Yes
Kaiser [19], 2015	Retrospective cohort	All neonates	89 exposed, 1,306 unexposed	<1.94 mmol/L (<35 mg/dL)	Universal newborn glucose screening at 1–3 h after birth; repeated after 1 h if hypoglycaemic	Laboratory glucose oxidase	Not specified	Intravenous dextrose or additional feeding	10 years	Fourth Grade Benchmark Examination (literacy and mathematics)	Yes	Yes
Goode [18], 2016	Retrospective cohort	Preterm and low birth weight	461 exposed/ 282 unexposed	<2.49 mmol/L (<45 mg/dL)	Not specified	Dextrostix or plasma glucose, method not specified	Not specified	Not specified	5, 8, and 18 years	Cognitive: Stanford-Binet, Peabody Picture Vocabulary Test, Wechsler Intelligence Scale for Children, Wechsler Abbreviated Scale of Intelligence. Academic: Woodcock-Johnson Tests of Achievement Behaviour: Child Behaviour Checklist, Youth Report Behaviour Surveillance System	No	Yes
McKinlay [13], 2017	Prospective cohort	Term and moderate to late preterm infants born at risk of hypoglycaemia	280 exposed, 197 unexposed	<2.6 mmol/L (<47 mg/dL)	1 h, then before feeds 3–4 h for first 24 h, then 6–8 h for next 24 h and until hypoglycaemia no longer a clinical concern. More frequent if hypoglycaemic or receiving intravenous dextrose	Capillary whole blood; laboratory glucose oxidase method; masked continuous interstitial glucose monitoring	≥2.6 mmol/L (47 mg/dL)	Additional feeding, buccal dextrose gel, intravenous dextrose	4.5 years	Cognitive: Wechsler Preschool and Primary Scale of Intelligence (version 3) Executive: a battery of five graded tasks, Behaviour Rating Inventory of Executive Function Motor: Movement Assessment Battery for Children (version 2) and Beery Buktenica Developmental Test of Visual Motor Integration (version 6) (BBV-MI-6) Vision: visual screening using six assessment categories, visual processing subscale of BBV-MI-6, random dot kinetograms of varying coherence Auditory processing: auditory subscale of the Phelps Kindergarten Readiness Scale Emotional and behaviour: Strengths and Difficulties Questionnaire, Child Behaviour Checklist	Yes	Yes

SGA, small for gestational age; ACTH, adrenocorticotropin.

Table 2. Risk of bias assessment

Author and date	Domain					
	selection of comparison groups	confounding	ascertainment of exposures	measurement of outcomes	missing data	reporting of results
Griffiths [22], 1971	uncertain	uncertain	high	low	uncertain	low
Koivisto [20], 1972	uncertain	uncertain	high	uncertain	low	uncertain
Pildes [15], 1974	low	high	uncertain	low	high	uncertain
Haworth [14], 1976	uncertain	high	low	low	uncertain	low
Stenninger [21], 1998	uncertain	high	low	low	high	low
Duvanel [17], 1999	uncertain	uncertain	uncertain	uncertain	uncertain	uncertain
Brand [16], 2005	low	uncertain	low	low	high	uncertain
Kerstjens [23], 2012	low	low	uncertain	low	uncertain	low
McKinlay [3], 2015	low	low	low	low	uncertain	uncertain
Kaiser [19], 2015	low	low	low	low	uncertain	low
Goode [18], 2016	low	low	uncertain	low	uncertain	low
McKinlay [13], 2017	low	low	low	low	uncertain	uncertain

Assessed using a modified version of the ROBINS-I tool for non-randomized studies of interventions [11].

23]. No study was at low risk of bias across all domains. Seven studies were small with fewer than 100 participants and had very imprecise estimates of exposure effect.

Early Childhood (2–5 Years)

Primary Outcomes

The risk of neurodevelopmental impairment in early childhood did not differ between those who were and were not exposed to neonatal hypoglycaemia (6 studies, 1,657 infants; 25.8 vs. 16.6%; OR = 1.16, 95% CI = 0.86–1.57; $p = 0.34$; $I^2 = 16\%$) [3, 13–16, 20, 23]. Four out of the 6 studies contributing data to this meta-analysis were at high risk of bias in one or more domains (Table 2). In 2 studies, exposure to neonatal hypoglycaemia was associated with increased risk of visual-motor impairment (508 infants; 4.6 vs. 1.5%; OR = 3.46, 95% CI = 1.13–10.57; $p = 0.03$; $I^2 = 0\%$) [13, 14]. One of these studies was at high risk of bias for confounding but contributed few data to the meta-analysis [14]. In 1 study, there was an association between neonatal hypoglycaemia and executive dysfunction (463 infants; 10.6 vs. 4.7%; OR = 2.50, 95% CI = 1.20–5.22; $p = 0.01$). This study had a low to uncertain risk of bias [13]. There were insufficient data to undertake the planned sensitivity analyses.

Secondary Outcomes

In early childhood, those exposed to neonatal hypoglycaemia compared with those not so exposed had similar rates of any cognitive impairment (3 studies, 746 infants, 15.4 vs. 15.9%; OR = 1.11, 95% CI = 0.73–1.69; $p = 0.63$; $I^2 = 28\%$), mild cognitive impairment (3 studies, 746 infants, 12.8 vs. 13.7%; OR = 0.86, 95% CI = 0.55–1.35; $p = 0.52$; $I^2 = 61\%$) and moderate-severe cognitive impairment (3 studies, 746 infants, 2.6 vs. 2.1%; OR = 1.57, 95% CI = 0.55–4.48; $p = 0.40$, $I^2 = 34\%$) [13, 20, 22]. Two of these 3 studies were at high risk of bias in one or more domains. The risk of epilepsy in early childhood did not differ between those exposed and not exposed to neonatal hypoglycaemia (4 studies, 772 infants, 4.2 vs. 2.1%; OR = 1.93, 95% CI = 0.76–4.85; $p = 0.16$, $I^2 = 0\%$) [13, 14, 20, 22]. Three of these 4 studies were at high risk of bias in one or more domains. The risk of emotional-behavioural difficulty did not differ between those exposed and not exposed to neonatal hypoglycaemia (3 studies, 587 infants, 18.9 vs. 19.0%; OR = 1.00, 95% CI = 0.66–1.53; $p = 0.98$, $I^2 = 0\%$) [13, 14, 22]. One of these studies was at low or uncertain risk of bias while 2 were at high risk of bias in one or more domains. The risk of visual impairment in early childhood did not differ between those exposed or not exposed to neonatal hypoglycaemia (2 studies, 616 infants, 5.0 vs. 1.7%; OR = 2.14, 95% CI = 0.70–6.53; $p = 0.18$, $I^2 = 0\%$) [13, 20]. One of these studies was at high risk of bias in one or more domains and contributed the most data to the meta-analysis [20]. In 1 study, the rate of hearing impairment in early childhood did not differ between those exposed or not exposed to neonatal hypoglycaemia (477 infants, 0 vs. 0.5%; OR = 0.23, 95% CI = 0.01–5.76; $p = 0.37$) [13]. This study had a low to uncertain risk

of bias. The risk of motor impairment in early childhood did not differ between those who were and were not exposed to neonatal hypoglycaemia (4 studies, 777 infants, 17.5 vs. 17.8%; OR = 1.06, 95% CI = 0.70–1.60; $p = 0.79$, $I^2 = 6\%$) [13, 14, 20, 22]. Three out of 4 of these studies were at high risk of bias in one or more domains. One study reported higher rates of low language/literacy in those exposed to neonatal hypoglycaemia compared with those not so exposed but results were imprecise and not statistically significant (37 infants, 16 vs. 0%; OR = 5.23, 95% CI = 0.26–105.50; $p = 0.28$) [14]. This study had an uncertain to high risk of bias. One study reported on rates of cerebral palsy and found no difference between those exposed and not exposed to neonatal hypoglycaemia (401 infants, 0.9 vs. 1.1%; OR = 0.81, 95% CI = 0.11–6.07; $p = 0.84$) [3]. This study was at a low to uncertain risk of bias. None of the included studies reported on abnormal brain imaging, highest education level, death or measures of general health and health care utilization in early childhood.

Quality of Evidence

For the primary outcomes in early childhood, the quality of evidence was either low or very low (Table 3). For the selected secondary outcomes of any cognitive impairment, epilepsy, and low language/literacy, the quality of evidence was also very low (Table 3).

Mid-Childhood (6–11 Years)

Primary Outcomes

In 2 small studies, those exposed to neonatal hypoglycaemia compared with those not so exposed had a higher risk of neurodevelopmental impairment (54 infants, 47.8 vs. 22.6%; OR = 3.62, 95% CI = 1.05–12.42; $p = 0.04$, $I^2 = 0\%$) [15, 21]. Both of these studies were at an uncertain to high risk of bias in one or more domains. None of the included studies reported on visual-motor impairment or executive dysfunction in mid-childhood. There were insufficient data to undertake the planned sensitivity analyses.

Secondary Outcomes

In 1 study, the risk of emotional-behavioural difficulty in mid-childhood was non-significantly increased in those exposed to neonatal hypoglycaemia than those not so exposed (28 infants, 30.8 vs. 6.7%; OR = 6.22, 95% CI = 0.60–64.97; $p = 0.13$) but rates of motor impairment were similar (28 infants, 15.4 vs. 13.3%; OR = 1.18, 95% CI = 0.14–9.83; $p = 0.88$) [21]. This study had an uncertain to high risk of bias in one or more domains. In another study, those exposed to neonatal hypoglycaemia compared with those not so exposed had an increased risk of low language/literacy (1,395 infants, 67.4 vs. 43.0%; OR = 2.04, 95% CI = 1.20–3.47; $p = 0.008$) [19] and low numeracy (1,395 infants, 53.9 vs. 34.0%; OR = 2.04, 95% CI = 1.21–3.44; $p = 0.007$) in mid-childhood [19]. This study had a low to uncertain risk of bias.

None of the included studies reported on any cognitive impairment, mild cognitive impairment, moderate-severe cognitive impairment, epilepsy, abnormal brain imaging, visual impairment, hearing impairment, highest educational level, death, and measures of general health and health care utilization in mid-childhood.

Quality of Evidence

For the primary outcome of neurodevelopmental impairment in mid-childhood the quality of the evidence was very low (Table 3). For the selected secondary outcomes of low language/literacy and low numeracy, the quality of the evidence was low (Table 3).

Adolescence (12–18 Years)

None of the included studies reported on primary or secondary outcomes in adolescence.

Discussion

Neonatal hypoglycaemia is the most common metabolic condition in newborn infants [4] and has been associated with widespread changes in the developing brain [24], yet the impact of neonatal hypoglycaemia on long-term neurodevelopment is widely debated [25]. We undertook this systematic review to determine the relationship between neonatal hypoglycaemia and neurodevelopment throughout childhood. We found low-quality evidence that in early childhood (2–5 years) neonatal hypoglycaemia is associated with specific cognitive deficits, including a two- to threefold increased risk of visual-motor impairment and executive dysfunction. In later childhood (6–11 years), we found low-quality evidence that neonatal hypoglycaemia is associated with a twofold increased risk of literacy and numeracy problems, and very low-quality evidence of an increased risk of general cognitive impairment. No data were available on outcomes in adolescence.

Visual-motor integration is the coordination of visual perception, the ability to extract and organize visual information from the environment, and motor skills, especially fine motor ones [26]. It allows the use of eyes and

Table 3. GRADE summary of quality of evidence for effect of neonatal hypoglycaemia on neurodevelopmental outcomes

Outcome	Exposure effect OR (95% CI)	Participants (studies)	Certainty/ quality of evidence	Comments
Early childhood (2–5 years)				
Neurodevelopmental impairment	1.16 (0.86–1.57)	1,657 (6)	Very low	Initial level low. Downgraded as 4 studies were at high risk of bias in several domains, and only 2 studies adjusted for confounding
Visual-motor impairment	3.46 (1.13–10.57)	508 (2)	Low	Initial level low. Downgraded as results were imprecise. Upgraded 1 level due to large treatment effect
Executive dysfunction	2.50 (1.20–5.22)	463 (1)	Low	Initial level low. Downgraded as there was only a single study. Upgraded 1 level due to large treatment effect
Any cognitive impairment	1.11 (0.73–1.69)	746 (3)	Very low	Initial level low. Downgraded as two studies were at high risk of bias, and only one study adjusted for confounding
Epilepsy	1.93 (0.76–4.85)	772 (4)	Very low	Initial level low. Downgraded as 2 studies were at high risk of bias, results were imprecise, and only 1 study adjusted for confounding
Low language/literacy	5.23 (0.26–105.50)	37 (1)	Very low	Initial level low. Large treatment effect but downgraded as there was only 1 study at high risk of bias with imprecise results
Mid-childhood (6–11 years)				
Neurodevelopmental impairment	3.62 (1.05–12.42)	54 (2)	Very low	Initial level low. Large treatment effect but downgraded as both studies were at high risk of bias with imprecise results
Visual-motor impairment	–	–	–	No data
Executive dysfunction	–	–	–	No data
Any cognitive impairment	–	–	–	No data
Epilepsy	–	–	–	No data
Low language/literacy	2.04 (1.20–3.47)	1,395 (1)	Low	Initial level low. Downgraded as there was only a single study. Upgraded 1 level due to large treatment effect
Low numeracy	2.04 (1.21–3.44)	1,395 (1)	Low	Initial level low. Downgraded as there was only a single study. Upgraded 1 level due to large treatment effect

Evaluated using the GRADE approach [12].

hands in a coordinated and efficient way, enabling, for example, one to perceive and copy shapes, letters, and numbers. Thus, visual-motor integration is important for learning and academic achievement including reading, writing, and mathematics [27, 28].

The development of visual and motor systems is closely related [29], and coordination of visual-motor function is thought to occur within the ventral and dorsal cortical visual streams. The ventral stream supports form processing and object recognition, and includes the occipital primary visual cortex and the inferior temporal lobe. The dorsal stream is responsible for motion perception and visually guided motor function and includes the occipital primary visual cortex, middle temporal lobe, and poste-

rior parietal lobe. In the neonatal period, these cortical areas appear to be particularly susceptible to injury from neuroglycopenia, possibly because of higher metabolic activity [9, 30–32]. This provides a possible pathophysiological basis for the association between neonatal hypoglycaemia and impaired visual-motor integration in early childhood.

Executive function is the collective capacity for problem-solving, planning, attention control, and goal-directed behaviour [33]. Children with impaired executive control have difficulty remembering and carrying out instructions, staying focused, and planning and monitoring progress with a specific task, which can affect not only daily activities but also learning. The prefrontal cortex is responsible for the proper development of executive function, and increased activation of this region is associated with better performance on executive function tasks, as well as academic outcomes [34, 35]. The development of the prefrontal cortex and executive capacity is continuous from childhood through adolescence and into early adulthood [36, 37], and any abnormality in this region can result in executive function difficulties. Although neonatal hypoglycaemia has traditionally been associated with posterior brain injury, recent studies have suggested that its effects on the brain may be more widespread and include the frontal cortex [24, 38], potentially interfering with the normal development of executive capacity.

Demands on visual-motor and executive function increase with age, but we could not determine whether the changes seen in early childhood after neonatal hypoglycaemia persist or worsen over time due to the lack of longer-term outcome data. However, the finding of a twofold increased risk of literacy and numeracy problems in mid-childhood suggests a trajectory of worsening function in skills that are important for learning [39, 40]. The fact that neonatal hypoglycaemia was associated with general cognitive impairment in mid-childhood but not in early childhood supports this hypothesis. Importantly, this systematic review shows that tests of general development in infancy are unlikely to adequately assess the effects of neonatal hypoglycaemia on brain development. Thus, intervention studies will require longer-term end points, at least into mid-childhood, including specific tests of visual-motor and executive function.

It is more than a decade since Boluyt et al. [7] conducted the first systematic review of neurodevelopmental outcomes after neonatal hypoglycaemia. They concluded that there were insufficient data to quantify the effect of neonatal hypoglycaemia on neurodevelopment and provided recommendations about an optimal study design. Our systematic review identified 3 subsequent studies, but only 1 that followed these recommendations [3, 13], including prospective cohort design, nested randomized trial of treatment, gold standard glucose measurements, standardized neurodevelopmental assessment and sufficient sample size [7]. This is somewhat surprising given the recognition of neonatal hypoglycaemia as a priority research area and calls from the National Institute of Child Health and Human Development for further high-quality studies [1].

There are several differences between our systematic review and that of Boluyt et al. [7]. We excluded case series because without contemporaneous controls it is not possible to account for confounding, especially relating to the reasons that babies were considered at risk of hypoglycaemia and socio-economic factors. We also excluded studies that assessed outcomes at less than 2 years of age, due to the limited predictive value of very early developmental assessment [41], and studies that primarily included infants with congenital hyperinsulinism. We assessed not only the methodological quality of individual studies, but also the overall strength of the evidence for key outcomes using the GRADE approach.

Even with optimal study design, several challenges remain in determining the effect of neonatal hypoglycaemia on later neurodevelopment. As with any cohort study, the possibility of residual confounding cannot be excluded. Although neuroglycopenia can cause irreversible brain injury, other mechanisms may underlie associations between episodes of hypoglycaemia and neurodevelopmental impairment. For example, genetic polymorphisms of ATP-dependent potassium channels could affect both pancreatic β-cells and neuronal function [42].

In addition, the relationship between the severity, frequency, and duration of neonatal hypoglycaemic episodes and cerebral energy supply and utilization remains unclear [43], and thus the best measure of exposure for use in analyses is uncertain [25]. This is complicated by different approaches to screening, diagnosis, and treatment of hypoglycaemia, making characterization of the degree of exposure challenging. Further, masked continuous interstitial glucose monitoring has shown that the burden of hypoglycaemia in the early newborn period may be substantially greater than is detected by serial glucose measurements, even with frequent screening [3]. These undetected and thus untreated episodes may have an important influence on long-term outcomes [13]. However, there are few data on the effect of different approaches to treatment on glucose concentrations after hypoglycaemia [44].

Limitations

A key limitation of this systematic review is that only a limited number of studies were identified that met the inclusion criteria, leading to imprecise estimates of effect, and that data were not available for all prespecified outcomes at each epoch. There are several possible reasons for this including the difficulty of recruiting large cohorts around the time of birth, and the cost and complexity of long-term neurodevelopmental follow-up throughout childhood. Of note, only 3 of the included studies contributed data beyond 5 years of age [18, 21, 23]. Another limitation is the lack of adjustment for potential confounding factors, with only half of the included studies attempting to control for this potential source of bias. Finally, the description of hypoglycaemic management and treatment targets was generally poor. This may be important, as there is emerging evidence both in animals and humans that glucose reperfusion injury may exacerbate oxidative stress associated with hypoglycaemia if the correction is too rapid or too high, even within the normal glucose range [3, 45, 46].

Recommendations for Research

Studies are needed to determine the efficacy and cost-effectiveness of different strategies for improving long-term outcomes in neonates born at risk of hypoglycaemia. Future studies should involve large prospective cohorts with nested randomized trials of different approaches to treatment, or large randomized trials of different approaches to prevention or screening and diagnosis of hypoglycaemia in neonates considered at risk. All studies require the use of gold standard glucose assay methods [25, 47] and long-term follow-up at least to school age, with attention to visual-motor and executive function, and educational achievement. Consideration should be given to the use of masked continuous glucose monitoring to aid in the interpretation of study results, although retrospective point-to-point recalibration against all laboratory blood glucose values is important for accurate interstitial measurements in babies [48].

Conclusion

This systematic review found that neonatal hypoglycaemia is associated with a two- to threefold increased risk of specific cognitive deficits in early childhood (2–5 years), including visual-motor impairment and executive dysfunction, and general cognitive impairment and literacy and numeracy problems in later childhood (6–11 years). Although the overall quality of evidence was low to very low, this review nevertheless suggests that neonatal hypoglycaemia may have important long-lasting adverse effects on neurodevelopment. Carefully designed intervention trials are needed to determine the optimal management of neonates at risk of hypoglycaemia to improve long-term outcomes.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Hay WW Jr, Raju TN, Higgins RD, Kalhan SC, Devaskar SU. Knowledge gaps and research needs for understanding and treating neonatal hypoglycemia: workshop report from Eunice Kennedy Shriver National Institute of Child Health and Human Development. J Pediatr. 2009 Nov;155(5):612–7.

2 Rozance PJ, Hay WW. Hypoglycemia in newborn infants: features associated with adverse outcomes. Biol Neonate. 2006;90(2):74–86.

3 McKinlay CJ, Alsweiler JM, Ansell JM, Anstice NS, Chase JG, Gamble GD, et al.; CHYLD Study Group. Neonatal glycemia and neurodevelopmental outcomes at 2 years. N Engl J Med. 2015 Oct;373(16):1507–18.

4 Harris DL, Weston PJ, Harding JE. Incidence of neonatal hypoglycemia in babies identified as at risk. J Pediatr. 2012 Nov;161(5):787–91.

5 Adamkin DH; Committee on Fetus and Newborn. Postnatal glucose homeostasis in late-preterm and term infants. Pediatrics. 2011;127(3):575–9.

6 Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al.; Pediatric Endocrine Society. Recommendations from the pediatric endocrine society for evaluation and management of persistent hypoglycemia in neonates, infants, and children. J Pediatr. 2015 Aug;167(2):238–45.

7 Boluyt N, van Kempen A, Offringa M. Neurodevelopment after neonatal hypoglycemia: a systematic review and design of an optimal future study. Pediatrics. 2006 Jun;117(6):2231–43.

8 Adamkin DH. Neonatal hypoglycemia. Semin Fetal Neonatal Med. 2017 Feb;22(1):36–41.

9 Paudel N, Chakraborty A, Anstice N, Jacobs RJ, Hegarty JE, Harding JE, et al.; CHYLD Study Group. Neonatal hypoglycaemia and visual development: a review. Neonatology. 2017;112(1):47–52.

10 Rozance PJ. Update on neonatal hypoglycemia. Curr Opin Endocrinol Diabetes Obes. 2014 Feb;21(1):45–50.

11 Bradford BF, Thompson JM, Heazell AE, Mccowan LM, McKinlay CJ. Understanding the associations and significance of fetal movements in overweight or obese pregnant women: a systematic review. Acta Obstet Gynecol Scand. 2018 Jan;97(1):13–24.

12 Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401–6.

13 McKinlay CJ, Alsweiler JM, Anstice NS, Burakevych N, Chakraborty A, Chase JG, et al.; Children With Hypoglycemia and Their Later Development (CHYLD) Study Team. Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. JAMA Pediatr. 2017 Oct;171(10):972–83.
14 Haworth JC, McRae KN, Dilling LA. Prognosis of infants of diabetic mothers in relation to neonatal hypoglycaemia. Dev Med Child Neurol. 1976 Aug;18(4):471–9.
15 Pildes RS, Cornblath M, Warren I, Page-El E, Di Menza S, Merritt DM, et al. A prospective controlled study of neonatal hypoglycemia. Pediatrics. 1974 Jul;54(1):5–14.
16 Brand PL, Molenaar NL, Kaaijk C, Wierenga WS. Neurodevelopmental outcome of hypoglycaemia in healthy, large for gestational age, term newborns. Arch Dis Child. 2005 Jan; 90(1):78–81.
17 Duvanel CB, Fawer CL, Cotting J, Hohlfeld P, Matthieu JM. Long-term effects of neonatal hypoglycemia on brain growth and psychomotor development in small-for-gestational-age preterm infants. J Pediatr. 1999 Apr; 134(4):492–8.
18 Goode RH, Rettiganti M, Li J, Lyle RE, Whiteside-Mansell L, Barrett KW, et al. Developmental outcomes of preterm infants with neonatal hypoglycemia. Pediatrics. 2016 Dec; 138(6):55–55.
19 Kaiser JR, Bai S, Gibson N, Holland G, Lin TM, Swearingen CJ, et al. Association between transient newborn hypoglycemia and fourth-grade achievement test proficiency: a population-based study. JAMA Pediatr. 2015 Oct;169(10):913–21.
20 Koivisto M, Blanco-Sequeiros M, Krause U. Neonatal symptomatic and asymptomatic hypoglycaemia: a follow-up study of 151 children. Dev Med Child Neurol. 1972 Oct;14(5): 603–14.
21 Stenninger E, Flink R, Eriksson B, Sahlèn C. Long-term neurological dysfunction and neonatal hypoglycaemia after diabetic pregnancy. Arch Dis Child Fetal Neonatal Ed. 1998 Nov;79(3):F174–9.
22 Griffiths AD, Bryant GM. Assessment of effects of neonatal hypoglycaemia. A study of 41 cases with matched controls. Arch Dis Child. 1971 Dec;46(250):819–27.
23 Kerstjens JM, Bocca-Tjeertes IF, de Winter AF, Reijneveld SA, Bos AF. Neonatal morbidities and developmental delay in moderately preterm-born children. Pediatrics. 2012 Aug; 130(2):e265–72.
24 Burns CM, Rutherford MA, Boardman JP, Cowan FM. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. Pediatrics. 2008 Jul;122(1):65–74.
25 Harding JE, Harris DL, Hegarty JE, Alsweiler JM, McKinlay CJ. An emerging evidence base for the management of neonatal hypoglycaemia. Early Hum Dev. 2017 Jan;104:51–6.
26 Beery KE. The Beery-Buktenica developmental test of visual-motor integration: Beery VMI, with supplemental developmental tests of visual perception and motor coordination, and stepping stones age norms from birth to age six. MN: NCS Pearson Minneapolis; 2004.
27 Barnhardt C, Borsting E, Deland P, Pham N, Vu T. Relationship between visual-motor integration and spatial organization of written language and math. Optom Vis Sci. 2005 Feb; 82(2):138–43.
28 Taylor Kulp M. Relationship between visual motor integration skill and academic performance in kindergarten through third grade. Optom Vis Sci. 1999 Mar;76(3):159–63.
29 Thompson B, McKinlay CJ, Chakraborty A, Anstice NS, Jacobs RJ, Paudel N, et al.; CHYLD Study Team. Global motion perception is associated with motor function in 2-year-old children. Neurosci Lett. 2017 Sep; 658:177–81.
30 Aslan Y, Dinc H. MR findings of neonatal hypoglycemia. AJNR Am J Neuroradiol. 1997 May;18(5):994–6.
31 Barkovich AJ, Ali FA, Rowley HA, Bass N. Imaging patterns of neonatal hypoglycemia. AJNR Am J Neuroradiol. 1998 Mar;19(3): 523–8.
32 Spar JA, Lewine JD, Orrison WW Jr. Neonatal hypoglycemia: CT and MR findings. AJNR Am J Neuroradiol. 1994 Sep;15(8):1477–8.
33 Anderson P. Assessment and development of executive function (EF) during childhood. Child Neuropsychol. 2002 Jun;8(2):71–82.
34 Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, Gogtay N, et al. Intellectual ability and cortical development in children and adolescents. Nature. 2006 Mar;440(7084):676–9.
35 Tamnes CK, Østby Y, Walhovd KB, Westlye LT, Due-Tønnessen P, Fjell AM. Neuroanatomical correlates of executive functions in children and adolescents: a magnetic resonance imaging (MRI) study of cortical thickness. Neuropsychologia. 2010 Jul;48(9):2496–508.
36 Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C. Microstructural maturation of the human brain from childhood to adulthood. Neuroimage. 2008 Apr;40(3):1044–55.
37 Tamnes CK, Østby Y, Fjell AM, Westlye LT, Due-Tønnessen P, Walhovd KB. Brain maturation in adolescence and young adulthood: regional age-related changes in cortical thickness and white matter volume and microstructure. Cereb Cortex. 2010 Mar;20(3):534–48.
38 Cakmakci H, Usal C, Karabay N, Kovanlikaya A. Transient neonatal hypoglycemia: cranial US and MRI findings. Eur Radiol. 2001; 11(12):2585–8.
39 Bull R, Espy KA, Wiebe SA. Short-term memory, working memory, and executive functioning in preschoolers: longitudinal predictors of mathematical achievement at age 7 years. Dev Neuropsychol. 2008;33(3):205–28.
40 Sortor JM, Kulp MT. Are the results of the Beery-Buktenica Developmental Test of Visual-Motor Integration and its subtests related to achievement test scores? Optom Vis Sci. 2003 Nov;80(11):758–63.
41 Harris SR, Langkamp DL. Predictive value of the Bayley mental scale in the early detection of cognitive delays in high-risk infants. J Perinatol. 1994 Jul-Aug;14(4):275–9.
42 Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes. 2005 Sep;54(9): 2503–13.
43 Mujsce DJ, Christensen MA, Vannucci RC. Regional cerebral blood flow and glucose utilization during hypoglycemia in newborn dogs. Am J Physiol. 1989 Jun;256(6 Pt 2):H1659–66.
44 Harris DL, Gamble GD, Weston PJ, Harding JE. What happens to blood glucose concentrations after oral treatment for neonatal hypoglycemia? J Pediatr. 2017 Nov;190:136–41.
45 Ennis K, Dotterman H, Stein A, Rao R. Hyperglycemia accentuates and ketonemia attenuates hypoglycemia-induced neuronal injury in the developing rat brain. Pediatr Res. 2015 Jan;77(1-1):84–90.
46 Suh SW, Gum ET, Hamby AM, Chan PH, Swanson RA. Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. J Clin Invest. 2007 Apr;117(4):910–8.
47 Glasgow MJ, Harding JE, Edlin R; for the CHYLD Study Team. Cost analysis of cot-side screening methods for neonatal hypoglycaemia. Neonatology. 2018;114(2):155–62.
48 McKinlay CJ, Chase JG, Dickson J, Harris DL, Alsweiler JM, Harding JE. Continuous glucose monitoring in neonates: a review. Matern Health Neonatol Perinatol. 2017 Oct; 3(1):18.

Neurodevelopmental Outcomes in Infants With Birth Weight ≤500 g at 3 Years of Age

Hirosuke Inoue, MD, PhD,[a,b] Masayuki Ochiai, MD, PhD,[a,b] Yasunari Sakai, MD, PhD,[a,b] Kazuaki Yasuoka, MD,[a,b] Koichi Tanaka, MD,[a,b] Masako Ichiyama, MD,[a,b] Hiroaki Kurata, MD,[a,b] Junko Fujiyoshi, MD,[a,b] Yuki Matsushita, MD, PhD,[a,b] Satoshi Honjo, MD, PhD,[c] Kazuaki Nonaka, DDS, PhD,[b] Tomoaki Taguchi, MD, PhD, FACS,[b] Kiyoko Kato, MD, PhD,[b] Shouichi Ohga, MD, PhD,[a,b] on behalf of the Neonatal Research Network of Japan

abstract

OBJECTIVES: To determine neurodevelopmental outcomes at 3 years of age in children born with a birth weight (BW) of ≤500 g.

METHODS: Infants who were born with a BW of ≤500 g from 2003 to 2012 in the Neonatal Research Network of Japan and survived to discharge from the NICU were eligible in this study. The study population consisted of 460 children (56.7% of 811 surviving infants) who were evaluated at 36 to 42 months of age. Neurodevelopmental impairment (NDI) was defined as having cerebral palsy, visual impairment, hearing impairment, or a developmental quotient score of <70.

RESULTS: The overall proportion of NDI was 59.1% (95% confidence interval [CI]: 54.6%–63.5%). The trend revealed no significant change during the study period. In a multivariate modified Poisson regression analysis, NDI was associated with severe intraventricular hemorrhage (adjusted risk ratio [RR]: 1.42; 95% CI: 1.19–1.68; $P < .01$), cystic periventricular leukomalacia (adjusted RR: 1.40; 95% CI: 1.13–1.73; $P < .01$), severe necrotizing enterocolitis (adjusted RR: 1.31; 95% CI: 1.07–1.60; $P < .01$), surgical ligation for patent ductus arteriosus (adjusted RR: 1.29; 95% CI: 1.09–1.54; $P < .01$), and male sex (adjusted RR: 1.19; 95% CI: 1.01–2.40; $P = .04$).

CONCLUSIONS: This cohort showed that neurodevelopmental outcomes of infants with a BW of ≤500 g have not improved from 2003 to 2012. Multivariate analysis revealed that severe intracranial hemorrhage and cystic periventricular leukomalacia were the strongest risk factors for NDIs. Our data suggested that measures aimed at reducing neurologic morbidities will be important for improving outcomes of infants with a BW of ≤500 g.

WHAT'S KNOWN ON THIS SUBJECT: The survival of infants born with a birth weight (BW) of ≤500 g has improved considerably in Japan; however, few studies have revealed the neurodevelopmental outcomes of survivors born with a BW of ≤500 g.

WHAT THIS STUDY ADDS: Neurodevelopmental outcomes in early childhood among surviving infants born with a BW of ≤500 g did not improve in a recent decade.

[a]*Department of Pediatrics, Graduate School of Medical Sciences and* [b]*Comprehensive Maternity and Perinatal Care Center, Kyushu University, Fukuoka, Japan; and* [c]*Department of Pediatrics, National Hospital Organization Fukuoka National Hospital, Fukuoka, Japan*

Dr Inoue conceptualized and designed the study, contributed to the analysis and interpretation of the data, and drafted the initial manuscript; Drs Ochiai, Sakai, and Ohga conceptualized and designed the study, contributed to the data analysis, and critically reviewed and revised the manuscript; Drs Yasuoka, Tanaka, Ichiyama, Kurata, Fujiyoshi, and Matsushita made substantial contributions to the analysis and interpretation of the data and reviewed and revised the manuscript; Dr Honjo supervised and supported the statistical analyses for the completion of the study and reviewed and revised the manuscript; Drs Nonaka, Taguchi, and Kato supervised the study design, contributed to the interpretation of the data, and critically reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

DOI: https://doi.org/10.1542/peds.2017-4286

Accepted for publication Sep 13, 2018

To cite: Inoue H, Ochiai M, Sakai Y, et al. Neurodevelopmental Outcomes in Infants With Birth Weight ≤500 g at 3 Years of Age. *Pediatrics*. 2018;142(6):e20174286

Downloaded from www.aappublications.org/news by guest on November 16, 2018

Advances in perinatal and neonatal intensive care have improved the survival rate of periviable, or extremely preterm, infants near the limit of viability.[1–3] On the other hand, researchers in recent studies have reported decreasing,[4] unchanged,[5] or increasing rates[6,7] in neurodevelopmental disabilities among periviable infants who survive compared with previous decades. These variable data lead to a concern that increased survival may come at the cost of later neurodevelopmental disabilities among survivors.[8] Thus, providing accurate data on both mortality and long-term neurodevelopmental outcomes is important for families and professionals in neonatal care in making appropriate decisions for these high-risk infants.[9]

Gestational age (GA) and birth weight (BW), alone or combined, are the critical factors that affect the survival rate of extremely preterm and small infants.[9–11] Early ultrasound assessment provides an accurate estimation of GA in most cases, but it is also known to cause an error as high as ±15%.[12] On the other hand, BW is a direct measurement of the body size of offspring. We have recently shown favorable results of survival rates for infants with a BW of ≤500 g in Japan, whereas in-hospital morbidity rates have remained high in the last decade.[13] We extended this study because the long-term outcomes of the infants with a BW of ≤500 g were currently unavailable. In the current study, we investigate neurodevelopmental outcomes at 3 years of age for surviving children born with a BW of ≤500 g using the nationwide cohort database in Japan.

METHODS

Study Population and Data Collection

The Neonatal Research Network of Japan (NRNJ) database prospectively registered all of the clinical information of infants with a BW of ≤1500 g admitted to the 204 participating NICUs, accounting for 54.3% of 376 secondary and tertiary-level NICUs in Japan. The participants in this study were born between January 1, 2003, and December 31, 2012. Decisions regarding the pursuit of active treatment versus comfort care were made by neonatologists on the basis of fetal information, the status of the infants at birth, and their communications with the parents. In general, attending neonatologists attempt to save the lives of neonates at a GA of ≥23 weeks, and this principle has not changed during the study period. The perinatal data were collected as previously described.[13] Children who were born with a BW of ≤500 g and survived to discharge from the NICU were included in this study. We excluded the infants who had died in the operating or delivery room before admission to the NICU, those transferred to other hospitals, those born at a GA of <22 weeks, and those without available records of mortality during the NICU stay. Chorioamnionitis was diagnosed clinically. Antenatal use of corticosteroids was defined as the administration of corticosteroids to the mother at any time before delivery. In most cases, GA was calculated from the date of the last menstrual period and verified by the fetal crown-rump length on ultrasonography in the first trimester. However, their menstrual histories or ultrasonographic findings were not recorded in the database. Small for gestational age (SGA) and severely SGA were defined as a BW but not intrauterine fetal weight of less than the 10th and third percentiles for the GA, respectively.[14] Major congenital abnormalities were defined as chromosomal abnormalities, congenital heart defects, intestinal atresia, renal hypoplasia, skeletal dysplasia, and inborn errors of metabolism. Nitric oxide inhalation was applied for pulmonary hypertension. Systemic postnatal corticosteroid was used to treat bronchopulmonary dysplasia (BPD) or refractory hypotension. Moderate to severe BPD was defined as the need for supplemental oxygen or positive pressure at 36 weeks' postmenstrual age.[15] Treatment with indomethacin or surgical ligation was conducted for closures of patent ductus arteriosus (PDA) diagnosed clinically and by echocardiography. Severe intraventricular hemorrhage (IVH) was defined as grade III or IV.[16] Cystic periventricular leukomalacia (cPVL) was diagnosed by cranial ultrasound or head MRI. Sepsis was defined as culture-proven septicemia or bacteremia during the NICU stay. Severe necrotizing enterocolitis (NEC) was defined as pneumoperitoneum or Bell stage ≥2.[17] Retinopathy of prematurity (ROP) was coded if treatment was required with laser coagulation or cryocoagulation. This study was approved by the Internal Review Board of Tokyo Women's Medical University. Written informed consent was obtained from the parents or guardians of all infants in the NRNJ.

Outcomes and Neurodevelopmental Assessments

Our primary objective in this study was to determine neurodevelopmental impairment (NDI) in surviving children with a BW of ≤500 g at 3 years of age. NDI was defined as the following conditions: cerebral palsy (CP), visual impairment, hearing impairment, or cognitive impairment. Comprehensive neurodevelopmental and growth assessments were performed on the surviving children at 36 to 42 months of chronological age at each participating institute. According to the protocol of the Japanese Society for Follow-up Study of High-Risk Infants,[18] the postnatal development and growth of the surviving children were assessed at 18 months of corrected age and at 3, 6, and 9 years of chronological age. During these periods, parents were advised to follow periodic

Downloaded from www.aappublications.org/news by guest on November 16, 2018

check-ups for their children. CP was defined as a nonprogressive central nervous system disorder characterized by abnormal muscle tone in at least 1 extremity and abnormal control of movement and posture,[19] and it was diagnosed by board-certified pediatricians who specialize in child neurology. Visual impairment included unilateral or bilateral blindness or severe myopia requiring corrective lenses diagnosed by ophthalmologists. Hearing impairment was diagnosed by otolaryngologists when serial test results indicated the loss of auditory functions. Cognitive impairment was defined as a developmental quotient (DQ) score of <70 with the Kyoto Scale of Psychological Development, a standard development-scoring system written in Japanese.[20] The test was available at all participating institutes and was performed by certified psychologists who were trained to assess childhood development under blinding to perinatal histories at each institute.[21,22] Interscorer reliability was not examined. According to the protocol, developmental function was categorized as delayed (DQ <70), subnormal (DQ 70–84), and normal (DQ ≥85). No disability was defined as a DQ score of ≥85 with no CP or visual or hearing impairment. Height, weight, head circumference (HC), and BMI SD scores were calculated by using Japanese reference values for sex and age.[23,24]

Statistical Analyses

Data were analyzed by using JMP 11.0 (SAS Institute, Inc, Cary, NC) and Stata 14.2 (Stata Corp, College Station, TX). Results with a 2-sided *P* value of <.05 were considered significant. Continuous and categorical variables were compared by using Wilcoxon rank sum tests and χ^2 tests, respectively. The Cochran-Armitage test was used to assess temporal trends. Risks of NDI were calculated with modified Poisson regression analysis.[25] We included in the regression model all factors of neonatal characteristics, in-hospital interventions, and neonatal morbidities from univariate analysis with a *P* value of <.2. Adjusted risk ratios (with 95% confidence intervals [CIs]) were calculated.

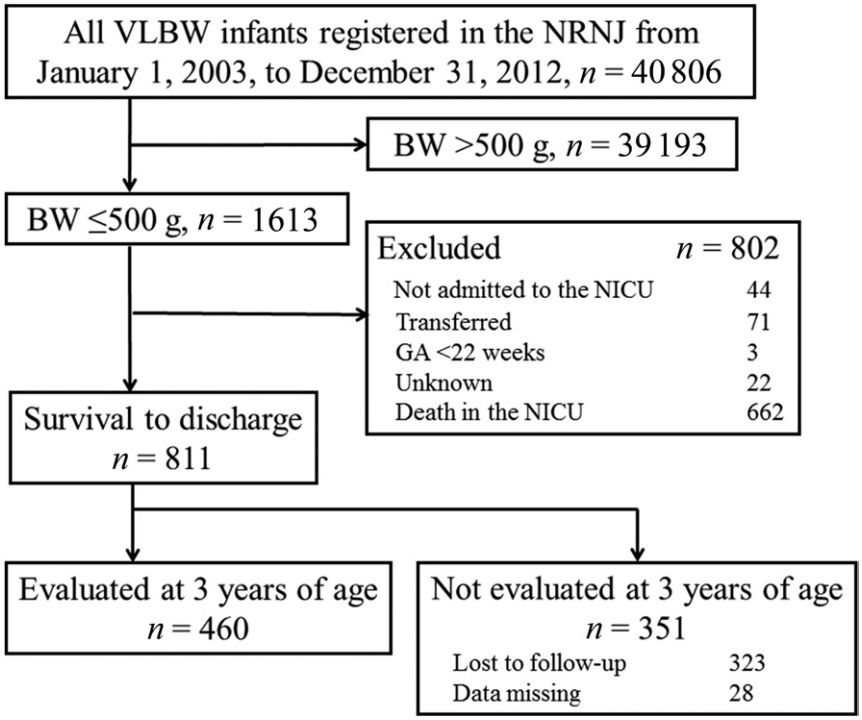


FIGURE 1
Patient enrollment. Our study population consisted of all children with a BW of ≤500 g and GA of ≥22 weeks who survived from the affiliated NICUs. VLBW, very low birth weight.

RESULTS

Clinical Characteristics

A total of 1613 live-born infants with a BW of ≤500 g were registered in the NRNJ database.[13] Of these, the following infants were excluded: 44 infants who were not admitted to the NICU, 71 infants who were transferred to other hospitals, 3 infants born at a GA of <22 weeks, and 22 infants without records of mortality status. Of 1473 live-born infants with a BW of ≤500 g at a GA of ≥22 weeks who were treated in the NICUs, 662 infants were excluded because of death during the NICU stay. Consequently, 811 children discharged from 113 NICUs were considered eligible to participate in a follow-up evaluation at age 3 years. These children were born at a median GA of 24 6/7 (range: 22 0/7–33 2/7) weeks and BW of 462 (267–500) g. The study population finally consisted of 460 children (56.7%) in 78 NICUs who had recorded data of NDI at 3 years of age (median 37 months; Fig 1). The NRNJ database includes information concerning visual and hearing impairments (presence or absence) for 442 (96.1%) and 393 (85.4%) children, respectively. Drop-off children included 323 who were lost during the follow-up period and 28 whose follow-up data were missing at age 3 years.

To determine if the study population represented the whole eligible population, baseline characteristics from the study population (*n* = 460) were compared with the nonevaluated children (*n* = 351; Table 1). There were significant but small differences in the proportions

Downloaded from www.aappublications.org/news by guest on November 16, 2018

TABLE 1 Demographics for Surviving Infants With a BW of ≤500 g

	Study Population, $N = 460$	Not Evaluated, $N = 351$	P
Maternal			
Age ≥35 y	140 of 432 (32.4)	122 of 340 (35.9)	.31
Singleton	413 of 460 (89.8)	310 of 344 (90.1)	.88
Clinical chorioamnionitis	83 of 450 (18.4)	48 of 331 (14.5)	.15
Antenatal corticosteroids	232 of 458 (50.7)	178 of 345 (51.6)	.79
Cesarean delivery	359 of 459 (78.2)	268 of 345 (77.7)	.86
Neonatal			
GA, wk	24 6/7, 22 0/7–32 2/7	24 6/7, 22 0/7–33 2/7	.79
BW, g	462, 276–500	462, 267–500	.96
SGA	342 of 460 (74.3)	259 of 351 (73.8)	.86
Severely SGA	298 of 460 (64.8)	223 of 351 (63.5)	.71
Boy	187 of 460 (40.7)	158 of 351 (45.0)	.21
Apgar score ≥4 at 5 min	385 of 447 (86.1)	282 of 334 (84.4)	.51
Major congenital abnormalities	11 of 460 (2.4)	2 of 350 (0.6)	.04
In-hospital interventions			
Surfactant	371 of 457 (81.2)	266 of 341 (78.0)	.27
High-frequency ventilation	313 of 453 (69.1)	211 of 340 (62.1)	.04
Inhaled nitric oxide	40 of 443 (9.0)	31 of 332 (9.3)	.88
Indomethacin	319 of 458 (69.7)	243 of 344 (70.6)	.76
Postnatal corticosteroids	247 of 392 (63.0)	182 of 300 (60.7)	.53
Antibiotics	384 of 452 (85.0)	306 of 341 (89.7)	.047
Neonatal morbidities			
Any of the following morbidities	365 of 454 (80.4)	283 of 342 (82.7)	.40
Moderate to severe BPD	255 of 450 (56.7)	197 of 335 (58.8)	.55
PDA ligation	65 of 458 (14.2)	39 of 343 (11.4)	.24
Severe IVH	30 of 456 (6.6)	31 of 341 (9.1)	.19
cPVL	18 of 457 (3.9)	13 of 344 (3.8)	.91
Sepsis	92 of 457 (20.1)	71 of 344 (20.6)	.86
Severe NEC	35 of 460 (7.6)	30 of 351 (8.5)	.63
Treatment of ROP	225 of 452 (49.8)	157 of 339 (46.3)	.33

Data presented as the number, number with available information (percentage), or as the median and range. *P* values are obtained by using the Wilcoxon rank-sum test (continuous variables) and χ^2 test (dichotomous variables).

of major congenital abnormalities, high-frequency ventilation, and antibiotics use.

Outcomes

We analyzed neurodevelopmental and growth outcomes of the study population ($n = 460$) and compared these profiles between the subgroups with BWs of ≤400 g ($n = 65$) and 401 to 500 g ($n = 395$; Table 2). The GA did not differ between the BW ≤400 g (median: 25 0/7 weeks; range: 22 0/7–30 2/7 weeks) and 401 to 500 g (24 6/7 weeks, 22 0/7–32 2/7 weeks) groups ($P = .09$). The overall proportion of NDI was 59.1% (95% CI: 54.6%–63.5%), whereas this rate was not different between the 2 subgroups. We classified 113 NICUs into the following 4 categories according to the number of live-discharged infants ($n = 811$): <5 ($n = 110$ in 52 NICUs), 5 to 10 (267 in 36), 11 to 20 (219 in 16), and ≥21 (215 in 9). The proportions of NDI in the study population ($n = 460$) were not different among these 4 categories (69.6%, 52.4%, 62.6%, and 57.3%, respectively; $P = .14$). The DQ scores were missing in 20 (4.3%) of 460 eligible subjects. Children with a BW of 401 to 500 g had a lower proportion of cognitive impairment than those with a BW of ≤400 g, whereas other neurodevelopmental outcomes did not differ between them. Overall, 80 children (17.4%) of the 460 subjects had no disability. Among 375 children who had information on disabilities, 101 (26.9%), 57 (15.2%), 28 (7.5%), and 1 (0.3%) had a single, double, triple, and full disabilities, respectively (Supplemental Table 6). The profiles of 3 children with a BW of ≤300 g are shown in Supplemental Table 7. NDI and no disability remained unchanged in their prevalence ($P = .70$ and .78 for trend, respectively; Fig 2). The proportions of children with <−2 SDs of weight, height, HC, and BMI were 69.9%, 69.6%, 40.6%, and 42.9%, respectively (Table 2). There was no difference in anthropometric measurements between the BW subgroups.

Factors Associated With NDI

In Table 3, we present the perinatal and neonatal characteristics of children with NDI ($n = 272$) and non-NDI ($n = 188$). The median GA (24 4/7 weeks) of children with NDI was lower than that of children with non-NDI (25 2/7 weeks; $P = .04$). The children with NDI included significantly higher proportions of boys and all neonatal morbidities. Other characteristics such as BW, SGA, severe SGA, congenital abnormalities, and in-hospital interventions did not differ between them. Multivariate regression analysis revealed that NDI was significantly associated with severe IVH, cPVL, severe NEC, PDA ligation, and male sex (Table 4). We found the essentially same relations between the clinical variable and risk for developing multiple disabilities (Supplemental Table 8). Among 442 children without severe IVH or cPVL, 256 (57.9%) infants developed NDI. Anthropometric data revealed that 47.4% (82 of 173) of children with NDI had an HC of <−2 SDs, which was higher than that (29.5%, 31 of 105) of children with non-NDI ($P <$ .01). Proportions of weight <−2 SDs (children with NDI: 72.3% [159 of 220] vs non-NDI: 66.2% [96 of 145]; $P = .22$), height <−2 SDs (72.8% [163 of 224] vs 64.9% [96 of 148]; $P = .10$), and BMI <−2 SDs (43.7% [83 of 190] vs 41.7% [50 of 120]; $P = .73$) did not differ between the 2 groups. Among 113 children with an HC of <−2 SDs, 12 (10.6%) and 10 (8.8%) had severe IVH and cPVL, respectively.

Downloaded from www.aappublications.org/news by guest on November 16, 2018

TABLE 2 Outcomes of Survivors With a BW of ≤500 g at 3 Years of Age

Outcomes	Total, *N* = 460		≤400 g, *N* = 65		401–500 g, *N* = 395		*P*
	n[a]	% (95% CI)	*n*[a]	%	*n*[a]	%	
NDI	272 of 460	59.1 (54.6–63.5)	44 of 65	67.7	228 of 395	57.7	.13
CP	100 of 451	22.2 (18.6–26.2)	18 of 62	29.0	82 of 389	21.1	.16
Visual impairment	94 of 442	21.3 (17.7–25.3)	16 of 62	25.8	78 of 380	20.5	.35
Hearing impairment	17 of 393	4.3 (2.7–6.8)	3 of 52	5.8	14 of 341	4.1	.58
Cognitive impairment	212 of 440	48.2 (43.5–52.8)	37 of 61	60.7	175 of 379	46.2	.04
No disability	80 of 460	17.4 (14.2–21.1)	8 of 65	12.3	72 of 395	18.2	.24
Anthropometric measurements							
Body wt <−2 SDs	255 of 365	69.9 (65.0–74.3)	42 of 56	75.0	213 of 309	68.9	.36
Body length <−2 SDs	259 of 372	69.6 (64.8–74.1)	44 of 57	77.2	215 of 315	68.3	.18
HC <−2 SDs	113 of 278	40.6 (35.0–46.5)	18 of 38	47.4	95 of 240	39.6	.36
BMI <−2 SDs	133 of 310	42.9 (37.5–48.5)	26 of 48	54.2	107 of 262	40.8	.09

NDI is defined as any of the following: CP, visual impairment, hearing impairment, or cognitive impairment. Cognitive impairment is defined as a DQ score of <70. No disability is defined as a DQ score of ≥85 with no CP or visual or hearing impairment. *P* values are obtained by using the χ^2 test.

[a] Number or number with available information.

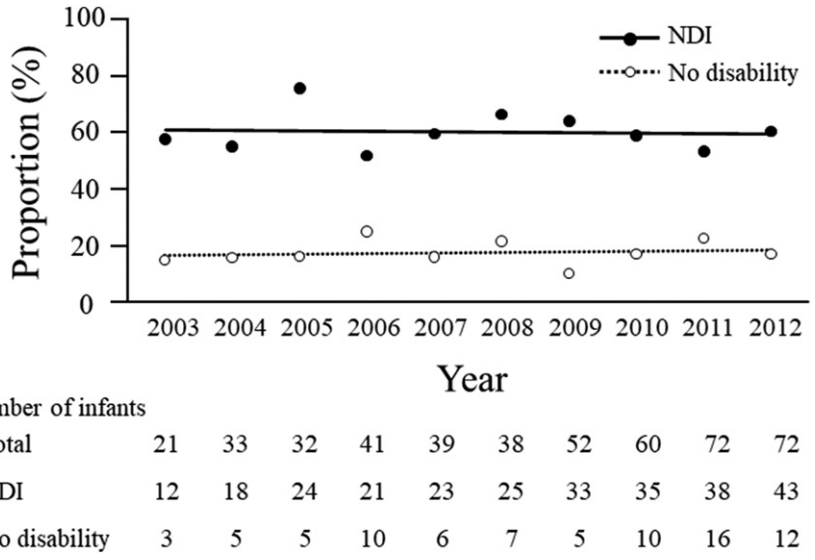


FIGURE 2
Neurodevelopmental outcomes at 3 years of age in children with a BW of ≤500 g. Trends in the proportions of NDI (solid line) and no disability (dotted line) in children with BWs of ≤500 g during the 10 years of the study period (2003–2012) are represented by regression lines. The number of infants per year is shown below the graphic chart.

DISCUSSION

In our nationwide cohort, the overall proportion of NDI at age 3 years for surviving children with a BW of ≤500 g born from 2003 to 2012 was estimated to be 59.1% (95% CI: 54.6%–63.5%). The proportions of children with NDI as well as no disability remained unchanged over the 10 years of the study period. Multivariate analysis revealed that NDI was significantly associated with severe IVH, cPVL, severe NEC, PDA ligation, and male sex.

We reviewed the studies on the long-term outcomes of surviving infants born with a BW of ≤500 g using the PubMed database (Table 5).[26–30] The prevalence of neurodevelopmental outcomes varied considerably in these reports. One of the reasons for the variable results is that each study used its own respective scale for neurologic assessments. For example, Vohr et al[28] defined normal neurologic examination as no abnormalities in the physical assessments, and Keir et al[30] defined no or minimal disability as a DQ of >1 SD. Cognitive impairment was assessed with the Stanford-Binet Intelligence Scale,[26] Bayley Scales of Infant Development-II,[28] Kaufmann Assessment Battery for Children,[29] or an unknown scale.[30] This heterogeneity has limited the direct comparison of data on neurodevelopmental outcomes across studies. The variability may also be related to population coverage (single institute versus nationwide cohort), health insurance system (universal versus private), and socioeconomic conditions. A universal health care system, which is available in Japan and Canada but not in the United States, may provide equal access to medical care for all the eligible newborns irrespective of socioeconomic status. Different ethnicity and homogeneous (Japan and Germany) or diverse ethnic backgrounds (United States and Canada) might be also critical for their neurodevelopmental outcomes.[9,28,31,32] However, neurodevelopmental outcomes among infants with a BW of ≤500 g seem not to improve over the 3 decades.

Brain injury is a risk for neurodevelopmental sequelae.[31,33] We confirmed that severe IVH and cPVL had great impact on neurodevelopmental outcomes among selected variables. As we previously reported,[13] unchanged

Downloaded from www.aappublications.org/news by guest on November 16, 2018

TABLE 3 Characteristics for NDI and Non-NDI Survivors With a BW of ≤500 g at 3 Years of Age

Characteristics	NDI, *N* = 272	Non-NDI, *N* = 188	*P*
Maternal			
Age ≥35 y	78 of 258 (30.2)	62 of 174 (35.6)	.24
Singleton	242 of 272 (89.0)	171 of 188 (91.0)	.49
Clinical chorioamnionitis	53 of 266 (19.9)	30 of 184 (16.3)	.33
Antenatal corticosteroids	138 of 271 (50.9)	94 of 187 (50.3)	.89
Cesarean section	207 of 272 (76.1)	152 of 187 (81.3)	.19
Neonatal			
GA, wk	24 4/7, 22 0/7–32 2/7	25 2/7, 22 0/7–29 3/7	.04
BW, g	456, 276–500	467, 286–500	.14
SGA	199 of 272 (73.2)	143 of 188 (76.1)	.48
Severely SGA	170 of 272 (62.5)	128 of 188 (68.1)	.22
Boy	124 of 272 (45.6)	63 of 188 (33.5)	<.01
Apgar score ≥4 at 5 min	218 of 261 (83.5)	167 of 186 (89.8)	.06
Major congenital abnormalities	6 of 272 (2.2)	5 of 188 (2.7)	.75
In-hospital interventions			
Surfactant	222 of 272 (81.6)	149 of 185 (80.5)	.77
High-frequency ventilation	189 of 269 (70.3)	124 of 184 (67.4)	.52
Inhaled nitric oxide	25 of 265 (9.4)	15 of 178 (8.4)	.72
Indomethacin	191 of 272 (70.2)	128 of 186 (68.8)	.75
Postnatal corticosteroids	155 of 236 (65.7)	92 of 156 (59.0)	.18
Antibiotics	234 of 270 (86.7)	150 of 182 (82.4)	.22
Neonatal morbidities			
Any of the following morbidities	233 of 270 (86.3)	132 of 184 (71.7)	<.01
Moderate to severe BPD	165 of 268 (61.6)	90 of 182 (49.5)	.01
PDA ligation	48 of 272 (17.6)	17 of 186 (9.1)	.01
Severe IVH	28 of 271 (10.3)	2 of 185 (1.1)	<.01
cPVL	16 of 271 (5.9)	2 of 186 (1.1)	<.01
Sepsis	64 of 272 (23.5)	28 of 185 (15.1)	.03
Severe NEC	28 of 272 (10.3)	7 of 188 (3.7)	<.01
Treatment of ROP	150 of 270 (55.6)	75 of 182 (41.2)	<.01

Data presented as the number, number with available information (percentage), or as the median and range. *P* values are obtained by using the Wilcoxon rank-sum test (continuous variables) and χ^2 test (dichotomous variables).

TABLE 4 Factors Related to NDI in Infants With a BW of ≤500 g at 3 Years of Age

	Crude RR (95% CI)	*P*	Adjusted RR (95% CI)	*P*
Severe IVH	1.64 (1.44–1.86)	<.01	1.42 (1.19–1.68)	<.01
cPVL	1.53 (1.28–1.84)	<.01	1.40 (1.13–1.73)	<.01
Severe NEC	1.39 (1.16–1.68)	<.01	1.31 (1.07–1.60)	<.01
PDA ligation	1.30 (1.09–1.53)	<.01	1.29 (1.09–1.54)	<.01
Boy	1.22 (1.05–1.42)	<.01	1.19 (1.01–1.40)	.04
Moderate to severe BPD	1.23 (1.04–1.44)	.01	1.20 (0.99–1.45)	.06
Apgar score ≤3 at 5 min	1.22 (1.02–1.48)	.03	1.10 (0.88–1.37)	.40
Treatment of ROP	1.26 (1.08–1.47)	<.01	1.09 (0.93–1.29)	.29
Postnatal corticosteroids	1.12 (0.94–1.34)	.19	1.08 (0.91–1.29)	.38
Sepsis	1.22 (1.04–1.44)	.02	1.05 (0.88–1.26)	.58
GA (per wk)	0.97 (0.93–1.01)	.09	1.01 (0.97–1.06)	.63
BW (per 100 g)	0.86 (0.74–1.00)	.06	0.92 (0.78–1.07)	.27

Risk ratios and *P* values are obtained from univariate and multivariate modified Poisson regression analyses. Multivariate analyses include 372 subjects for whom clinical variables are completely available, and adjusted risk ratios are obtained after adjusting for all covariates listed here. RR, risk ratio.

morbidities of brain injury among infants with a BW of ≤500 g might underlie the unimproved neurodevelopmental outcomes during the 10 years of our study period. cPVL, a severe form of cerebral white matter injury, has been observed in a small portion of very preterm infants. By contrast, noncystic or diffuse periventricular leukomalacia is a predominant form of brain lesion and is not readily detected by neuroimaging.[33] Therefore, early detection and prevention of white matter injuries will be the next targets to control in perinatal medicine.

HCs of <−2 SDs at age 3 years were significantly associated with NDI in this study, as previously reported.[34] HC can be considered a proxy for brain volume,[35] and head growth is affected by nutritional and nonnutritional factors.[34] Diffuse periventricular leukomalacia is associated with volumetric deficits of the cerebral cortex and thalamus and delayed cortical maturation.[33] These findings support the ongoing need for more extensive investigations into whether the children's postnatal nutrition and/or environmental distress, besides perinatal brain injury, are associated with their small HC.

We have recently reported that the survival rate of infants with a BW of ≤500 g has greatly improved from 2003 to 2012 in Japan.[13] Their survival was associated with several factors, including higher GA and BW, and the increasing rate of antenatal steroids and cesarean delivery contributed to improving their survival rate.[13] Notably, these advantageous factors were not shown to reduce the risk of NDI for survivors with BWs of ≤500 g. The combined outcomes of mortality or morbidity can be determined for all births or live births and are associated with GA and BW.[9–11] However, neurologic outcomes can only be determined for survivors to the age of the follow-up assessment. Thus, analysis of NDI might mask the effects of GA or BW.[32] The dissociated results are inevitable because severely affected infants are at high risk for death before discharge. Moreover, attrition in this study might reduce the power to detect effects of GA or BW. Because we selected surviving infants as eligible subjects, we were able to add variables of in-hospital interventions and neonatal complications in the current study. Consequently, severe IVH and cPVL were identified

Downloaded from www.aappublications.org/news by guest on November 16, 2018

TABLE 5 Neurodevelopmental Outcomes of Infants With a BW of ≤500 g

Ref. No.	Location (Type of Study)	Year of Birth	Population *n*[a]						Neurodevelopmental Outcomes[b]					
				n[c]	Follow-up Rate[d]	SGA[e]	Age at Evaluation, y	NDI	CP	Visual Impairment	Hearing Impairment	Cognitive Impairment	No Disability
25	Canada (Regional cohort)	1983–1994	≤500 g 113	13	72.2	94.4[f]	3	69.2	46.2	15.3	7.7	61.5	30.8
26	Unites States (Single center)	1989–2009	≤500 g 212	44	72.1	90.2[f]	2	nd	15.9	9.1	22.7	nd	nd
27	Unites States (Multicenter)	1993–1994	401–500 g nd	14	77.8	73.3	1.5	nd	28.6	21.4	7.1	35.7	57.1
28	Germany (3 centers)	1998–2001	<501 g 48	19	65.5	94.7	5	52.6	36.8	26.3	10.5	41.2	26.3
29	Australia (Single center)	2005–2010	≤500 g 26	9	64.3	88.9	3	88.9	33.3	22.2	11.1	33.3	11.1
This study	Japan (Nationwide cohort)	2003–2012	≤500 g 1473	460	56.7	74.3	3	59.1	22.2	21.3	4.3	48.2	17.4

nd, not described.

[a] Number of live-born infants who are admitted to NICUs.

[b] Neurodevelopmental outcomes data are expressed as the percentage of evaluated infants. Note that the definitions of each outcome are different in each study.

[c] Number of surviving and evaluated infants.

[d] Follow-up rates are expressed as percentages and calculated by dividing the number of surviving and evaluated infants by the total number of survivals to discharge from NICUs.

[e] Data are expressed as percentages of surviving and evaluated infants.

[f] Data are expressed as percentages of all surviving infants.

among the survivors with BWs of ≤500 g as the highest risk factors for NDI but not GA, BW, or obstetric intervention. Given the improving survival rates[13] and the unchanged neurodevelopmental outcomes, the number of both survivors with NDI and those without apparent disabilities were likely to increase in Japan. Nonetheless, these data provide useful information to guide the treatment of extremely small infants and social consensus, which is a goal of future research.

This study has several limitations. First, the follow-up rate was 56.7%. Because of the low follow-up rate, we failed to count the number of infants who died after discharge. In fact, the rates in drop-off were commonly high (30%–36%) in previous NRNJ studies.[21,22] One of the reasons might be that not all infants were followed-up at the same hospitals from which they were discharged. Therefore, their follow-up data were not recovered to a level comparable to those in former reports from other countries. Three perinatal parameters (congenital abnormalities, high-frequency ventilation, and antibiotics use) revealed significant differences between the study population and the nonevaluated survivors (Table 1). Two of these differences suggest the study population is sicker than the population without follow-up; therefore, the rate of NDI may be overestimated for the former. Second, there is no information on receiving the mode of active treatment. Therefore, neurodevelopmental outcomes in this study might be better than those for all births or live births. The third limitation is the lack of comparison with term-born infants, which may introduce expectation bias. However, the NDI rate at age 3 years in this study was higher than those of children with a BW of >500 g (501–750 g, 36.3%; 751–1000 g, 20.1%) in a previous NRNJ study.[21] Lastly, other

Downloaded from www.aappublications.org/news by guest on November 16, 2018

prognostic factors for neurodevelopmental disability such as parental education level, erythropoietin treatment, aggressive nutritional support, and specific strategies of ventilation and steroids administration[32] were not analyzed. Identifying the key environmental factors among these variables will further unveil the potential targets of intervention for extremely small infants.

CONCLUSIONS

Neurodevelopmental outcomes in early childhood among surviving infants with a BW of ≤500 g did not improve in a recent decade. In this large-population study, we verified that perinatal brain injury was an unfavorable risk factor for NDI. Changes in medical management that are able to ameliorate brain injury would be expected to reduce adverse neurodevelopmental burdens for extremely small infants.

ACKNOWLEDGMENTS

Institutions enrolled in the study of the NRNJ were as follows: Sapporo City General Hospital, Asahikawa Kosei General Hospital, Engaru-Kosei General Hospital, Kushiro Red Cross Hospital, Obihiro-Kosei General Hospital, Tenshi Hospital, NTT Higashinihon Sapporo Hospital, Nikko Memorial Hospital, Nayoro City General Hospital, Sapporo Medical University, Asahikawa Medical University, Aomori Prefectural Central Hospital, Iwate Medical University, Iwate Prefectural Ofunato Hospital, Iwate Prefectural Kuji Hospital, Iwate Prefectural Ninohe Hospital, Sendai Red Cross Hospital, Akita Red Cross Hospital, Tsuruoka Municipal Shonai Hospital, Yamagata University, Yamagata Prefectural Central Hospital, Fukushima Medical University, Takeda General Hospital, Fukushima National Hospital, Tsukuba University, Tsuchiura Kyodo Hospital, Ibaraki Children's Hospital, Dokkyo Medical University, Jichi Medical University, Ashikaga Red Cross Hospital, Gunma Children's Medical Center, Kiryu Kosei General Hospital, Fuji Heavy Industries Health Insurance Society Ota Memorial Hospital, Gunma University, Saitama Children's Medical Center, Nishisaitama-chuo National Hospital, Saitama Medical University Saitama Medical Center, Kawaguchi Municipal Medical Center, Jichi Medical University Saitama Medical Center, Asahi General Hospital, Chiba Kaihin Municipal Hospital, Kameda Medical Center, Tokyo Women's Medical University Yachiyo Medical Center, Juntendo University Urayasu Hospital, Tokyo Metropolitan Children's Medical Center, Tokyo Women's Medical University, Aiiku Hospital, Nihon University Itabashi Hospital, National Center for Global Health and Medicine, Tokyo Medical University, Teikyo University, Showa University, Japan Red Cross Medical Center, National Center for Child Health and Development, Tokyo Metropolitan Otsuka Hospital, Toho University, Tokyo Metropolitan Bokuto Hospital, Tokyo Jikei Medical University, Tokyo Medical and Dental University, Saint Luke's International Hospital, Juntendo University, Sanikukai Hospital, Katsushika Red Cross Hospital, Yokohama Rosai Hospital, Yokohama City University Medical Center, St. Marianna University School of Medicine Hospital, Kanagawa Children's Medical Center, Tokai University, Kitazato University, Odawara Municipal Hospital, Nippon Medical School Musashi Kosugi Hospital, Saiseikai Yokohamashi Tobu Hospital, National Hospital Organization Yokohama Medical Center, Yamanashi Prefectural Central Hospital, Nagano Children's Hospital, Shinshu University, Iida Municipal Hospital, National Hospital Organization Shinshu Ueda Medical Center, Saku General Hospital, Niigata University, Niigata Prefectural Central Hospital, Niigata Municipal Hospital, Nagaoka Red Cross Hospital, Koseiren Takaoka Hospital, Toyama Prefectural Central Hospital, Toyama University, Ishikawa Medical Center for Maternal and Child Health, Kanazawa Medical University, Kanazawa Medical Center, Fukui Prefectural Hospital, Fukui University, Gifu Prefectural General Medical Center, National Hospital Organization Nagara Medical Center, Takayama Red Cross Hospital, Seirei Hamamatsu Hospital, Shizuoka Saiseikai Hospital, Shizuoka Children's Hospital, Hamamatsu Medical University, Numazu Municipal Hospital, Yaizu City Hospital, Fujieda Municipal General Hospital, Nagoya Red Cross Daini Hospital, Nagoya University, Nagoya Red Cross Daiichi Hospital, Toyohashi Municipal Hospital, Nagoya City West Medical Center, Anjo kosei Hospital, Tosei General Hospital, Komaki Municipal Hospital, TOYOTA Memorial Hospital, Okazaki Municipal Hospital, Konan Kosei Hospital, National Mie Central Medical Center, Ise Red Cross Hospital, Yokkaichi Municipal Hospital, Otsu Red Cross Hospital, Shiga University of Medical Science Hospital, Nagahama Red Cross Hospital, Uji Tokushukai Hospital, The Japan Baptist Hospital, Kyoto University, Kyoto Red Cross Daiichi Hospital, National Maizuru Medical Center, Fukuchiyama City Hospital, Kyoto Prefectural University of Medicine Hospital, Kyoto City Hospital, Mitsubishi Kyoto Hospital, Yodogawa Christian Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka University, Takatsuki General Hospital, Kansai Medical University, Osaka City General Hospital, Osaka City Sumiyoshi Hospital, Aizenbashi Hospital, Toyonaka Municipal Hospital, National Cerebral and Cardiovascular Center, Kitano Hospital, Saiseikai Suita Hospital, Chifune Hospital,

Downloaded from www.aappublications.org/news by guest on November 16, 2018

Bellland General Hospital, Rinku General Medical Center, Osaka Red Cross Hospital, Yao Municipal Hospital, Osaka General Medical Center, Osaka City University, Hyogo Prefectural Kobe Children's Hospital, Kobe University, Kakogawa West City Hospital, Saiseikai Hyogoken Hospital, Kobe City Medical Center General Hospital, Hyogo College of Medicine Hospital, Himeji Red Cross Hospital, Toyooka Public Hospital, Hyogo Prefectural Awaji Medical Center, Nara Medical University, Wakayama Medical University, Tottori Prefectural Central Hospital, Tottori University, Shimane Prefectural Central Hospital, Matsue Red Cross Hospital, Kurashiki Central Hospital, Tsuyama Central Hospital, Kawasaki Medical School Hospital, National Hospital Organization Okayama Medical Center, Okayama Red Cross Hospital, Hiroshima City Hiroshima Citizens Hospital, Hiroshima Prefectural Hospital, Hiroshima University, Tsuchiya General Hospital, National Hospital Organization Kure Medical Center, Yamaguchi University, Yamaguchi Grand Medical Center, Tokushima University, Tokushima Municipal Hospital, Kagawa University, National Hospital Organization Kagawa Children's Hospital, Matsuyama Red Cross Hospital, Ehime Prefectural Central Hospital, Kochi Health Science Center, St. Mary's Hospital, National Kyushu Medical Center, Kurume University, Kitakyushu Municipal Medical Center, University of Occupational and Environmental Health, Fukuoka University, Kyushu University, Iizuka Hospital, National Hospital Organization Kokura Medical Center, National Hospital Organization Saga Hospital, National Hospital Organization Nagasaki Medical Center, Kumamoto City Hospital, Kumamoto University, Oita Prefectural Hospital, Almeida Memorial Hospital, Nakatsu Municipal Hospital, Miyazaki University, National Hospital Organization Miyakonojo Medical Center, Kagoshima City Hospital, Imakiire General Hospital, Okinawa Prefectural Nanbu Medical Center and Children's Medical Center, Okinawa Prefectural Chubu Hospital, Naha City Hospital, Okinawa Red Cross Hospital.

ABBREVIATIONS

BPD: bronchopulmonary dysplasia
BW: birth weight
CI: confidence interval
CP: cerebral palsy
cPVL: cystic periventricular leukomalacia
DQ: developmental quotient
GA: gestational age
HC: head circumference
IVH: intraventricular hemorrhage
NDI: neurodevelopmental impairment
NEC: necrotizing enterocolitis
NRNJ: Neonatal Research Network of Japan
PDA: patent ductus arteriosus
ROP: retinopathy of prematurity
SGA: small for gestational age

Address correspondence to Hirosuke Inoue, MD, PhD, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: hinoue@pediatr.med.kyushu-u.ac.jp

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2018 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Funded in part by JSPS KAKENHI grant JP17K16300 (Dr Inoue), JP15K09717 (Dr Ochiai), and JP16K19688 (Dr Fujiyoshi).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

1. Stoll BJ, Hansen NI, Bell EF, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993-2012. *JAMA*. 2015;314(10):1039–1051
2. Patel RM, Kandefer S, Walsh MC, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Causes and timing of death in extremely premature infants from 2000 through 2011. *N Engl J Med*. 2015;372(4):331–340
3. Younge N, Goldstein RF, Bann CM, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Survival and neurodevelopmental outcomes among periviable infants. *N Engl J Med*. 2017;376(7):617–628
4. Younge N, Smith PB, Gustafson KE, et al. Improved survival and neurodevelopmental outcomes among extremely premature infants born near the limit of viability. *Early Hum Dev*. 2016;95:5–8
5. Tommiska V, Heinonen K, Lehtonen L, et al. No improvement in outcome of nationwide extremely low birth weight infant populations between 1996-1997 and 1999-2000. *Pediatrics*. 2007;119(1):29–36
6. Hintz SR, Kendrick DE, Vohr BR, Poole WK, Higgins RD; National Institute of Child Health and Human Development Neonatal Research Network. Changes in neurodevelopmental outcomes at 18 to 22 months' corrected age among infants of less than 25 weeks'

Downloaded from www.aappublications.org/news by guest on November 16, 2018

gestational age born in 1993-1999. *Pediatrics*. 2005;115(6):1645–1651

7. Hintz SR, Kendrick DE, Wilson-Costello DE, et al; NICHD Neonatal Research Network. Early-childhood neurodevelopmental outcomes are not improving for infants born at <25 weeks' gestational age. *Pediatrics*. 2011;127(1):62–70
8. Wilson-Costello D, Friedman H, Minich N, Fanaroff AA, Hack M. Improved survival rates with increased neurodevelopmental disability for extremely low birth weight infants in the 1990s. *Pediatrics*. 2005;115(4):997–1003
9. Ecker JL, Kaimal A, Mercer BM, et al; American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine. Periviable birth: interim update. *Am J Obstet Gynecol*. 2016;215(2):B2–B12.e1
10. Salihu HM, Salinas-Miranda AA, Hill L, Chandler K. Survival of pre-viable preterm infants in the United States: a systematic review and meta-analysis. *Semin Perinatol*. 2013;37(6):389–400
11. Tyson JE, Parikh NA, Langer J, Green C, Higgins RD; National Institute of Child Health and Human Development Neonatal Research Network. Intensive care for extreme prematurity–moving beyond gestational age. *N Engl J Med*. 2008;358(16):1672–1681
12. American College of Obstetricians and Gynecologists. ACOG practice bulletin no. 101: ultrasonography in pregnancy. *Obstet Gynecol*. 2009;113(2, pt 1):451–461
13. Inoue H, Ochiai M, Yasuoka K, et al; Neonatal Research Network of Japan (NRNJ). Early mortality and morbidity in infants with birth weight of 500 grams or less in Japan. *J Pediatr*. 2017;190(11):112–117.e3
14. Itabashi K, Miura F, Uehara R, Nakamura Y. New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr Int*. 2014;56(5):702–708
15. Ehrenkranz RA, Walsh MC, Vohr BR, et al; National Institutes of Child Health and Human Development Neonatal Research Network. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics*. 2005;116(6):1353–1360
16. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr*. 1978;92(4):529–534
17. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187(1):1–7
18. Kono Y, Mishina J, Sato N, Watanabe T, Honma Y. Developmental characteristics of very low-birthweight infants at 18 months' corrected age according to birthweight. *Pediatr Int*. 2008;50(1):23–28
19. Bax MC. Terminology and classification of cerebral palsy. *Dev Med Child Neurol*. 1964;6:295–297
20. Kono Y, Yonemoto N, Kusuda S, et al. Developmental assessment of VLBW infants at 18 months of age: a comparison study between KSPD and Bayley III. *Brain Dev*. 2016;38(4):377–385
21. Kono Y, Mishina J, Yonemoto N, Kusuda S, Fujimura M. Outcomes of very-low-birthweight infants at 3 years of age born in 2003-2004 in Japan. *Pediatr Int*. 2011;53(6):1051–1058
22. Ishii N, Kono Y, Yonemoto N, Kusuda S, Fujimura M; Neonatal Research Network, Japan. Outcomes of infants born at 22 and 23 weeks' gestation. *Pediatrics*. 2013;132(1):62–71
23. Suwa S, Tachibana K. Standard growth charts for height and weight of Japanese children from birth to 17 years based on a cross-sectional survey of national data. *Clin Pediatr Endocrinol*. 1993;2(2):87–97
24. Kato N, Takimoto H, Sudo N. The cubic functions for spline smoothed L, S and M values for BMI reference data of Japanese children. *Clin Pediatr Endocrinol*. 2011;20(2):47–49
25. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective studies with correlated binary data. *Stat Methods Med Res*. 2013;22(6):661–670
26. Sauve RS, Robertson C, Etches P, Byrne PJ, Dayer-Zamora V. Before viability: a geographically based outcome study of infants weighing 500 grams or less at birth. *Pediatrics*. 1998;101(3, pt 1):438–445
27. Upadhyay K, Pourcyrous M, Dhanireddy R, Talati AJ. Outcomes of neonates with birth weight≤500 g: a 20-year experience. *J Perinatol*. 2015;35(9):768–772
28. Vohr BR, Wright LL, Dusick AM, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993-1994. *Pediatrics*. 2000;105(6):1216–1226
29. Rieger-Fackeldey E, Blank C, Dinger J, Steinmacher J, Bode H, Schulze A. Growth, neurological and cognitive development in infants with a birthweight <501 g at age 5 years. *Acta Paediatr*. 2010;99(9):1350–1355
30. Keir A, McPhee A, Wilkinson D. Beyond the borderline: outcomes for inborn infants born at ≤500 grams. *J Paediatr Child Health*. 2014;50(2):146–152
31. Jarjour IT. Neurodevelopmental outcome after extreme prematurity: a review of the literature. *Pediatr Neurol*. 2015;52(2):143–152
32. Linsell L, Malouf R, Morris J, Kurinczuk JJ, Marlow N. Prognostic factors for poor cognitive development in children born very preterm or with very low birth weight: a systematic review. *JAMA Pediatr*. 2015;169(12):1162–1172
33. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 2009;8(1):110–124
34. Raghuram K, Yang J, Church PT, et al; Canadian Neonatal Network; Canadian Neonatal Follow-Up Network Investigators. Head growth trajectory and neurodevelopmental outcomes in preterm neonates. *Pediatrics*. 2017;140(1):e20170216
35. Cheong JL, Hunt RW, Anderson PJ, et al. Head growth in preterm infants: correlation with magnetic resonance imaging and neurodevelopmental outcome. *Pediatrics*. 2008;121(6). Available at: www.pediatrics.org/cgi/content/full/121/6/e1534

Downloaded from www.aappublications.org/news by guest on November 16, 2018

Social gradient of birthweight in England assessed using the INTERGROWTH-21st gestational age-specific standard

Jane E Hirst,[1] Hannah Ellin Knight,[2] Eric O Ohuma,[3,4] Terence Dwyer,[5] Benjamin D Hennig,[6] Aris T Papageorghiou,[1] Leila Cheikh Ismail,[7] José Villar,[1] Stephen H Kennedy[1]

[1]Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK
[2]Department of Health Services Research and Policy, London School of Hygiene & Tropical Medicine, London, UK
[3]Centre for Statistics in Medicine, Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Oxford, UK
[4]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[5]The George Institute for Global Health UK, University of Oxford, Oxford, UK
[6]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland
[7]Clinical Nutrition and Dietetics Department, University of Sharjah College of Health Sciences, Sharjah, UAE

Correspondence to
Dr Jane E Hirst, Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford OX3 9DU, UK; jane.hirst@wrh.ox.ac.uk

Received 5 April 2018
Revised 8 October 2018
Accepted 9 October 2018

Check for updates

© Author(s) (or their employer(s)) 2018. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Hirst JE, Knight HE, Ohuma EO, *et al*. *Arch Dis Child Fetal Neonatal Ed* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/archdischild-2018-315295

ABSTRACT

Objective To determine the socioeconomic gradient of birthweights in England with reference to the prescriptive INTERGROWTH-21st Birthweight Standard.

Design National cross-sectional study using data from Hospital Episode Statistics.

Setting National Health Service in England.

Participants All singleton babies, live born between 34 weeks' gestation and 42 weeks' gestation, between 1 April 2011 and 31 March 2012.

Main outcome measures Birthweight distribution of babies with a birthweight of <10th centile or >90th centile, that is, small for gestational age (SGA) or large for gestational age (LGA) using Index of Multiple Deprivation quintiles as a proxy for socioeconomic status.

Results Of 508 230 babies born alive between 1 April 2011 and 31 March 2012, 38 838 (7.6%) were SGA and 81 026 (15.9%) were LGA. Median birthweight was 3405 g, median z-score was 0.25 (SD 1.06). Birthweight z-score demonstrated a social gradient, from 0.26 (SD 1.1) in the most deprived areas to 0.53 (1.0) in the least deprived. Women in the most deprived areas were twice as likely to have SGA babies using the INTERGROWTH-21st chart (OR 1.94; 95% CI 1.87 to 2.01) compared with those in the least deprived areas. If all women had the same rate of SGA equivalent to those living in the least deprived areas, approximately 12 410 (30%) fewer babies would be born SGA in England each year.

Conclusions This study gives a measure of the social gradient in singleton SGA and LGA babies across England using an international standard of newborn size at birth.

What is already known on this topic?

- Low birthweight for gestational age, which is associated with adverse short-term and long-term health outcomes, is more common in women from a lower socioeconomic background.

What this study adds?

- Large regional differences in birthweight exist across England with evidence of a social gradient.
- Women living in the most deprived areas of the country are most likely to have low birthweight babies across all gestational ages and more small for gestational age babies.
- The mean birthweight of babies born in England is greater than the optimal international standard.

INTRODUCTION

Size at birth indicates the quality of the intrauterine environment and identifies babies born at greater risk of adverse immediate and future outcomes. Being born small for gestational age (SGA) increases the risk of perinatal mortality,[1 2] infections in childhood[3] and has been linked to lifelong disparities in cardiovascular and metabolic health,[4] shorter adult stature[5] and decreased economic productivity.[6] Being born large for gestational age (LGA) places both mother and baby at higher risk of complications during birth, such as shoulder dystocia and caesarean section,[7] and in some populations has been associated with childhood overweight and obesity.[8 9]

In 2014, the INTERGROWTH-21st Consortium published the first prescriptive, international standards for fetal growth[10] and newborn size at birth (weight, length and head circumference) for gestational age and sex,[11] based on WHO recommendations for the construction of such standards.[12] The standards describe optimal intrauterine growth and size at birth for babies born to healthy, well-nourished women receiving adequate antenatal care and living in environments across the world with minimal constraints on growth. Under such conditions, babies grow similarly in utero and achieve a similar size at birth irrespective of their mothers' ancestry, skin colour or geographical location.[13] The findings justify the use in routine clinical practice of a single set of international standards for all populations around the world. As these standards perfectly complement the existing WHO Child Growth Standards,[14] there is now a unified approach to measuring growth and development from early pregnancy to childhood.[15]

Graded health inequalities are present throughout life. Ensuring a healthy start to life was a key policy objective in the 2010 report *Fair Society, Healthy Lives (the Marmot Review)*.[16] The report emphasised interventions aimed only at the most deprived

Arch Dis Child Fetal Neonatal Ed: first published as 10.1136/archdischild-2018-315295 on 21 November 2018. Downloaded from http://fn.bmj.com/ on 22 November 2018 by guest. Protected by copyright.

Table 1 Distribution of birthweights

Exposure	Total babies	Birthweight (grams) Median	SD	Z-score	SD of z-score	Centile
Gestational age at birth (completed weeks)						
34	3563	2240	472	−0.12	1.11	45
35	5676	2495	465	−0.11	1.10	46
36	11 240	2720	483	−0.049	1.14	48
37	26 081	2950	470	0.041	1.11	52
38	65 426	3175	463	0.20	1.09	58
39	118 857	3340	443	0.23	1.03	59
40	149 799	3490	444	0.29	1.03	61
41	105 900	3640	454	0.36	1.04	64
42	21 688	3710	473	0.32	1.08	62
Deprivation quintile						
1	72 750	3480	498	0.40	1.01	65
2	79 070	3455	509	0.35	1.03	64
3	92 343	3430	513	0.30	1.05	62
4	116 087	3400	520	0.22	1.06	59
5	147 980	3340	527	0.11	1.08	54
Total	508 230	3405	518	0.25	1.06	60

in society will miss many others who could also benefit from better health outcomes. It is known that birthweight displays a social gradient,[17–20] although many studies have failed to consider preterm babies (born less than 37 weeks' gestation) separately from those that are born small because of impaired fetal growth.

We aimed to determine the distribution of birthweights in England and the proportion of babies born SGA and LGA using the INTERGROWTH-21st BirthWeight Standard for gestational age and sex, relative to the mother's residential area as a marker of her socioeconomic status.

METHODS

The study was a retrospective analysis of routinely collected national data on babies born in England between 1 April 2011 and 31 March 2012. Records were extracted from the Hospital Episode Statistics (HES) database to identify all births that took place in English National Health Service trusts (acute hospital organisations) during the study period. The HES database contains pseudonymised patient demographics, clinical information and administrative data for every inpatient episode of care. Episodes related to labour and birth capture additional information, including the baby's sex, gestational age in completed weeks and birthweight, in supplementary data fields known as the HES 'maternity tail'. Birth records were identified as any episode containing information about the mode of birth in either the procedure field (OPCS-4 codes R17–R25) or the maternity tail.

The study was limited to singleton, live-born babies with complete data on sex, weight and gestational age at birth and maternal postcode district. The analysis was limited to late preterm and term babies born between 34 weeks' gestation and 42 weeks' gestation as these represent the overwhelming majority of births.

The birthweight centile and z-score for gestational age and sex were calculated for each baby according to the INTERGROWTH-21st standard.[11] To determine the proportion of SGA and LGA, all newborns were categorised as: (1) SGA (birthweight <10th centile); (2) appropriate for gestational age (birthweight between 10th and 90th centile inclusive) or (3) LGA (birthweight >90th centile). Although the INTERGROWTH-21st standard presents centiles and z-scores in days throughout pregnancy, gestational age at birth is only recorded in the HES database by completed week of gestation: therefore, 3 days (ie, half a week) were added to each recorded gestational age to minimise potential misclassification bias of SGA and LGA babies.

The exposures studied were the baby's sex, gestational age at birth and an estimate of maternal social deprivation derived from the Index of Multiple Deprivation (IMD), a measure that combines economic, social and housing indicators based on postcodes.[21] Deprivation scores were based on data from 32 480 Lower Super Output Areas in England in 2010. The IMD score is presented in quintiles (quintile 1 being the least deprived, and quintile 5 being the most deprived).

The distribution of birthweights and the proportion of SGA and LGA babies were compared across gestational ages, sexes and IMD quintiles. Univariable and multivariable logistic regression models were used to estimate crude and adjusted effects of gestational age, sex and IMD quintile on the SGA and LGA rates.

All statistical tests were two-sided, and the level of significance was set at $p<0.05$. All analyses were performed in Stata V.13.

The presentation of the results was realised using a novel approach to spatial data visualisation. This was applied to allow a different geographic interpretation than is possible in conventional maps. A density-equalising cartogram transformation based on Gastner and Newman's algorithm was used.[22 23] Each postcode area was resized according to the total number of babies born there. Areas with a low number of births are proportionally reduced in size. This makes these maps different from a land area map where the least populated areas (and those with usually much lower absolute numbers of births) are proportionally over-represented. Onto these maps the relative LGA and SGA rates were overlaid. This cartogram visualisation technique made it possible to highlight the varying birth rates across the geographic areas where most births occurred.

RESULTS

From the 673 595 births during the study period, 508 230 singleton babies met the inclusion criteria with 260 103 (51.2%)

Arch Dis Child Fetal Neonatal Ed: first published as 10.1136/archdischild-2018-315295 on 21 November 2018. Downloaded from http://fn.bmj.com/ on 22 November 2018 by guest. Protected by copyright.

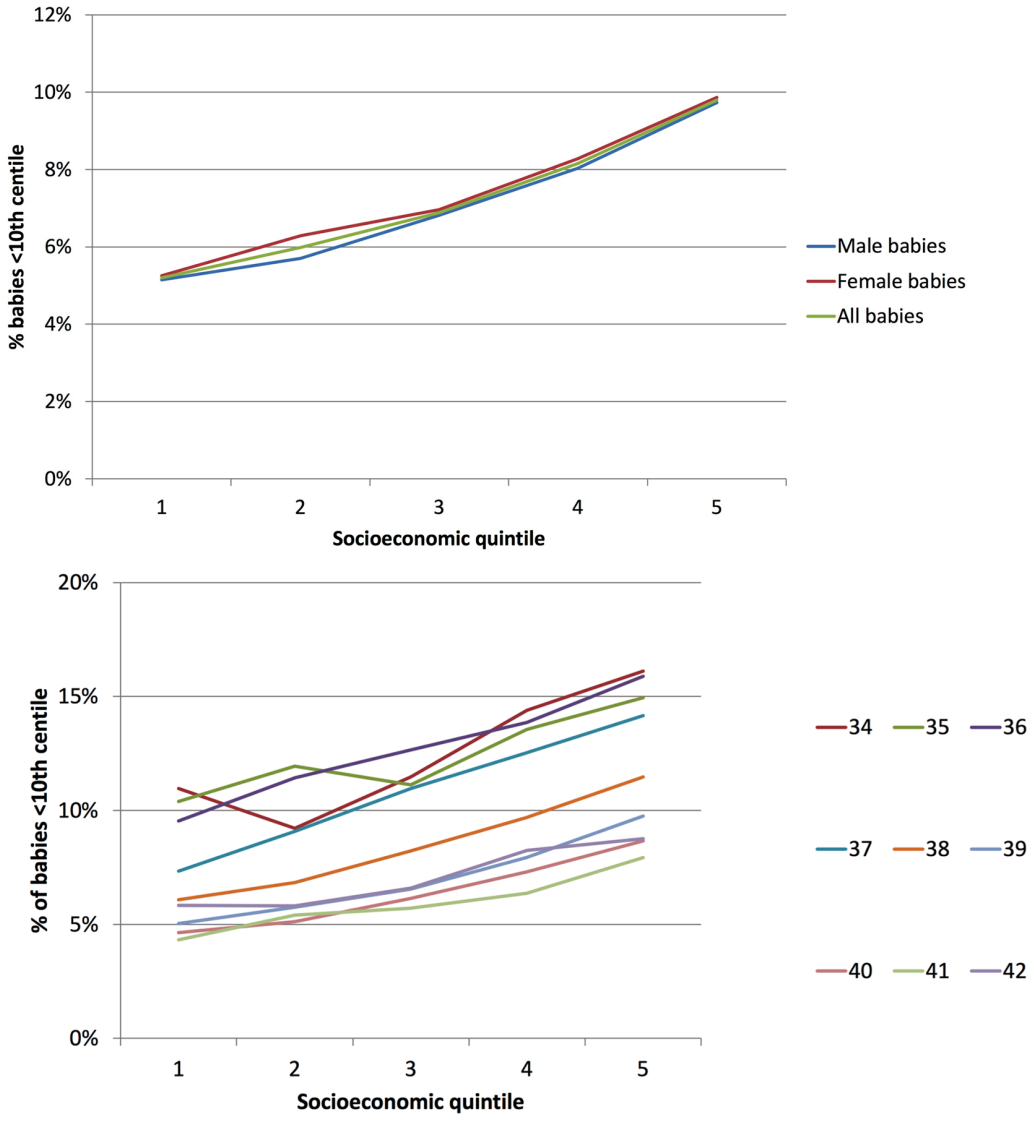


Figure 1 Proportion of babies born small for gestational age by socioeconomic quintile and sex (A) and by gestational age at birth (B).

males. The overall median birthweight was 3405 g, corresponding to the 60th centile and z-score of 0.25 of the INTERGROWTH-21st standard. Birthweight displayed a gradient across the IMD groups with the median weight of babies in the highest quintile 140 g heavier than those born in the lowest quintile (table 1). There were 38 838 (7.6%) babies born SGA and 81 026 (15.9%) babies born LGA.

There was a social gradient in the proportion of babies born SGA, with a similar trend observed between boys and girls (figure 1A). Among babies born to mothers in the least deprived areas, 5.2% were SGA, compared with 9.8% in the most deprived, p<0.001. Median z-score differed from +0.4 in the least deprived group to +0.11 in the most deprived.

The social gradient was also observed for the proportion of babies born SGA by week of birth, with the highest rates observed for babies born at 34 weeks' gestation (figure 1B). While the highest proportion of SGA babies were both preterm and born to mothers living in the most deprived areas: 16.1% of babies born at 34 weeks' gestation were SGA in socioeconomic quintile 5 (most deprived) compared with 11.0% in quintile 1 at the same gestational age; this difference did not reach statistical significance, p=0.39.

After adjustment for sex and gestational age, mothers in the most deprived areas were twice as likely to give birth to an SGA baby (OR 1.94; 95% CI 1.87 to 2.01) compared with those in

Table 2 Relationship between SGA and LGA and IMD deprivation score

	Deprivation score	Adjusted OR*	95% CI
SGA	1	Reference	–
	2	1.16	1.11 to 1.21
	3	1.34	1.28 to 1.40
	4	1.60	1.54 to 1.66
	5	1.94	1.87 to 2.01
LGA	1	Reference	–
	2	0.95	0.93 to 0.98
	3	0.88	0.86 to 0.91
	4	0.80	0.78 to 0.82
	5	0.69	0.68 to 0.71

*Adjusted for gestational age at birth and sex.
IMD, Index of Multiple Deprivation; LGA, large for gestational age; SGA, small for gestational age.

Arch Dis Child Fetal Neonatal Ed: first published as 10.1136/archdischild-2018-315295 on 21 November 2018. Downloaded from http://fn.bmj.com/ on 22 November 2018 by guest. Protected by copyright.

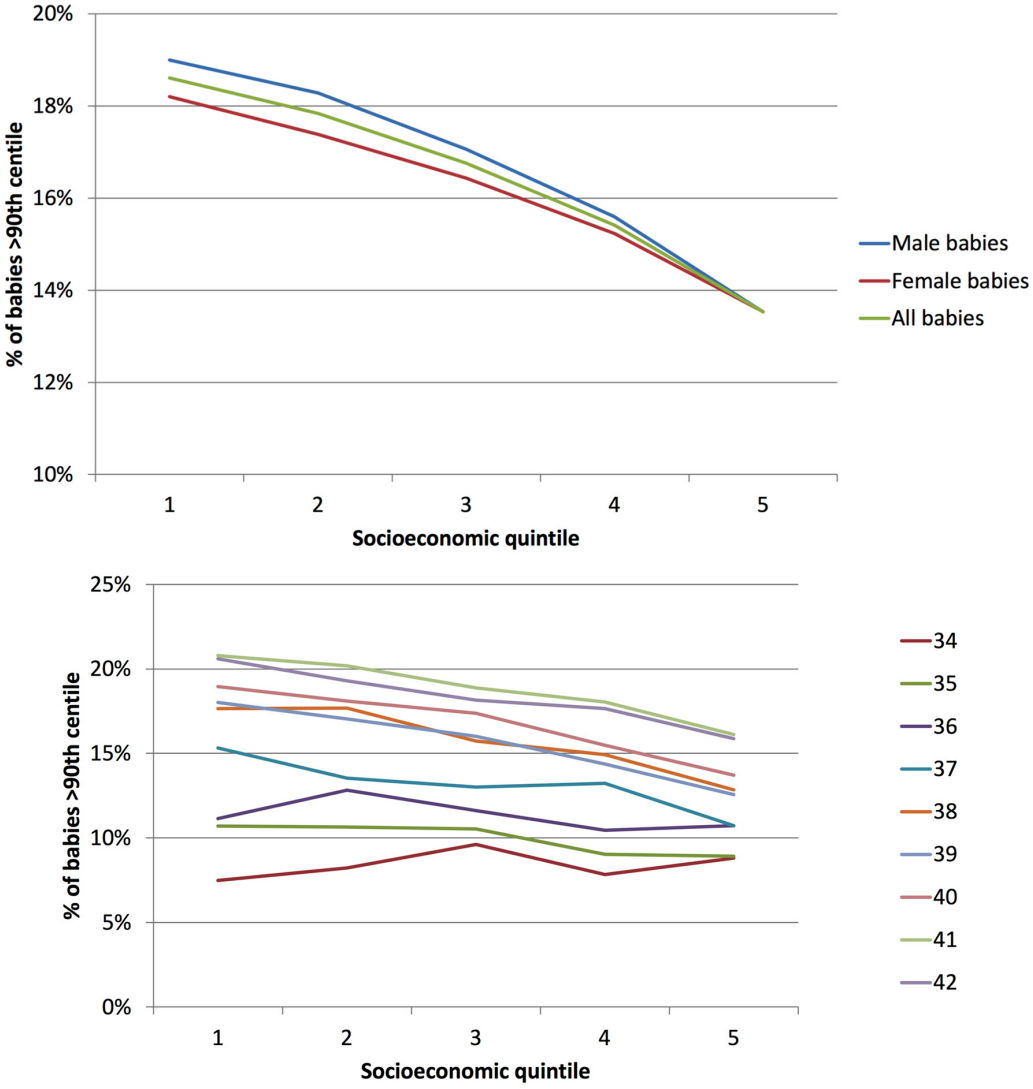


Figure 2 Proportion of large for gestational age babies by socioeconomic quintile and sex (A) and gestational age at birth (B).

the least deprived areas (table 2), with a dose–response trend observed across the social groups.

In contrast, more LGA babies were born to mothers in the least deprived areas (18.6% vs 13.5% in the most deprived areas, p value <0.001). A small differential effect was observed between the sexes for LGA, with boys more frequently LGA than girls in quintiles 1–4; however, no sex difference was observed in quintile 5 (figure 2A). There was an increase in the proportion of LGA babies across gestational age groups, with 8% of babies born at 34 weeks' gestation LGA, rising to 18% of those born at 42 weeks' gestation LGA. While this trend was observed across all socioeconomic groups, differences between the groups were only apparent after 37 weeks' gestation (figure 2B).

After adjustment for sex and gestational age, mothers in the most deprived areas were less likely to give birth to an LGA baby (OR 0.68; 95% CI 0.67 to 0.70) than those in the least deprived areas (table 2), with evidence of the reverse gradient to what was observed for SGA babies.

SGA and LGA rates differed by individual postcode area across England (figure 3A,B). Density equalising cartograms of live births combined with SGA and LGA rates demonstrate regional differences in their quantitative dimension. The predominantly urban areas with the highest number of births are emphasised with this cartographic technique. It shows the relatively higher LGA prevalence in large parts of the North and South West and higher SGA trends in the Midlands and other central urban areas, including in deprived parts of the North of England and East London.

DISCUSSION

We present the first description of birthweight for babies born in England compared with an international standard. The observation that deprived women have smaller babies is consistent with previous reports. We have quantified the socioeconomic gradient in size at birth that exists in England, highlighting that deprived women have twice the rate of SGA babies compared with the least deprived. We estimate that if pregnancy outcomes for women across the entire population of England were equal to that of women living in the most well-off areas, 12 410 (30%) fewer babies would have been born SGA in 2011–2012.

We also present evidence that birthweights in England are, on the whole, higher than those defined by the optimal birth weight standard. This has been observed in other western countries that have compared population birthweight distributions to the INTERGROWTH-21st standard.[24 25] When examined by gestational week, the proportion of babies born LGA increases

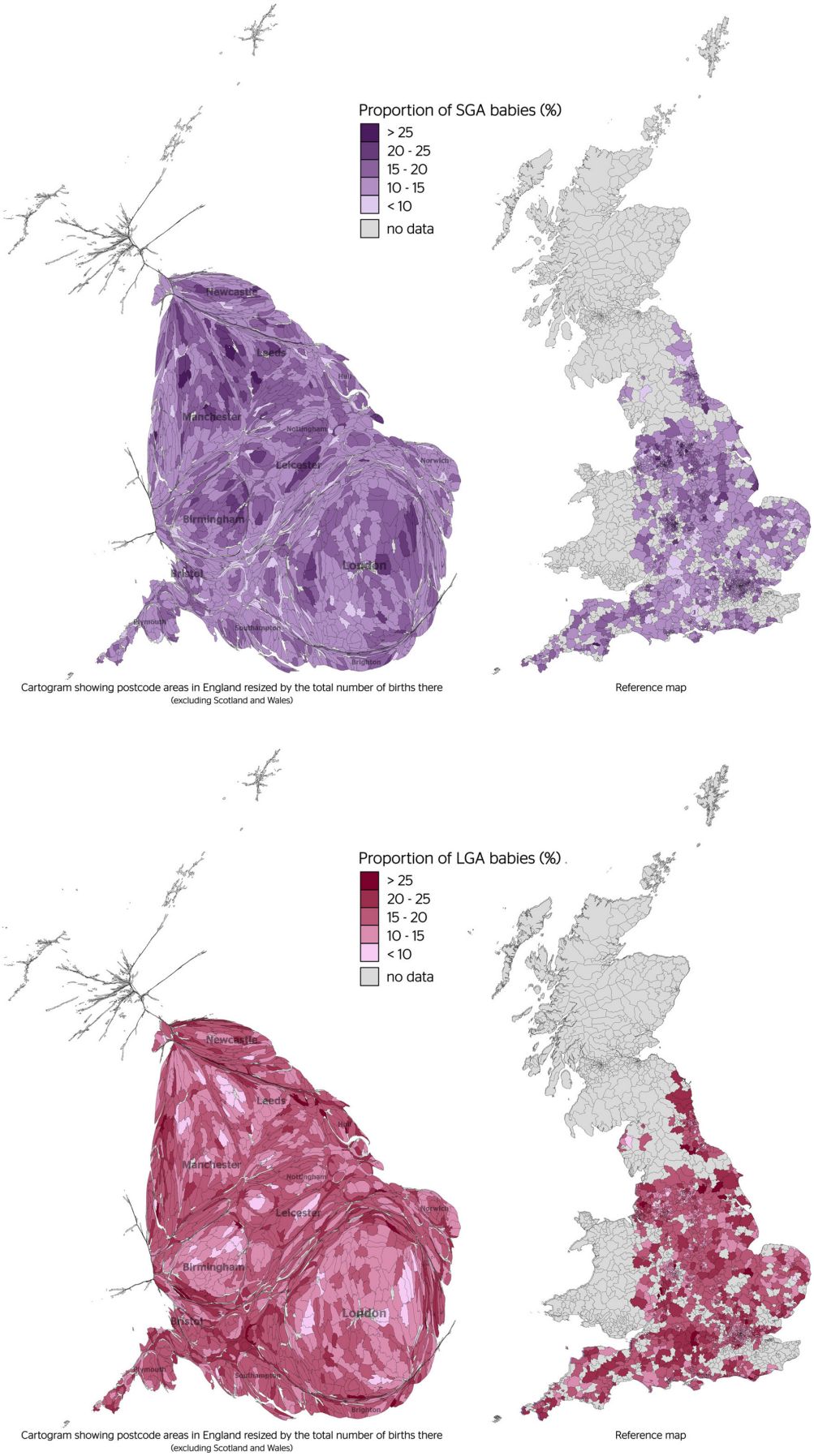


Figure 3 Cartograms showing the postcode areas in England resized by the total number of SGA births (A) and LGA births (B) with reference maps. LGA, large for gestational age; SGA, small for gestational age.

particularly after 37 weeks' gestation, correlating with the period of maximal fetal fat deposition.[26] The significance of a higher proportion of LGA babies in the population compared with the standard is not known. Given the current high rates of childhood overweight and obesity, this observation merits further analysis.

In contrast, preterm babies were more likely to be SGA, with a graded social relationship between both SGA and being born preterm. While it is plausible that a proportion of this could be explained by higher rates of risk factors such as smoking and adolescent pregnancy in more deprived groups, in practice, most cases of preterm birth and SGA do not have obvious risk factors and identifying direct causal factors can be challenging, particularly among minority groups.[27] Combinations of stress, poverty, subclinical infections, environmental pollution, poor nutrition, inadequate housing and barriers to seeking care may also be important contributory factors in reducing rates of SGA and will require a multisectoral approach.

Strengths and weaknesses of study

This study has several strengths: (1) it is population-based and nationally representative; (2) birthweight was compared with a gestational age and sex-specific standard, which avoids the confounding effect from prematurity when birthweight alone is used; and (3) birthweight in England is compared against an optimal standard for the first time. However, we also acknowledge several limitations. First, we relied on the accuracy of the recorded gestational age in the database (which was recorded to the nearest week only) and were unable to confirm the basis of this information (ie, early or late ultrasound or maternal recall of last menstrual period). To reduce the potential for a misclassification bias when calculating SGA and LGA, we elected to add 3 days to the gestational age in full weeks. This meant our estimation of the prevalence of LGA was more conservative than our previous publication[28]; however, the effect of place of birth of SGA and LGA remained unchanged. The categorisation of social standing by IMD group is based on postcode, and while a validated indicator,[29] it cannot account for individual level factors with socioeconomic status a multidimensional construct.[30] More information is needed to understand the implications of these findings to guide public health interventions and clinical practice. It is also possible that clinician interventions may have influenced the proportion of SGA babies delivered at term, with current Royal College of Obstetricians and Gynaecologists (RCOG) guidelines recommending planned delivery after 37 weeks' gestation.[31]

We defined SGA and LGA using the historical thresholds of the 10th and 90th centiles. This may be overly inclusive, given that the INTERGROWTH-21st standard was based on an optimal population. These traditional definitions relied on observations of increased perinatal mortality rate among babies within these groups, based on the observed weights and mortalities of babies born in Colorado in the 1960s.[32] True pathological growth may be better identified by the 3rd or 97th centile, corresponding to a z-score of +1.88 or −1.88, as recommended in child anthropometry.[14] In the fetus and newborn, growth may be better approximated by a change in growth centile over time[33] or body composition at birth[34] rather than a cross-sectional assessment of size at birth. Further work is urgently needed to demonstrate increased risks of adverse outcomes in childhood at different thresholds in order to inform clinical practice guidelines. A further limitation is that we present data for 1 year only. Populations are dynamic, and the point of comparing against a standard is to detect temporal changes. Future comparisons between years will now be possible as we work towards reducing inequality in society. We also focused only on singleton births, as these are the babies for whom the INTERGROWTH-21st standards were developed.

Several important pregnancy outcomes have been associated with socioeconomic status, measured by IMD, education and employment status, including perinatal mortality,[35 36] cerebral palsy[37] and preterm birth.[38] Interventions to flatten the social gradient and improve pregnancy outcomes will be complex, and include adopting a life course approach to health, nutrition, education, social justice and the rights of women and girls. While pregnancy care plays an important role, true change will require a societal shift.

Implications for policy makers and clinicians

We highlight the ongoing importance of social inequalities on pregnancy and birth outcomes in England, with clear evidence of a graded effect across social groups for babies born throughout the late third trimester. Childhood obesity has become a national epidemic, with the UK estimated to have the highest rates in Europe, with evidence of higher prevalence in more deprived groups.[39] It is recognised that SGA babies born to deprived parents are more likely to become overweight by age 7 years,[40] possibly due to poor early feeding choices and environmental factors.[41] Poor growth in utero is also linked to a number of epigenetic changes that may predispose to obesity and metabolic syndromes later in life. Correctly identifying SGA babies at birth is important to ensure parents are given the support they need to breast feed effectively and exclusively for 6 months and thereafter initiate appropriate supplementary feeding. Ultimately, however, we should try and prevent these disparities at birth occurring, and work towards ensuring that all children have the best possible start in life. We believe it is time to hold governments accountable for narrowing the gap in pregnancy outcomes within and between societies. Reporting and comparing birthweight disparities using the INTERGROWTH-21st standard is a simple yet powerful indicator to advocate for accelerated change.

Contributors JEH, SHK, HEK, ATP, EOO and JV devised the original concept. JEH wrote the original manuscript draft with subsequent conceptual and editing input from HEK, EOO, LCI, ATP, TD, JV, BDH and SHK. HEK and EOO performed the analysis. BDH performed the cartographic analysis and interpretation. All authors approved the final version prior to submission.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 McIntire DD, Bloom SL, Casey BM, *et al*. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med* 1999;340:1234–8.
2 McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 1985;312:82–90.
3 Samuelsen SO, Magnus P, Bakketeig LS. Birth weight and mortality in childhood in Norway. *Am J Epidemiol* 1998;148:983–91.
4 Harville EW, Srinivasan S, Chen W, *et al*. Is the metabolic syndrome a small baby syndrome?: the bogalusa heart study. *Metab Syndr Relat Disord* 2012;10:413–21.
5 Araújo de França GV, De Lucia Rolfe E, Horta BL, *et al*. Associations of birth weight, linear growth and relative weight gain throughout life with abdominal fat depots in adulthood: the 1982 Pelotas (Brazil) birth cohort study. *Int J Obes* 2016;40:14–21.
6 Victora CG, Adair L, Fall C, *et al*. Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* 2008;371:340–57.

7 Weissmann-Brenner A, Simchen MJ, Zilberberg E, *et al*. Maternal and neonatal outcomes of large for gestational age pregnancies. *Acta Obstet Gynecol Scand* 2012;91:844–9.
8 Mehta SH, Kruger M, Sokol RJ. Being too large for gestational age precedes childhood obesity in African Americans. *Am J Obstet Gynecol* 2011;204:265.e1–5.
9 Hawkins SS, Cole TJ, Law C, *et al*. An ecological systems approach to examining risk factors for early childhood overweight: findings from the UK Millennium Cohort Study. *J Epidemiol Community Health* 2009;63:147–55.
10 Papageorghiou AT, Ohuma EO, Altman DG, *et al*. International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project. *Lancet* 2014;384:869–79.
11 Villar J, Cheikh Ismail L, Victora CG, *et al*. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* 2014;384:857–68.
12 WHO Working Group on Infant Growth. An evaluation of infant growth: the use and interpretation of anthropometry in infants. *Bull World Health Organ* 1995;73:165–74.
13 Villar J, Papageorghiou AT, Pang R, *et al*. The likeness of fetal growth and newborn size across non-isolated populations in the INTERGROWTH-21st Project: the Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. *Lancet Diabetes Endocrinol* 2014;2:781–92.
14 de Onis M, Garza C, Onyango AW, *et al*. WHO Child Growth Standards. *Acta Paediatr Suppl* 2006;450:1–101.
15 Villar J, Papageorghiou AT, Pang R, *et al*. Monitoring human growth and development: a continuum from the womb to the classroom. *Am J Obstet Gynecol* 2015;213:494–9.
16 Marmot M, Goldblatt P, Allen J, *et al*. *Fair society, healthy lives (The Marmot Review)*: UCL Institute of Health Equity, 2010.
17 Glinianaia SV, Ghosh R, Rankin J, *et al*. No improvement in socioeconomic inequalities in birthweight and preterm birth over four decades: a population-based cohort study. *BMC Public Health* 2013;13:345.
18 Spencer N, Bambang S, Logan S, *et al*. Socioeconomic status and birth weight: comparison of an area-based measure with the Registrar General's social class. *J Epidemiol Community Health* 1999;53:495–8.
19 Astone NM, Misra D, Lynch C. The effect of maternal socio-economic status throughout the lifespan on infant birthweight. *Paediatr Perinat Epidemiol* 2007;21:310–8.
20 Dibben C, Sigala M, Macfarlane A. Area deprivation, individual factors and low birth weight in England: is there evidence of an "area effect"? *J Epidemiol Community Health* 2006;60:1053–9.
21. The English Indices of Deprivation. Communities and Local Government Neighbourhoods, 2010. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/6320/1870718.pdf
22 Hennig BD. *Rediscovering the World: Map Transformations of Human and Physical Space*. Heidelberg/New York/Dordrecht/London: Springer, 2013.
23 Gastner MT, Newman ME. From the cover: diffusion-based method for producing density-equalizing maps. *Proc Natl Acad Sci U S A* 2004;101:7499–504.
24 Anderson NH, Sadler LC, McKinlay CJD, *et al*. INTERGROWTH-21st vs customized birthweight standards for identification of perinatal mortality and morbidity. *Am J Obstet Gynecol* 2016;214:509.e1–7.
25 Liu S, Metcalfe A, León JA, *et al*. Evaluation of the INTERGROWTH-21st project newborn standard for use in Canada. *PLoS One* 2017;12:e0172910.
26 Villar J, Puglia FA, Fenton TR, *et al*. Body composition at birth and its relationship with neonatal anthropometric ratios: the newborn body composition study of the INTERGROWTH-21st project. *Pediatr Res* 2017;82:305–16.
27 Uphoff EP, Pickett KE, Wright J. Social gradients in health for Pakistani and White British women and infants in two UK birth cohorts. *Ethn Health* 2016;21:452–67.
28 Hirst JE, Villar J, Papageorghiou AT, *et al*. Preventing childhood obesity starts during pregnancy. *Lancet* 2015;386:1039–40.
29 Gordon D. Census based deprivation indices: their weighting and validation. *J Epidemiol Community Health* 1995;49:S39–44.
30 Braveman PA, Cubbin C, Egerter S, *et al*. Socioeconomic status in health research: one size does not fit all. *JAMA* 2005;294:2879–88.
31 Royal College of Obstetricians and Gynaecologists. *The investigation and management of the small-for-gestational-age fetus: Green Top Guideline No. 31*. 2nd Edn, 2013.
32 Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. *J Pediatr* 1967;71:159–63.
33 Sovio U, White IR, Dacey A, *et al*. Screening for fetal growth restriction with universal third trimester ultrasonography in nulliparous women in the Pregnancy Outcome Prediction (POP) study: a prospective cohort study. *Lancet* 2015;386:2089–97.
34 Carberry AE, Raynes-Greenow CH, Turner RM, *et al*. Is body fat percentage a better measure of undernutrition in newborns than birth weight percentiles? *Pediatr Res* 2013;74:730–6.
35 Hollowell J, Kurinczuk JJ, Brocklehurst P, *et al*. Social and ethnic inequalities in infant mortality: a perspective from the United kingdom. *Semin Perinatol* 2011;35:240–4.
36 Zeitlin J, Mortensen L, Prunet C, *et al*. Socioeconomic inequalities in stillbirth rates in Europe: measuring the gap using routine data from the Euro-Peristat Project. *BMC Pregnancy Childbirth* 2016;16:15.
37 Dolk H, Pattenden S, Bonellie S, *et al*. Socio-economic inequalities in cerebral palsy prevalence in the United Kingdom: a register-based study. *Paediatr Perinat Epidemiol* 2010;24:149–55.
38 Gray R, Bonellie SR, Chalmers J, *et al*. Social inequalities in preterm birth in Scotland 1980-2003: findings from an area-based measure of deprivation. *BJOG* 2008;115:82–90.
39 Ells LJ, Hancock C, Copley VR, *et al*. Prevalence of severe childhood obesity in England: 2006-2013. *Arch Dis Child* 2015;100:631–6.
40 Morgen CS, Andersen PK, Mortensen LH, *et al*. Socioeconomic disparities in birth weight and body mass index during infancy through age 7 years: a study within the Danish National Birth Cohort. *BMJ Open* 2017;7:e011781.
41 Goodell LS, Wakefield DB, Ferris AM. Rapid weight gain during the first year of life predicts obesity in 2-3 year olds from a low-income, minority population. *J Community Health* 2009;34:370–5.

POPULATION STUDY ARTICLE

Capturing the statewide incidence of neonatal abstinence syndrome in real time: the West Virginia experience

Amna Umer[1], Sean Loudin[2], Stefan Maxwell[3], Christa Lilly[4], Meagan E. Stabler[5], Lesley Cottrell[1], Candice Hamilton[1], Janine Breyel[6], Christina Mullins[7] and Collin John[1]

BACKGROUND: Neonatal abstinence syndrome (NAS) is one of the consequences at birth affecting the newborn after discontinuation of prenatal drug exposure to mainly opioids. The objective of this study was to determine the extent of the problem in the state of West Virginia (WV) using a real-time statewide surveillance system.
METHODS: Project WATCH is a surveillance tool that since 1998 collects data on all infants born in the state of WV. NAS surveillance item was added to the tool in October 2016. This study examined all births ($N = 23,667$) in WV from October to December 2017. The data from six WV birthing facilities were audited for 1 month to evaluate how well this tool was capturing NAS data using κ-statistics.
RESULTS: The 2017 annual incidence rate of NAS was 51.3 per 1000 live births per year for all births and 50.6 per 1000 live births per year for WV residents only. The κ-coefficient between the hospital medical records and Project WATCH data was 0.74 (95% confidence interval: 0.66–0.82) for NAS.
CONCLUSION: The study provides justification to develop effective systems of care for the mother–infant dyad affected by substance use, especially targeting pregnant women in rural communities.

Pediatric Research _####################_; https://doi.org/10.1038/s41390-018-0172-z

INTRODUCTION

Neonatal abstinence syndrome (NAS) is a multi-system withdrawal syndrome of the newborn that presents shortly after birth when in utero exposure to illegal or prescription drugs (classically opioids) is suddenly discontinued at delivery.[1,2] Some of the clinical signs that may appear within the first few days after birth and may include excessive sweating, tremors, high-pitched cry, poor feeding, watery stools, and excessive weight loss.[3,4] Negative long-term outcomes are also evident as these children have an increased risk of social and behavior abnormalities in early years,[5] as well as poorer school performances than their peers.[6]

In the United States (US), the incidence rate of NAS in number of cases per 1000 live births per year has increased from 1.2 in 2000 to 3.4 in 2009[7] to 5.8 in 2012.[8] In 2012, the estimated cost of admissions for infants diagnosed with NAS was nearly US$316 million nationally.[9] The aggregate hospital charges for NAS totaled US$1.5 billion.[8] Moreover, nearly 80% of the infants diagnosed with NAS are born to families whose health insurance coverage is through state-funded Medicaid programs,[7,9] thus escalating the burden on the already strained healthcare system.[10]

The incidence of NAS is disproportionately higher in rural counties compared to urban counties and increasing much more rapidly in rural areas compared to urban areas.[11] There are geographical disparities in NAS rates based on Appalachian mountain regional status as well; in the states of Kentucky and Tennessee, the rates of NAS were more in the Appalachian regions compared to the non-Appalachian regions.[12,13]

With the rise in the opioid epidemic nationally, the state of West Virginia (WV) has experienced a much higher rate of substance use due to the constellation of several socio-demographic factors that negatively impact health behaviors. WV is the only state that is entirely within the Appalachian mountain region and nearly half of the people in the state live in rural areas.[14,15] WV also has the highest age-adjusted drug overdose death rate in the nation.[16] A study in 2009 showed that 19% of mothers used drugs or drank alcohol during pregnancy in WV.[17] Stabler et al.[18] examined the 2007–2013 WV Health Care Authority and Uniform Billing Data for WV and found that between 2007 to 2013 the incidence of NAS increased from 7.74 per 1000 live birth per year to 31.56 per 1000 live birth per year.[18]

Most studies use administrative data based on billing and coding from medical documentation to obtain NAS estimates, which leads to a few years of lag in presenting current NAS estimates.[18–21] With the steep increase of substance use in the state of WV, this lag may lead to misrepresentation of the current crisis. The objective of the manuscript was to present the most current rate of NAS for WV as well as evaluate how well Project WATCH surveillance tool was capturing NAS data in real time for the state. Given the rise in opioid epidemic and the parallel surge in infants with NAS nationally as well as in the state of WV, the results of this study have the potential to contribute to addressing this critical public health problem in one of the hardest hit states in the country by informing policy makers as to the true extent of the problem in real time.

[1]Department of Pediatrics, School of Medicine, West Virginia University, Morgantown, WV 26506, USA; [2]Department of Pediatrics, School of Medicine, Marshall University, Huntington, WV 25701, USA; [3]PEDIATRIX Medical Group, Department of Pediatrics, WV School of Medicine (Charleston division), Women and Children's Hospital, Charleston, SC, USA; [4]Department of Biostatistics, School of Public Health, West Virginia University, Morgantown, WV 26506, USA; [5]Department of Epidemiology, The Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA; [6]West Virginia Perinatal Partnership, Department of Obstetrics and Gynecology, School of Medicine, West Virginia University, Morgantown, WV 26506, USA and [7]Office of Maternal, Child and Family Health, West Virginia Department of Health and Human Resources, Charleston, WV, USA
Correspondence: Amna Umer (amumer@hsc.wvu.edu)

Received: 9 May 2018 Revised: 20 August 2018 Accepted: 23 August 2018
Published online: 04 October 2018

© International Pediatric Research Foundation, Inc 2018

METHODS

The study used data from Project WATCH (Working in Appalachia to identify at-risk infants, Critical congenital heart disease, and Hearing loss). This project as a statewide mandate since 1998 (House Bill 2388[22]) focuses on collecting surveillance data on every infant born in WV birthing hospitals/facilities. More information about this project can be found elsewhere.[23] In October 2016, Project WATCH collaborated with The West Virginia Perinatal Partnership and the WV Department of Health and Human Resources to expand its surveillance tool to include real-time information on substance use during pregnancy and presence of NAS at the time of infant discharge from the hospital. Given that Project WATCH has been used by all WV birthing hospitals for more than 20 years, the addition of a new surveillance item to this tool that captures real-time data appeared to be an ideal way to identify infants with NAS and accurately estimate the extent of the problem in WV.

The nurses at each WV birth facility completed the questionnaire form for Project WATCH before hospital discharge. Intrauterine substance exposure (IUSE) was assessed using several possible sources (self-report, documented in medical record, or/and positive drug screening test). In September 2014, WV neonatologists, pediatricians, hospital coders, and members of the West Virginia Perinatal Partnership met to develop a standardized definition for NAS as well as guidance for documenting exposure and withdrawal in newborns. The definition that was agreed upon was as follows: NAS is diagnosed when a baby has intrauterine exposure to a neuro-active substance, and exhibited clinical signs of withdrawal, regardless of whether or not pharmacological treatment is required. Training sessions on the standardized definition for NAS were conducted statewide at all birth facilities in 2015–2016. Following the statewide training efforts, the IUSE and NAS surveillance items were added to the Project WATCH surveillance tool on 1 October 2016. Infants who had IUSE were assessed for signs of NAS consistent with the agreed upon statewide definition

This study examined all births in WV over a 15-month time period (1 October 2016 to 31 December 2017). Nearly 16% of infants were born to mothers who live in the surrounding states of Kentucky, Maryland, Ohio, Pennsylvania, and Virginia, but gave birth in WV. The data were analyzed for all births as well as births for WV residents only. Within WV, the 55 counties were clustered into six regions. These regions are based on the sub-state regions defined by the 2012–2014 National Survey on Drug Use and Health (NSDUH) conducted by the Substance Abuse and Mental Health Services Administration (SAMHSA). These regions were created to understand the geographic variability of substance use within WV, which in turn provided vital information for policy efforts.[24] Most substance use data are analyzed using these regions, rather than using county-level data since not all counties have the same level of resources and people cross county lines to access services. Regions were compared on both the prevalence of IUSE and the incidence of NAS.[25,26]

One-month data collection audit

Since Project WATCH started collecting data on substance use and NAS in October 2016, the research team wanted to assess the quality of data being recorded in Project WATCH database. Subsequently, a chart audit was performed on all births from six large birthing hospitals in WV for the month of January 2017. Detailed and standardized review instructions were provided to all hospital personnel who were to be responsible for data abstraction. Data abstraction was not blinded but data were de-identified before analysis. All births were reviewed to examine what was reported in the medical chart, which ICD-10 code was entered and what was reported by Project WATCH. The ICD-10-CM codes included P04.1–P04.4, P04.8, P04.9, and P96.1.[27] The κ-coefficient was calculated to examine the level of agreement between the hospital medical records and the data from Project WATCH.

RESULTS

The results showed that in the select project period there were 23,667 births in the state of WV. The rate of IUSE and NAS for all births and for WV residents only (84%) is shown in Fig. 1. For the WV residents only, the incidence of NAS was 52.6 per 1000 live births per year (Fig. 1). The breakdown of the data by year 2016 (3 months) and 2017 (12 months) as well as the total for the 15-month period is shown in Table 1.

The results of IUSE and NAS rates according to the six predefined SAMHSA regions are displayed in Table 2 and Fig. 2. Region 1 had one of the highest prevalence of IUSE and the highest incidence of NAS in the state. Similar patterns were not found in other regions. For example, in region 4 the prevalence of the IUSE was the lowest, but the incidence of the NAS was the second highest in the state (Table 2).

The information on IUSE was gathered from multiple sources and could include more than one source per person. Of those with IUSE ($n = 3350$), nearly two-thirds (65.76%; confidence interval (CI):

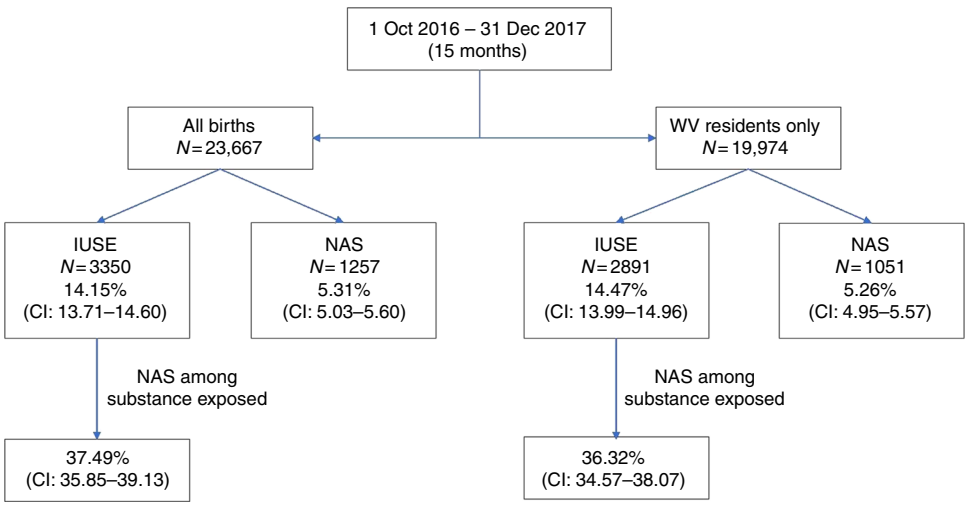


Fig. 1 Prevalence of intra-uterine substance exposure (IUSE) and incidence of neonatal abstinence syndrome (NAS) in West Virginia from 1 October 2016 to 31 December 2017 for all births ($N = 23,667$) and for West Virginia residents only ($N = 19,974$)

Table 1. Prevalence of intra-substance use exposure (IUSE) and incidence of neonatal abstinence syndrome (NAS) in West Virginia by year 2016 and 2017

Total	2016 Oct to Dec (3 months) (*N*=4,870)		2017 Jan to Dec (12 months) (*N*=18,787)		Total Oct 2016 to Dec 2017 (15 months) (23,667)	
	n	Percent (95% CI)	*n*	Percent (95% CI)	*n*	Percent (95% CI)
IUSE	720	14.78 (13.79 – 15.78)	2630	13.99 (13.50 – 14.49)	3350	14.15 (13.71 – 14.60)
NAS	295	6.06 (5.39 – 6.73)	962	5.12 (4.80 – 5.43)	1257	5.31 (5.03 – 5.60)
West Virginia only						
	(*N*=4131)		(*N*=15,843)		(*N*=19,974)	
IUSE	626	15.15 (14.06–16.25)	2265	14.30 (13.75–14.84)	2891	14.47 (13.99–14.96)
NAS	249	6.03 (5.30–6.75)	820	5.06 (4.72–5.40)	1051	5.26 (4.95–5.57)

Table 2. Prevalence of intra-substance use exposure (IUSE) and incidence of neonatal abstinence syndrome (NAS) in West Virginia (October 2016 to 31 Dec 2017) by SAMHSA sub-state regional classification system, $N = 19{,}974$

Regions	Population frequency (%)	IUSE % (95% CI)	Rank	NAS % (95% CI)	Rank	NAS % among IUSE (95% CI)	Rank
1	1379 (6.92%)	18.49 (16.44–20.54)	1	8.41 (6.95–9.88)	1	45.49 (39.38–51.60)	1
2	1854 (9.30%)	15.32 (13.68–16.96)	2	4.21 (3.29–5.12)	5	27.46 (22.27–32.66)	5
3	1801 (9.04%)	14.99 (13.34–16.64)	3	2.67 (1.92–3.41)	6	17.78 (13.22–22.34)	6
4	4871 (24.44%)	13.49 (12.53–14.45)	6	5.79 (5.13–6.45)	2	42.77 (38.99–46.55)	2
5	6171 (30.96%)	13.79 (12.93–14.65)	5	5.46 (4.89–6.03)	3	39.60 (36.31–42.89)	3
6	3853 (19.33%)	14.69 (13.57–15.81)	4	4.88 (4.20–5.56)	4	33.22 (29.34–37.10)	4
Total	19,929	14.47 (13.98–14.95)		5.26 (4.95–5.57)		36.35 (34.60–38.11)	
Missing	45	45		45		8	

64.15–67.37) had a positive drug screen during their labor and delivery hospital admission and nearly half of the women self-reported (52.72%; CI: 51.03–54.14) or had a positive substance use information in their past medical records (50.90%; CI: 49.20–52.59).

One-month data collection audit

There were 789 births in the six hospitals where the chart audits were performed. This represented 52% of 1521 births in Project WATCH database for all birth in January 2017 in WV. Out of 133 IUSE cases identified in the hospital medical records, 107 (81%) were identified as IUSE cases by the Project WATCH surveillance data tool (κ-coefficient = 0.85 (CI: 0.83–0.91)). For the NAS data, out of 79 cases identified in the hospital medical records, 56 (71%) were identified as NAS cases by the Project WATCH surveillance tool (κ-coefficient = 0.74 (CI: 0.66–0.82). (Table 3). The ICD-10-CM code for NAS was recorded by less than half (47.25%) of the infants diagnosed with NAS in the combined data for the six hospitals.

DISCUSSION

The study used a real-time surveillance system to assess the extent of NAS in a state that has been significantly impacted by the current opioid crisis. The rate of NAS was 53 cases per 1000 live births per year, which was much higher than rate of NAS reported in earlier studies in 2013, that is, nearly 30 per 1000 live births per year.[18,19] This rise was expected, as national trends show a significant increase in NAS diagnoses as well. However, based on the discordance between coding data and Project WATCH data, there is a level of concern that the earlier rates may have been grossly underestimated. WV is a rural Appalachian state and the results from this study showed that NAS rate was seven times higher in WV than what was found in rural regions of the country in 2013 (i.e., nearly 7 per 1000 live births per year).[11] Moreover, this study demonstrated that the rate of NAS in WV is one of the highest in the country and nearly 10-fold the national estimate of 5.8 per 1000 births live births per year in 2012.[8]

The rate of IUSE in WV was 14%, which is consistent with a study conducted in 2009.[17] Stitely et al.[17] examined umbilical cord tissues of women who gave birth in 1 month in eight birth hospitals in WV and found 14% of mothers were positive for drug use. Based on the rising opioid epidemic in the state, we hypothesized that in 2017 the prevalence would be much higher than what was observed in 2009. One of the likely reasons for the underestimated prevalence in our study is the fact that data on IUSE was gathered through self-report, previous documentation, or drug testing at certain hospitals compared to the earlier study that used universal umbilical cord tissue samples.[17] WV does not have a mandated maternal drug-screening program.

Geographical disparities in the rates of IUSE and NAS were also observed. The northern panhandle (region 1) has one of the highest rates of IUSE as well as NAS incidence. The northern central region 4 has the lowest prevalence of IUSE, but the second highest NAS rates. We postulate that the inconsistency in this particular region likely arises from a combination in obtaining accurate drug history data from mother as well as the possibility that physicians in region 4 may be more conservative in diagnosing NAS. Interestingly, our regional results are not consistent with the results from Stabler et al.,[18] as they found the southern region 4 and region 5 had the highest rates of NAS in 2013.[18] Some of the reasons for this discrepancy may be, (1) the use of administrative data source (ICD-9-CM codes) compared to the current study that uses the diagnosis made at the hospital and recorded by the nurses before hospital discharge, and (2) the likely steep increase of incidence between 2013 and 2017. Furthermore, in 2017 the high-intensity drug trafficking areas (HIDTAs) in WV include regions 1, 5, and 6, which may explain the high prevalence of IUSE and NAS in region 1, but nonetheless fails to explain the lower incidence in regions 5 and 6 in the current study.[28]

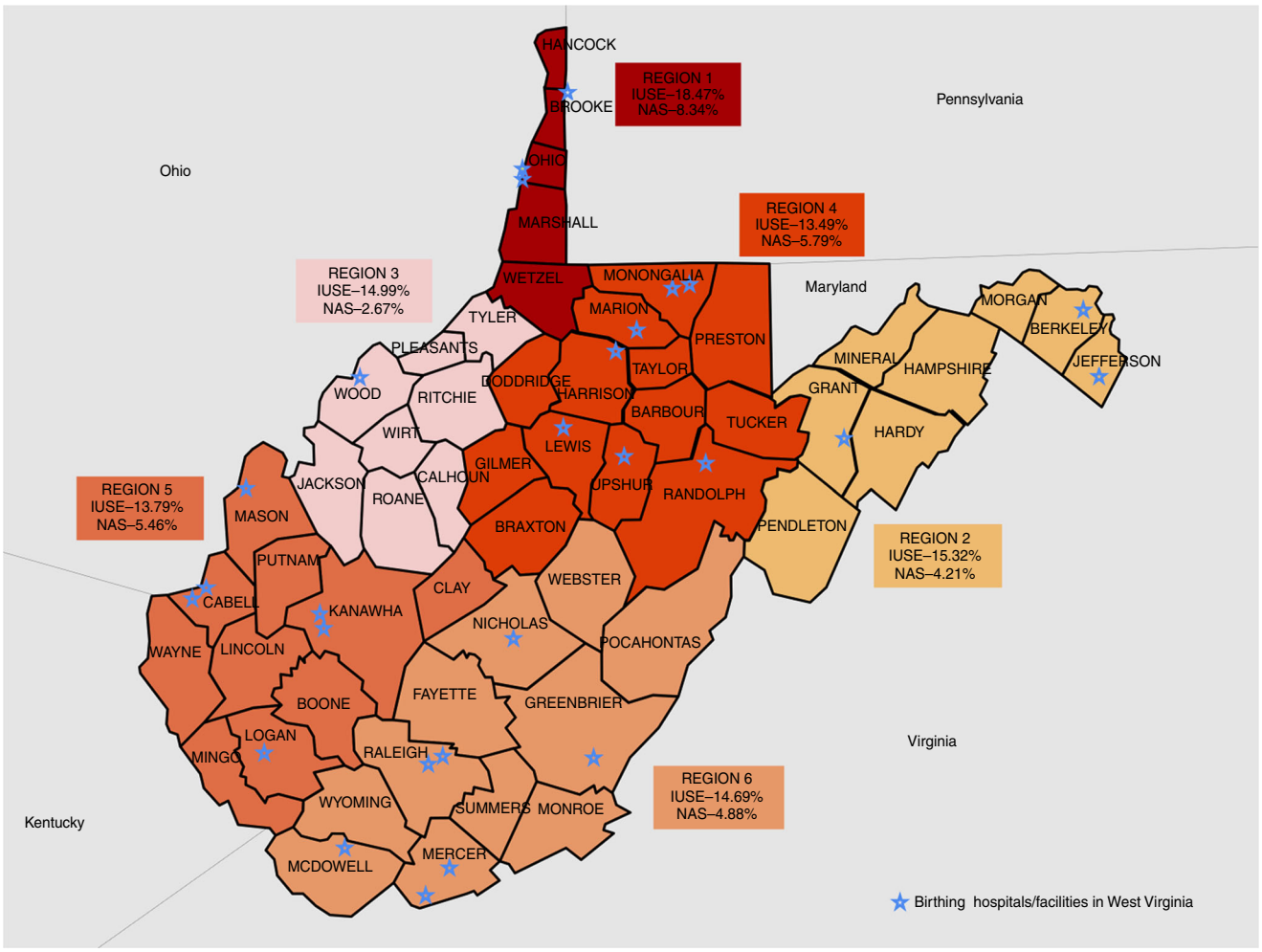


Fig. 2 Project WATCH surveillance data on prevalence of intra-uterine substance exposure (IUSE) and neonatal abstinence syndrome (NAS) in West Virginia by SAMHSA sub-state regional classification system, $N = 20{,}002$

Table 3. One-month (January 2017) audit data for six hospitals in the state of WV comparing the project WATCH data with the hospital medical records and the ICD-10-CM codes ($N = 789$)

Data source	Frequency	Percent (95% CI)
Intrauterine substance exposure		
Hospital data	134	17.07 (14.36–19.60)
Birth Score data	111	14.14 (11.64–16.49)
ICD-10-CM code in chart—code P04.x	57	7.23 (5.42–9.03)
Neonatal abstinence syndrome		
Hospital data	79	10.1 (7.92–12.11)
Birth Score data	67	8.55 (6.55–10.44)
ICD-10-CM code in chart—code P96.1	43	5.46 (3.87–7.03)

The results of the audit found that Project WATCH was capturing the prevalence of IUSE and incidence of NAS reasonably well. However, the agreement between the hospital chart review and the Project WATCH data for IUSE was higher than the agreement between the Project WATCH data and NAS. One likely explanation for this observation is that one of the six hospitals, which incidentally had one of the highest contributions of birth data to the audit, ultimately contributed a higher rate of NAS, which may be reflective of biases from the chart reviewers. Project WATCH had already used this audit data for quality improvement of its data collection process by training nurses on data entry and report. The audit data also revealed that only half of the infants diagnosed with NAS were identified utilizing the ICD-10-CM codes. Other studies have also shown that administrative data diagnostic codes under-represent the actual number of NAS cases diagnosed in the hospital.[20]

Based on our results, Project WATCH appears to capture IUSE and NAS diagnoses via a novel, accurate, and cost-effective electronic surveillance tool. Although these data does not include births delivered outside the hospital (~0.01%), Project WATCH collects data on every infant born in WV birthing hospitals/facilities. This new data source shows improved ability to capture NAS cases compared to the traditional method of utilizing electronic claims data via ICD codes. Additionally, Project WATCH data are captured in real time allowing for timely surveillance of a leading public health issue in the state of WV. WV is one of a few states that collects surveillance data on NAS; four additional states (Florida, Georgia, Kentucky, and Tennessee) have legally mandated NAS as a reportable condition and have a passive surveillance system in place.[29] States without a specific surveillance system for NAS report the condition via traditional methods such as diagnostic codes and hospital billing records (e.g., the State Hospital Discharge Database or State All Payer Claims data). Traditional methods used to survey the burden of NAS may have

several months of lag from data collection to analysis.[18–21] A real-time estimation of NAS has the potential to guide targeted resources and interventions in a timely manner.

Some of the limitations include the fact that Project WATCH does not gather information on the type(s) of specific substances that the infant is exposed to in utero. It is likely that many of the infants in this study were exposed to multiple substances simultaneously. Although the effect of polysubstance use on the occurrence of NAS is controversial,[30] results from a large population-based cohort study showed significant increases in the risk of NAS associated with opioid use along with co-exposure to antidepressants, benzodiazepines, and gabapentin compared with opioids alone.[31] However, given the main purpose of the study was to document the incidence of NAS, this was not felt to be a significant limitation. Moreover, despite a standardized statewide definition of NAS, there is a degree of subjectivity and individual variability in approaches when making NAS diagnosis and these differences in identification approaches are difficult to assess.[32] Data collection for Project WATCH started in October 2016 and therefore cannot be retrospectively compared to traditional hospital billing data. Future research comparing Project WATCH and hospital billing data utilizing ICD codes is warranted. Moreover, non-punitive policies that enable early identification of NAS, improve access to comprehensive prenatal care and opioid replacement therapy, and increase funding for child welfare systems are also warranted. Additional resources are needed, especially to those sub-state regions with the highest rates of substance use and NAS. In addition, other states may benefit from a similar NAS surveillance system used by Project WATCH in WV.

ACKNOWLEDGEMENTS

The West Virginia WATCH/Birth Score Program is funded under an agreement with the West Virginia Department of Health and Human Resources, Bureau for Public Health, Office of Maternal, Child, and Family Health.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Kocherlakota, P. Neonatal abstinence syndrome. *Pediatrics* **134**, e547–e561 (2014).
2. Convertino, I. et al. Neonatal adaptation issues after maternal exposure to prescription drugs: withdrawal syndromes and residual pharmacological effects. *Drug Saf.* **39**, 903–924 (2016).
3. Logan, B. A., Brown, M. S. & Hayes, M. J. Neonatal abstinence syndrome: treatment and pediatric outcomes. *Clin. Obstet. Gynecol.* **56**, 186–192 (2013).
4. McQueen, K. & Murphy-Oikonen, J. Neonatal abstinence syndrome. *N. Engl. J. Med* **375**, 2468–2479 (2016).
5. Klinger, G. et al. Long-term outcome following selective serotonin reuptake inhibitor induced neonatal abstinence syndrome. *J. Perinatol.* **31**, 615–620 (2011).
6. Oei, J. L. et al. Neonatal abstinence syndrome and high school performance. *Pediatrics* **139**, e20162651 (2017).
7. Patrick, S. W. et al. Neonatal abstinence syndrome and associated health care expenditures: United States, 2000–2009. *JAMA* **307**, 1934–1940 (2012).
8. Patrick, S. W., Davis, M. M., Lehmann, C. U. & Cooper, W. O. Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009 to 2012. *J. Perinatol.* **35**, 650–655 (2015).
9. Corr, T. E. & Hollenbeak, C. S. The economic burden of neonatal abstinence syndrome in the United States. *Addiction* **112**, 1590–1599 (2017).
10. Ridic, G., Gleason, S. & Ridic, O. Comparisons of health care systems in the United States, Germany and Canada. *Mater. Sociomed.* **24**, 112–120 (2012).
11. Villapiano, N. L., Winkelman, T. N., Kozhimannil, K. B., Davis, M. M. & Patrick, S. W. Rural and urban differences in neonatal abstinence syndrome and maternal opioid use, 2004 to 2013. *JAMA Pediatr.* **171**, 194–196 (2017).
12. Brown, J. D., Goodin, A. J. & Talbert, J. C. Rural and appalachian disparities in neonatal abstinence syndrome incidence and access to opioid abuse treatment. *J. Rural Health* **34**, 6–13 (2017).
13. Warren, M. D. et al. Implementation of a statewide surveillance system for neonatal abstinence syndrome—Tennessee, 2013. *Morb. Mortal. Wkly Rep.* **64**, 125–128 (2015).
14. Kaiser Family Foundation. *KFF Medicaid's Role in West Virginia* (Kaiser Family Foundation, Washington, 2018).
15. Griffith, B. N., Lovett, G. D., Pyle, D. N. & Miller, W. C. Self-rated health in rural Appalachia: health perceptions are incongruent with health status and health behaviors. *BMC Public Health* **11**, 229 (2011).
16. Hedegaard, H., Warner, M. & Minino, A. M. Drug overdose deaths in the United States, 1999–2015. *NCHS Data Brief* 1–8 (2017).
17. Stitely, M. L., Calhoun, B., Maxwell, S., Nerhood, R. & Chaffin, D. Prevalence of drug use in pregnant West Virginia patients. *W V Med. J.* **106**, 48–52 (2010).
18. Stabler, M. E. et al. Neonatal abstinence syndrome in West Virginia Substate Regions, 2007–2013. *J. Rural Health* **33**, 92–101 (2017).
19. Ko, J. Y. et al. Incidence of neonatal abstinence syndrome—28 States, 1999–2013. *Morb. Mortal. Wkly Rep.* **65**, 799–802 (2016).
20. Burns, L. & Mattick, R. P. Using population data to examine the prevalence and correlates of neonatal abstinence syndrome. *Drug Alcohol Rev.* **26**, 487–492 (2007).
21. Traylor, J., Miller, A. & Warren, M. D. Evaluation of the web-based neonatal abstinence syndrome surveillance system in Tennessee. In *Conference: 2014 Council of State and Territorial Epidemiologists Annual Conference* (2014). https://www.wvdhhr.org/birthscore/.
22. Article 22B. Birth Score Program (1998). https://www.wvdhhr.org/birthscore/.
23. Mullett, M. D., Britton, C. M., John, C. & Hamilton, C. W. WV birth score: maternal smoking and drugs of abuse. *W V Med. J.* **106**, 16–18, 20 (2010).
24. Zhang, Z., Infante, A., Meit, M. & English, N. *An Analysis of Mental Health and Substance Abuse Disparities and Access to Treatment Services in the Appalachian Region. CHAPTER 2: Substance Use, Mental Disorders, and Access to Treatment Services in Household Surveys* (2008).
25. The Governor's Initiative on Substance Abuse (2017). http://governorssubstanceabusetaskforceswv.com.
26. SAMHSA 2016 State Reports From the 2014 National Survey on Drug Use and Health (NSDUH). https://www.samhsa.gov/data/nsduh/reports-detailed-tables-2016-NSDUH.
27. ICD-10-CM Codes. (2018). https://www.icd10data.com/ICD10CM/Codes.
28. HIDTA 2017 High Intensity Drug Trafficking Areas Program (HIDTA), ONDCP. Available at https://www.dea.gov/ops/HIDTA_2017_map.pdf. Accessed on 21 Jan 2018.
29. Ko, J. Y. et al. CDC grand rounds: public health strategies to prevent neonatal abstinence syndrome. *Morb. Mortal. Wkly Rep.* **66**, 242–245 (2017).
30. American Academy of Pediatrics Committee on Drugs. Neonatal drug withdrawal. *Pediatrics* **101**,1079–1088 (1998).
31. Huybrechts, K. F. et al. Risk of neonatal drug withdrawal after intrauterine co-exposure to opioids and psychotropic medications: cohort study. *BMJ* **358**, j3326 (2017).
32. Sarkar, S. & Donn, S. M. Management of neonatal abstinence syndrome in neonatal intensive care units: a national survey. *J. Perinatol.* **26**, 15–17 (2006).

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

Modulators of inflammation in Bronchopulmonary Dysplasia

Rashmin C. Savani

Center for Pulmonary & Vascular Biology, Division of Neonatal-Perinatal Medicine, The Department of Pediatrics, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9063, USA

ARTICLE INFO

ABSTRACT

Over 50 years after its first description, Bronchopulmonary Dysplasia (BPD) remains a devastating pulmonary complication in preterm infants with respiratory failure and develops in 30–50% of infants less than 1000-gram birth weight. It is thought to involve ventilator- and oxygen-induced damage to an immature lung that results in an inflammatory response and ends in aberrant lung development with dysregulated angiogenesis and alveolarization. Significant morbidity and mortality are associated with this most common chronic lung disease of childhood. Thus, any therapies that decrease the incidence or severity of this condition would have significant impact on morbidity, mortality, human costs, and healthcare expenditure. It is clear that an inflammatory response and the elaboration of growth factors and cytokines are associated with the development of BPD. Numerous approaches to control the inflammatory process leading to the development of BPD have been attempted. This review will examine the anti-inflammatory approaches that are established or hold promise for the prevention or treatment of BPD.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Bronchopulmonary Dysplasia is a complex condition that has a multifactorial pathogenesis. Key contributors include oxygen toxicity, ventilator-induced lung injury, inflammation and an arrest of lung vascular development.[1,2] Inflammation, an accumulation of neutrophils and macrophages into the injured preterm lung, results in the elaboration of pro-inflammatory and pro-fibrotic growth factors, an imbalance of the proteolytic activity in the lung and increased vascular permeability.[3] Neutrophil and macrophage accumulation commences early in the first week of life and counts remain elevated in infants that develop BPD.[4] Coordinated development of the distal epithelial and vascular compartments is a necessity for the final ability to conduct adequate gas exchange. Lung microvascular development is critical to normal secondary septation leading to alveolar formation. Multiple animal models and examination of autopsy samples of infants with BPD have confirmed an arrest of vascular development and a simplification of the distal lung architecture as a result of preterm birth and associated lung injury.[5] Indeed, alveolar septation can be preserved in animal models by decreasing the effects of contributors to BPD pathogenesis, including inflammation, oxygen and mechanical ventilation.[6] The pathogenesis of BPD involves the effects of these multiple pathologic mechanisms on lung development.

A number of models of BPD have been developed. Two large animal models, the sheep and baboon preterm birth and ventilation models, recapitulate the essential features of the human condition, including preterm birth, oxygen exposure, mechanical ventilation, inflammation and arrested lung

E-mail address: rashmin.savani@utsouthwestern.edu

https://doi.org/10.1053/j.semperi.2018.09.009
0146-0005/© 2018 Elsevier Inc. All rights reserved.

development.[7] However, these models do not support genetic studies to identify the molecular determinants of BPD. Therefore, rodent models of newborn lung injury, that involve either hyperoxia or hypoxia exposure for up to 14 days and result in pathology that mimics BPD, were established.[8–10] Further, a model in which bleomycin, administered intraperitoneally to neonatal rodents, also recapitulates the features of BPD.[11] While these rodent models do not involve preterm birth or ventilation, several groups have confirmed changes in inflammation, cytokines, oxidative stress, and vascular defects in association with alveolar simplification that validate the use of these models in the study of BPD.[8–11] The fact that alveolar development is entirely a postnatal event occurring in the first 2–3 weeks of life in rodents, provides an appropriate window to examine the effects of injury on the alveolar phase of the developing lung. More recently, the use of high oxygen exposures during the first 14 days of life has been questioned since human preterm infants are exposed to these conditions only during the saccular phase of lung development.[12,13] Thus, more appropriate oxygen exposure in the first four to five days of life in rodents have been developed and also demonstrate inflammatory changes and alveolar defects.[14,15]

The recruitment of inflammatory cells to the injured lung requires activation of bone marrow-derived cells, circulating leukocytes and the endothelium. Initiation of injury results in initial rolling, then firm adhesion of inflammatory cells to the activated endothelium. This is followed by transmigration of leukocytes through the endothelium and their accumulation in areas of injury. Initially recruited neutrophils undergo apoptosis and alveolar macrophages engulf these cells. This process results in the production of active transforming growth factor beta (TGFß) which promotes fibrosis. A number of key molecules have been identified in the process of inflammatory cell recruitment and include selectins, integrins, cytokines and growth factors, the innate immune system including Toll-Like Receptors (TLRs), Surfactant Protein A (SPA) and SPD, as well as components of the extracellular matrix.[16,17] Macrophage subsets demonstrating differential functions have been identified. Classically activated macrophages (CAMs), also called M1 macrophages, result from TLR stimulation, bacterial infection and interferon-gamma stimulation. Alternatively activated macrophages (AAMs), also called M2 macrophages, result from Th2 cytokine (e.g. interleukin or IL4 and IL13) stimulation.[18] Specific markers for M1 and M2 macrophages have emerged. For instance, M1 macrophages can be identified by the expression of inducible nitric oxide synthase (iNOS) and CD11c, whereas M2 macrophages can be identified by the expression of Arginase 1 or YM1 (also known as chitinase-like 3 or Chil3).[19,20] While M1 macrophages have potent microbicidal properties and promote Th1 responses, M2 macrophages play a role in the resolution of inflammation, with high endocytic clearance capacity and reduced pro-inflammatory cytokine secretion. Given the large predominance of inflammation in the pathogenesis of BPD, considerable efforts have been made to develop anti-inflammatory strategies, ranging from glucocorticoids to newer more novel therapeutic targeting of components of the innate immune system. The pathogenesis of BPD and the multiple anti-inflammatory strategies discussed in this review are summarized in Fig. 1.

Non-invasive respiratory support

The contribution of intubation and mechanical ventilation to inflammation and the incidence and severity of BPD has been established in animal models and in human preterm infants.[21,22] Mouse and lamb models show that mechanical ventilation alone without hyperoxia or lipopolysaccharide (LPS) exposure is sufficient for the development of lung inflammation, as well as altered alveolarization and lung function.[23,24] Indeed, respiratory management with continuous positive airway pressure (CPAP) in baboons and mice, or high frequency, non-invasive ventilation in lambs is associated with less inflammation and better alveolarization.[25–27] In large clinical trials, the incidence of BPD in infants randomized to either non-invasive respiratory support or selective intubation and surfactant therapy were found to be equivalent, findings that have pushed changes in neonatal practice significantly in favor of non-invasive approaches to respiratory management.[28–30]

Corticosteroids

First described in sheep in 1969[31] and in a human trial in 1972, antenatal glucocorticoid therapy to enhance fetal lung maturity was associated with decreased incidence of Respiratory Distress Syndrome (RDS) and death.[32] Indeed, this therapy Is also associated with decreased intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and early sepsis.[33] Given the early descriptions of inflammation in infants with BPD, postnatal glucocorticoid therapy to decrease or abrogate the inflammatory response, improve lung function and increase the likelihood of extubation failure has been examined since the early 1970's. While benefits of early (in the first week of life) corticosteroid therapy in preterm infants at risk of developing BPD include decreased incidence of BPD at 28 days and at 36 weeks postmenstrual age (PMA), decreased rates of failure of extubation and decreased incidence of patent ductus arteriosus (PDA) and retinopathy of prematurity (ROP), significant adverse events have been noted including hyperglycemia, hypertension, hypertrophic cardiomyopathy, growth failure, intestinal perforation and bleeding, as well as increased incidence of cerebral palsy.[34,35] The use of postnatal steroids in infants that remain intubated past 7–14 days of life has also been reviewed recently.[36] Similar benefits with respect to decreased rates of death or BPD at 28 days and 36 weeks PMA and adverse outcomes including hyperglycemia and hypertension were found, but longer term cerebral palsy or neurodevelopmental complications were not significantly different from those of controls.[35] However, given the considerable adverse effects described, the current recommendations are for judicious use of postnatal corticosteroid therapy. Further, although difficult to demonstrate in preterm infants, dexamethasone treatment of newborn rodents is associated with an arrest of alveolarization that persists into adulthood.[37–39]

Caffeine

The methylxanthines caffeine, aminophylline and theophylline bind to adenosine receptors and act as non-selective adenosine

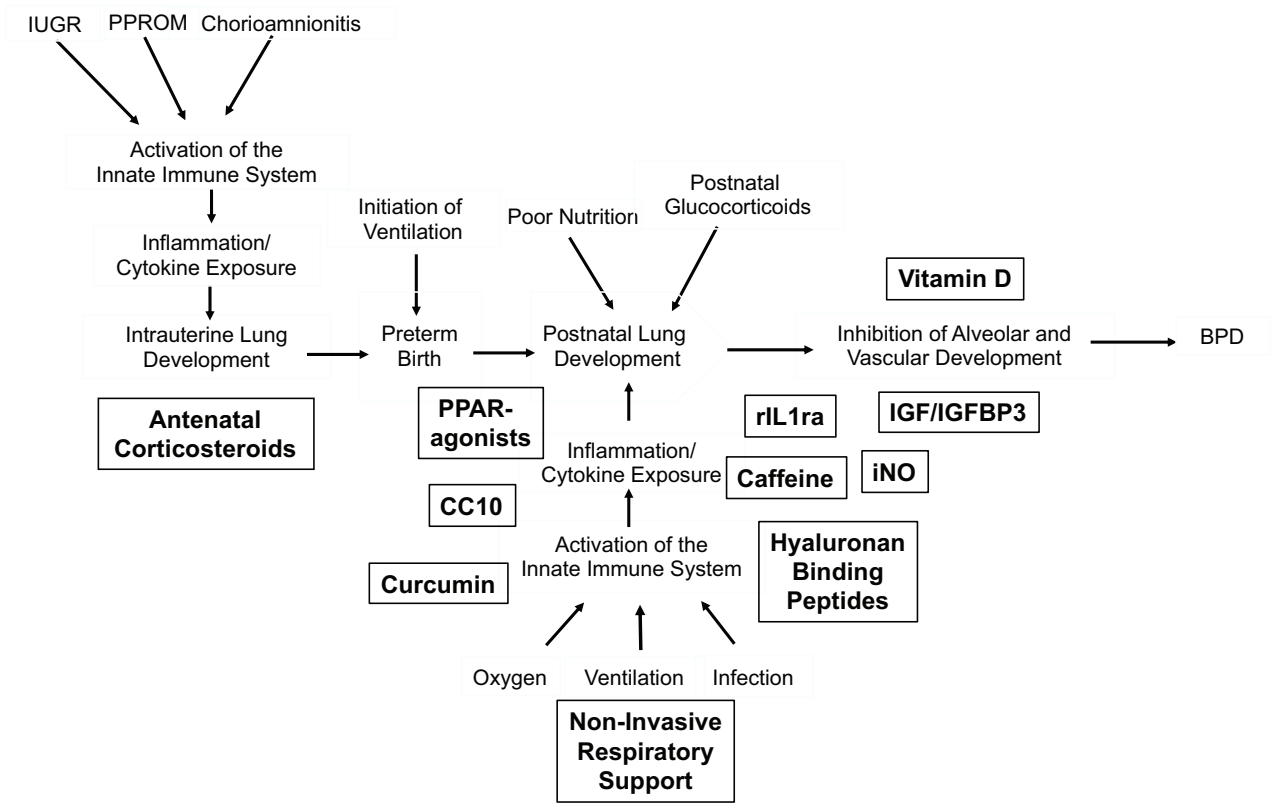


Fig. 1 – Pathogenesis of BPD and anti-inflammatory interventions. Lung development occurs in five distinct but overlapping phases. Several antenatal factors can adversely influence lung development and include intrauterine growth restriction (IUGR), prolonged preterm rupture of membranes (PPROM) and chorioamnionitis. These factors all appear to activate the innate immune system and increase the expression of cytokines that result in inflammation. Among a multitude of effects, antenatal corticosteroid therapy accelerates lung development, in particular, the surfactant system. Preterm birth is often associated with initiation of ventilation and exposure to oxygen and an increased incidence of infections, all of which also result in the activation of the innate immune system and the elaboration of cytokines that drive inflammation. Lung development now has to occur ex-utero, is influenced by this inflammatory milieu, and results in an inhibition of alveolar and vascular development recognized as BPD. A number of strategies and approaches have been investigated to abrogate or dampen the inflammatory response. These are indicated in bold boxes in the figure and are discussed in the review. For additional abbreviations, please see the text.

antagonists.[40] Caffeine also inhibits phosphodiesterases leading to the accumulation of cAMP and cGMP.[41] These two mechanisms likely explain the different neurologic and respiratory effects of caffeine respectively.[40] Despite reports of potential harmful effects of caffeine including tachycardia, growth failure, neurologic energy failure and long-term behavioral problems,[42] this drug was widely used to stimulate the respiratory center of preterm infants in the treatment of apnea of prematurity. The Caffeine for Apnea of Prematurity or CAP Trial established, in a randomized controlled manner, that caffeine therapy in preterm infants 500–1250 g birth weight was associated with a decrease in the incidence of BPD and PDA, and although a lower weight gain was noted in treated infants at two weeks of age, this was not sustained and there were no differences in mortality, head ultrasound findings of injury or NEC.[43] Indeed, long-term follow up of caffeine-treated infants in this trial has demonstrated a reduced incidence of motor impairment and no effect on academic or behavioral outcomes at 11 years of age.[44]

While an increased respiratory drive most likely contributed to the favorable outcomes in caffeine-treated infants, other mechanisms for the effects of caffeine have been proposed. Thus, caffeine can increase diaphragmatic contractility,[45] has a diuretic effect,[46] can protect neuronal injury,[47,48] and can even transcriptionally induce SP-B.[49,50] Interestingly, however, caffeine has anti-inflammatory effects in newborn animals. Thus, hyperoxia exposure of six-day old rat pups induced cytokine and inflammatory cell recruitment, both of which were inhibited by administration of caffeine.[51] Additionally, premature rabbits exposed to hyperoxia and treated with caffeine had less inflammation and improved alveolarization compared to hyperoxia-exposed controls.[52] Indeed, postnatal inflammation driven by LPS-induced amnionitis was also abrogated by caffeine treatment, although alveolar simplification was not.[53] Thus, caffeine has significant anti-inflammatory effects though the mechanisms of these effects are unclear to date.

Nitric oxide (NO)

NO, generated by nitric oxide synthases (neuronal or nNOS, endothelial or eNOS and iNOS) upon the conversion of L-arginine to L-citrulline, plays a key role in developmental and physiological processes in the lung.[54,55] Studies in the mature airway indicate that the epithelium is the primary source of NO[56] and that NO mediates neurotransmission, smooth muscle relaxation, bacteriostasis, ciliary motility, mucin secretion, and plasma exudation.[54,55,57] Elegant *in vitro* studies from the Shaul/Mineo laboratories implicate NO in anti-inflammatory processes such as inhibition of monocyte adhesion to activated endothelium and platelet aggregation.[58,59] Indeed, inhaled NO (iNO) attenuates pulmonary inflammation in a neonatal rat hyperoxia model.[60]

NO plays an important role in pulmonary vascularization, alveolarization, and airway branching.[61–66] NO also has a well-recognized role in mediating pulmonary vasomotor tone around the time of birth.[67] In studies of lungs from fetal baboons, pulmonary NOS expression is upregulated during normal fetal development. However, in the baboon model of BPD, expression of nNOS and eNOS was significantly decreased, and iNO given at 5ppm improved pulmonary artery pressures, pulmonary mechanics, lung elastin deposition and was associated with modest increases in secondary septation.[68] Based on these and other animal and early stage human studies,[22,64,69,70] multiple trials of iNO therapy have been performed in human preterm infants to prevent BPD.[71–74] These have yielded mixed results and the use of iNO is not currently recommended for the prevention of BPD.

Vitamin D

Vitamin D or cholecalciferol is a fat-soluble vitamin that is synthesized from 7-dehydrocholesterol in the skin after ultraviolet B exposure or absorbed from the diet. Vitamin D is converted to 25-hydroxy-vitamin D in the liver and to the biologically active 1,25-hydroxy-vitamin D or calcitriol in the kidney. The effects of 1,25-OH-vitamin D are mediated by a nuclear receptor, the vitamin D receptor (VDR) that binds to Vitamin D-responsive elements to regulate the transcription of a wide number of genes. Vitamin D deficiency is highly prevalent, particularly in pregnancy and has been associated with a number of adverse outcomes such as poor maternal weight gain, preeclampsia, increased rates of operative delivery and shorter gestation.[75] In addition, the impact of vitamin D deficiency on lung development and health is evident in VDR knockout mice that have an imbalance of matrix metalloproteinases and develop emphysema and inflammation.[76,77]

More recently, direct demonstration of the importance of Vitamin D in pregnancy and BPD has been obtained from experiments in which antenatal Vitamin D supplementation was associated with improved placental vascularization and fetal growth.[78] Postnatal Vitamin D supplementation was associated with an improvement in alveolarization in rats that were exposed to intra-amniotic LPS,[79] and Vitamin D induced downregulation of TLR4 and therefore decreased downstream cytokines such as IL1ß in neonatal rats exposed to hyperoxia.[80] The effects of Vitamin D on the activation of eNOS likely contribute to the beneficial observations with Vitamin D both on the placenta and postnatal vascular and alveolar growth.[81] Interestingly, a recent report demonstrated beneficial effects of nebulized Vitamin D to enhance lung maturation.[82]

Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists

PPAR-γ is a key anti-inflammatory nuclear hormone receptor that regulates a wide array of downstream effects.[83] When ligands bind PPAR-γ, the receptor dimerizes with retinoid X receptors (RXRs) and this heterodimer translocates to the nucleus, binding to PPAR response elements of target genes that stimulate the transcription of genes regulating key pathways, including lipid metabolism, glucose homeostasis and cell differentiation. A number of ligands for PPAR-γ have been described and include nitrated fatty acids and anti-diabetic glitazones.[84,85] Importantly, PPAR-γ ligands inhibit transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and signal transducer and activator of transcription 1 (STAT1), and decrease the induction of pro-inflammatory molecules such as iNOS.[86,87] Thus, a key feature of PPAR-γ signaling is the inactivation of macrophages. Proof of concept that PPAR-γ agonists are useful therapeutic agents in models of neonatal lung injury include prevention of aberrant TGFß and Wnt signaling, dampening of inflammation and improvement of secondary septation changes in models of hyperoxia-mediated neonatal lung injury.[88,89] Indeed, antenatally administered and postnatally nebulized PPAR-γ agonists have also shown promise in preventing the changes seen in hyperoxia-induced neonatal lung injury.[90–92]

Curcumin

Curcumin or diferuloylmethane, a yellow pigment found in turmeric that is obtained from the turmeric plant *Curcuma longa*, is a dietary spice used in Indian curry and in Ayurvedic medicine.[93] Curcumin has been evaluated in a wide range of pulmonary diseases including chronic obstructive pulmonary disease (COPD), acute lung injury and fibrosis.[94–96] A wide range of molecular actions have been ascribed to curcumin but, in the case of lung inflammatory diseases, they largely fall into three categories. First, it blocks activation of NFκB, a key transcription factor that activates inflammatory pathways,[97] it acts as an antioxidant targeting superoxide among others,[98] and inhibits key growth factors such as TGFß that contribute to fibrosis.[99] Another proposed mechanism, that curcumin acts as a PPAR-γ agonist, remains controversial.[100,101]

Curcumin has been evaluated in neonatal hyperoxia models of BPD.[102–104] Neonatal hyperoxia in rodents is associated with increased expression of pro-inflammatory cytokines and growth factors, an inflammatory response, increased myofibroblasts and decreased alveolarization. Curcumin effectively reverses these observations and promotes improved lung architecture.[102–104] The major issue with the development of

curcumin as a drug is its poor solubility, poor bioavailability and rapid metabolism.[105] A number of alternate approaches have been explored, including co-administration of piperine to enhance bioavailability, and the development of nanoparticle delivery devices.[106,107]

Club cell protein 16 (CC16; Clara cell or CC10)

CC10, also known as uteroglobin, is a 10kDa homodimeric secretory protein produced by Clara cells in the airways and in the urogenital tract, This protein possesses a number of *in vitro* anti-inflammatory properties including blocking of N-Formyl-methionyl-leucyl—phenylalanine (fLMP)-stimulated neutrophil and monocyte chemotaxis,[108] inhibition of phospholipase A_2 (PLA_2),[109] inhibition of platelet aggregation,[110] and suppression of IL2-stimulated production of tumor necrosis factor alpha (TNFα), IL1ß and interferon (IFN)γ in lymphocytes.[111] CC10 knockout mice exposed to hyperoxia,[112,113] or ozone,[114] demonstrate an increased inflammatory response consisting of an exaggerated pro-inflammatory cytokine profile including IL1ß and an accumulation of inflammatory cells.

Given the low concentrations of CC10 that are found in tracheal aspirate samples obtained from premature infants born at 28–32 weeks, its anti-inflammatory properties, and its ability to inhibit PLA_2, an enzyme that inactivates surfactant, it was natural to propose recombinant human CC10 (rhCC10) as a potential therapeutic agent to limit the incidence and severity of BPD.[115] Safety of rhCC10 was demonstrated in several animal models of lung injury, including neonatal pigs[116], rabbits[117] and lambs.[118,119] Intratracheal administration of rhCC10 in a Phase I trial demonstrated that, while endogenous CC10 concentrations did not rise in tracheal aspirate samples of control infants until 72 h of life, rhCC10 increased the concentration in tracheal aspirates as early as 12 h of life and showed systemic absorption and urinary excretion at 6–12 h of life. Treatment with rhCC10 was associated with a lower neutrophil count, lower protein concentration and lower IL6 concentrations.[115] These encouraging results in 22 infants have prompted a larger study involving over 80 patients, but the results have yet to be published.

Insulin-like growth factor 1 (IGF-1) / IGF-binding protein 3 (IGFBP3)

IGF-1 is an important contributor to fetal growth and development and low serum concentrations of IGF-1 have been reported in premature infants in association with ROP,[120] IVH,[121] NEC[122] and BPD.[123] In fact, IGF-1 has a number of properties that define it as a reparative growth factor. For example, IGF-1 promotes the endothelial survival signaling of vascular endothelial growth factor (VEGF),[124] and regulates glucose metabolism in the brain.[125] Interestingly, IGF-1 suppresses neonatal immune responses to phorbol ester stimulation in cord blood monocytes in association with decreased DNA binding of NFκB, suggesting profound anti-inflammatory properties.[126] Collectively, these findings suggest that IGF-1 replacement in preterm neonates could hold benefit in a number of diseases, but most likely in ROP and BPD.

Indeed, following pharmacokinetic studies of continuous rhIGF-1 and its binding partner rhIGFBP3,[127,128,129] a drug called SHP607 (Shire Pharmaceuticals), a Phase I/IIa trial was conducted to decrease the incidence and/or severity of ROP. The results of this trial have been announced as a press release. While this trial did not meet this goal, substantial decreases in the severity of BPD and severe IVH were noted. Further confirmation of these findings is currently being pursued.

Nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome blockade

IL1ß, a key initiator of inflammation, has a complex two-step activation system, and has been implicated in inflammatory disorders in humans. In the first step, endogenous danger-associated molecular patterns (DAMPs) stimulate pattern recognition receptors (PRR) such as Toll-Like Receptors (TLRs) to activate NFκB, resulting in transcriptional increases the expression of pro-IL1ß.[130] Further elucidation of this pathway revealed the existence of a family of exclusively intracellular proteins called NLRs that also bind to DAMPs.[131] One such NLR, NLRP3, forms a protein complex with the adaptor molecule *Apoptosis-associated Speck-like protein containing a CARD* (ASC) and pro-caspase1 to form the *NLRP3 inflammasome*.[132,133] Thus, the second signal is the activation of the purinergic receptor P2 × 7 by extracellular ATP that results in the formation of the NLRP3 inflammasome,[134,135] a response that has been demonstrated after hyperoxia exposure in adult mice[135] and in our studies of neonatal mice exposed to hyperoxia.[136] Activation of this complex cleaves pro-caspase1 to caspase1 (p20), which in turn proteolytically cleaves pro-IL1ß to produce mature IL1ß. This cytokine is released and interacts with its receptor, IL1R, to signal inflammatory pathways. Importantly, Interleukin 1 receptor antagonist (IL1ra) is an endogenous blocker of IL1R, and its recombinant form, rIL1ra is used in monogenic disorders with elevated IL1ß as well as several rheumatologic disorders.[137,138]

Elevated concentrations of IL1ß in amniotic fluid[139] and postnatally[4] have been associated with the development of BPD. Indeed, in preterm infants at risk of BPD, there is a balance of mature IL1ß to IL1ra in the first few days of life, but the ratio of IL1ß to IL1ra is increased by days 5-7 in infants that subsequently developed BPD.[140] Further, in fetal and neonatal mice, conditional transgenic overexpression of IL1ß in Clara cells, even in the absence of hyperoxia, results in inflammation, airway remodeling and distal alveolar simplification, changes reminiscent of BPD.[141,142]

A number of reports have now demonstrated at least partial efficacy of rIL1ra treatment in neonatal rodent models of BPD. Thus, rIL1ra treatment of neonatal rats exposed to 60% O_2 resulted in decreased inflammation, improved alveolarization, and partially restored vessel density.[143] Nold et al., using a model of *in utero* LPS exposure followed by postnatal O_2 exposure, showed that rIL1ra treatment up to 28 days of life rescued inflammation and alveolarization at least at lower concentrations, and less so at high concentrations of O_2.[144] Our own studies show that the NLRP3 inflammasome is critically involved in the neonatal hyperoxia model of BPD, and

that the NLRP3 inflammasome is activated in the preterm baboon model of BPD. Importantly, tracheal aspirate IL1ß concentrations in the first three days of life are independently associated with death or BPD at 36 weeks PMA in preterm infants with respiratory failure.[136] Further development of this strategy holds promise for the ability to block the effects of the NLRP3 inflammasome (Fig. 2).

Hyaluronan binding peptides

Hyaluronan (hyaluronic acid, HA), a glycosaminoglycan component of the extracellular matrix, is a polymer made up of repeating disaccharide units of glucuronic acid and N-acetyl glucosamine, and is an early and important mediator of inflammation.[145,146] HA regulation of inflammation is both dose- and molecular size-dependent. HA, at doses of 1 mg/ml or greater and in high molecular weight (HMW) form, inhibits inflammatory cell chemotaxis,[147,148] phagocytosis,[149] elastase release,[150] and respiratory burst activity.[149] On the other hand, at lower concentrations and at lower molecular weights, fragmented HA (LMW HA) promotes monocyte maturation into macrophages as measured by production of IGF-1[151] and IL1ß.[152,153]

An increased recovery of HA in bronchoalveolar lavage (BAL) has been found in various disease states such as sarcoidosis,[154] occupational disorders[155] and acute RDS (ARDS),[156] and after acute lung injury induced by intratracheal bleomycin instillation in rodents.[157–159] Further, the increased recovery of HA temporally correlates with an influx of inflammatory cells.[160] We have previously demonstrated that alveolar macrophages from bleomycin-injured animals are more motile than those from control animals and that an HA-binding peptide (HABP) is able to completely inhibit this increased motility.[161] Further, systemic administration of this HABP to animals prior to injury resulted in decreased macrophage accumulation and fibrosis.[161] These data suggest that HA is upstream of and critical for the inflammatory response to lung injury.

Preterm infants destined to develop BPD have increased superoxide and peroxynitrite levels early in their postnatal course.[162,163] Both superoxide and peroxynitrite cause chemical fragmentation of HA to generate HA fragments (LMW HA) that act as endogenous danger signals that interact with HA and TLRs to activate the innate immune system.[164–168] Interestingly, we and others have demonstrated that HA fragments are able to activate the NLRP3 inflammasome,[152,153] thereby providing direct rationale to study the effects of HA-binding

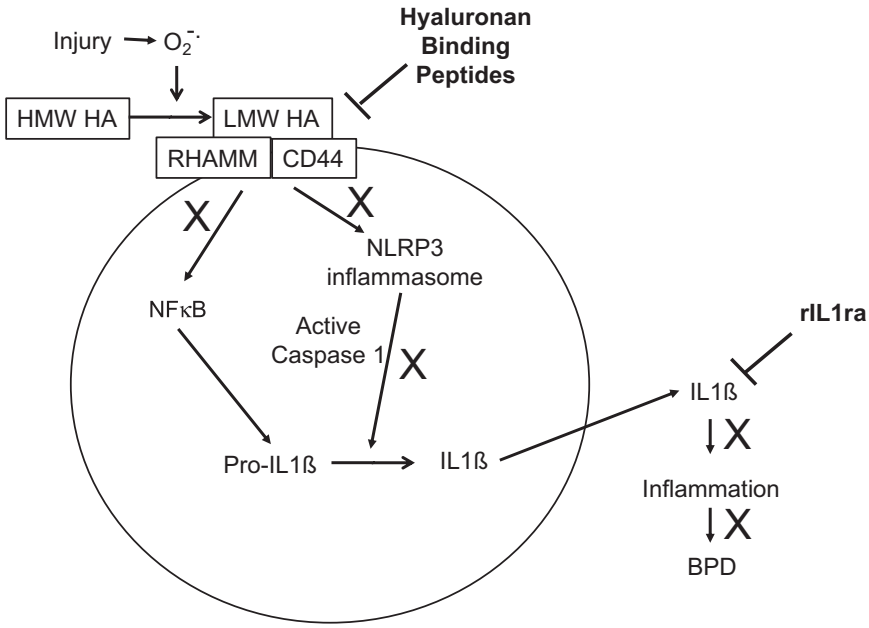


Fig. 2 – Activation of the NLRP3 inflammasome and the utility of its blockade in the prevention of BPD. Interleukin 1 beta (IL1ß) is a master regulator of inflammation. Endogenous danger associated molecular patterns (DAMPs) activate the innate immune system to elaborate IL1ß. One such DAMP is low molecular weight hyaluronan (LMW HA), which is formed by the fragmentation of HMW HA by oxidative stress. Acting through the HA receptors RHAMM and CD44, LMW HA activates the transcription factor NFκB to transcriptionally upregulate pro-IL1ß. At the same time, the NLRP3 inflammasome is also activated which results in the formation of active caspase 1 which cleaves pro-IL1ß to IL1ß to initiate inflammation. The development of HA-binding peptides holds promise in interfering with the LMW HA-RHAMM-CD44 complex to prevent activation of the NLRP3 inflammasome system, which would be predicted to prevent the formation of IL1ß. Recombinant IL1 receptor antagonist will block the effects of IL1ß to also prevent its downstream actions. For additional abbreviations, please see the text.

peptides in models of BPD, and eventually in preterm infants at risk of developing BPD. These concepts are depicted in Fig. 2.

HA interacts with specific cell-associated receptors including CD44 and Receptor for HA-Mediated Motility (RHAMM, CD168). Both of these ubiquitously expressed proteins have been implicated in acute lung injury. [145] CD44 expression is increased after bleomycin injury,[169,170] and acute lung injury in CD44 knockout (KO) mice results in the accumulation of inflammatory cells and excess HA accumulation, suggesting that CD44 is necessary for the resolution of inflammation.[171] RHAMM is expressed at the cell surface, in the cytoplasm and in the nucleus, and regulates cell locomotion and proliferation.[172–174] RHAMM expression is increased in macrophages,[146] fibroblasts,[175] epithelial cells,[167] and smooth muscle cells[174] responding to injury. After intratracheal bleomycin-induced acute lung injury, RHAMM expression is increased in macrophages responding to injury, and the increased motility of these cells is entirely dependent on RHAMM.[161,176] Interestingly, antibody blockade of RHAMM *in vivo* decreases the accumulation of macrophages into the bleomycin-injured lung, suggesting that RHAMM-HA interactions are crucial for the recruitment of these cells to the site of injury.[161,176] In order to examine the role of RHAMM in acute lung injury using genetic models, we recently studied the response to intratracheal bleomycin in RHAMM KO mice and in mice that had transgenic overexpression of RHAMM specifically in macrophages.[177] As expected, RHAMM KO mice had less respiratory distress, decreased inflammatory cell accumulation and less fibrosis than littermate controls. On the other hand, bleomycin injury of mice with transgenic overexpression of RHAMM in macrophages resulted in worse respiratory distress, increased inflammation and severe fibrosis compared to littermate wild type animals injured with bleomycin.[177] Therefore, RHAMM is a potential novel therapeutic target to control inflammation after lung injury. We propose that LMW HA uses RHAMM and CD44 as a complex to allow signaling to activate the NLRP3 inflammasome (Fig. 2).

Summary

Despite over 50 years of efforts that have resulted in significant improvements in the care of premature infants, including antenatal glucocorticoid treatment, exogenous surfactant administration, gentler ventilation techniques, judicious use of fluids, prevention of infections and improved nutrition, the incidence of BPD has remained essentially unchanged. Given its importance in the pathogenesis of BPD, there continues to be an intense focus on the mechanisms of inflammation in this disorder. Significant progress has been made with the use of animal models with promising therapies emerging. Translational of these promising avenues into clinical use that will decrease the incidence and/or severity of BPD will be challenging and will require careful investigation and substantial support from public, private and commercial sources.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

The author is mindful that this review is not intended to be all encompassing and any omission of work that would be relevant to the topic of this review is not intentional. The author is a co-founder of Eravon Therapeutics, Inc. that is focused on RHAMM-HA-based therapeutics. Funding for the studies described that were conducted in the Savani laboratory was from NIH R01 awards HL62868, HL62472 and HL093535, and U01 award HL075900. Additional funding was obtained from the William Buchanan Chair in Pediatrics and a Children's Hospital Foundation Dallas grant (#137).

REFERENCES

1. Ambalavanan N, Carlo WA. Bronchopulmonary dysplasia: new insights. *Clin Perinatol*. 2004;31(3):613–628.
2. Bhandari A, Bhandari V. Pathogenesis, pathology and pathophysiology of pulmonary sequelae of bronchopulmonary dysplasia in premature infants. *Front Biosci*. 2003;8:e370–e380.
3. Speer CP. Inflammation and bronchopulmonary dysplasia. *Semin Neonatol*. 2003;8(1):29–38.
4. Rindfleisch MS, Hasday JD, Taciak V, Boderick K, Viscardi RM. Potential role of interleukin-1 in the development of bronchopulmonary dysplasia. *J Interf Cytokine Res*. 1996;16:365–373.
5. Stenmark KR, Abman SH. Lung vascular development: implications for the pathogenesis of bronchopulmonary dysplasia. *Annu Rev Physiol*. 2005;67:623–661.
6. Thebaud B. Angiogenesis in lung development, injury and repair: implications for chronic lung disease of prematurity. *Neonatology*. 2007;91(4):291–297.
7. Coalson JJ. Experimental models of bronchopulmonary dysplasia. *Biol Neonate*. 1997;71(Suppl 1):35–38.
8. Nakanishi H, Sugiura T, Streisand JB, Lonning SM, Roberts JD Jr. TGF-beta-neutralizing antibodies improve pulmonary alveologenesis and vasculogenesis in the injured newborn lung. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(1):L151–L161.
9. ter Horst SA, Fijlstra M, Sengupta S, Walther FJ, Wagenaar GT. Spatial and temporal expression of surfactant proteins in hyperoxia-induced neonatal rat lung injury. *BMC Pulm Med*. 2006;6:8.
10. Ambalavanan N, Nicola T, Hagood J, et al. Transforming growth factor-beta signaling mediates hypoxia-induced pulmonary arterial remodeling and inhibition of alveolar development in newborn mouse lung. *Am J Physiol Lung Cell Mol Physiol*. 2008;295(1):L86–L95.
11. Tourneux P, Markham N, Seedorf G, Balasubramaniam V, Abman SH. Inhaled nitric oxide improves lung structure and pulmonary hypertension in a model of bleomycin-induced bronchopulmonary dysplasia in neonatal rats. *Am J Physiol Lung Cell Mol Physiol*. 2009;297(6):L1103–L1111.
12. Nardiello C, Mizikova I, Morty RE. Looking ahead: where to next for animal models of bronchopulmonary dysplasia. *Cell Tissue Res*. 2017;367(3):457–468.
13. Berger J, Bhandari V. Animal models of bronchopulmonary dysplasia. The term mouse models. *Am J Physiol Lung Cell Mol Physiol*. 2014;307(12):L936–L947.
14. Yee M, Chess PR, McGrath-Morrow SA, et al. Neonatal oxygen adversely affects lung function in adult mice without altering surfactant composition or activity. *Am J Physiol Lung Cell Mol Physiol*. 2009;297(4):L641–L649.

15. Lingappan K, Jiang W, Wang L, Moorthy B. Sex-specific differences in neonatal hyperoxic lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2016;311(2):L481–L493.
16. Ley K. Molecular mechanisms of leukocyte recruitment in the inflammatory process. *Cardiovasc Res*. 1996;32(4):733–742.
17. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007;7(9):678–689.
18. Cuisset L, Jeru I, Dumont B, et al. Mutations in the autoinflammatory cryopyrin-associated periodic syndrome gene: epidemiological study and lessons from eight years of genetic analysis in France. *Ann Rheumatic Dis*. 2011;70(3):495–499.
19. Herlin T, Sorensen N, Veirum J. [Hereditary periodical fever syndromes]. *Ugeskr Laeger*. 2011;173(13):968–973.
20. Lachmann HJ, Quartier P, So A, Hawkins PN. The emerging role of interleukin-1beta in autoinflammatory diseases. *Arthritis Rheum*. 2011;63(2):314–324.
21. Van Marter LJ, Allred EN, Pagano M, et al. Do clinical markers of barotrauma and oxygen toxicity explain interhospital variation in rates of chronic lung disease? The neonatology committee for the developmental network. *Pediatrics*. 2000;105(6):1194–1201.
22. Bland RD. Neonatal chronic lung disease in the post-surfactant era. *Biol Neonate*. 2005;88(3):181–191.
23. Mokres LM, Parai K, Hilgendorff A, et al. Prolonged mechanical ventilation with air induces apoptosis and causes failure of alveolar septation and angiogenesis in lungs of newborn mice. *Am J Physiol Lung Cell Mol Physiol*. 2010;298(1):L23–L35.
24. Polglase GR, Hillman NH, Ball MK, et al. Lung and systemic inflammation in preterm lambs on continuous positive airway pressure or conventional ventilation. *Pediatric research*. 2009;65(1):67–71.
25. Thomson MA, Yoder BA, Winter VT, et al. Treatment of immature baboons for 28 days with early nasal continuous positive airway pressure. *Am J Respir Crit Care Med*. 2004;169(9):1054–1062.
26. Reyburn B, Di Fiore JM, Raffay T, et al. The effect of continuous positive airway pressure in a mouse model of hyperoxic neonatal lung injury. *Neonatology*. 2016;109(1):6–13.
27. Reyburn B, Li M, Metcalfe DB, et al. Nasal ventilation alters mesenchymal cell turnover and improves alveolarization in preterm lambs. *Am J Respir Crit Care Med*. 2008;178(4):407–418.
28. Morley CJ, Davis PG, Doyle LW, et al. Nasal CPAP or intubation at birth for very preterm infants. *N Engl J Med*. 2008;358(7):700–708.
29. Network SSGotEKSNNRFiner NN, Carlo WA, et al. Early CPAP versus surfactant in extremely preterm infants. *N Engl J Med*. 2010;362(21):1970–1979.
30. Bhandari V. Noninvasive respiratory support in the preterm infant. *Clin Perinatol*. 2012;39(3):497–511.
31. Liggins GC. Premature delivery of foetal lambs infused with glucocorticoids. *J Endocrinol*. 1969;45(4):515–523.
32. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*. 1972;50(4):515–525.
33. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*. 2017;3:CD004454.
34. Doyle LW, Ehrenkranz RA, Halliday HL. Early (< 8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. *Cochrane Database Syst Rev*. 2014(5):CD001146.
35. Halliday HL. Update on postnatal steroids. *Neonatology*. 2017;111(4):415–422.
36. Doyle LW, Ehrenkranz RA, Halliday HL. Late (>7 days) postnatal corticosteroids for chronic lung disease in preterm infants. *Cochrane Database Syst Rev*. 2014(5):CD001145.
37. Massaro D, Massaro GD. Invited review: pulmonary alveoli: formation, the "call for oxygen," and other regulators. *Am J Physiol Lung Cell Mol Physiol*. 2002;282(3):L345–L358.
38. Massaro D, Massaro GD. Critical period for alveologenesis and early determinants of adult pulmonary disease. *Am J Physiol Lung Cell Mol Physiol*. 2004;287(4):L715–L717.
39. Garber SJ, Zhang H, Foley JP, et al. Hormonal regulation of alveolarization: structure-function correlation. *Respir Res*. 2006;7:47.
40. Fredholm BB. Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine. *Pharmacol Toxicol*. 1995;76(2):93–101.
41. Choi OH, Shamim MT, Padgett WL, Daly JW. Caffeine and theophylline analogues: correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. *Life Sci*. 1988;43(5):387–398.
42. Millar D, Schmidt B. Controversies surrounding xanthine therapy. *Semin Neonatol*. 2004;9(3):239–244.
43. Schmidt B, Roberts RS, Davis P, et al. Caffeine therapy for apnea of prematurity. *N Engl J Med*. 2006;354(20):2112–2121.
44. Schmidt B, Roberts RS, Anderson PJ, et al. Academic performance, motor function, and behavior 11 years after neonatal caffeine citrate therapy for apnea of prematurity: an 11-year follow-up of the cap randomized clinical trial. *JAMA Pediatr*. 2017;171(6):564–572.
45. Parikka V, Beck J, Zhai Q, Leppasalo J, Lehtonen L, Soukka H. The effect of caffeine citrate on neural breathing pattern in preterm infants. *Early Hum Dev*. 2015;91(10):565–568.
46. Pacifici GM. Clinical pharmacology of theophylline in preterm infants: effects, metabolism and pharmacokinetics. *Curr Pediatr Rev*. 2014;10(4):297–303.
47. Back SA, Craig A, Luo NL, et al. Protective effects of caffeine on chronic hypoxia-induced perinatal white matter injury. *Ann Neurol*. 2006;60(6):696–705.
48. Endesfelder S, Weichelt U, Strauss E, et al. Neuroprotection by caffeine in hyperoxia-induced neonatal brain injury. *Int J Mol Sci*. 2017;18(1).
49. Fehrholz M, Hutten M, Kramer BW, Speer CP, Kunzmann S. Amplification of steroid-mediated SP-B expression by physiological levels of caffeine. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(1):L101–L109.
50. Fehrholz M, Bersani I, Kramer BW, Speer CP, Kunzmann S. Synergistic effect of caffeine and glucocorticoids on expression of surfactant protein B (SP-B) mRNA. *PLoS One*. 2012;7(12):e51575.
51. Weichelt U, Cay R, Schmitz T, et al. Prevention of hyperoxia-mediated pulmonary inflammation in neonatal rats by caffeine. *Eur Respir J*. 2013;41(4):966–973.
52. Nagatomo T, Jimenez J, Richter J, et al. Caffeine prevents hyperoxia-induced functional and structural lung damage in preterm rabbits. *Neonatology*. 2016;109(4):274–281.
53. Koroglu OA, MacFarlane PM, Balan KV, et al. Anti-inflammatory effect of caffeine is associated with improved lung function after lipopolysaccharide-induced amnionitis. *Neonatology*. 2014;106(3):235–240.
54. Barnes PJ. Nitric oxide and airway disease. *Ann Med*. 1995;27(3):389–393.
55. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. *Am J Respir Crit Care Med*. 1994;149(2 Pt 1):538–551.
56. Dweik RA, Laskowski D, Abu-Soud HM, et al. Nitric oxide synthesis in the lung. Regulation by oxygen through a kinetic mechanism. *J Clin Invest*. 1998;101(3):660–666.
57. Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun*. 1993;191(1):83–88.
58. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. *Circ Res*. 2006;98(11):1352–1364.

59. Mineo C, Shaul PW. Modulation of endothelial NO production by high-density lipoprotein. *Cold Spring Harb Symp Quant Biol*. 2002;67:459–469.
60. ter Horst SA, Walther FJ, Poorthuis BJ, Hiemstra PS, Wagenaar GT. Inhaled nitric oxide attenuates pulmonary inflammation and fibrin deposition and prolongs survival in neonatal hyperoxic lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(1):L35–L44.
61. Balasubramaniam V, Tang JR, Maxey A, Plopper CG, Abman SH. Mild hypoxia impairs alveolarization in the endothelial nitric oxide synthase-deficient mouse. *Am J Physiol Lung Cell Mol Physiol*. 2003;284(6):L964–L971.
62. Edwards YS, Sutherland LM, Murray AW. NO protects alveolar type II cells from stretch-induced apoptosis. A novel role for macrophages in the lung. *Am J Physiol Lung Cell Mol Physiol*. 2000;279(6):L1236–L1242.
63. Holopainen R, Aho H, Laine J, Halkola L, Kaapa P. Nitric oxide inhalation inhibits pulmonary apoptosis but not inflammatory injury in porcine meconium aspiration. *Acta Paediatr*. 1999;88(10):1147–1155.
64. Tang JR, Seedorf G, Balasubramaniam V, Maxey A, Markham N, Abman SH. Early inhaled nitric oxide treatment decreases apoptosis of endothelial cells in neonatal rat lungs after vascular endothelial growth factor inhibition. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(5):L1271–L1280.
65. Tang ZL, Wasserloos KJ, Liu X, et al. Nitric oxide decreases the sensitivity of pulmonary endothelial cells to LPS-induced apoptosis in a zinc-dependent fashion. *Mol Cell Biochem*. 2002;234-235(1-2):211–217.
66. Young SL, Evans K, Eu JP. Nitric oxide modulates branching morphogenesis in fetal rat lung explants. *Am J Physiol Lung Cell Mol Physiol*. 2002;282(3):L379–L385.
67. Shaul PW. Nitric oxide in the developing lung. *Adv Pediatr*. 1995;42:367–414.
68. Afshar S, Gibson LL, Yuhanna IS, et al. Pulmonary NO synthase expression is attenuated in a fetal baboon model of chronic lung disease. *Am J Physiol Lung Cell Mol Physiol*. 2003;284(5):L749–L758.
69. McCurnin DC, Pierce RA, Chang LY, et al. Inhaled NO improves early pulmonary function and modifies lung growth and elastin deposition in a baboon model of neonatal chronic lung disease. *Am J Physiol Lung Cell Mol Physiol*. 2005;288(3):L450–L459.
70. Tang JR, Markham NE, Lin YJ, et al. Inhaled nitric oxide attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor. *Am J Physiol Lung Cell Mol Physiol*. 2004;287(2):L344–L351.
71. Ballard RA, Truog WE, Cnaan A, et al. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. *N Engl J Med*. 2006;355(4):343–353.
72. Kinsella JP, Cutter GR, Walsh WF, et al. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med*. 2006;355(4):354–364.
73. Schreiber MD, Gin-Mestan K, Marks JD, Huo D, Lee G, Srisuparp P. Inhaled nitric oxide in premature infants with the respiratory distress syndrome. *N Engl J Med*. 2003;349(22):2099–2107.
74. Van Meurs KP, Wright LL, Ehrenkranz RA, et al. Inhaled nitric oxide for premature infants with severe respiratory failure. *N Engl J Med*. 2005;353(1):13–22.
75. Christesen HT, Falkenberg T, Lamont RF, Jorgensen JS. The impact of vitamin D on pregnancy: a systematic review. *Acta Obstet Gynecol Scand*. 2012;91(12):1357–1367.
76. Lykkedegn S, Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(7):L587–L602.
77. Sundar IK, Hwang JW, Wu S, Sun J, Rahman I. Deletion of vitamin D receptor leads to premature emphysema/COPD by increased matrix metalloproteinases and lymphoid aggregates formation. *Biochem Biophys Res Commun*. 2011;406(1):127–133.
78. Cookson MW, Ryan SL, Seedorf GJ, Dodson RB, Abman SH, Mandell EW. Antenatal vitamin d preserves placental vascular and fetal growth in experimental chorioamnionitis due to intra-amniotic endotoxin exposure. *Am J Perinatol*. 2018.
79. Mandell E, Seedorf G, Gien J, Abman SH. Vitamin D treatment improves survival and infant lung structure after intra-amniotic endotoxin exposure in rats: potential role for the prevention of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(5):L420–L428.
80. Yao L, Shi Y, Zhao X, et al. Vitamin D attenuates hyperoxia-induced lung injury through downregulation of Toll-like receptor 4. *Int J Mol Med*. 2017;39(6):1403–1408.
81. Molinari C, Uberti F, Grossini E, et al. 1alpha,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. *Cell Physiol Biochem*. 2011;27(6):661–668.
82. Taylor SK, Sakurai R, Sakurai T, Rehan VK. Inhaled Vitamin D: A Novel Strategy to Enhance Neonatal Lung Maturation. *Lung*. 2016;194(6):931–943.
83. Wang S, Dougherty EJ, Danner RL. PPARgamma signaling and emerging opportunities for improved therapeutics. *Pharmacol Res*. 2016;111:76–85.
84. Narala VR, Subramani PA, Narasimha VR, Shaik FB, Panati K. The role of nitrated fatty acids and peroxisome proliferator-activated receptor gamma in modulating inflammation. *Int Immunopharmacol*. 2014;23(1):283–287.
85. Moraes LA, Piqueras L, Bishop-Bailey D. Peroxisome proliferator-activated receptors and inflammation. *Pharmacol Ther*. 2006;110(3):371–385.
86. Ricote M, Huang JT, Welch JS, Glass CK. The peroxisome proliferator-activated receptor(PPARgamma) as a regulator of monocyte/macrophage function. *J Leukoc Biol*. 1999;66(5):733–739.
87. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature*. 1998;391(6662):79–82.
88. Dasgupta C, Sakurai R, Wang Y, et al. Hyperoxia-induced neonatal rat lung injury involves activation of TGF-{beta} and Wnt signaling and is protected by rosiglitazone. *Am J Physiol Lung Cell Mol Physiol*. 2009;296(6):L1031–L1041.
89. Rehan VK, Wang Y, Patel S, Santos J, Torday JS. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, prevents hyperoxia-induced neonatal rat lung injury in vivo. *Pediatr Pulmonol*. 2006;41(6):558–569.
90. Sakurai R, Lee C, Shen H, Waring AJ, Walther FJ, Rehan VK. A combination of the aerosolized PPAR-gamma agonist pioglitazone and a synthetic surfactant protein B peptide mimic prevents hyperoxia-induced neonatal lung injury in rats. *Neonatology*. 2018;113(4):296–304.
91. Morales E, Sakurai R, Husain S, et al. Nebulized PPARgamma agonists: a novel approach to augment neonatal lung maturation and injury repair in rats. *Pediatr Res*. 2014;75(5):631–640.
92. Rehan VK, Sakurai R, Corral J, et al. Antenatally administered PPAR-gamma agonist rosiglitazone prevents hyperoxia-induced neonatal rat lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2010;299(5):L672–L680.
93. Lelli D, Sahebkar A, Johnston TP, Pedone C. Curcumin use in pulmonary diseases: state of the art and future perspectives. *Pharmacol Res*. 2017;115:133–148.
94. Yuan J, Liu R, Ma Y, Zhang Z, Xie Z. Curcumin attenuates airway inflammation and airway remolding by inhibiting NF-

kappaB signaling and COX-2 in cigarette smoke-induced COPD mice. *Inflammation*. 2018.
95. Suresh MV, Wagner MC, Rosania GR, et al. Pulmonary administration of a water-soluble curcumin complex reduces severity of acute lung injury. *Am J Respir Cell Mol Biol*. 2012;47(3):280–287.
96. Smith MR, Gangireddy SR, Narala VR, et al. Curcumin inhibits fibrosis-related effects in IPF fibroblasts and in mice following bleomycin-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2010;298(5):L616–L625.
97. Shishodia S, Potdar P, Gairola CG, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-kappaB activation through inhibition of Ikappa-Balpha kinase in human lung epithelial cells: correlation with suppression of COX-2, MMP-9 and cyclin D1. *Carcinogenesis*. 2003;24(7):1269–1279.
98. Kunwar A, Barik A, Sandur SK, Indira Priyadarsini K. Differential antioxidant/pro-oxidant activity of dimethoxycurcumin, a synthetic analogue of curcumin. *Free Radic Res*. 2011;45(8):959–965.
99. Kumari A, Dash D, Singh R. Curcumin inhibits lipopolysaccharide (LPS)-induced endotoxemia and airway inflammation through modulation of sequential release of inflammatory mediators (TNF-alpha and TGF-beta1) in murine model. *Inflammopharmacology*. 2017;25(3):329–341.
100. Lin J, Chen A. Activation of peroxisome proliferator-activated receptor-gamma by curcumin blocks the signaling pathways for PDGF and EGF in hepatic stellate cells. *Lab Invest*. 2008;88(5):529–540.
101. Narala VR, Smith MR, Adapala RK, et al. Curcumin is not a ligand for peroxisome proliferator-activated receptor-gamma. *Gene Ther Mol Biol*. 2009;13(1):20–25.
102. Sakurai R, Li Y, Torday JS, Rehan VK. Curcumin augments lung maturation, preventing neonatal lung injury by inhibiting TGF-beta signaling. *Am J Physiol Lung Cell Mol Physiol*. 2011;301(5):L721–L730.
103. Sakurai R, Villarreal P, Husain S, et al. Curcumin protects the developing lung against long-term hyperoxic injury. *Am J Physiol Lung Cell Mol Physiol*. 2013;305(4):L301–L311.
104. Wei H, Zhang Y, Liu W, Zhang XP, Feng L. [Effect of curcumin on bronchopulmonary dysplasia induced by 600 ml/L oxygen in neonatal rats]. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2014;45(2):225–229: 244.
105. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm*. 2007;4(6):807–818.
106. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*. 1998;64(4):353–356.
107. Wang W, Zhu R, Xie Q, et al. Enhanced bioavailability and efficiency of curcumin for the treatment of asthma by its formulation in solid lipid nanoparticles. *Int J Nanomedicine*. 2012;7:3667–3677.
108. Vasanthakumar G, Manjunath R, Mukherjee AB, Warabi H, Schiffmann E. Inhibition of phagocyte chemotaxis by uteroglobin, an inhibitor of blastocyst rejection. *Biochem Pharmacol*. 1988;37(3):389–394.
109. Levin SW, Butler JD, Schumacher UK, Wightman PD, Mukherjee AB. Uteroglobin inhibits phospholipase A2 activity. *Life Sci*. 1986;38(20):1813–1819.
110. Manjunath R, Levin SW, Kumaroo KK, et al. Inhibition of thrombin-induced platelet aggregation by uteroglobin. *Biochem Pharmacol*. 1987;36(5):741–746.
111. Dierynck I, Bernard A, Roels H, De Ley M. The human Clara cell protein: biochemical and biological characterisation of a natural immunosuppressor. *Mult Scler*. 1996;1(6):385–387.
112. Johnston CJ, Mango GW, Finkelstein JN, Stripp BR. Altered pulmonary response to hyperoxia in Clara cell secretory protein deficient mice. *Am J Respir Cell Mol Biol*. 1997;17(2):147–155.
113. Reynolds SD, Mango GW, Gelein R, Boe IM, Lund J, Stripp BR. Normal function and lack of fibronectin accumulation in kidneys of Clara cell secretory protein/uteroglobin deficient mice. *Am J Kidney Dis*. 1999;33(3):541–551.
114. Mango GW, Johnston CJ, Reynolds SD, Finkelstein JN, Plopper CG, Stripp BR. Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. *Am J Physiol*. 1998;275(2 Pt 1):L348–L356.
115. Levine CR, Gewolb IH, Allen K, et al. The safety, pharmacokinetics, and anti-inflammatory effects of intratracheal recombinant human Clara cell protein in premature infants with respiratory distress syndrome. *Pediatr Res*. 2005;58(1):15–21.
116. Chandra S, Davis JM, Drexler S, et al. Safety and efficacy of intratracheal recombinant human Clara cell protein in a newborn piglet model of acute lung injury. *Pediatric research*. 2003;54(4):509–515.
117. Miller TL, Shashikant BN, Melby JM, Pilon AL, Shaffer TH, Wolfson MR. Recombinant human Clara cell secretory protein in acute lung injury of the rabbit: effect of route of administration. *Pediatr Crit Care Med*. 2005;6(6):698–706.
118. Miller TL, Shashikant BN, Pilon AL, Pierce RA, Shaffer TH, Wolfson MR. Effects of recombinant Clara cell secretory protein (rhCC10) on inflammatory-related matrix metalloproteinase activity in a preterm lamb model of neonatal respiratory distress. *Pediatr Crit Care Med*. 2007;8(1):40–46.
119. Wolfson MR, Funanage VL, Kirwin SM, et al. Recombinant human Clara cell secretory protein treatment increases lung mRNA expression of surfactant proteins and vascular endothelial growth factor in a premature lamb model of respiratory distress syndrome. *Am J Perinatol*. 2008;25(10):637–645.
120. Hellstrom A, Engstrom E, Hard AL, et al. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. *Pediatrics*. 2003;112(5):1016–1020.
121. Hellstrom A, Ley D, Hansen-Pupp I, et al. Role of insulinlike growth factor 1 in fetal development and in the early postnatal life of premature infants. *Am J Perinatol*. 2016;33(11):1067–1071.
122. Tian F, Liu GR, Li N, Yuan G. Insulin-like growth factor I reduces the occurrence of necrotizing enterocolitis by reducing inflammatory response and protecting intestinal mucosal barrier in neonatal rats model. *Eur Rev Med Pharmacol Sci*. 2017;21(20):4711–4719.
123. Lofqvist C, Hellgren G, Niklasson A, et al. Low postnatal serum IGF-I levels are associated with bronchopulmonary dysplasia (BPD). *Acta Paediatr*. 2012;101(12):1211–1216.
124. Hellstrom A, Perruzzi C, Ju M, et al. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc Natl Acad Sci U S A*. 2001;98(10):5804–5808.
125. Cheng CM, Reinhardt RR, Lee WH, Joncas G, Patel SC, Bondy CA. Insulin-like growth factor 1 regulates developing brain glucose metabolism. *Proc Natl Acad Sci USA*. 2000;97(18):10236–10241.
126. Puzik A, Rupp J, Troger B, Gopel W, Herting E, Hartel C. Insulin-like growth factor-I regulates the neonatal immune response in infection and maturation by suppression of IFN-gamma. *Cytokine*. 2012;60(2):369–376.
127. Chung JK, Hallberg B, Hansen-Pupp I, et al. Development and verification of a pharmacokinetic model to optimize physiologic replacement of rhIGF-1/rhIGFBP-3 in preterm infants. *Pediatr Res*. 2017;81(3):504–510.

128. Hansen-Pupp I, Hellstrom A, Hamdani M, et al. Continuous longitudinal infusion of rhIGF-1/rhIGFBP-3 in extremely preterm infants: Evaluation of feasibility in a phase II study. *Growth Horm IGF Res*. 2017;36:44–51.
129. Hellstrom A, Ley D, Hallberg B, et al. IGF-1 as a drug for preterm infants: a step-wise clinical development. *Curr Pharm Des*. 2017;23(38):5964–5970.
130. Gasse P, Mary C, Guenon I, et al. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. *J Clin Invest*. 2007;117(12):3786–3799.
131. Ye Z, Ting JP. NLR, the nucleotide-binding domain leucine-rich repeat containing gene family. *Curr Opin Immunol*. 2008;20(1):3–9.
132. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol*. 2007;7(1):31–40.
133. Petrilli V, Dostert C, Muruve DA, Tschopp J. The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol*. 2007;19(6):615–622.
134. Mariathasan S, Weiss DS, Newton K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature*. 2006;440(7081):228–232.
135. Kolliputi N, Shaik RS, Waxman AB. The inflammasome mediates hyperoxia-induced alveolar cell permeability. *J Immunol*. 2010;184(10):5819–5826.
136. Liao J, Kapadia VS, Brown LS, et al. The NLRP3 inflammasome is critically involved in the development of bronchopulmonary dysplasia. *Nat Commun*. 2015;6:8977.
137. Goldbach-Mansky R, Dailey NJ, Canna SW, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N Engl J Med*. 2006;355(6):581–592.
138. Sibley CH, Plass N, Snow J, et al. Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease treated with anakinra: a cohort study to determine three- and five-year outcomes. *Arthritis Rheum*. 2012;64(7):2375–2386.
139. Yoon BH, Romero R, Jun JK, et al. Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. *Am J Obstet Gynecol*. 1997;177(4):825–830.
140. Kakkera DK, Siddiq MM, Parton LA. Interleukin-1 balance in the lungs of preterm infants who develop bronchopulmonary dysplasia. *Biol Neonate*. 2005;87(2):82–90.
141. Lappalainen U, Whitsett JA, Wert SE, Tichelaar JW, Bry K. Interleukin-1beta causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. *Am J Respir Cell Mol Biol*. 2005;32(4):311–318.
142. Bry K, Whitsett JA, Lappalainen U. IL-1beta disrupts postnatal lung morphogenesis in the mouse. *Am J Respir Cell Mol Biol*. 2007;36(1):32–42.
143. Johnson BH, Yi M, Masood A, et al. A critical role for the IL-1 receptor in lung injury induced in neonatal rats by 60% O2. *Pediatric Res*. 2009;66(3):260–265.
144. Nold MF, Mangan NE, Rudloff I, et al. Interleukin-1 receptor antagonist prevents murine bronchopulmonary dysplasia induced by perinatal inflammation and hyperoxia. *Proc Natl Acad Sci U S A*. 2013;110(35):14384–14389.
145. Savani RC, DeLisser HM. Hyaluronan and its receptors in lung health and disease. In: Garg HG, Roughley PJ, Hales CA, et al., eds. *Proteoglycans and Lung Disease*, New York: Marcel Dekker; 2003. 73–106.
146. Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. *Physiol Rev*. 2011;91(1):221–264.
147. Tamoto K, Nochi H, Tada M, et al. High-molecular weight hyaluronic acids inhibit chemotaxis and phagocytosis but not lysosomal enzyme release induced by receptor-mediated stimulations in guinea pig phagocytes. *Microbiol Immunol*. 1994;38(1):73–80.
148. Foley JP, Lam D, Jiang H, et al. Toll-like receptor 2 (TLR2), transforming growth factor-beta, hyaluronan (HA), and receptor for HA-mediated motility (RHAMM) are required for surfactant protein A-stimulated macrophage chemotaxis. *J Biol Chem*. 2012;287(44):37406–37419.
149. Suzuki Y, Yamaguchi T. Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release. *Agents Actions*. 1993;38:32–37.
150. Akatsuka M, Yamamoto Y, Tobetto K, Yasui K, Ando T. Suppressive effects of hyaluronic acid on elastase release from rat peritoneal leucocytes. *J Pharm Pharmacol*. 1993;45:110–114.
151. Noble PW, Lake FR, Henson PM, Riches DW. Hyaluronate activation of CD44 induces insulin-like growth factor-1 expression by a tumor necrosis factor-alpha-dependent mechanism in murine macrophages. *J Clin Invest*. 1993;91(6):2368–2377.
152. Østerholt HC, Dannevig I, Wyckoff MH, et al. Antioxidant protects against increases in low molecular weight hyaluronan and inflammation in asphyxiated newborn pigs resuscitated with 100% oxygen. *PLoS One*. 2012;7(6):e38839.
153. Yamasaki K, Muto J, Taylor KR, et al. NLRP3/cryopyrin is necessary for interleukin-1{beta} (IL-1{beta}) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury. *J Biol Chem*. 2009;284(19):12762–12771.
154. Hällgren R, Eklund A, Engstrom-Laurent A, Schmekel B. Hyaluronate in bronchoalveolar lavage fluid: a new marker in sarcoidosis reflecting pulmonary disease. *Br Med J*. 1985;290:1778–1781.
155. Bjermer L, Engstrom-Laurent A, Lundgren R, Rosenhall L, Hallgren R. Hyaluronate and type III procollagen peptide concentrations in bronchoalveolar lavage fluid as markers of disease activity in farmer's lung. *Br Med J*. 1987;295:803–806.
156. Hällgren R, Samuelsson T, Laurent TC, Modig J. Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome. *Am Rev Respir Dis*. 1989;139:682–687.
157. Bray BA, Sampson PM, Osman M, Giandomenico A, Turino GM. Early changes in lung tissue hyaluronan (hyaluronic acid) and hyaluronidase in bleomycin-induced alveolitis in hamsters. *Am Rev Resp Dis*. 1991;143:284–288.
158. Nettelbladt O, Bergh J, Schenholm M, Tengblad A, Hallgren R. Accumulation of hyaluronic acid in the alveolar interstitial tissue in bleomycin-induced alveolitis. *Am Rev Respir Dis*. 1989;139:759–762.
159. Nettelbladt O, Hallgren R. Hyaluronan (hyaluronic acid) in bronchoalveolar fluid during the development of bleomycin-induced alveolitis in the rat. *Am Rev Respir Dis*. 1989;140:1028–1032.
160. Giri SN, Hyde DM, Nakashima JM. Analysis of bronchoalveolar lavage fluid from bleomycin-induced pulmonary fibrosis in hamsters. *Toxicol Pathol*. 1986;14(2):149–157.
161. Savani RC, Hou G, Liu P, et al. A role for hyaluronan in macrophage accumulation and collagen deposition after bleomycin-induced lung injury. *Am J Respir Cell Mol Biol*. 2000;23(4):475–484.
162. Banks BA, Ischiropoulos H, McClelland M, Ballard PL, Ballard RA. Plasma 3-nitrotyrosine is elevated in premature infants who develop bronchopulmonary dysplasia. *Pediatrics*. 1998;101(5):870–874.
163. Davis JM. Role of oxidant injury in the pathogenesis of neonatal lung disease. *Acta Paediatr Suppl*. 2002;91(437):23–25.
164. Deguine V, Menasche M, Ferrari P, Fraisse L, Pouliquen Y, Robert L. Free radical depolymerization of hyaluronan by Maillard reaction products: role in liquefaction of aging vitreous. *Int J Biol Macromol*. 1998;22(1):17–22.

165. Hrabarova E, Juranek I, Soltes L. Pro-oxidative effect of peroxynitrite regarding biological systems: a special focus on high-molar-mass hyaluronan degradation. *Gen Physiol Biophys*. 2011;30(3):223–238.
166. Li M, Rosenfeld L, Vilar RE, Cowman MK. Degradation of hyaluronan by peroxynitrite. *Arch Biochem Biophys*. 1997;341(2):245–250.
167. Manzanares D, Monzon ME, Savani RC, Salathe M. Apical oxidative hyaluronan degradation stimulates airway ciliary beating via RHAMM and RON. *Am J Respir Cell Mol Biol*. 2007;37(2):160–168.
168. Moseley R, Waddington RJ, Embery G. Degradation of glycosaminoglycans by reactive oxygen species derived from stimulated polymorphonuclear leukocytes. *Biochimica et biophysica acta*. 1997;1362(2-3):221–231.
169. Kasper M, Bierhaus A, Whyte A, Binns RM, Schuh D, Muller M. Expression of CD44 isoforms during bleomycin- or radiation-induced pulmonary fibrosis in rats and mini-pigs. *Histochem Cell Biol*. 1996;105:221–230.
170. Teder P, Nettelbladt O, Heldin P. Characterization of the mechanism involved in bleomycin-induced increased hyaluronan production in rat lung. *Am J Respir Cell Mol Biol*. 1995;12:181–189.
171. Teder P, Vandivier RW, Jiang D, et al. Resolution of lung inflammation by CD44. *Science*. 2002;296(5565):155–158.
172. Hardwick C, Hoare K, Owens R, et al. Molecular cloning of a novel Hyaluronan receptor that mediates tumor cell motility. *J Cell Biol*. 1992;117(6):1343–1350.
173. Hall CL, Yang B, Yang X, et al. Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for H-ras transformation. *Cell*. 1995;82(1):19–26.
174. Savani RC, Wang C, Yang B, et al. Migration of bovine aortic smooth muscle cells after wounding injury. The role of hyaluronan and RHAMM. *J Clin Invest*. 1995;95(3):1158–1168.
175. Hall CL, Collis LA, Bo AJ, et al. Fibroblasts require protein kinase C activation to respond to hyaluronan with increased locomotion. *Matrix Biol*. 2001;20(3):183–192.
176. Zaman A, Cui Z, Foley JP, et al. Expression and role of the hyaluronan receptor RHAMM in inflammation after bleomycin injury. *Am J Respir Cell Mol Biol*. 2005;33(5):447–454.
177. Cui Z, Liao J, Cheong N, et al. The receptor for hyaluronan-mediated motility (CD168) promotes inflammation and fibrosis after acute lung injury. *Matrix Biol*. 2018 Aug 8. https://doi.org/10.1016/j.matbio.2018.08.002. pii: S0945-053X(18)30029-5.

Seminars in Perinatology 42 (2018)

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

Surfactant, steroids and non-invasive ventilation in the prevention of BPD

Vikramaditya Dumpa[a], and Vineet Bhandari[b]*

[a]Division of Neonatology, Department of Pediatrics, NYU Winthrop Hospital, 259 First Street, Mineola, NY 11501, United States
[b]Section of Neonatal-Perinatal Medicine, Department of Pediatrics, St. Christopher's Hospital for Children, Drexel University College of Medicine, 160 East Erie Avenue, Philadelphia, PA 19134, United States

ARTICLE INFO

Key Words:
Newborn, Surfactant
Antenatal Steroids
Postnatal Corticosteroids
NIPPV
Noninvasive Ventilation
Lung
Chronic Lung Disease
Bronchopulmonary Dysplasia

ABSTRACT

Bronchopulmonary dysplasia (BPD) is a complex disorder with multiple factors implicated in its etiopathogenesis. Despite the scientific advances in the field of neonatology, the incidence of BPD has remained somewhat constant due to increased survival of extremely premature infants. Surfactant deficiency in the immature lung, exposure to invasive mechanical ventilation leading to volutrauma, barotrauma and lung inflammation are some of the critical contributing factors to the pathogenesis of BPD. Hence, strategies to prevent BPD in the postnatal period revolve around mitigation of this injury and inflammation. This article reviews the progress made in the last 5 years in the development of new preparations of surfactant, use of corticosteroids and non-invasive ventilation in the prevention of BPD. Emerging techniques of surfactant delivery through minimally invasive and non-invasive routes are also discussed.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Bronchopulmonary dysplasia (BPD) is a common cause of mortality and morbidity in extreme preterm infants.[1] The adverse effects of BPD extend well into childhood and adulthood with associated significant health care costs.[2] Even though there is currently a better understanding of the pathogenesis of BPD, its incidence has remained somewhat constant because of the increased survival of extreme preterm infants.[3,4] These infants are born at the late canalicular–saccular stage of lung development with resulting surfactant inadequacy. Early surfactant administration in this population permits the use of less aggressive ventilation strategies including non-invasive ventilation, thus reducing the risk of BPD development.[5] Lung inflammation is another important risk factor in the development of BPD, providing the rationale for treating patients at risk for BPD with steroids. This review focuses on studies involving the use of newer surfactants, use of corticosteroids and non-invasive ventilation published in the last 5 years, with the primary outcome of BPD (Fig. 1). For the purpose of this review, BPD has been defined using the National Institutes of Health consensus definition.[6]

Surfactant and BPD

While early exogenous surfactant therapy is effective in the prevention and treatment of respiratory distress syndrome (RDS), it has not been shown to decrease the incidence of BPD. A multicenter randomized controlled trial (RCT) evaluat-

* Corresponding author.
E-mail addresses: vineet.bhandari@americanacademic.com, vb366@drexel.edu (V. Bhandari).

https://doi.org/10.1053/j.semperi.2018.09.006
0146-0005/© 2018 Elsevier Inc. All rights reserved.

ing the outcomes of infants who were on mechanical ventilation and received late surfactant administration at 2 weeks did not show a difference in the outcome of severe BPD/death. However, the surfactant treated group had lesser respiratory morbidity at 1 year of age.[7]

Efforts are ongoing to develop a synthetic surfactant that has comparable efficacy to animal derived surfactant because of the potential limitations of the latter including limited supply, theoretical risk of infection and batch to batch variability in function. A comprehensive review on the evolution of surfactant therapy, different types of surfactant treatment and development of newer-generation surfactants up until 2013 was published by El-Gendy et al.[8] A Cochrane review was published in 2015 with a meta-analysis of RCTs done between 1993 and 2005 comparing the effect of animal derived vs. synthetic surfactant in preterm infants at risk of developing RDS. The analysis showed a slight decrease in the risk of BPD or mortality associated with the use of animal derived surfactant preparations (typical relative risk or RR 0.95, 95% confidence intervals or CI 0.91–1.00; typical risk difference or RD −0.03, 95% CI −0.06 to 0.00; 6 studies, 3811 infants).[9]

Recent studies on the use of synthetic surfactants are showing promising results. CHF5633 (Chiesi Farmaceutici S.p.A., Parma, Italy) is a synthetic surfactant preparation consisting of phosphatidylcholine and phosphatidylglycerol, enriched by peptide analogues of both human surfactant proteins (SP)-B and SP-C. In animal studies, it was shown to decrease inflammation in immature newborn lambs with surfactant deficiency, comparable to animal derived surfactant Survanta® (AbbVie Inc., North Chicago, IL, USA).[10] In a study on preterm rabbits with severe surfactant deficiency, CHF5633 was shown to be as efficient as Curosurf® (Chiesi Farmaceutici S.p.A., Parma, Italy) and a clear dose-dependent improvement of lung function was observed for CHF5633, with the dose of 200 mg/kg being the most efficient one.[11] Rey-Santano et al. showed that use of CHF 5633 resulted in improvement of pulmonary status in preterm lambs with better lung and brain injury scores than controls and favorable cerebral hemodynamics, comparable to those with Curosurf® treatment.[12] After encouraging results in these animal studies,[10–12] a pilot human trial using CHF 5633 was conducted in 40 preterm infants with gestational age ranging between 27 $^{0/7}$ and 33 $^{6/7}$ weeks to assess its safety and efficacy. CHF5633 was noted to be well tolerated at both doses of 100 mg/kg and 200 mg/kg with improvement of RDS. The rate of BPD was low in this study (10%) as would be expected due to the relatively low risk study population.[13] A RCT is in progress (ClinTrials.gov NCT02452476) comparing CHF5633 to Curosurf® in a larger population with extremely preterm infants and with BPD as one of the primary outcomes.

The cost of the animal derived surfactants is a limitation for its large-scale use in developing countries. A new method of extraction and purification of a natural porcine-derived surfactant was developed by an institute in Sao Paulo, Brazil (Butanan surfactant) with a reduced production cost. A multicenter, RCT was done comparing the outcomes of infants who received Butanan surfactant to those who received Survanta® and Curosurf®. There was no difference in the primary outcomes of mortality at 72 h and 28 days but the use of oxygen at 28 days was higher in the group of infants who received Butanan surfactant.[14] We were unable to find any additional studies on the use of this surfactant. In another study comparing synthetic surfactants composed of different combinations of surfactant components, it was shown that Synsurf-3 containing both SP-B and SP-C synthetic analogs had comparable and better efficacy than commercially used Surfacten® in *in vitro* experiments and an *in vivo* rabbit model, respectively.[15] The alternate routes of surfactant administration are discussed later in this review. Table 1 shows the surfactants developed or under development in the last 5 years.

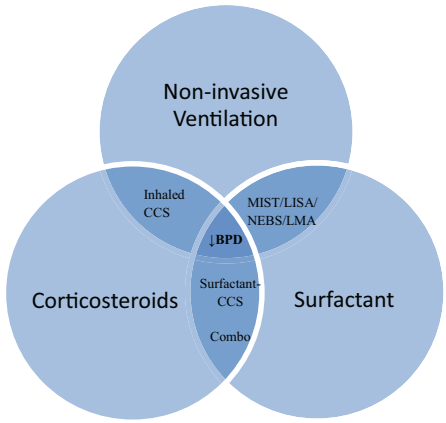


Fig. 1 – The figure shows the interplay between surfactant, steroids and non-invasive ventilation in decreasing BPD.

CCS: corticosteroids; MIST: minimally invasive surfactant administration; LISA: less invasive surfactant administration; NEBS: nebulized; LMA: laryngeal mask airway; Combo: combination.

Steroids and BPD

The use of postnatal glucocorticoids in the prevention and management of BPD has been an area of controversy mainly due to the potential long-term adverse effects on neurodevelopmental outcomes. Recent Cochrane reviews on the use of early (<8 days) and late (>7 days) systemic corticosteroids for prevention of BPD in preterm infants showed a reduction in the outcome of BPD and BPD/death. However, the authors concluded that the benefits of corticosteroid treatment may not outweigh the adverse effects, particularly with the use of dexamethasone.[16,17]

The effect of early low-dose hydrocortisone on BPD was investigated in the PREMILOC trial.[18] It was a large, multicenter, randomized, placebo controlled trial designed to look at the effect of early low-dose hydrocortisone on survival without BPD in extremely preterm infants with gestation age between 24 $^{0/7}$ to 27 $^{6/7}$ weeks. Hydrocortisone was administered at a dose of 1 mg/kg per day divided into two doses per day on days 1–7, followed by one dose of 0.5 mg/kg per day for the next 3 days. Of the 255 infants assigned to hydrocortisone, 153 (60%) survived without BPD, compared to 136 (51%) of 266 infants assigned to placebo. The odds ratio, adjusted for gestational age group and interim analyses was 1.48 (95% CI 1.02–2.16, $p = 0.04$).[18] Long-term follow-up results are encouraging with no significant difference in

Table 1 – Surfactants developed or under development in the last 5 years.

Surfactant source	Composition	Surfactant proteins	Surfactant name
Animal-porcine	Minced lung extract	SP-B, SP-C	Butanan surfactant
Synthetic	Synthetic DPCC, PG	Analogs of SP-B, SP-C	CHF 5633
Synthetic	Synthetic DPCC, PG, PA	KL-4	Aerosurf (Inhaled form of Lucinactant)
Synthetic	Synthetic DPCC, PG, PA	Analogs of SP-B, SP-C	Synsurf-3

DPCC: Dipalmitoyl Phosphatidylcholine; PG: Phosphatidylglycerol; PA: Palmitic acid; SP-B: Surfactant protein B; SP-C: Surfactant protein-C; KL-4: synthetic peptide developed to resemble the amino acid pattern of SP-B.

neurodevelopmental outcomes between infants in the 2 groups at the 2 year follow-up.[19] Follow up study done by stratifying infants according to gestational age showed a statistically significant improvement in neurodevelopmental outcomes in infants born at 24 and 25 weeks of gestation in the hydrocortisone group.[20]

Inhaled glucocorticosteroids with their potential for less systemic toxicity have been used in the management of BPD. The effect of early inhaled corticosteroids on BPD was investigated in the NEUROSIS trial.[21] Inhaled budesonide vs. placebo was administered to extremely premature infants (23 $^{0/7}$ weeks to 27 $^{6/7}$ weeks gestational age) starting within 24 h after birth and continuing until the infants no longer needed supplemental oxygen and positive-pressure support or reached a postmenstrual age of 32 $^{0/7}$ weeks. Budesonide was administered at a dose of 2 puffs (200 micrograms per puff) every 12 hours in the first 14 days of life and one puff administered every 12 hours from day 15 until the last dose of the study drug had been administered. There was a reduction in composite outcome of BPD/death in the budesonide group. Infants who received budesonide had a lower rate of BPD (27.8% vs. 38%) but a higher death rate (16.9% vs. 13.6%).[21] This finding of increased mortality among infants who received budesonide was also noted at the 2 year follow up.[22]

Another recent RCT evaluated the effect of early inhaled budesonide on preterm infants (gestational age 23–28 weeks, $n = 70$) starting 12 h after birth and continued for a maximum of 7 days. Even though the use of inhaled budesonide decreased the incidence of BPD (defined as oxygen requirement for 28 days), the difference was not statistically significant. (31.4% vs. 54.3%, $p = 0.053$).[23] A meta-analysis of published trials concluded that inhaled steroids (beclomethasone, budesonide, fluticasone, flunisolide, and dexamethasone) were associated with a significant reduction in BPD (RR = 0.77, 95% CI 0.65–0.91).[24] However, further long-term studies are needed before the use of early inhaled corticosteroids can be routinely recommended as part of BPD management.

A RCT by Yeh et al. reported the effect of intratracheal corticosteroid-surfactant combination on BPD.[25] In this study, very low birth weight (VLBW) infants ($n = 265$) with severe RDS requiring mechanical ventilation and fraction of inspired oxygen (FiO_2) ≥ 0.50 were randomized to receive either intratracheal budesonide (0.25 mg/kg) combined with surfactant (Survanta® 100 mg/kg) or surfactant (Survanta® 100 mg/kg) alone within four hours of birth. The intervention group had a significantly lower incidence of BPD or death (42.0% vs. 66%; RR 0.58; 95% CI 0.44–0.77; $p < 0.001$; number needed to treat or NNT, 4.1; 95% CI 2.8–7.8).[25] A meta-analysis of two RCTs demonstrated that intratracheal budesonide-surfactant mixture was associated with a decreased risk of BPD (RR: 0.57; 95% CI: 0.43–0.76, NNT = 5) and the composite outcome of death/BPD (RR: 0.60; 95%CI: 0.49–0.74, NNT = 3) in VLBW infants.[26] These results are promising and further large-scale RCTs are needed to confirm the efficacy and establish the safe side effect profile of the steroid-surfactant combination.

Ongoing trials: The Minidex trial is an ongoing multicenter RCT evaluating the effect of low-dose dexamethasone (13 doses of 0.015 ml/kg/day) in preterm infants <30 weeks gestational age between postnatal days 10–24 (https://www.npeu.ox.ac.uk/minidex). The STOP–BPD study is another multicenter randomized double blind placebo controlled trial being conducted in the Europe investigating if systemic hydrocortisone started between postnatal days 7–14 decreases the combined outcome of death/BPD in ventilated preterm infants with gestational age <30 weeks and/or birth weight less than 1250 g. Hydrocortisone (cumulative dose 72.5 mg/kg) or placebo is being administered with a 22 day tapering schedule in this study and the 2 year follow up is expected to be completed in 2019.[27]

Non-invasive ventilation and BPD

As it is increasingly being recognized that endotracheal mechanical ventilation is associated with increased risk of developing BPD, use of non-invasive ventilation techniques in preterm infants is gaining popularity.[28] A recent meta-analysis comparing prophylactic continuous positive airway pressure (CPAP) to assisted ventilation with or without surfactant in preterm infants less than 32 weeks gestation demonstrated that use of CPAP resulted in a reduction in the incidence of BPD (RR 0.89, 95% CI 0.79–0.99, $p = 0.04$) and death or BPD (RR 0.89, 95% CI 0.81–0.97).[29] Another meta-analysis investigating the effect of strategies that avoid endotracheal mechanical ventilation (i.e. strategies employing non-invasive ventilation) in preterm infants less than 30 weeks gestation demonstrated a decrease in BPD/death with odds ratio of 0.83(0.71–0.96, $p = 0.01$).[30]

The type of non-invasive ventilation strategy does not seem to have a significant impact on BPD. In a RCT ($n = 987$ analyzed) comparing the use of nasal intermittent positive pressure ventilation (NIPPV) to CPAP in preterm infants less than 30 weeks gestation and birth weight less than 1000 g, the incidence of BPD did not differ significantly between the 2 groups (33.9% vs. 31%, OR 1.14 95% CI 0.84–1.54, $p = 0.32$).[31] A

secondary, non-randomized analysis of the infants in the NIPPV arm in this trial was done comparing the outcomes of infants who received bi-level CPAP (Bi-PAP) vs. NIPPV via a conventional mechanical ventilator. There was no significant difference in the composite outcome of death/BPD between infants in the two groups [adjusted OR 0.88 (95% CI 0.57−1.35)]. The rate of BPD was lower in the infants who received Bi-PAP; however, this difference was not statistically significant (30% vs. 37%) [adjusted OR 0.64 (95% CI 0.41 to 1.02)]. More deaths occurred in infants receiving Bi-PAP (9.4%) than in NIPPV (2.3%) (adjusted OR 5.01: 95% CI 1.74−14.4).[32] A meta-analysis from the recent Cochrane reviews did not demonstrate a significant reduction in BPD with the use of NIPPV compared to CPAP (typical RR 0.94, 95% CI 0.80−1.10; typical RR 0.78, 95% CI 0.58−1.06).[33,34] A recent systematic review noted that use of NIPPV was associated with higher rates of successful extubation compared to CPAP.[35]

The duration of mechanical ventilation is another significant predictor of the development and severity of BPD. In infants who are intubated, the risk of developing BPD increases with the duration of mechanical ventilation. In a large retrospective cohort study of extremely LBW (ELBW) infants, it was noted that the risk of developing BPD increased with the cumulative duration of mechanical ventilation, but it was not related to the number of ventilation courses. Based on the results of the study, the authors recommend that extubation be trialed once low ventilator settings are reached, even if extubation success is not guaranteed, as the sooner an extubation attempt is made, the lower the risk of BPD.[36] Robbins et al. reported in a cohort of 224 patients born at <27 weeks' gestation that the age at first extubation attempt correlated directly with endotracheal mechanical ventilation days and length of stay. In this study, the median mechanical ventilation days were 32 and 65% of patients needed re-intubation. Furthermore, they reported that the earlier an extubation attempt was made, the lower the rate of BPD.[37]

The above 2 studies essentially re-affirmed the findings of an earlier retrospective study of 262 preterm infants with gestational age ≤28 weeks and who were intubated on day of life 1, where it was noted that delaying extubation beyond the first 7 days was associated with an increased risk of BPD and BPD/death. Infants who were extubated early but had to be re-intubated later were noted to have a lower incidence of BPD/death compared to infants who remained intubated and were extubated later.[38]

Prompt determination of extubation readiness helps to minimize or prevent extubation failures. Pilot studies utilizing data on heart rate variability to determine extubation readiness in extremely preterm infants met with success and a RCT is ongoing to develop an automated prediction tool for extubation readiness in extremely preterm infants.[39,40]

Alternate routes of surfactant delivery

Perhaps the most significant progress in the prevention/reduction of BPD in the last decade has been made in developing techniques to administer exogenous surfactant through non-invasive or minimally invasive routes. Some of the routes of surfactant administration other than conventional endotracheal tube instillation include aerosolized delivery through a nebulizer, surfactant delivery through laryngeal mask airway (LMA) and administration via a thin catheter introduced endotracheally. All these strategies obviate the need for endotracheal tube ventilation in the at-risk preterm population, reducing the potential for lung injury and subsequent development of BPD. A comprehensive review on minimally invasive surfactant therapy and non-invasive respiratory support was published by Kribs.[41] Table 2 summarizes the clinical studies on surfactant administration through alternate routes published in the last 5 years.

Aerosol delivery of surfactant continues to be an area of active research. Multiple small studies in animal models and neonates demonstrated the safety and feasibility of nebulized surfactants but their superiority over intratracheally administered surfactants needs to be proven in large trials.

Two studies done in animal models confirmed the feasibility of surfactant administration through the nebulization technique utilizing the eFlow® nebulizer – a customized vibrating perforated membrane nebulizer (Neonatal Nebulizer system; PARI Pharma, Starnberg, Germany).[42,43] Recently, a phase 2b clinical trial (n = 221 born between 28 and 32 weeks gestation) using Aerosurf (synthetic peptide KL4 based lung surfactant) delivered through a vibrating nebulizer mesh showed that Aerosurf had a similar level of efficacy compared to the CPAP only controls. (http://windtreetx.investorroom.com/2017-06-29-Windtree-Announces-Top-Line-Results-from-AEROSURF-R-Phase-2b-Clinical-Trial-for-the-Treatment-of-Respiratory-Distress-Syndrome-RDS-in-Premature-Infants).

The CureNeb study by Minocchieri et al. is a pragmatic, stratified, double blind RCT evaluating the impact of nebulized surfactant on intubation requirement in preterm infants with RDS. 64 infants with GA ranging from 29 $^{0/7}$ to 33 $^{6/7}$ weeks were randomized within the first four hours after birth to receive either CPAP or aerosolized surfactant and CPAP. The surfactant (poractant alfa, 200 mg/kg) was delivered via eFlow®. The primary outcome of need for intubation within 72 h of birth was less in the nebulization group compared to the CPAP alone group [RR (95% CI) = 0.526 (0.292−0.950)]. Notably, this difference was observed only in the 32 $^{0/7}$−33 $^{6/7}$ GA group but not in the 29 $^{0/7}$−31 $^{6/7}$ GA stratification group. None of the infants in the study had the outcome of BPD. The study was closed to recruitment earlier than planned due to insufficient funding/personnel.[44]

The terms less invasive surfactant administration (LISA) and minimally invasive surfactant therapy (MIST) are sometimes used interchangeably in literature. Originally LISA was used to refer to surfactant administration through a feeding catheter introduced endotracheally using a Magill forceps. MIST was used to refer to surfactant administration through a semi-rigid vascular catheter placed endotracheally without the use of Magill forceps. Researchers have published studies with slight modifications to these two techniques resulting in varied nomenclature.

Another strategy to decrease lung injury involves intubation and surfactant administration followed by immediate extubation (INSURE) technique. A recent meta-analysis demonstrated no significant difference in the outcomes of BPD/death with prophylactic INSURE vs. CPAP.[45] A new modified

Table 2 – Clinical studies published in the last 5 years on surfactant administration through alternate routes.

Author/ref	Type	N	Control	Intervention	Outcomes
Aerosolized					
Minocchieri et al.[44]	RCT	64	CPAP alone	CPAP+Curosurf via vibrating membrane nebulizer	Decreased need for intubation in intervention group. No difference in BPD
Segal et al.	RCT	Ongoing	CPAP alone	CPAP+Lucinactant (3 doses)	Pending
Sood et al.	RCT	Ongoing	Survanta 100 vs. 200 mg/kg	Survanta;100 vs. 200 mg/kg via nebulizer	Pending
LMA					
Attridge et al.[51]	RCT	26	CPAP alone	Surfactant by LMA followed by CPAP	Decrease in supplemental oxygen requirement. No difference in BPD
Sadeghnia et al.[52]	RCT	70	INSURE	Surfactant by supraglottic airway (iGel) administration followed by CPAP	Higher A-a DO_2 after surfactant in LMA group ($p = 0.001$)
Pinheiro et al.[53]	RCT	61	INSURE	Surfactant via LMA followed by CPAP	Failure rate of surfactant resulting in MV: 77% in control group vs. 30% in LMA group ($p < 0.001$). No difference in BPD
Roberts et al.[54]	RCT	103	CPAP alone	Surfactant via LMA followed by CPAP	Decreased rate of intubation and MV in LMA group (38% vs. 64%, $p = 0.006$) No difference in BPD
Barbosa et al.[55]	RCT	48	Surfactant via ETT	Surfactant via LMA followed by CPAP	FiO_2 requirement similar in both groups. No difference in BPD
LISA/MIST					
Dargaville et al.[56]	Open feasibility study	158	Historic controls who received CPAP	MIST (16 G vascular catheter)-Hobart technique	Decreased MV with MIST. No difference in BPD
Klebermass-Shrehoff et al.[57]	Nonrandomized study	406	Historic controls	LISA (4 Fr feeding catheter)	No significant difference in BPD: Decreased oxygen at day 28 and CLD/Death with LISA. 40% vs. 51% ($p = 0.03$)
Mirnia et al.[58]	RCT	80	INSURE	LISA (5 Fr catheter)	Shorter CPAP duration and lower NEC with LISA. No difference in BPD
Kanmaz et al.[59]	RCT	200	INSURE	LISA (5 Fr catheter)- Take Care technique	BPD, need for MV, duration of CPAP and MV lower with LISA
Aguar et al.[60]	Prospective cohort study	75	Historic controls who received INSURE	MIST (3.5–4 Fr nasogastric catheter)	Higher requirement for second dose of surfactant with MIST. No difference in BPD
Kribs et al.[61]	RCT	211	Surfactant via ETT	LISA (4 Fr catheter)	No difference in survival without BPD but survival without major complications higher with LISA
Krajewski et al.[62]	Prospective cohort study	52	Historic controls who received INSURE	MIST (0.04 ch feeding tube)	Less BPD with MIST
Mohammadizadeh et al.[63]	RCT	38	Surfactant via ETT	LISA (4 Fr feeding tube)	Shorter duration of oxygen therapy with LISA. No difference in BPD.
Bao et al.[64]	RCT	90	INSURE	MIST (16 G vascular catheter)	Shorter duration of MV and CPAP with LISA
Gopel et al.[65]	Prospective cohort study	2206	Matched controls	LISA	Lower rates of MV and BPD with LISA
		47		MIST (16 G vascular catheter)	

Table 2 (*continued*)

Author/ref	Type	N	Control	Intervention	Outcomes
Canals Candela et al.[66]	Retrospective observational study		Historic controls who failed NIV and needed Surfactant via ETT		Lesser need for ETT intubation with MIST. No difference in BPD
Tomar et al.[67]	Prospective observational study	132	Historic controls who received INSURE	MIST (5 Fr orogastric tube)	Shorter duration of MV and CPAP with MIST. No difference in BPD
Olivier et al.[68]	RCT	45	CPAP. Surfactant via ETT if needed	MIST (5 Fr feeding tube)	Need for MV and rate of pneumothoraces requiring chest tube lower with MIST
Lau et al.[69]	Meta-analysis	328	INSURE	LISA	Decreased need for MV within first 72 h, duration of MV, CPAP, and supplemental oxygen noted with LISA. A trend towards a reduction in BPD noted with LISA ($p = 0.141$)
Aldana-Aguirre et al.[70]	Meta-analysis	895	Surfactant via ETT	LISA	Decreased need for MV within 72 h, need for MV, death/BPD with LISA
Hartel et al.[49]	Observational study	6319	Surfactant via ETT	LISA	Decreased BPD, death, BPD/death but increased focal intestinal perforation with LISA
Dargaville et al OPTIMIST-A trial.[71]	RCT	Ongoing	Sham treatment	MIST (16 G vascular catheter)	
Intra-amniotic instillation					
Agrawal et al.[72]	Randomized pilot study	40	28–34 week GA infants	Intra-amniotic surfactant instillation 2–8 h before delivery in 28–34 week GA infants	Decreased severe RDS and need for postnatal surfactant in intervention group

RCT: randomized controlled trial; CPAP: continuous positive airway pressure; LMA: laryngeal mask airway; INSURE: intubate-surfactant-extubate; BPD: bronchopulmonary dysplasia; MV: mechanical ventilation; A-a DO_2: Alveolar-arterial oxygen gradient; ETT: Endotracheal tube; FiO_2: fraction of inspired oxygen; CLD: chronic lung disease; LISA/MIST: less invasive surfactant administration/minimally invasive surfactant administration; NIV: non-invasive ventilation; NEC: Necrotizing enterocolitis.

technique with alveolar recruitment maneuver before surfactant administration (IN-REC-SUR-E) is currently being investigated.[46]

A meta-analysis comparing 7 different ventilation strategies for preterm infants including CPAP alone, INSURE, LISA, NIPPV, nebulized surfactant administration, surfactant administration via LMA, and mechanical ventilation concluded that the use of LISA was associated with the lowest likelihood of the composite outcome of death or BPD at 36 weeks postmenstrual age. However, the authors conclude that these findings were limited by the overall low quality of evidence and lack of robustness in higher-quality trials.[47] Another meta-analysis evaluating 6 RCTs concluded that LISA decreases the risk of BPD (RR = 0.71 [0.52–0.99]; NNT = 21), death or BPD (RR = 0.74 [0.58–0.94] NNT = 15) and early CPAP failure or invasive ventilation requirements (RR = 0.67 [0.53–0.84]; NNT = 8 and RR = 0.69 [0.53–0.88]; NNT = 6).[48] Hartel et al. recently published an observational cohort study from a large neonatal network comparing the outcomes of infants who received surfactant via ETT (n = 3695) to those who received LISA (n = 2624). In this study, LISA was associated with a reduced risk of BPD [OR 0.55 (95% CI: 0.49–0.62), $p < 0.001$], death [OR 0.66 (95% CI: 0.51–0.84), $p < 0.001$] and BPD/death [OR 0.5 (95% CI: 0.44–0.57), $p < 0.001$], after adjusting for various factors in a multivariable logistic regression model. However, there was an increased incidence of focal intestinal perforation in the LISA group [OR 1.49 (95% CI: 1.14–1.95), $p = 0.003$] in a multivariable logistic regression model, primarily noted in the VLBW infants born at less than 26 weeks. Future trials should focus on the safety of LISA in the subgroup of extremely premature infants.[49]

In a RCT by Oncel et al. (n = 200, preterm infants 26–32 weeks gestational age) comparing early NIPPV and NCPAP with in the MIST approach, NIPPV decreased the need for invasive mechanical ventilation within the first 72 h of life and reduced the surfactant requirement. Infants in the NIPPV group had significantly decreased moderate-to-severe BPD (7% vs. 16%; $p = 0.046$), although this difference was not observed on multivariate analysis.[50]

Conclusions

Based on the current available evidence in the arena of BPD prevention, it can be recommended that an attempt be made to extubate the infant early (within the first 72 h of life) and to use non-invasive ventilation strategies and MIST/LISA whenever feasible. While the use of postnatal corticosteroids still remains controversial, early use of low-dose hydrocortisone in an at-risk preterm population showed a decreased incidence of BPD without significant neurodevelopmental impairment on long-term follow-up. The use of intratracheal surfactant-corticosteroid combination is promising but needs to be evaluated in further large-scale trials. Research on the use of synthetic and/or nebulized surfactants is ongoing and if proven to have a comparable or higher efficacy than animal derived surfactants, will be a boon in the management of RDS and BPD worldwide.

REFERENCES

1. Bhandari A, Bhandari V. Pitfalls, problems, and progress in bronchopulmonary dysplasia. *Pediatrics*. 2009;123:1562–1573.
2. Underwood MA, Danielsen B, Gilbert WM. Cost, causes and rates of rehospitalization of preterm infants. *J Perinatol Offic J Calif Perinat Assoc*. 2007;27:614–619.
3. Smith VC, Zupancic JA, McCormick MC, et al. Trends in severe bronchopulmonary dysplasia rates between 1994 and 2002. *J Pediatr*. 2005;146:469–473.
4. Zysman-Colman Z, Tremblay GM, Bandeali S, Landry JS. Bronchopulmonary dysplasia - trends over three decades. *Paediatr Child Health*. 2013;18:86–90.
5. Stevens TP, Harrington EW, Blennow M, Soll RF. Early surfactant administration with brief ventilation vs. selective surfactant and continued mechanical ventilation for preterm infants with or at risk for respiratory distress syndrome. *Cochrane Database Syst Rev*. 2007:Cd003063.
6. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723–1729.
7. Hascoet JM, Picaud JC, Ligi I, et al. Late surfactant administration in very preterm neonates with prolonged respiratory distress and pulmonary outcome at 1 year of age: a randomized clinical trial. *JAMA Pediatr*. 2016;170:365–372.
8. El-Gendy N, Kaviratna A, Berkland C, Dhar P. Delivery and performance of surfactant replacement therapies to treat pulmonary disorders. *Ther Deliv*. 2013;4:951–980.
9. Ardell S, Pfister RH, Soll R. Animal derived surfactant extract versus protein free synthetic surfactant for the prevention and treatment of respiratory distress syndrome. *Cochrane Database Syst Rev*. 2015;8:CD000144.
10. Sato A, Ikegami M. SP-B and SP-C containing new synthetic surfactant for treatment of extremely immature lamb lung. *PLoS One*. 2012;7:e39392.
11. Ricci F, Murgia X, Razzetti R, Pelizzi N, Salomone F. In vitro and in vivo comparison between poractant alfa and the new generation synthetic surfactant CHF5633. *Pediatr Res*. 2017;81:369–375.
12. Rey-Santano C, Mielgo VE, Murgia X, et al. Cerebral and lung effects of a new generation synthetic surfactant with SP-B and SP-C analogs in preterm lambs. *Pediatr Pulmonol*. 2017;52:929–938.
13. Sweet DG, Turner MA, Stranak Z, et al. A first-in-human clinical study of a new SP-B and SP-C enriched synthetic surfactant (CHF5633) in preterm babies with respiratory distress syndrome. *Arch Disease Childhood Fetal Neonat Ed*. 2017;102:F497–f503.
14. Rebello CM, Precioso AR, Mascaretti RS. A multicenter, randomized, double-blind trial of a new porcine surfactant in premature infants with respiratory distress syndrome. *Einstein (Sao Paulo, Brazil)*. 2014;12:397–404.
15. Choi YS, Chung SH, Bae CW. A combination of short and simple surfactant protein b and c analogues as a new synthetic surfactant: in vitro and animal experiments. *Yonsei Med J*. 2017;58:823–828.
16. Doyle LW, Cheong JL, Ehrenkranz RA, Halliday HL. Early (< 8 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants. *Cochrane Database Syst Rev*. 2017;10:Cd001146.
17. Onland W, Offringa M, van Kaam A. Late (>/= 7 days) inhalation corticosteroids to reduce bronchopulmonary dysplasia in preterm infants. *Cochrane Database Syst Rev*. 2017;8:Cd002311.
18. Baud O, Maury L, Lebail F, et al. Effect of early low-dose hydrocortisone on survival without bronchopulmonary dysplasia in extremely preterm infants (PREMILOC): a double-blind, placebo-controlled, multicentre, randomised trial. *Lancet*. 2016;387:1827–1836.

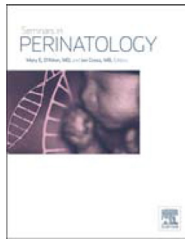
19. Baud O, Trousson C, Biran V, Leroy E, Mohamed D, Alberti C. Association between early low-dose hydrocortisone therapy in extremely preterm neonates and neurodevelopmental outcomes at 2 years of age. *JAMA*. 2017;317:1329–1337.
20. Baud O, Trousson C, Biran V, Leroy E, Mohamed D, Alberti C. Two-year neurodevelopmental outcomes of extremely preterm infants treated with early hydrocortisone: treatment effect according to gestational age at birth. *Arch Diseas Childhood Fetal Neonat Ed*. 2018 Jan 10. pii: fetalneonatal-2017-313756. https://doi.org/10.1136/archdischild-2017-313756. [Epub ahead of print].
21. Bassler D, Plavka R, Shinwell ES, et al. Early inhaled budesonide for the prevention of bronchopulmonary dysplasia. *N Engl J Med*. 2015;373:1497–1506.
22. Bassler D, Shinwell ES, Hallman M, et al. Long-term effects of inhaled budesonide for bronchopulmonary dysplasia. *New England J Med*. 2018;378:148–157.
23. Sadeghnia A, Beheshti BK, Mohammadizadeh M. The effect of inhaled budesonide on the prevention of chronic lung disease in premature neonates with respiratory distress syndrome. *Int J Prev Med*. 2018;9:15.
24. Shinwell ES, Portnov I, Meerpohl JJ, Karen T, Bassler D. Inhaled corticosteroids for bronchopulmonary dysplasia: a meta-analysis. *Pediatrics*. Dec; 2016;138(6): pii: e20162511.
25. Yeh TF, Chen CM, Wu SY, et al. Intratracheal Administration of Budesonide/Surfactant to Prevent Bronchopulmonary Dysplasia. *Am J Respir Crit Care Med*. 2016;193:86–95.
26. Venkataraman R, Kamaluddeen M, Hasan SU, Robertson HL, Lodha A. Intratracheal administration of budesonide-surfactant in prevention of bronchopulmonary dysplasia in very low birth weight infants: a systematic review and meta-analysis. *Pediatr Pulmonol*. 2017;52:968–975.
27. Onland W, Merkus MP, Nuytemans DH, Jansen-van der Weide MC, Holman R, van Kaam AH. Systemic Hydrocortisone To Prevent Bronchopulmonary Dysplasia in preterm infants (the SToP-BPD study): statistical analysis plan. *Trials*. 2018;19:178.
28. Bhandari V. The potential of non-invasive ventilation to decrease BPD. *Semin Perinatol*. 2013;37:108–114.
29. Subramaniam P, Ho JJ, Davis PG. Prophylactic nasal continuous positive airway pressure for preventing morbidity and mortality in very preterm infants. *Cochrane Database Syst Rev*. 2016 Jun 14; (6):CD001243. https://doi.org/10.1002/14651858.CD001243.pub3.
30. Fischer HS, Buhrer C. Avoiding endotracheal ventilation to prevent bronchopulmonary dysplasia: a meta-analysis. *Pediatrics*. 2013;132:e1351–e1360.
31. Kirpalani H, Millar D, Lemyre B, et al. A trial comparing noninvasive ventilation strategies in preterm infants. *N Engl J Med*. 2013;369:611–620.
32. Millar D, Lemyre B, Kirpalani H, Chiu A, Yoder BA, Roberts RS. A comparison of bilevel and ventilator-delivered non-invasive respiratory support. *Arch Diseas Childh Fetal Neonat Ed*. 2016;101:F21–F25.
33. Lemyre B, Davis PG, De Paoli AG, Kirpalani H. Nasal intermittent positive pressure ventilation (NIPPV) versus nasal continuous positive airway pressure (NCPAP) for preterm neonates after extubation. *Cochrane Database Syst Rev*. 2017;2:Cd003212.
34. Lemyre B, Laughon M, Bose C, Davis PG. Early nasal intermittent positive pressure ventilation (NIPPV) versus early nasal continuous positive airway pressure (NCPAP) for preterm infants. *Cochrane Database Syst Rev*. 2016;12:Cd005384.
35. Ferguson KN, Roberts CT, Manley BJ, Davis PG. Interventions to improve rates of successful extubation in preterm infants: a systematic review and meta-analysis. *JAMA Pediatr*. 2017;171:165–174.
36. Jensen EA, DeMauro SB, Kornhauser M, Aghai ZH, Greenspan JS, Dysart KC. Effects of multiple ventilation courses and duration of mechanical ventilation on respiratory outcomes in extremely low-birth-weight infants. *JAMA Pediatr*. 2015;169:1011–1017.
37. Robbins M, Trittmann J, Martin E, Reber KM, Nelin L, Shepherd E. Early extubation attempts reduce length of stay in extremely preterm infants even if re-intubation is necessary. *J Neonat Perinat Med*. 2015;8:91–97.
38. Berger J, Mehta P, Bucholz E, Dziura J, Bhandari V. Impact of early extubation and reintubation on the incidence of bronchopulmonary dysplasia in neonates. *Am J Perinatol*. 2014;31:1063–1072.
39. Kaczmarek J, Chawla S, Marchica C, Dwaihy M, Grundy L, Sant'Anna GM. Heart rate variability and extubation readiness in extremely preterm infants. *Neonatology*. 2013;104:42–48.
40. Shalish W, Kanbar LJ, Rao S, et al. Prediction of Extubation readiness in extremely preterm infants by the automated analysis of cardiorespiratory behavior: study protocol. *BMC Pediatr*. 2017;17:167.
41. Kribs A. Minimally invasive surfactant therapy and noninvasive respiratory support. *Clin Perinatol*. 2016;43:755–771.
42. Hutten MC, Kuypers E, Ophelders DR, et al. Nebulization of Poractant alfa via a vibrating membrane nebulizer in spontaneously breathing preterm lambs with binasal continuous positive pressure ventilation. *Pediatr Res*. 2015;78:664–669.
43. Linner R, Perez-de-Sa V, Cunha-Goncalves D. Lung deposition of nebulized surfactant in newborn piglets. *Neonatology*. 2015;107:277–282.
44. Minocchieri S, Berry CA, Pillo JJ, Nebulised surfactant to reduce severity of respiratory distress: a blinded, parallel, randomised controlled trial. *Arch Diseas Childh Fetal Neonat Ed*. 2018 Jul 26. pii: fetalneonatal-2018-315051. https://doi.org/10.1136/archdischild-2018-315051. [Epub ahead of print].
45. Isayama T, Chai-Adisaksopha C, McDonald SD. Noninvasive ventilation with vs without early surfactant to prevent chronic lung disease in preterm infants: a systematic review and meta-analysis. *JAMA Pediatr*. 2015;169:731–739.
46. Vento G, Pastorino R, Boni L, et al. Efficacy of a new technique – INtubate-RECruit-SURfactant-Extubate – "IN-REC-SUR-E" – in preterm neonates with respiratory distress syndrome: study protocol for a randomized controlled trial. *Trials*. 2016;17:414.
47. Isayama T, Iwami H, McDonald S, Beyene J. Association of Noninvasive Ventilation Strategies With Mortality and Bronchopulmonary Dysplasia Among Preterm Infants: A Systematic Review and Meta-analysis. *JAMA*. 2016;316:611–624.
48. Rigo V, Lefebvre C, Broux I. Surfactant instillation in spontaneously breathing preterm infants: a systematic review and meta-analysis. *Eur J Pediatr*. 2016;175:1933–1942.
49. Hartel C, Paul P, Hanke K, et al. Less invasive surfactant administration and complications of preterm birth. *Sci Rep*. 2018;8:8333.
50. Oncel MY, Arayici S, Uras N, et al. Nasal continuous positive airway pressure versus nasal intermittent positive-pressure ventilation within the minimally invasive surfactant therapy approach in preterm infants: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed*. 2016;101:F323–F328.
51. Attridge JT, Stewart C, Stukenborg GJ, Kattwinkel J. Administration of rescue surfactant by laryngeal mask airway: lessons from a pilot trial. *Am J Perinatol*. 2013;30:201–206.
52. Sadeghnia A, Tanhaei M, Mohammadizadeh M, Nemati M. A comparison of surfactant administration through i-gel and ET-tube in the treatment of respiratory distress syndrome in newborns weighing more than 2000 grams. *Adv Biomed Res*. 2014;3:160.
53. Pinheiro JM, Santana-Rivas Q, Pezzano C. Randomized trial of laryngeal mask airway versus endotracheal intubation for surfactant delivery. *J Perinatol Offic J Califor Perin Assoc*. 2016;36:196–201.

54. Roberts KD, Brown R, Lampland AL, et al. Laryngeal mask airway for surfactant administration in neonates: a randomized, controlled trial. *J Pediatr*. 2018;193:40–46.e41.
55. Barbosa RF, Simoes ESAC, Silva YP. A randomized controlled trial of the laryngeal mask airway for surfactant administration in neonates. *J Pediatr*. 2017;93:343–350.
56. Dargaville PA, Aiyappan A, De Paoli AG, et al. Minimally-invasive surfactant therapy in preterm infants on continuous positive airway pressure. *Arch Dis Child Fetal Neonatal Ed*. 2013;98: F122–F126.
57. Klebermass-Schrehof K, Wald M, Schwindt J, et al. Less invasive surfactant administration in extremely preterm infants: impact on mortality and morbidity. *Neonatology*. 2013;103:252–258.
58. Mirnia K, Heidarzadeh M, Hoseini MB, et al. Surfactant administration via thin catheter during spontaneous breathing: randomized controlled trial in Alzahra hospital. *Iran J Neonatol IJN*. 2013;4:5–9.
59. Kanmaz HG, Erdeve O, Canpolat FE, Mutlu B, Dilmen U. Surfactant administration via thin catheter during spontaneous breathing: randomized controlled trial. *Pediatrics*. 2013;131: e502–e509.
60. Aguar M, Cernada M, Brugada M, Gimeno A, Gutierrez A, Vento M. Minimally invasive surfactant therapy with a gastric tube is as effective as the intubation, surfactant, and extubation technique in preterm babies. *Acta Paediatr (Oslo, Norway: 1992)*. 2014;103:e229–e233.
61. Kribs A, Roll C, Gopel W, et al. Nonintubated surfactant application vs conventional therapy in extremely preterm infants: a randomized clinical trial. *JAMA Pediatr*. 2015;169:723–730.
62. Krajewski P, Chudzik A, Strzalko-Gloskowska B, et al. Surfactant administration without intubation in preterm infants with respiratory distress syndrome – our experiences. *J Mater Fetal Neonat Med Offic J Eur Assoc Perinat Med Feder Asia Ocean Perinat Soc Int Soc Per Obst*. 2015;28:1161–1164.
63. Mohammadizadeh M, Ardestani AG, Sadeghnia AR. Early administration of surfactant via a thin intratracheal catheter in preterm infants with respiratory distress syndrome: feasibility and outcome. *J Res Pharm Pract*. 2015;4:31–36.
64. Bao Y, Zhang G, Wu M, Ma L, Zhu J. A pilot study of less invasive surfactant administration in very preterm infants in a Chinese tertiary center. *BMC Pediatr*. 2015;15:21.
65. Gopel W, Kribs A, Hartel C, et al. Less invasive surfactant administration is associated with improved pulmonary outcomes in spontaneously breathing preterm infants. *Acta Paediatr (Oslo, Norway: 1992)*. 2015;104:241–246.
66. Canals Candela FJ, Vizcaino Diaz C, Ferrandez Berenguer MJ, Serrano Robles MI, Vazquez Gomis C, Quiles Dura JL. [Surfactant replacement therapy with a minimally invasive technique: experience in a tertiary hospital]. *Anal Pediatr (Barcelona, Spain :2003)*. 2016;84:79–84.
67. Tomar RS, Ghuliani R, Yadav D. Effect of surfactant therapy using orogastric tube for tracheal catheterization in preterm newborns with respiratory distress. *Indian J Pediatr*. 2017;84:257–261.
68. Olivier F, Nadeau S, Belanger S, et al. Efficacy of minimally invasive surfactant therapy in moderate and late preterm infants: a multicentre randomized control trial. *Paediatr Child Health*. 2017;22:120–124.
69. Lau CSM, Chamberlain RS, Sun S. Less invasive surfactant administration reduces the need for mechanical ventilation in preterm infants: a meta-analysis. *Glob Pediatr Health*. 2017;4: 2333794 × 17696683.
70. Aldana-Aguirre JC, Pinto M, Featherstone RM, Kumar M. Less invasive surfactant administration versus intubation for surfactant delivery in preterm infants with respiratory distress syndrome: a systematic review and meta-analysis. *Arch Disease Childhood Fetal Neonat Ed*. 2017;102:F17–f23.
71. Dargaville PA, Kamlin CO, De Paoli AG, et al. The OPTIMIST-A trial: evaluation of minimally-invasive surfactant therapy in preterm infants 25–28 weeks gestation. *BMC Pediatr*. 2014;14:213.
72. Agarwal N, Bathwal S, Kriplani A, Deorari A, Bhatla N. Intra-amniotic instillation of surfactants for the prevention of neonatal respiratory distress syndrome following preterm delivery. *Int J Gynaecol Obstetr Offic Organ Int Feder Gynaecol Obstetr*. 2016;135:196–199.

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com



An update on the diagnosis and management of bronchopulmonary dysplasia (BPD)-associated pulmonary hypertension

Sara K. Berkelhamer[a]*, Karen K. Mestan[b], and Robin Steinhorn[c]

[a]Jacobs School of Medicine, University at Buffalo, Buffalo, NY, USA
[b]Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[c]George Washington University, Washington, DC, USA

ARTICLE INFO

Keywords:
bronchopulmonary dysplasia
pulmonary hypertension
chronic lung disease
pulmonary vascular disease
diagnosis
management

ABSTRACT

The past decade of neonatal care has been highlighted by increased survival rates in smaller and more premature infants. Despite reduction in mortality associated with extreme prematurity, long term pulmonary morbidities remain a concern, with growing recognition of the clinical burden attributable to infants with bronchopulmonary dysplasia (BPD)-associated pulmonary hypertension (PH). Recent publications shed light on the critical contributions of maternal placental pathology and compromised intrauterine growth to fetal pulmonary vascular development. A body of literature has further clarified postnatal risk factors for PH, most notably the severity of BPD but surprisingly the additional presence of non-pulmonary morbidities including necrotizing enterocolitis (NEC). Limitations of current diagnostics persist with growing consideration of novel echocardiographic approaches as well as complementary non-invasive biomarkers to better identify infants at risk. In 2015, a joint report published by the American Heart Association and American Thoracic Society provided the first guidelines for the care of children with PH with limited content to address BPD-associated PH. These guidelines were expanded upon in an expert consensus report produced by the Pediatric Pulmonary Hypertension Network (PPHNet). These recommendations encouraged the use of standardized screening protocols and emphasized the importance of evaluation and treatment of comorbidities when PH is identified. Cardiac catheterization was recommended prior to initiation of therapy for more accurate quantification of pulmonary pressures, clarification of anatomy and guidance in the use of pharmacotherapy. Despite these guidelines, significant practice variation persists and gaps remain with respect to optimal evaluation and management of BPD-associated PH.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Advances in neonatal care, including routine use of antenatal steroids and surfactant, coupled with improved ventilation and nutritional strategies, have resulted in increasing rates of survival of extremely premature infants.[1] Despite increased use of gentle and non-invasive ventilation strategies, these infants remain at significant risk of prolonged pulmonary morbidity, commonly suffering from compromised lung

* Corresponding author.
E-mail address: saraberk@buffalo.edu (S.K. Berkelhamer).

https://doi.org/10.1053/j.semperi.2018.09.005
0146-0005/© 2018 Elsevier Inc. All rights reserved.

development with prolonged need for respiratory support and/or supplemental oxygen, meeting the clinical definition of bronchopulmonary dysplasia (BPD). Despite improved rates of survival of extremely premature infants, there has been relatively limited progress over the past two decades in reducing rates of BPD with a relatively stable incidence of approximately 40% in surviving infants less than or equal to 28 weeks gestation.[1–4]

While early characterization of BPD mainly focused on parenchymal lung injury,[5] there is growing appreciation for the contribution of a dysmorphic pulmonary bed as well as the impact of postnatal insults on pulmonary vascular remodeling and development. Ultimately, these vascular findings can result in elevated pulmonary resistance with increased pulmonary pressures, resulting in compensatory right ventricular hypertrophy and a clinical diagnosis of pulmonary hypertension (PH). The presence of PH markedly impacts clinical outcomes, with recent publications reporting mortality rates ranging from 14 -38%.[6–8] Meta-analysis of studies which have looked specifically at death before discharge report a cumulative mortality rate of 16% while those that followed patients out to 2 years estimated 40% mortality.[9] Beyond the increased risk of mortality, PH is associated with significant pulmonary morbidity with added concern for the impact on neurodevelopmental outcomes.[10,11] While progress has been made in the diagnosis and management of BPD-associated PH, the natural course and long-term outcomes of the disease remain poorly characterized and numerous gaps remain in defining optimal care.

Prevalence

Several recent publications have defined rates of occurrence of PH by BPD classification. While there is considerable heterogeneity with respect to timing of screening and definitions, a recent meta-analysis of over 1400 patients from 25 publications confirmed the association of PH with severity of pulmonary disease with cumulative prevalence of PH in 6%, 12%, and 39% of infants with mild, moderate and severe BPD, respectively (Figure 1).[9] While these data help to characterize the overall risk for PH, a wide range within the included studies was noted, with single center studies reporting prevalence rates ranging from 15 to 64% in infants with severe BPD.[12,13]

Of interest, this meta-analysis also identified a cumulative prevalence of PH in 2% in extremely preterm infants in the absence of BPD. Indeed, several recent studies have raised concern that abnormal pulmonary vascular development may occur as a result of prematurity alone.[14–16]

Pathophysiology

Premature infants are susceptible to altered lung development and postnatal lung injury as a result of exposure to pulmonary care, including ventilation and oxygen therapy. These insults, along with infection, inflammation and compromised nutrition can lead to the phenotype of compromised alveolar development and vascular pruning characteristic of BPD.[17] A subset of infants with BPD will develop increased pulmonary vascular resistance with elevated pulmonary arterial pressures as a result of a combination of arteriovenous maldevelopment, vascular remodeling, increased vascular tone and/or altered vasoreactivity. While pulmonary vascular disease is commonly attributed to lung injury and the severity of lung disease, the presence of PH in premature infants who were *not* classified as having BPD suggests that additional risk factors or alternative pathophysiology contribute to the development of PH. Recent publications have advanced our understanding of the contributions of the maternal disease states and environmental exposures, compromised *in utero* fetal growth, and postnatal non-pulmonary morbidity to the development of neonatal pulmonary vascular disease.

Table 1 – Infant characteristics associated with the presence of pulmonary hypertension.

Characteristic	Infants with or without BPD RR(CI)	Infants with BPD RR(CI)
Male sex	1.1 (0.9, 1.3)	0.9 (0.7, 1.2)
SGA	1.8 (1.2, 2.)	0.5 (0.1, 1.8)
BPD	1.3 (1.1, 1.5)	NA
Severe BPD	2.7 (1.7, 4.2)	NA
NEC	1.8 (0.9, 3.9)	3.4 (1.1, 10.2)
PDA	1.3 (1.2, 1.5)	1.2 (1.0, 1.5)
PDA ligation	1.8 (1.3, 2.4)	NA
Severe IVH	1.7 (0.8, 3.4)	NA
ROP	1.9 (1.3, 2.7)	1.2 (0.8, 1.9)

BPD, bronchopulmonary dysplasia; RR, risk ratio; CI, confidence interval; NA, data not available;
SGA, small for gestational age; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis; PDA,
patent ductus arteriosus. Adapted from reference (9).

Maternal-fetal pathophysiology

While BPD and PH share many of the same risk factors, a growing body of evidence suggests that PH may have a distinct fetal pathophysiology that arises in part from maternal vascular disease during pregnancy. This is supported by clinical observations that many infants with severe BPD, despite the usual postnatal exposures and risk factors, never develop PH. In contrast, some babies with relatively mild disease and uncomplicated postnatal courses demonstrate evidence of early and persistent PH.[8,18,19] The presence of a "fixed" or prenatal risk of PH is supported by studies of maternal, placental and cord blood biomarkers that vary according to BPD and PH. Maternal preeclampsia and intrauterine growth restriction, particularly with severe features or accompanied by reversed or absent end diastolic flow, are common indications for medically indicated preterm delivery. These infants appear to be at highest risk for persistent PH, even when BPD is mild or absent.[20]

The mechanisms by which maternal and prenatal events impact fetal lung vascular development leading to PH remain unclear. An important mediator appears to be the placenta. At 28 weeks or earlier, placental changes of villus and vessel maldevelopment are prominent in preterm neonates who later develop BPD associated PH.[21,22] Moreover, circulating levels of pro-angiogenic growth factors are decreased at birth in cord blood of infants who have placental vascular

malperfusion and later develop BPD associated PH.[23] These findings contribute to the growing evidence of the fetal origins of PH in infants with BPD. The distinct mechanisms by which cord blood angiogenic factors play a role at birth and in the postnatal period remain to be elucidated. Possible mechanisms include fetal endothelial cell dysfunction secondary to placental hypoxia,[24] paracrine signaling by factors produced by the placenta,[25,26] or epigenetic changes at the cellular and/or tissue-specific level due to adverse intrauterine stressors that have lasting negative impact on the development of the fetal pulmonary vascular tree, neonatal lung vascular growth, or both.[27,28] These are key topics of ongoing investigation in BPD-PH research. Defining these interactions could enhance and refine recent developments in immune-mediated and cell-based therapies to attenuate and perhaps even prevent BPD-PH. [29–32]

Risk factors

Risk factors for BPD-associated PH can be compiled from numerous series, and a recent meta-analysis identified 12 non-overlapping studies of patients with and without BPD and took into consideration co-morbidities of BPD. The combined data provided by this meta-analysis suggest that PH is associated with lower birthweight in infants with and without BPD, but only with gestational age in BPD infants (Table 1). Similarly, SGA was associated with PH in infants with and without BPD (Relative Risk or RR 1.8; Confidence Interval or CI 1.2, 2.7), however this relationship was not present in BPD infants (Table 1).[9] Collectively, these findings raise concerns for smaller and more premature infants who may be impacted by abnormal pulmonary vascular development even before they are challenged by pulmonary insults associated with postnatal care.

Findings from the meta-analysis also support an association with non-pulmonary morbidities, specifically retinopathy of prematurity (ROP) and necrotizing enterocolitis (NEC), with a particularly strong association between NEC and PH in infants with BPD (RR 3.4; CI 1.1, 10.2). In addition, the presence of a patent ductus arteriosus (PDA) and need for ligation resulted in a weak association with PH.[9] In contrast, there was no association of severe intraventricular hemorrhage (IVH), gender, maternal hypertension, antenatal corticosteroids, chorioamnionitis and prolonged and preterm rupture of membranes (PPROM) with risk for PH. These associations suggest the possibility that BPD-PH is in part triggered by inflammation.

Diagnosis

Screening

The adoption of standardized clinical algorithms for evaluation of infants at risk of BPD-associated PH remains a challenge. In 2015, recommendations for care of pediatric PH were published by the American Heart Association (AHA) and American Thoracic Society (ATS).[33] While the guidelines provided content related to the screening and care of BPD-associated PH, more detailed recommendations were recently published by the Pediatric Pulmonary Hypertension Network (PPHNet), a multidisciplinary panel of PH experts.[34] This report provided practical clinical recommendations for the evaluation, diagnosis and management of PH in infants with BPD, classifying recommendations by both their potential benefit as well as the level of supportive evidence available.

With respect to screening, PPHNet recommendations include obtaining an echocardiogram for premature infants with severe hypoxic respiratory failure with minimal parenchymal disease, to identify those infants with life threatening acute PH that might benefit from therapy. Echocardiographic evaluation should also be considered for infants with continued need for ventilator support at 7 days or with significant sustained need for respiratory support at any age, and at the time of formal BPD diagnosis for all infants with moderate or severe BPD (36 weeks postmenstrual age). Infants with mild BPD can be monitored clinically, with echocardiographic screening reserved for those infants with clinical deterioration or failure to improve. Standardized echocardiographic parameters to be used with these evaluations were also provided with acknowledgement of the limitations of these indicators.[34]

These screening guidelines would miss some cases of PH in those the infants who only met criteria for mild BPD. Consideration of additional risk factors, including birthweight, fetal growth restriction, presence of high risk comorbidities (i.e. ROP or NEC) or placental vascular pathology might allow directed screening for higher risk infants. An evidence-based risk calculator to assist in the identification of appropriate cases of mild BPD to screen may be of clinical value. Such a tool has not yet been developed but could guide clinicians in management strategies and anticipation of resource utilization for a given patient.

A survey performed in 2017 (2 years after publication of the AHA guidelines) evaluated clinical practice of over 300 neonatologists who practice in tertiary care NICUs, most of whom had over 20 years of experience.[35] The majority of respondents noted an increasing number of extremely premature infants being diagnosed with PH, although reporting of formal echocardiography screening protocols varied significantly. Only 46% of respondents replied that institutional protocols were in place that aligned with the AHA recommendations of targeted PH screening for moderate and severe BPD, while 38% reported that existing institutional protocols screened all infants with BPD.

Echocardiogram

While echocardiography is the most common screening modality for PH in infants with BPD, there are significant limitations to its use. Tricuspid regurgitant jet velocity (TRJV) is the most frequently used measurement for evaluating PH, however many infants do not have a detectable tricuspid regurgitant jet, and lung hyperinflation may impact detection.[34] Further, numerous studies have identified that pulmonary arterial pressures as diagnosed by echocardiography poorly correlate with data from cardiac catheterization in infants with BPD.[36,37] Evaluation of additional

echocardiographic parameters, including flattened intraventricular septum, right ventricular (RV) dilation and/or hypertrophy and depressed function can improve PH detection. While inter-rater reliability of cardiologists revealed strong agreement on screening echocardiograms, standardized protocols are needed to optimize evaluation of PH.[38] More recently, advanced echocardiographic assessments, including tissue Doppler imaging (TDI) and speckle tracking echocardiography, have been suggested for identification of pathology not detected by conventional methods.[39] Specifically, decreased RV longitudinal strain (a RV systolic function parameter) has been identified by speckle tracking in infants and children with a history of PH, suggesting this techniques may be capable of identifying subclinical ventricular dysfunction where standard imaging was normal.[39,40]

With growing appreciation of the contribution of pulmonary vein stenosis to pulmonary vascular disease, pulsed Doppler interrogation of pulmonary veins should be performed with all BPD screening. Indeed, there has been increasing identification of acquired PV stenosis (PVS) resulting in obstructed pulmonary blood flow and elevated pulmonary pressures.[41,42] A recent series of 213 patients with severe BPD identified that 5% had some degree of PVS while 26% of infants catheterized in a Spanish registry were found to be affected.[42,43] Of concern are data implicating PVS in poor outcomes, with long term survival rates of only 43-50% in affected infants.[42,44] While echocardiography may be capable of identifying many cases, cardiac catheterization, CT angiography or MRI imaging are often required for a more comprehensive evaluation of the degree of vessel involvement.

Cardiac catheterization

While echocardiography remains first line for screening, invasive catheterization remains the gold standard for detection of elevated pulmonary pressures. The PPHNet recommendations state that cardiac catheterization should be considered prior to initiation of pulmonary vasodilator therapy, but in clinical practice, the procedure is often deferred at discretion of the care team.[34] Beyond providing a more reliable assessment of pulmonary pressures, cardiac catheterization assists in defining the reversible component of vasoconstriction when therapy is being considered. Further, catheterization assists in the detection of PVS, aortopulmonary collaterals (APC), and left heart dysfunction. Each of these may complicate clinical management, as use of pulmonary vasodilators in affected patients can result in pulmonary vascular congestion and disturbed gas exchange. However, catheterization also has risks, including need for intubation and sedation, risk of hemodynamic instability and vascular, thrombotic and infectious complications associated with an invasive procedure. A single center retrospective study in pediatric PH patients who underwent catheterization suggested a 6% risk of need for resuscitation or death; however, more recent reports have demonstrated much lower risks.[45,46] Nonetheless, CT angiography may be an appropriate consideration in infants who are suboptimal candidates for catheterization as this study can also provide insight into presence of PVS and APCs while characterizing parenchymal lung disease.

Biomarkers

Serum brain natriuretic peptide (BNP) and its prohormone, N-terminal pro-BNP (NT-pro-BNP) are released by the myocardium in response to stretch and have been proposed as biomarkers for monitoring right ventricular strain. Studies have correlated BNP and NT-pro-BNP with mean pulmonary artery pressure, pulmonary vascular resistance and right atrial pressures in adult and pediatric patients.[47–49] Age specific reference ranges have been defined, identifying that BNP levels peak at birth with an acute decline over the first few days of life.[50] Limited data have suggested that BNP and NT-pro BNP may aid in the diagnosis and monitoring of infants with BPD-associated PH.[51–53] However, as these tests are non-specific and not well validated, results should be used in conjunction with echocardiography and not replace more formal screening.[34] Numerous additional biomarkers have been explored in the adult and pediatric cardiac literature including some which are detectable and in urine samples alone.[54] Identification of novel biomarkers which reliably detect BPD-associated PH pathophysiology could greatly impact screening and facilitate clinical care.

Management

Evaluation

Similar to the wide variation in screening practices, there is significant variation in the clinical management of infants identified with BPD-associated PH. There has been growing appreciation for the complex and variable phenotypes of the disease, with recent evaluations carefully characterizing the coexistence of parenchymal and large airway disease with PH. In a cohort of 47 infants with severe BPD associated with PH (defined as bidirectional or right to left PDA shunt, systolic pulmonary artery pressures > 40 mmHg by TRJV or a flattened/bowed interventricular septum at the end of systole), 10% were also found to have large airway disease (tracheomalacia or bronchomalacia by bronchoscopy), 32% had coexisting severe parenchymal lung injury (defined as an Ochiai score of 8 or greater on chest computed tomography[55]) and 45% had evidence of both airway and severe lung disease .[56]

The PPHNet recommendations suggest that evaluation and treatment of comorbidities should be done before initiation of therapy for BPD-associated PH (Fig. 2).[9] This includes evaluation for intermittent hypoxemia, aspiration, gastroesophageal reflux, structural air disease, pulmonary artery or vein stenosis, APC and LV dysfunction.[34] Repeated episodes of hypoxemia, chronic airway micro-aspiration, or airway comorbidities, including subglottic stenosis, vocal cord movement abnormalities, laryngo- and bronchomalacia can all lead to airway obstruction or lung injury with CO_2 retention, contributing to the risk and severity of BPD-associated PH. Optimization of gas exchange and maintenance of adequate oxygen saturations (SpO_2 between 92–95%) should occur prior to consideration of vasodilator therapy for mild or moderate PH. Thresholds for initiation of vasodilators should include significant disease as characterized by TRJV > 3 m/second, estimated RV/LV systolic pressure >0.5, and septal flattening in absence of a significant left to right shunt. As mentioned above, cardiac catheterization should be

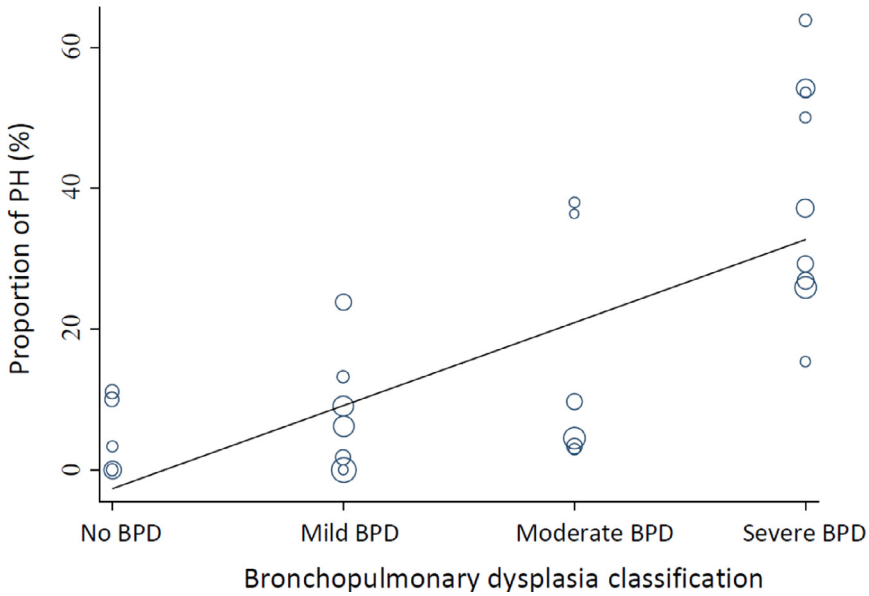


Fig. 1 – Pulmonary hypertension by BPD classification. Reprinted with permission from Wiley Publishing from Pediatric Perinatal Epidemiology, Arjaans et al. *Identification of gaps in the current knowledge on pulmonary hypertension in extremely preterm infants: A systematic review and meta–analysis*, 17 January 2018, 1–10.

considered prior to initiation of pharmacotherapy. Catheterization measurements considered significant include a ratio of pulmonary artery to systemic pressure of ≥ 0.5, indexed pulmonary vascular resistance (PVR) ≥ 3 wood units (WU) or pulmonary to systemic vascular resistance (Rp:Rs) of ≥ 0.5 and a normal wedge or left ventricle end diastolic pressure without evidence of significant PVS.[33,34]

Consistent with the reported variability in screening algorithms, a wide range of practice exists with respect to evaluation and treatment of BPD-associated PH. Most neonatologists report that they would formally consult cardiology (83%) and pediatric pulmonary (53%) for infants with BPD-associated PH. However, only 41% routinely evaluate these patients for micro-aspiration, 29% for airway obstruction, and even fewer (11%) pursue cardiac catheterization.[35] Obstacles commonly encountered in performing cardiac catheterization include need for transport to another center, need for intubation with procedure and concerns for clinical instability. Ninety % of responders to the survey reported that they used systemic PH medications (phosphodiesterase-5 or PDE5 inhibitors, endothelin receptor antagonists (ERAs) or prostacyclins), while only 8% responded that they "often" or "always" catheterize patients prior to their initiation.[35]

Pharmacotherapy

While experience with pharmacotherapy for BPD-associated PH is steadily growing, knowledge regarding long-term efficacy and safety remains limited. In addition, there is growing use of combined pharmacotherapy, with thoughts that additive effects may result in optimal outcomes at lower doses or augment response to treatment.[57] Dosing, common side effects and special considerations for specific agents have been suggested, although with the exception of nitric oxide (NO), none are FDA approved for treatment of infants (Table 2).[34]

Phosphodiesterase inhibitors: Phosphodiesterases hydrolyze and inactivate cyclic guanosine momophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), key regulators of intracellular calcium concentrations and pulmonary vasoconstriction.

- *Sildenafil*, a PDE5 inhibitor, is commonly used as a first line therapy for BPD-associated PH, in part because it is well tolerated and easily administered enterally. PDE5 is highly expressed in the lung, and not only uses cGMP as a substrate but also contains a specific cGMP binding domain that serves to activate its catabolic activity. As the primary enzyme regulating levels of cGMP, PDE5 is a critical controller of NO-mediated vasodilation.[58]

  Despite wide spread adult and pediatric use, clinical data remain limited regarding the efficacy of sildenafil with BPD-associated PH. Small, retrospective studies have suggested accelerated recovery of PH with potential improved right ventricular function and reduced mortality as compared to historical controls.[59–61] The U.S. Food and Drug Administration (FDA) issued a statement in 2012 recommending that sildenafil not be prescribed for children ages 1-17 with PAH, citing the results of the STARTS-2 trial, which showed a dose-dependent increase in mortality in pediatric patients with idiopathic PH on sildenafil as monotherapy.[62] However, whether these findings are relevant to the BPD-PH population is not known, and low dose sildenafil (2 mg/kg/day or less) is approved by the European Medication Authority for use in children. There are anecdotal reports that sildenafil may worsen gastroesophageal reflux. There was also concern that by enhancing angiogenesis, sildenafil could worsen ROP, but a recent report found that sildenafil did not result in ROP worsening in infants with BPD-PH.[63]

  A large retrospective cohort study using data from the Pediatric Health Information System (PHIS) identified that 17% of the 598 infants diagnosed with BPD-associated PH received sildenafil. Likelihood of treatment correlated with

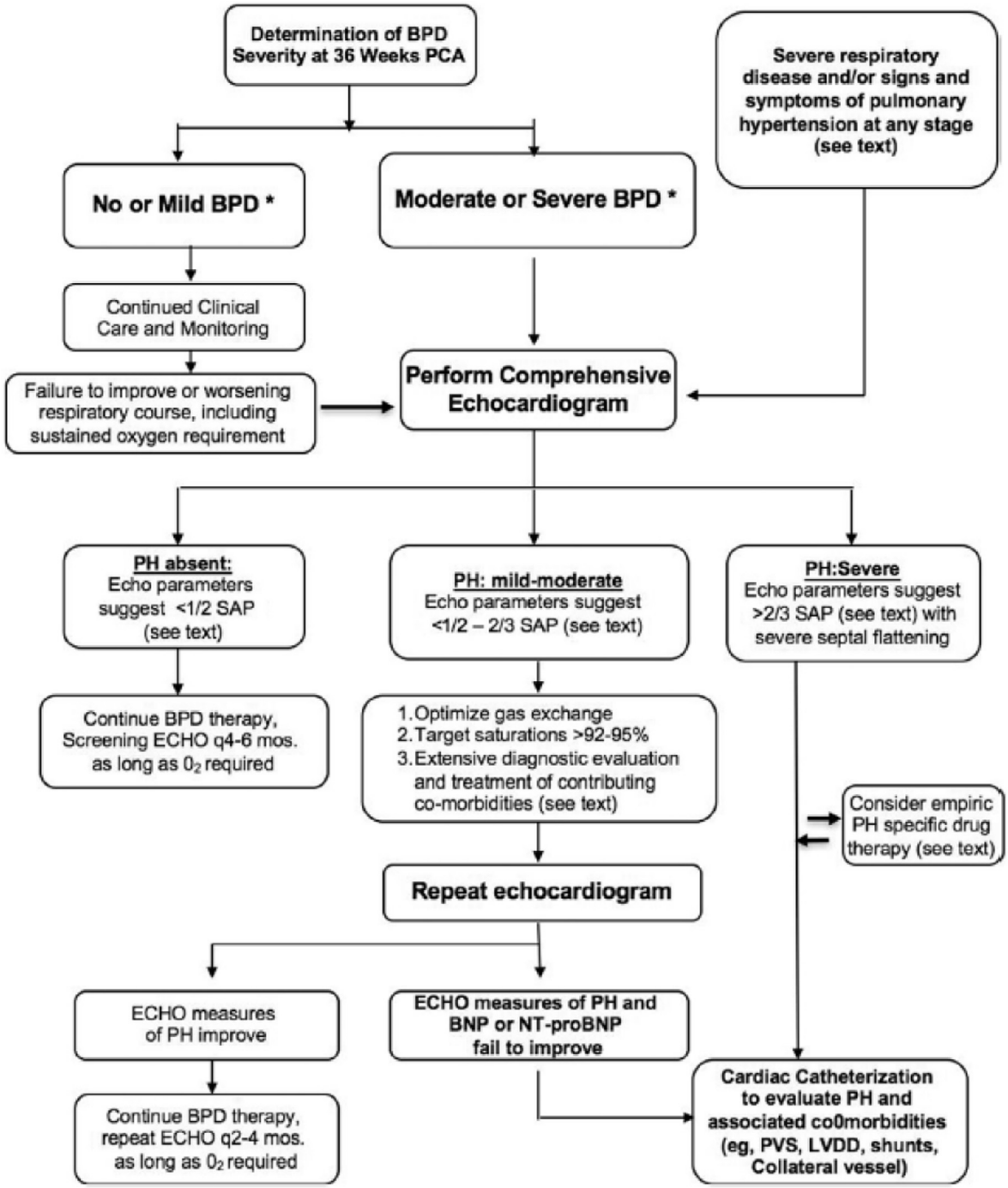


Fig. 2 – Clinical approach to the evaluation and treatment of pulmonary hypertension in BPD infants. ECHO, echocardiogram; LVDD, LV diastolic dysfunction; O2, oxygen; SAP, systemic arterial pressure. Permission obtained, 3.19.2018: Reprinted from The Journal of Pediatrics, Vol. 188, Krishnan U et al., *Evaluation and Management of Pulmonary Hypertension in Children with Bronchopulmonary Dysplasia*, 24–34. Copyright (2017), with permission from Elsevier.

gestation age, SGA and severity of BPD, and there was significant interinstitutional variation in its use.[64] A longer acting PDE5 inhibitor, tadalafil, has been approved for management of adult patients with PH since 2009. However, this drug remains to be evaluated in pediatric disease.

- *Milrinone*, a PDE3A inhibitor, increases cAMP levels in arterial smooth muscle cells and myocardium resulting in decreased pulmonary vascular resistance and increased cardiac contractility. In addition, milrinone has systemic vasodilatory properties, reducing afterload with potential for improved cardiac function. With these dual effects, milrinone may be an optimal therapy when PH is associated with ventricular dysfunction. Efficacy in neonates with PPHN has been reported, although randomized controlled trials are lacking.[65–67] A recent small series suggested that milrinone improved biventricular output and reduced RV

Table 2 – Pharmacotherapy of pulmonary hypertension in BPD.

Names	Dose/titration	Side effects	Comments
Sildenafil phiospho-diesterase-5 inhibitor	PO: 1 mg/kg 6–8 h; start with low dose (0.3–0.5 mg/kg/dose) and increase gradually to 1 mg/kg/dose as tolerated; slower as outpatient. Maximal dose of 10 mg q 8 h per EMA guidelines for infants. lntravenous: 0.25–0.5 mg/kg/dose q 6–8 h {titrate slowly and administer over 60 min.)	Hypotension, GER, irritability (headache), bronchospasm, nasal stuffiness, fever, rarely priapism	Monitor for adverse effects, lower the dose or switch to alternate therapy if not tolerated
Bosentan (Endothelin receptor antagonist)	1 mg/kg PO q 12 h as starting dose; may increase to 2 mg/kg BID in 2–4 wk, if tolerated and liver enzymes stable.	Liver dysfunction especially during viral infections, VO mismatch, hypotension, anemia (edema and airway issues rare in infants)	Monitor LFT s monthly (earlier with respiratory infections); monitor CBC quarterly. Teratogenicity precautions for caregivers
Inhaled lloprost	2.5–5 mcg every 2–4 h. Can be given as continuous inhalation during mechanical ventilation. Can titrate dose from 1 to 5 mcg and frequency from every 4 h to continuous.	Bronchospasm, hypotension, ventilator tube crystallization and clogging, pulmonary hemorrhage, prostanoid side effects (GI disturbances), may be teratogenic to caregivers.	Need close monitoring for clogged tubing, may need further dilution. May need bronchodilators or inhaled steroid pretreatment with bronchospasm.
Intravenous Epoprostenol (Flolan)	Start at 1–2 ng/kg/min, titrate up slowly every 4–6 h to 20 ng/kg/min; need to increase dose at regular intervals because of tachyphylaxis. Further increases as guided by clinical targets and avoiding adverse effects.	Hypotension, VO mismatch, GI disturbances. Needs dedicated line, very short half-life with high risk for rebound PH with brief interruption of therapy; line related complications include infection, clogging, breaks in line, thrombosis, arrhythmia)	Monitor closely if added to other vasodilator therapies, such as milrinone; careful attention to line care is essential.
Treprostinil (Remodulin) IV or Subcutaneous	Start at 2 ng/kg/min and titrate every 4–6 h up to 20 ng/kg/min, then slow increase dose as tolerated (dose often 1.5–2 times greater than equivalent epoprostenol dose, if switching medications)	SO: local site pain; IV: similar risks as with epoprostenol, but treprostinil has a longer half-life, which reduces risk for severe PH with interruption of infusion	Site pain managed with local and systemic measures
Milrinone (IV) (phosphodiesterase-3 inhibitor)	0.15–0.5 mcg/kg/min – lower dosage range when used with other vasodilators	Arrhythmogenic; systemic hypotension and high risk for decreased myocardial perfusion; caution with renal dysfunction	May need to add a presser, such as vasopressin, to mitigate effects of decrease in systemic pressures.

BID, twice a day; *CBC*, complete blood count; *EMA*, European Medicines Agency; *GER*, gastroesophageal reflux; *GI*, gastrointestinal; *IV*, intravenous; *kg*, kilogram; *LFT*, liver function tests; *mcg*, microgram; *ng*, nanogram; *PO*, oral; *SC*, subcutaneous; *SR*, sustained release; *VO*, ventilation-perfusion.

From The Journal of Pediatrics, Vol. 188, Krishnan U et al., Evaluation and Management of Pulmonary Hypertension in Children with Bronchopulmonary Dysplasia, 24–34. Copyright (2017), with permission from Elsevier.

pressures in NO resistant preterm infants with PH.[68] As milrinone can only be delivered by continuous infusion, its use is limited for children hospitalized with acute exacerbations of their disease.

Endothelin receptor antagonists: Endothelin-1 (ET-1) is a potent vasoconstrictor produced by endothelial cells in response to hypoxia. ET-1 promotes endothelial cell dysfunction, smooth muscle cell proliferation and remodeling, as well as inflammation and fibrosis.[69] Two receptor subtypes (ETA and ETB) have been characterized, with binding to the ETA receptor on smooth muscle cells resulting in vasoconstriction. Increased ET-1 production and altered ET receptor (ETR) activity have been reported in both animal models and clinical samples from adults with pulmonary hypertension.[70] *Bosentan,* an orally active non-selective ETA and ETB receptor antagonist, has been used extensively in adult patients with PH and is approved by the FDA for children 3 years of age and older. Experience with bosentan in pediatric patients with PH has been favorable with improved outcomes.[71,72] However, published experience in infants with BPD-associated PH remains limited,[73] although some institutions are now using this drug as their preferred initial therapy.[34] Liver functions need to be followed monthly and in the event of an intercurrent viral illness. A newer ETA receptor specific antagonist, ambrisentan, was approved by the FDA for use with adult PH in 2013 but remains to be studied in pediatric disease.

Prostacyclins: Prostacyclin (PGI_2) is a metabolite of arachidonic acid that is endogenously produced by the vascular endothelium. PGI_2 binds to its receptor (IP), stimulating adenylate cyclase to produce cAMP, resulting in smooth muscle relaxation via reduction in intracellular calcium concentrations. PH is associated with decreased synthesis of prostacyclin, reduced expression of the IP receptor, and increased synthesis of the vasoconstrictor prostanoid thromboxane A_2.[74,75] Several different prostanoid drug preparations are currently in clinical use.

- *Epoprostanol* was one of the earliest therapeutics used for PH. Its short half-life necessitates continuous infusion and depends on central line access. In adults and children with idiopathic and secondary pulmonary hypertension, epoprostenol has been shown to improve pulmonary hemodynamics, quality of life, exercise capacity, and survival.[76–81] These long-term improvements can occur in absence of acute pulmonary vasodilation, suggesting that the effect on platelet aggregation, inhibition of smooth muscle cell growth, or protection of right ventricular function may contribute to clinical improvement of PH.[82] The efficacy and safety of this therapy is still being explored in infants with BPD.[83] As a continuous intravenous (IV) medication, its use remains limited to inpatient care. Inhaled epoprostanol is commonly used for acute PH in adult patients, but there is little experience with this form of delivery in preterm infants.
- *Iloprost* is a more chemically stable prostacyclin analogue with a half-life of 20-30 minutes facilitating delivery by inhalation, which in turn reduces systemic side effects.[84] Iloprost has been reported to improve oxygenation in infants with persistent pulmonary hypertension of the newborn (PPHN)[85–87] and limited case studies suggest clinical improvement occurs in BPD-associated PH.[88] Data from neonatal test lung models suggest that optimal distribution can be achieved with placement of a proximal nebulizer and that delivery is feasible via high frequency ventilation.[89]
- *Treprostinil*, a longer acting prostacyclin analogue, is typically administered via inhalation every 4-6 hours. Alternatively, this drug can be delivered via continuous subcutaneous injection with bioavailability that is equivalent to IV delivery, and without safety concerns or significant problems with site pain.[90] Subcutaneous treprostinil has been used to transition some children who were chronically stable on IV epoprostenol,[91] and may be effective when added to other vasodilator therapies.[92] Preliminary studies of treprostinil imply an acceptable safety profile and clinical benefit in pediatric patients with PH,[93] and case series describe dramatic improvement in premature infants who were non-responsive to nitric oxide.[90,94]

Nitric oxide (NO) is a biological signaling gas molecule synthesized by the enzyme NO synthase (NOS). Alterations in NO signaling have been observed in conjunction with, or even as a cause of the vascular and lung injury characteristic of BPD.[95] Three isoforms of NOS are present in the lung, although endothelial NOS is regarded as the most important regulator of NO production in the lung vasculature. NO stimulates guanylate cyclase activity in vascular smooth muscle cells, leading to production of cGMP and vascular smooth muscle relaxation. Administration of inhaled NO (iNO) results in selective pulmonary vasodilation but requires continuous administration due to its short half-life. Mourani et al. showed that pulmonary pressures in patients with BPD improved to near-normal levels with acute exposure to iNO during cardiac catheterization, and that the effect was greater than pulmonary vasodilation induced by oxygen alone.[96] However, the benefit of prolonged exposure to iNO in BPD-associated PH has not been evaluated. The logistics of continuous treatment with an inhalational medication currently prevent chronic management with iNO in the outpatient setting; instead the drug is mainly used for acute PH exacerbations in the hospitalized infant.

Emerging therapeutics

Several newer or investigational drugs are worth mentioning, although their potential application to neonatal care and management of BPD-associated PH remain unknown. However, these therapies target a range of cellular mechanisms (Fig. 3)[57] and may represent novel strategies in the management of neonatal PH.

- *Soluble guanylate cyclase simulators or activators* (riociguat, a stimulator and cinaciguat, an activator) increase smooth muscle cell cGMP production and cause vasodilation. Riociguat was FDA approved in 2013 for use in adult PH but remains to be studied in pediatric PH. Preliminary data on cinaciguat from a newborn lamb model suggests it may be effective in inducing pulmonary vasodilation with PPHN.[97]
- *L-citrulline* is a precursor to L-arginine, which is a key substrate used by nitric oxide synthase in the production of NO. Low citrulline levels are associated with PH in preterm

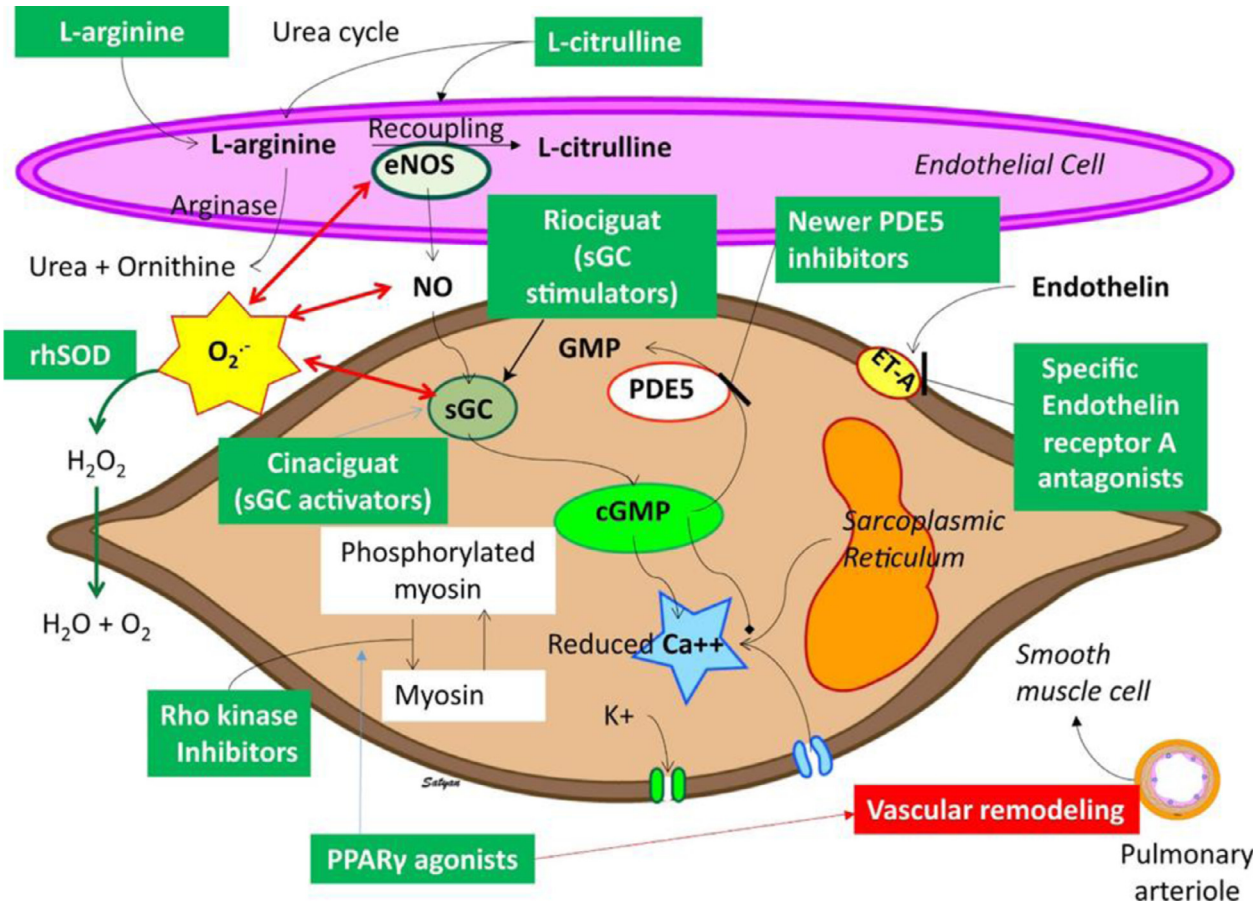


Fig. 3 – Emerging targets and therapies for PPHN. eNOS, extracellular nitric oxide synthase; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; rhSOD, recombinant human superoxide dismutase; H2O2, hydrogen peroxide; O2, oxygen; O2-, superoxide; ET-A, endothelin A, PPAR γ, peroxisome proliferator-activated receptor gamma. Permission obtained, 3.23.2018: Reprinted from Seminars in Perinatology, Vol. 40, Lakshminrusimha, et al., Pharmacologic strategies in neonatal pulmonary hypertension other than nitric oxide, 160-173. Copyright (2016), with permission from Elsevier.

infants and adult PH has been shown to improve with oral L-citrulline.[98,99] However, whether citrulline prevents or reverses BPD-PH in neonates remains unknown.

- *Rho-kinase inhibitors* (fasudil and Y27632) are capable of inhibiting vascular contraction by blocking phosphorylation of the myosin light chain in vascular smooth muscle cells. This class of inhibitors have shown efficacy in both fetal lamb and neonatal rat PH models.[100,101]
- *PPARγ agonists* (rosiglitazone) regulate smooth muscle cell proliferation as well as smooth muscle vasodilation through inhibition of Rho-kinase. Activation of PPARγ reduced RV pressures and vascular remodeling in a rat PH model, suggesting that these agonists may play a role in the management of neonatal PH.[102]

Interdisciplinary teams

Despite increasing use of pharmacotherapy for BPD-associated PH, evidence of drug efficacy and safety is limited and significant controversy regarding treatment practices remains.[33] In addition, there is growing appreciation of the highly variable pathophysiology and course of the disease. Guidelines recommend that PH specialists should be involved diagnostic evaluation, use of PH-targeted therapies, and aggressive management of the underlying lung disease.[34] This recommendation mirrors the reports of improved outcomes of BPD with interdisciplinary teams that optimize cardiopulmonary, nutritional, developmental and transitional care.[103,104] This disease-centered care model remains an important goal, as only 30% of institutions report having established PH clinics.[35] With a growing burden of post-NICU care attributable to infants impacted by BPD-associated PH, it is critical that additional efforts be placed on establishing interdisciplinary teams to assist in the management of these complex patients.

Conclusion

BPD-associated PH remains a serious problem, accounting for significant neonatal and early childhood morbidity and mortality. While several advances have been made in pharmacotherapeutics to treat PH disease in older children and adults, the role of such agents in the treatment of BPD-PH is relatively understudied. This is an important gap to fill, as neonates born extremely preterm and even older, former

preterm infants have distinct and diverse pathophysiology leading to PH disease. Clinical and translational studies are still urgently needed, not only to test the efficacy and safety of certain agents but also to place into context the combined use of clinical risk factors, novel biomarkers, screening and diagnostic tests that will help navigate timing, dosing and duration of therapy. Multidisciplinary efforts in both clinical care and research will enhance strategies for prevention and management.

REFERENCES

1. Stoll BJ, Hansen NI, Bell EF, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA*. 2015;314(10):1039–1051.
2. Zysman-Colman Z, Tremblay GM, Bandeali S, Landry JS. Bronchopulmonary dysplasia – trends over three decades. *Paediatr Child Health*. 2013;18(2):86–90.
3. Day CL, Ryan RM. Bronchopulmonary dysplasia: old becomes new again!. *Pediatr Res*. 2017;81(1-2):210–213.
4. Lapcharoensap W, Gage SC, Kan P, et al. Hospital variation and risk factors for bronchopulmonary dysplasia in a population-based cohort. *JAMA Pediatr*. 2015;169(2):e143676.
5. Northway WH Jr., Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med*. 1967;276(7):357–368.
6. Slaughter JL, Pakrashi T, Jones DE, South AP, Shah TA. Echocardiographic detection of pulmonary hypertension in extremely low birth weight infants with bronchopulmonary dysplasia requiring prolonged positive pressure ventilation. *J Perinatol*. 2011;31(10):635–640.
7. An HS, Bae EJ, Kim GB, et al. Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. *Korean Circ J*. 2010;40(3):131–136.
8. Kim DH, Kim HS, Choi CW, Kim EK, Kim BI, Choi JH. Risk factors for pulmonary artery hypertension in preterm infants with moderate or severe bronchopulmonary dysplasia. *Neonatology*. 2012;101(1):40–46.
9. Arjaans S, Zwart EAH, Ploegstra MJ, et al. Identification of gaps in the current knowledge on pulmonary hypertension in extremely preterm infants: a systematic review and meta-analysis. *Paediatr Perinat Epidemiol*. 2018;32(3):258–267. https://doi.org/10.1111/ppe.12444: Epub 2018 Jan 17.
10. Anderson PJ, Doyle LW. Neurodevelopmental outcome of bronchopulmonary dysplasia. *Semin Perinatol*. 2006;30(4):227–232.
11. Nakanishi H, Uchiyama A, Kusuda S. Impact of pulmonary hypertension on neurodevelopmental outcome in preterm infants with bronchopulmonary dysplasia: a cohort study. *J Perinatol*. 2016;36(10):890–896.
12. Mirza H, Ziegler J, Ford S, Padbury J, Tucker R, Laptook A. Pulmonary hypertension in preterm infants: prevalence and association with bronchopulmonary dysplasia. *J Pediatr*. 2014;165(5):909–914: e901.
13. Waruingi W, Mhanna MJ. Pulmonary hypertension in extremely low birth weight infants: characteristics and outcomes. *World J Pediatr*. 2014;10(1):46–52.
14. Mourani PM, Sontag MK, Younoszai A, et al. Early pulmonary vascular disease in preterm infants at risk for bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2015;191(1):87–95.
15. Weismann CG, Asnes JD, Bazzy-Asaad A, Tolomeo C, Ehrenkranz RA, Bizzarro MJ. Pulmonary hypertension in preterm infants: results of a prospective screening program. *J Perinatol*. 2017;37(5):572–577.
16. Beshish AG, Haraldsdottir K, Barton G, et al. Pulmonary vascular response to exercise and hypoxia in adult survivors of preterm birth. *Am J Resp Crit Care Med*. 2017;195:A3109.
17. Baraldi E, Filippone M. Chronic lung disease after premature birth. *N Engl J Med*. 2007;357(19):1946–1955.
18. Farquhar M, Fitzgerald DA. Pulmonary hypertension in chronic neonatal lung disease. *Paediatr Respir Rev*. 2010;11(3):149–153.
19. Khemani E, McElhinney DB, Rhein L, et al. Pulmonary artery hypertension in formerly premature infants with bronchopulmonary dysplasia: clinical features and outcomes in the surfactant era. *Pediatrics*. 2007;120(6):1260–1269.
20. Check J, Gotteiner N, Liu X, et al. Fetal growth restriction and pulmonary hypertension in premature infants with bronchopulmonary dysplasia. *J Perinatol*. 2013;33(7):553–557.
21. Mestan KK, Check J, Minturn L, et al. Placental pathologic changes of maternal vascular underperfusion in bronchopulmonary dysplasia and pulmonary hypertension. *Placenta*. 2014;35(8):570–574.
22. Yallapragada SG, Mestan KK, Palac H, et al. Placental villous vascularity is decreased in premature infants with bronchopulmonary dysplasia-associated pulmonary hypertension. *Pediatr Dev Pathol*. 2016;19(2):101–107.
23. Mestan KK, Gotteiner N, Porta N, Grobman W, Su EJ, Ernst LM. Cord blood biomarkers of placental maternal vascular underperfusion predict bronchopulmonary dysplasia-associated pulmonary hypertension. *J Pediatr*. 2017;185:33–41.
24. Su EJ, Xin H, Yin P, et al. Impaired fetoplacental angiogenesis in growth-restricted fetuses with abnormal umbilical artery doppler velocimetry is mediated by aryl hydrocarbon receptor nuclear translocator (ARNT). *J Clin Endocrinol Metab*. 2015;100(1):E30–E40.
25. Baker CD, Seedorf GJ, Wisniewski BL, et al. Endothelial colony-forming cell conditioned media promote angiogenesis in vitro and prevent pulmonary hypertension in experimental bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2013;305(1):L73–L81.
26. Pipp F, Heil M, Issbrucker K, et al. VEGFR-1-selective VEGF homologue PlGF is arteriogenic: evidence for a monocyte-mediated mechanism. *Circul Res*. 2003;92(4):378–385.
27. Madonna R, De Caterina R, Geng YJ. Epigenetic regulation of insulin-like growth factor signaling: a novel insight into the pathophysiology of neonatal pulmonary hypertension. *Vascul Pharmacol*. 2015;73:4–7.
28. Xu XF, Lv Y, Gu WZ, et al. Epigenetics of hypoxic pulmonary arterial hypertension following intrauterine growth retardation rat: epigenetics in PAH following IUGR. *Respir Res*. 2013;14:20.
29. Wallace B, Peisl A, Seedorf G, et al. Anti-sFlt-1 therapy preserves lung alveolar and vascular growth in antenatal models of bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2018;197(6):776–787.
30. Willis GR, Kourembanas S, Mitsialis SA. Toward exosome-based therapeutics: isolation, heterogeneity, and fit-for-purpose potency. *Front Cardiovasc Med*. 2017;4:63.
31. Chen CM, Lin W, Huang LT, Chou HC. Human mesenchymal stem cells ameliorate experimental pulmonary hypertension induced by maternal inflammation and neonatal hyperoxia in rats. *Oncotarget*. 2017;8(47):82366–82375.
32. Augustine S, Avey MT, Harrison B, et al. Mesenchymal stromal cell therapy in bronchopulmonary dysplasia: systematic review and meta-analysis of preclinical studies. *Stem Cells Transl Med*. 2017;6(12):2079–2093.
33. Abman SH, Hansmann G, Archer SL, et al. Pediatric pulmonary hypertension: guidelines from the american heart association and american thoracic society. *Circulation*. 2015;132(21):2037–2099.
34. Krishnan U, Feinstein JA, Adatia I, et al. Evaluation and management of pulmonary hypertension in children with bronchopulmonary dysplasia. *J Pediatr*. 2017;188:24–34: e21.

35. Altit G, Lee HC, Hintz S, Tacy TA, Feinstein JA, Bhombal S. Practices surrounding pulmonary hypertension and bronchopulmonary dysplasia amongst neonatologists caring for premature infants. *J Perinatol*. 2018;38(4):361–367.
36. Mourani PM, Sontag MK, Younoszai A, Ivy DD, Abman SH. Clinical utility of echocardiography for the diagnosis and management of pulmonary vascular disease in young children with chronic lung disease. *Pediatrics*. 2008;121(2):317–325.
37. Arcasoy SM, Christie JD, Ferrari VA, et al. Echocardiographic assessment of pulmonary hypertension in patients with advanced lung disease. *Am J Respir Crit Care Med*. 2003;167(5):735–740.
38. Carlton EF, Sontag MK, Younoszai A, et al. Reliability of echocardiographic indicators of pulmonary vascular disease in preterm infants at risk for bronchopulmonary dysplasia. *J Pediatr*. 2017;186:29–33.
39. Singh GK, Levy PT, Holland MR, Hamvas A. Novel methods for assessment of right heart structure and function in pulmonary hypertension. *Clin Perinatol*. 2012;39(3):685–701.
40. Kwon HW, Kim HS, An HS, et al. Long-Term outcomes of pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. *Neonatology*. 2016;110(3):181–189.
41. Latson LA, Prieto LR. Congenital and acquired pulmonary vein stenosis. *Circulation*. 2007;115(1):103–108.
42. Swier NL, Richards B, Cua CL, et al. Pulmonary vein stenosis in neonates with severe bronchopulmonary dysplasia. *Am J Perinatol*. 2016;33(7):671–677.
43. del Cerro MJ, Sabate Rotes A, Carton A, et al. Pulmonary hypertension in bronchopulmonary dysplasia: clinical findings, cardiovascular anomalies and outcomes. *Pediatr Pulmonol*. 2014;49(1):49–59.
44. Drossner DM, Kim DW, Maher KO, Mahle WT. Pulmonary vein stenosis: prematurity and associated conditions. *Pediatrics*. 2008;122(3):e656–e661.
45. Taylor CJ, Derrick G, McEwan A, Haworth SG, Sury MR. Risk of cardiac catheterization under anaesthesia in children with pulmonary hypertension. *Br J Anaesth*. 2007;98(5):657–661.
46. Hill KD, Lim DS, Everett AD, Ivy DD, Moore JD. Assessment of pulmonary hypertension in the pediatric catheterization laboratory: current insights from the Magic registry. *Catheter Cardiovasc Interv*. 2010;76(6):865–873.
47. Mauritz GJ, Rizopoulos D, Groepenhoff H, et al. Usefulness of serial N-terminal pro-B-type natriuretic peptide measurements for determining prognosis in patients with pulmonary arterial hypertension. *Am J Cardiol*. 2011;108(11):1645–1650.
48. Vijlbrief DC, Benders MJ, Kemperman H, van Bel F, de Vries WB. B-type natriuretic peptide and rebound during treatment for persistent pulmonary hypertension. *J Pediatr*. 2012;160(1):111–115: e111.
49. Reynolds EW, Ellington JG, Vranicar M, Bada HS. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. *Pediatrics*. 2004;114(5):1297–1304.
50. Nir A, Lindinger A, Rauh M, et al. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. *Pediatr Cardiol*. 2009;30(1):3–8.
51. Cuna A, Kandasamy J, Sims B. B-type natriuretic peptide and mortality in extremely low birth weight infants with pulmonary hypertension: a retrospective cohort analysis. *BMC Pediatr*. 2014;14:68.
52. Kim GB. Pulmonary hypertension in infants with bronchopulmonary dysplasia. *Korean J Pediatr*. 2010;53(6):688–693.
53. Joseph L, Nir A, Hammerman C, Goldberg S, Ben Shalom E, Picard E. N-terminal pro-B-type natriuretic peptide as a marker of bronchopulmonary dysplasia in premature infants. *Am J Perinatol*. 2010;27(5):381–386.
54. Minkin R, Sandhu G, Grosu H, et al. Desmosine and Isodesmosine as a novel biomarker for pulmonary arterial hypertension: a pilot study. *Am J Ther*. 2017;24(4):e399–e404.
55. Ochiai M, Hikino S, Yabuuchi H, et al. A new scoring system for computed tomography of the chest for assessing the clinical status of bronchopulmonary dysplasia. *J Pediatr*. 2008;152(1):90–95: e91–93.
56. Wu JE, White A, Wang Y, Biko D, Zhang H, Fraga M, Mercer-Rosa L, Kirpalani H. Phenotypes of Severe Bronchopulmonary Dysplasia. Philadelphia, PA: Abstract 111, Eastern Society for Pediatric Research; 2018.
57. Lakshminrusimha S, Mathew B, Leach CL. Pharmacologic strategies in neonatal pulmonary hypertension other than nitric oxide. *Semin Perinatol*. 2016;40(3):160–173.
58. Farrow KN, Steinhorn RH. Phosphodiesterases: emerging therapeutic targets for neonatal pulmonary hypertension. *Handb Exp Pharmacol*. 2011(204):251–277.
59. Mourani PM, Sontag MK, Ivy DD, Abman SH. Effects of long-term sildenafil treatment for pulmonary hypertension in infants with chronic lung disease. *J Pediatr*. 2009;154(3):379–384: e371–372.
60. Nyp M, Sandritter T, Poppinga N, Simon C, Truog WE. Sildenafil citrate, bronchopulmonary dysplasia and disordered pulmonary gas exchange: any benefits? *J Perinatol*. 2012;32(1):64–69.
61. Wardle AJ, Wardle R, Luyt K, Tulloh R. The utility of sildenafil in pulmonary hypertension: a focus on bronchopulmonary dysplasia. *Arch Dis Child*. 2013;98(8):613–617.
62. Barst RJ, Beghetti M, Pulido T, et al. STARTS-2: long-term survival with oral sildenafil monotherapy in treatment-naive pediatric pulmonary arterial hypertension. *Circulation*. 2014;129(19):1914–1923.
63. Aboudi D, Swaminathan N, Brumberg HL, et al. Sildenafil and retinopathy of prematurity in preterm infatns with bronchopulmonary dysplasia. *J Pediatr*. 2018;199:16–21. https://doi.org/10.1016/j.jpeds.2018.04.005. Epub 2018 May 9.
64. Backes CH, Reagan PB, Smith CV, Jadcherla SR, Slaughter JL. Sildenafil treatment of infants with bronchopulmonary dysplasia-associated pulmonary hypertension. *Hosp Pediatr*. 2016;6(1):27–33.
65. McNamara PJ, Laique F, Muang-In S, Whyte HE. Milrinone improves oxygenation in neonates with severe persistent pulmonary hypertension of the newborn. *J Crit Care*. 2006;21(2):217–222.
66. McNamara PJ, Shivananda SP, Sahni M, Freeman D, Taddio A. Pharmacology of milrinone in neonates with persistent pulmonary hypertension of the newborn and suboptimal response to inhaled nitric oxide. *Pediatr Crit Care Med*. 2013;14(1):74–84.
67. Bassler D, Kreutzer K, McNamara P, Kirpalani H. Milrinone for persistent pulmonary hypertension of the newborn. *Cochrane Database Syst Rev*. 2010;11:CD007802.
68. James AT, Corcoran JD, McNamara PJ, Franklin O, El-Khuffash AF. The effect of milrinone on right and left ventricular function when used as a rescue therapy for term infants with pulmonary hypertension. *Cardiol Young*. 2016;26(1):90–99.
69. Abman SH. Role of endothelin receptor antagonists in the treatment of pulmonary arterial hypertension. *Annu Rev Med*. 2009;60:13–23.
70. Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *New Engl J Med*. 1993;328:1732–1739.
71. Beghetti M. Current treatment options in children with pulmonary arterial hypertension and experiences with oral bosentan. *Eur J Clin Invest*. 2006;36(Suppl 3):16–24.
72. Maiya S, Hislop AA, Flynn Y, Haworth SG. Response to bosentan in children with pulmonary hypertension. *Heart*. 2006;92(5):664–670.

73. Rugolotto S, Errico G, Beghini R, Ilic S, Richelli C, Padovani EM. Weaning of epoprostenol in a small infant receiving concomitant bosentan for severe pulmonary arterial hypertension secondary to bronchopulmonary dysplasia. *Minerva Pediatr*. 2006;58(5):491–494.
74. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *New Engl J Med*. 1992;327(2):70–75.
75. Lakshminrusimha S, Porta NF, Farrow KN, et al. Milrinone enhances relaxation to prostacyclin and iloprost in pulmonary arteries isolated from lambs with persistent pulmonary hypertension of the newborn. *Pediatr Crit Care Med*. 2009;10(1):106–112.
76. Rosenzweig EB, Kerstein D, Barst RJ. Long-term prostacyclin for pulmonary hypertension with associated congenital heart defects. *Circulation*. 1999;99(14):1858–1865.
77. Barst RJ, Maislin G, Fishman AP. Vasodilator therapy for primary pulmonary hypertension in children. *Circulation*. 1999;99(9):1197–1208.
78. Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *New Engl J Med*. 1996;334(5):296–302.
79. Barst RJ, Rubin LJ, McGoon MD, Caldwell EJ, Long WA, Levy PS. Survival in primary pulmonary hypertension with long-term continuous intravenous prostacyclin. *Ann Intern Med*. 1994;121(6):409–415.
80. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. *J Am Coll Cardiol*. 2002;40(4):780–788.
81. Yung D, Widlitz AC, Rosenzweig EB, Kerstein D, Maislin G, Barst RJ. Outcomes in children with idiopathic pulmonary arterial hypertension. *Circulation*. 2004;110(6):660–665.
82. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med*. 1991;115(5):343–349.
83. Zaidi AN, Dettorre MD, Ceneviva GD, Thomas NJ. Epoprostenol and home mechanical ventilation for pulmonary hypertension associated with chronic lung disease. *Pediatr Pulmonol*. 2005;40(3):265–269.
84. Ewert R, Schaper C, Halank M, Glaser S, Opitz CF. Inhalative iloprost – pharmacology and clinical application. *Expert Opin Pharmacother*. 2009;10(13):2195–2207.
85. Ehlen M, Wiebe B. Iloprost in persistent pulmonary hypertension of the newborn. *Cardiol Young*. 2003;13(4):361–363.
86. Janjindamai W, Thatrimontrichai A, Maneenil G, Chanvitan P, Dissaneevate S. Effectiveness and safety of intravenous iloprost for severe persistent pulmonary hypertension of the newborn. *Indian Pediatr*. 2013;50(10):934–938.
87. Kahveci H, Yilmaz O, Avsar UZ, et al. Oral sildenafil and inhaled iloprost in the treatment of pulmonary hypertension of the newborn. *Pediatr Pulmonol*. 2014;49(12):1205–1213.
88. Hwang SK, O YC, Kim NS, Park HK, Yum MK. Use of inhaled iloprost in an infant with bronchopulmonary dysplasia and pulmonary artery hypertension. *Korean Circ J*. 2009;39(8):343–345.
89. DiBlasi RM, Crotwell DN, Shen S, Zheng J, Fink JB, Yung D. Iloprost drug delivery during infant conventional and high-frequency oscillatory ventilation. *Pulm Circ*. 2016;6(1):63–69.
90. Ferdman DJ, Rosenzweig EB, Zuckerman WA, Krishnan U. Subcutaneous treprostinil for pulmonary hypertension in chronic lung disease of infancy. *Pediatrics*. 2014;134(1):e274–e278.
91. Ivy DD, Claussen L, Doran A. Transition of stable pediatric patients with pulmonary arterial hypertension from intravenous epoprostenol to intravenous treprostinil. *Am J Cardiol*. 2007;99(5):696–698.
92. Levy M, Celermajer DS, Bourges-Petit E, Del Cerro MJ, Bajolle F, Bonnet D. Add-on therapy with subcutaneous treprostinil for refractory pediatric pulmonary hypertension. *J Pediatr*. 2011;158(4):584–588.
93. Krishnan U, Takatsuki S, Ivy DD, et al. Effectiveness and safety of inhaled treprostinil for the treatment of pulmonary arterial hypertension in children. *Am J Cardiol*. 2012;110(11):1704–1709.
94. Park BY, Chung SH. Treprostinil for persistent pulmonary hypertension of the newborn, with early onset sepsis in preterm infant: 2 Case reports. *Medicine (Baltimore)*. 2017;96(26):e7303.
95. Afshar S, Gibson LL, Yuhanna IS, et al. Pulmonary NO synthase expression is attenuated in a fetal baboon model of chronic lung disease. *Am J Physiol Lung Cell Mol Physiol*. 2003;284(5):L749–L758.
96. Mourani PM, Ivy DD, Gao D, Abman SH. Pulmonary vascular effects of inhaled nitric oxide and oxygen tension in bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2004;170(9):1006–1013.
97. Chester M, Seedorf G, Tourneux P, et al. Cinaciguat, a soluble guanylate cyclase activator, augments cGMP after oxidative stress and causes pulmonary vasodilation in neonatal pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2011;301(5):L755–L764.
98. O'Connor MG, Suthar D, Vera K, et al. Pulmonary hypertension in the premature infant population: analysis of echocardiographic findings and biomarkers. *Pediatr Pulmonol*. 2018;53(3):302–309.
99. Sharif Kashani B, Tahmaseb Pour P, Malekmohammad M, et al. Oral l-citrulline malate in patients with idiopathic pulmonary arterial hypertension and Eisenmenger Syndrome: a clinical trial. *J Cardiol*. 2014;64(3):231–235.
100. Chou HC, Huang LT, Yeh TF, Chen CM. Rho-kinase inhibitor Y-27632 attenuates pulmonary hypertension in hyperoxia-exposed newborn rats. *Acta Pharmacol Sin*. 2013;34(10):1310–1316.
101. Parker TA, Roe G, Grover TR, Abman SH. Rho kinase activation maintains high pulmonary vascular resistance in the ovine fetal lung. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(5):L976–L982.
102. Zhang D, Wang G, Han D, et al. Activation of PPAR−gamma ameliorates pulmonary arterial hypertension via inducing heme oxygenase-1 and p21(WAF1): an in vivo study in rats. *Life Sci*. 2014;98(1):39–43.
103. Abman SH, Collaco JM, Shepherd EG, et al. Interdisciplinary care of children with severe bronchopulmonary dysplasia. *J Pediatr*. 2017;181:12–28: e11.
104. Gien J, Kinsella J, Thrasher J, Grenolds A, Abman SH, Baker CD. Retrospective analysis of an interdisciplinary ventilator care program intervention on survival of infants with ventilator-dependent bronchopulmonary dysplasia. *Am J Perinatol*. 2017;34(2):155–163.

Accepted Manuscript

Review

Bronchopulmonary dysplasia: pathophysiology and potential anti-inflammatory therapies

Paris C. Papagianis, J.J. Pillow, Timothy J. Moss

PII: S1526-0542(18)30079-4
DOI: https://doi.org/10.1016/j.prrv.2018.07.007
Reference: YPRRV 1281

To appear in: *Paediatric Respiratory Reviews*

Please cite this article as: P.C. Papagianis, J.J. Pillow, T.J. Moss, Bronchopulmonary dysplasia: pathophysiology and potential anti-inflammatory therapies, *Paediatric Respiratory Reviews* (2018), doi: https://doi.org/10.1016/j.prrv.2018.07.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Bronchopulmonary dysplasia: pathophysiology and potential anti-inflammatory therapies.

Paris C. Papagianis[1,2], J J. Pillow[2] and Timothy J. Moss[1]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Department of Obstetrics and Gynecology, Monash University, Clayton, VIC 3168, Australia

[2]Human Sciences and Centre for Neonatal Research and Education, The University of Western Australia, Crawley, WA, Australia

PC Papagianis: paris.papagianis@monash.edu

JJ Pillow: jane.pillow@uwa.edu.au

TJ Moss: tim.moss@monash.edu

Correspondence to:

Paris Papagianis, BSci (Hons), PhD candidate

Email: paris.papagianis@monash.edu

Tel: +61 430 127 197

Declarations of interest: none

Funding: NHMRC Grants: 1057514, 1057759, 1077769, 1077691 and 1043294

Acknowledgements: The Victorian Government's Operational Infrastructure Support Program.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1 Introduction

Globally, 15 million infants are born preterm each year, representing more than 1 in 10 live births.[1] Extremely preterm infants (born before 28 weeks' gestational age (GA)) are the most vulnerable, most commonly complicated by the chronic lung disease, bronchopulmonary dysplasia (BPD).[2] BPD is the only complication of preterm birth for which the incidence is increasing at the same time that all other co-morbidities are decreasing[2].

There is no cure for BPD. The use of antenatal corticosteroids and exogenous surfactant administration has improved the acute respiratory complications of preterm birth dramatically, but these treatments do not prevent BPD.[3, 4] The early application of non-invasive respiratory support is an alternative strategy aimed at preventing BPD but is unsuccessful at reducing its incidence.[5]

Although BPD is clearly a respiratory disease of prematurity, BPD may not simply be a consequence of lung immaturity. The immature immune system of preterm neonates coupled with the immune modulating effects of factors that commonly accompany preterm birth (e.g. antenatal corticosteroids, intrauterine infection/inflammation and postnatal sepsis),[6] may be responsible for aberrant regulation of lung inflammation. Thus, targeting the chronic lung inflammation that underlies BPD with the use of new anti-inflammatory therapies offers the prospect of preventative and reparative treatment for infants with BPD.

2 Bronchopulmonary dysplasia

BPD was first described by Northway in the 1960s as a form of chronic lung disease in moderately preterm infants, characterised predominately by fibrosis.[7] Northway attributed the injurious effects of high oxygen levels and airway pressures used in mechanical ventilation as the main causes of the disease[7]. However, changes in respiratory management strategies for preterm infants has transformed BPD into a disease characterised by more subtle lung abnormalities, including alveolar hypoplasia (fewer and larger simplified alveoli),[8, 9] dysmorphic pulmonary vasculature and chronic pulmonary inflammation.[10-12] The lungs of infants with severe BPD exhibit a lack of septation within the developing airspace, resulting in reduced surface area for gas exchange in the lungs, hyperconstrictive vasculature that further compromises gas exchange, and impaired surfactant synthesis,[13] favouring atelectasis. The National Institute of Child Health and Human Development defines this "New BPD" as the requirement for at least 28 days of supplemental oxygen, with the severity of BPD indicated by the level of respiratory support required at 36 weeks postmenstrual age.[14]

3 The aetiology of BPD

3.1 Prenatal inflammation

Inflammation within the uterus during pregnancy is a common antecedent of preterm birth that manifests as chorioamnionitis; inflammation of the chorion and amnion. Chorioamnionitis is commonly classified as clinical or histological: clinical chorioamnionitis is diagnosed prior to labour, when women present with symptoms including fever, a tender uterus and preterm rupture of membranes (PROM) with purulent liquor; histological chorioamnionitis is an asymptomatic inflammation of the membranes.[15] Histological chorioamnionitis is more common than clinical chorioamnionitis but may be undiagnosed because post-partum histological examination

ACCEPTED MANUSCRIPT

of the placenta is required.[15] Histological chorioamnionitis complicates between 30–70 % of preterm births with PROM and spontaneous labour, with incidence inversely related to GA. Thus, rates of histological chorioamnionitis exceed 70 % for infants at highest risk of BPD (23 weeks GA).[16]

Prenatal inflammation alters fetal lung development, with consequences that may be detrimental or beneficial for preterm newborns. Histological chorioamnionitis reduces the risk of respiratory distress syndrome (RDS),[16-18] likely due to elevated surfactant production in the lungs.[19-21] However, despite lower risk of RDS, infants exposed to chorioamnionitis may require longer term respiratory support and have higher rates of BPD and persistent pulmonary hypertension of the newborn (PPHN).[22] Although increases[18, 23] or decreases[16,24] in the incidence of BPD are reported after exposure to chorioamnionitis, the relationship is complicated by low-birth-weight and postnatal events, such as sepsis. The avoidance of prolonged mechanical ventilation in low-birth-weight newborns exposed to chorioamnionitis is associated with decreased incidence of BPD, compared to newborns exposed to chorioamnionitis who received prolonged ventilation.[25] It is unclear whether a prenatal (chorioamnionitis) or postnatal (ventilator-induced) origin of lung inflammation is the greater contributor to the pathogenesis of BPD.

Fetal sheep exposed to inflammation induced by intra-amniotic (IA) injection of lipopolysaccharide (LPS) have lung abnormalities like those observed in infants who die of BPD: alveolar hypoplasia[19, 26] and decreased septation,[19, 27] impaired surfactant secretion (despite increased surfactant protein),[19] and impaired pulmonary vascular development and function.[28] The similarities in the lungs of fetal lambs exposed to inflammation and the pathological features of BPD support a prenatal origin of BPD. Although preterm infants exposed to chorioamnionitis have less RDS, experimental intrauterine inflammation does not reduce the postnatal respiratory support required by preterm baboons[29] and lambs.[30]

3.2 Respiratory Support

Mechanical ventilation initiates an influx of neutrophils and macrophages into the alveoli.[31] These cells produce cytokines,[31] which can disrupt lung development and may be used as biomarkers in serum and tracheal aspirates to identify infants at risk of BPD.[10-12] Infants who develop BPD have persistently elevated pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) in tracheal aspirates and blood, compared to infants who recover from initial RDS.[32] Preterm infants may become increasingly dependent on respiratory support, which exacerbates pulmonary inflammation, inducing the production of reactive oxygen species (ROS).[33] ROS promote inflammation and epithelial cell death in the lungs via the cleavage, and thus activation, of caspase-1 (Figure 1).[34]

In preterm lambs, 2 hours of mechanical ventilation initiates inflammation within the lungs resulting in similar upregulation of IL-1β, IL-6 and IL-8, the same biomarkers as infants that are ventilated or were exposed to chorioamnionitis.[35, 36] Longer ventilation of preterm lambs (3-4 weeks) increases neutrophil and macrophage infiltration into the lungs and causes non-uniform inflation patterns and abnormal lung vascular development, similar to that observed in infants who die from BPD.[37]

4 Long-term pulmonary consequences of BPD

The long-term sequelae of new BPD are described by very few studies. Autopsies reveal its major pathological features: abnormal alveolar architecture (alveolar hypoplasia) and impaired pulmonary vascular development,

ACCEPTED MANUSCRIPT

where vessels are distant from airspaces.[8, 9] Pulmonary gas exchange is impaired in 2-year-old infants with BPD compared to non-BPD controls; however, alveolar volume was normal in both cohorts, suggesting a lower alveolar surface area, consistent with an alveolar hypoplasia lung phenotype.[38] Persistent abnormalities in lung parenchyma have long-term functional consequences: 7-to-8-year-olds who had BPD and received surfactant during the perinatal period had lower forced expiratory volume and higher airway resistance, compared to age- and sex- matched controls without BPD, indicative of increased work of breathing.[39]

5 Steroidal approaches to prevent pulmonary inflammation

5.1 Antenatal corticosteroids

Antenatal corticosteroids administered to women at risk of preterm delivery accelerate fetal lung maturation to prevent RDS but do not prevent BPD.[3] Antenatal corticosteroids cause remodeling of the lung parenchyma, which improves gas exchange but results ultimately in fewer, larger alveoli,[40] like the lungs of fetal sheep exposed to prenatal inflammation.[8, 19] Antenatal corticosteroids alter immune activity by suppressing lymphocytes but increasing neutrophils in preterm infants,[41] and altering immune cell function,[42] which may underlie an increased risk of early-onset sepsis.[3]

Antenatal corticosteroid treatment can suppress lung inflammation (induced by IA injection of LPS) in fetal sheep but the reduction of LPS-induced inflammation is transient.[43] Thus, the timing of antenatal corticosteroid administration in humans may influence the lung inflammation that accompanies chorioamnionitis. The optimal timing, dose, and frequency of administration of glucocorticoids to women at risk of preterm birth are unknown. While chorioamnionitis is not a contraindication to the use of antenatal corticosteroids, their interaction likely affects lung development differently to either in isolation;[19, 44] the impact of this interaction on rates of BPD is not known.

Few studies investigate any effect of antenatal glucocorticoids on postnatal ventilator requirements and lung inflammation. In adult animals, pretreatment with corticosteroids reduces ventilation-induced lung injury.[45, 46] Preterm lambs ventilated following exposure to antenatal glucocorticoids have less lung injury and inflammation in comparison to ventilated preterm lambs exposed to antenatal saline.[47] The maturational and anti-inflammatory effects of antenatal glucocorticoids in the preterm lungs appear to be maintained for the initial ventilation period, consistent with lower RDS incidence. However, meta-analyses show that antenatal corticosteroids do not reduce BPD.[3]

5.2 Postnatal glucocorticoids

Postnatal glucocorticoid use peaked in the late 1990s[48] after observations of improved extubation rates in a small trial.[49] Later meta-analyses revealed reduced BPD incidence in preterm infants who received postnatal glucocorticoids, but increased incidence of cerebral palsy and death.[50-53] Thus, postnatal glucocorticoids are now used reluctantly in infants with intractable BPD: fewer than 10 % of preterm infants receive them.[2]

Ventilator management of preterm infants has evolved since the 1990s, aiming for respiratory management with lower airway pressures and inspired oxygen concentrations, and avoidance of prolonged periods of intubation.[5, 54] The practice of more 'gentle' ventilation coupled with significantly less postnatal steroid exposure may result

ACCEPTED MANUSCRIPT

in infants receiving longer periods of respiratory support than may have been used prior to adoption of these changes in practice. It is unclear whether the increasing BPD incidence is attributable to longer periods of respiratory support and/or the decrease in steroid use. Typically, infants who receive postnatal steroids are those who require prolonged periods of invasive ventilation, indicating a stronger predisposition for BPD development, and complicating any assessment of the impact of postnatal steroids on BPD incidence.

Early studies of postnatal high-dose dexamethasone therapy (~1 mg/kg/day over 42 d)[49] focused on immediate respiratory outcomes, at the expense of neurological follow-up. Optimal postnatal steroid dosing regimens are undefined, contributing to reluctance for their clinical use. Early administration of lower dexamethasone doses may be safe and effective in infants at high-risk of developing BPD.[53]

Despite the key role of inflammation in the pathogenesis of BPD, few studies of postnatal steroids include inflammation as an outcome. Small studies describe a reduction in neutrophils[55, 56] and IL-1 concentration in bronchoalveolar lavage (BAL) of ventilated preterm infants receiving dexamethasone.[55, 57] Similarly, IL-1β expression is lower in the lungs of preterm lambs receiving a single dexamethasone dose (0.5 mg/kg) immediately before initiation of ventilation compared to placebo controls.[47] No animal studies assess the ability of postnatal dexamethasone to treat established lung inflammation and injury.

Neurological impairment is associated with high dose postnatal steroids. The risk of cerebral palsy increases by 40 % for every 1 mg/kg increase in dexamethasone dose.[58] The most premature infants are not the worst affected (despite presumably more immature organ systems); treatment after 33 weeks' postmenstrual age is associated with greatest neurological deficit at follow-up.[58] Adverse clinical neurological outcomes associated with dexamethasone are consistent with animal studies.[59-61] Other complications of high-dose postnatal steroid use include stunted growth[62], intraventricular haemorrhage (IVH),[53, 63] gastrointestinal bleeding,[52] sepsis and hypertension.[51-53, 64] Combined use of dexamethasone and indomethacin (for closure of the ductus arteriosus) increases the likelihood of gastrointestinal perforation three-fold.[62]

The DART trial aimed to investigate the ability of a low-dose 10-day tapered course of postnatal dexamethasone (0.89 mg/kg cumulative over 10 days) to prevent BPD in preterm infants born before 28 weeks GA.[65] The trial was terminated because of low (10 %) recruitment,[65] but infants who received dexamethasone spent less time intubated on mechanical ventilation; although this did not reach statistical significance. Major disability and cerebral palsy were not different between dexamethasone-treated and placebo-treated preterm infants at 2 years follow-up.[66]

6 Non-steroidal approaches to prevent pulmonary inflammation

Inflammation associated with BPD involves the activation or over-expression of a number of inflammatory cytokines and pro-inflammatory mediators (Figure 1) providing opportunity for multiple therapeutic targets to prevent lung inflammation in newborn infants. Inhibition of cell signaling that exacerbates inflammation or inhibition of specific pro-inflammatory cytokines in the lungs may prevent BPD progression.

ACCEPTED MANUSCRIPT

6.1 Suppressing inflammation at various sites: Pentoxifylline, NLRP3 inhibition, IL-1Ra and Adenosine Monophosphate Proteins

6.1.1 Pentoxifylline

Pentoxifylline is a synthetic theobromine derivative, structurally similar to caffeine.[67] Pentoxifylline is a an immunological agent sometimes used in septic shock due to its ability to lower blood viscosity and improve tissue perfusion.[67] Pentoxifylline acts by inhibiting erythrocyte phosphodiesterase, which increases expression of the anti-inflammatory protein adenosine monophosphate protein kinase (AMPK), suppressing neutrophils and pro-inflammatory cytokines[67, 68] and likely preventing chronic inflammation.

Pentoxifylline is well tolerated by neonates, in whom it is predominately used during sepsis. Pentoxifylline infusion (5 mg/kg/hour for 6 hours) over six days lowers plasma IL-6 and TNF in preterm infants with sepsis, when compared to placebo.[69] Infants receiving pentoxifylline had less hypotension and overall improved clinical course,[69] highlighting its immuno- and vasculo-modulatory effects. However, comparison of pentoxifylline and dexamethasone in low-birth-weight infants with oxygen requirements >30 % at 72 hours of age revealed neither treatment impacted BPD incidence.[70]

It is unclear if pentoxifylline is beneficial for BPD prevention. Current studies are limited by small sample size and poor design (e.g. no blinding is apparent in any of the studies). Hypotension, arrhythmia[67] and more rarely, IVH, are noted in adult trials using pentoxifylline.[71, 72] It is unclear whether pentoxifylline may have particular side effects in infants with BPD. Preclinical studies using pentoxifylline for BPD are rare, and animal studies are warranted to ensure effective translation of pentoxifylline into larger randomised controlled trials (RCTs). One RCT is currently recruiting preterm infants to receive either pentoxifylline or placebo for preventing sepsis and necrotising enterocolitis (NEC), with BPD as a secondary outcome (ACTRN: 12616000405415). No RCTs using pentoxifylline assess BPD as a primary outcome.

In newborn rats exposed to hyperoxia, pentoxifylline administration increased survival and expression of lung vasculogenic markers compared to normoxic controls.[73] However, hyperoxia produces a classic fibrotic BPD phenotype, not contemporary BPD. Adult rats subjected to intratracheal hydrochloric acid (HCl) have lung inflammation and develop acute RDS.[74] Prophylactic, but not rescue, pentoxifylline reduces HCl-induced lung inflammation and normalises alveolar architecture,[74] indicating the timing of pentoxifylline may be important when considering its application in preterm infants.

6.1.2 NLRP3 inflammasome

The NLRP3 inflammasome is part of the innate immune system, responsible for sensing pathogens and initiating inflammation.[75] The NLRP3 inflammasome is activated by numerous stimuli,[76] forming a complex with other molecules, including procaspase-1.[75, 77] Procaspase-1 is activated upon formation of the NLRP3 complex and induces IL-1β maturation.[75, 77] Expression of IL-1β is tightly regulated by activation of the NLRP3 complex.

NLRP3 is implicated in adult ventilator-induced lung injury[78] and is upregulated in BAL after 5 hours of ventilation.[78] There are no data suggesting activation of NLRP3 in neonatal ventilation, however NLRP3 is part of the innate immune system and should be present at birth.

ACCEPTED MANUSCRIPT

The NLRP3 inflammasome can be activated by ROS (through injurious ventilation), or TLR activation (through LPS; Figure 1).[79] Ventilation using low or high tidal volumes both stimulate NLRP3 and IL-1β expression in mice lungs.[78] Overexpression of NLRP3 interrupts alveolar formation and leads to abnormal lung morphogenesis of mice.[80] NLRP3-deficient mice are protected from ventilator-induced lung injury and have low IL-1β in their lungs.[78, 81]

NLRP3 activity is altered in the presence of glucocorticoids and LPS, which may compromise NLPR3 blockade for BPD prevention in preterm infants. Cultured human and mouse macrophages pre-treated with LPS have elevated NLRP3 and IL-1β following exposure to dexamethasone, despite glucocorticoids being anti-inflammatory (dexamethasone alone blocks IL-1β).[79] The use of postnatal steroids may be less effective in reducing NLRP3-induced inflammation in preterm infants exposed to both chorioamnionitis and ventilation. Targeting inflammatory inhibition downstream of NLRP3 may be more appropriate in these infants.

Other avenues for NLRP3 suppression include administration of the antidiabetic drug glibenclamide and IL-1 inhibitors [see section 6.1.3]. In ventilated adult mice receiving glibenclamide, compared to placebo, NLRP3 and IL-1β in the lungs was reduced.[78] Glibenclamide is used antenatally in mothers with gestational diabetes[82] and in neonates with permanent neonatal diabetes mellitus,[83] but not in neonatal lung inflammation.

6.1.3 IL-1 receptor antagonist

IL-1 plays a crucial role in inflammation.[84] IL-1α and IL-1β enhance their own upregulation and recruit other pro-inflammatory cytokines, including IL-6 and IL-8, to aggravate inflammation.[84, 85] Upregulation of IL-1 is apparent in tracheal aspirates of preterm infants who were exposed to chorioamnionitis[86] or who have BPD.[32] Elevated IL-1 in tracheal aspirates between days 1-3 of life may better predict BPD than GA alone for infants born <27 weeks GA.[81]

Imbalance between IL-1 and its endogenous IL-1 receptor antagonist (Ra) may be involved in the pathogenesis of BPD. Preterm infants <30 weeks GA have elevated IL-1 and lower IL-1Ra,[87, 88] an imbalance that can persist for the first month of life.[87] However, levels of IL-1 and IL-1Ra are both higher than non-BPD controls,[88] indicating an inability to inhibit IL-1β by IL-1Ra in preterm infants at risk of BPD. Increases in IL-1:IL-1Ra, favouring inflammation, may contribute to prolonged pulmonary inflammation and BPD development. Elevated IL-1 in tracheal aspirates preceded increased macrophage activity between 7-10 days of life in preterm infants who develop BPD, indicating a hyperactive immune system.

The ratio of IL-1:IL-1Ra increases exponentially in tracheal aspirates of baboons delivered at 70 % of full gestation and ventilated for 2, 6 or 14 days,[81] suggesting an ongoing inflammatory response. Synthetic IL-1Ra prevents BPD-like lung pathology in mice and rats by reducing pulmonary inflammation and normalising alveolar development.[89,90] Synthetic IL-1Ra reduces lung inflammation and suppresses IL-1β in fetal lambs exposed to LPS. [91]

IL-1Ra has a well-established safety profile for therapeutic use in adults,[92] but 100-fold levels of IL-1Ra to IL-1 are required for functional inhibition of IL-1.[92] Synthetic IL-1Ra is used in neonatal-onset multisystem inflammatory disease to control relapsing inflammation.[93, 94] However, clinical use for BPD prevention is not reported. There are no guidelines for IL-1Ra use in the neonate and only one IL-1Ra (Anakinra) has shown to be

ACCEPTED MANUSCRIPT

safe in patients less than 2 years old.[95, 96] Other FDA-approved IL-1 inhibitors have not been tested in neonates.[97,98]

6.1.4 Adenosine monophosphate proteins

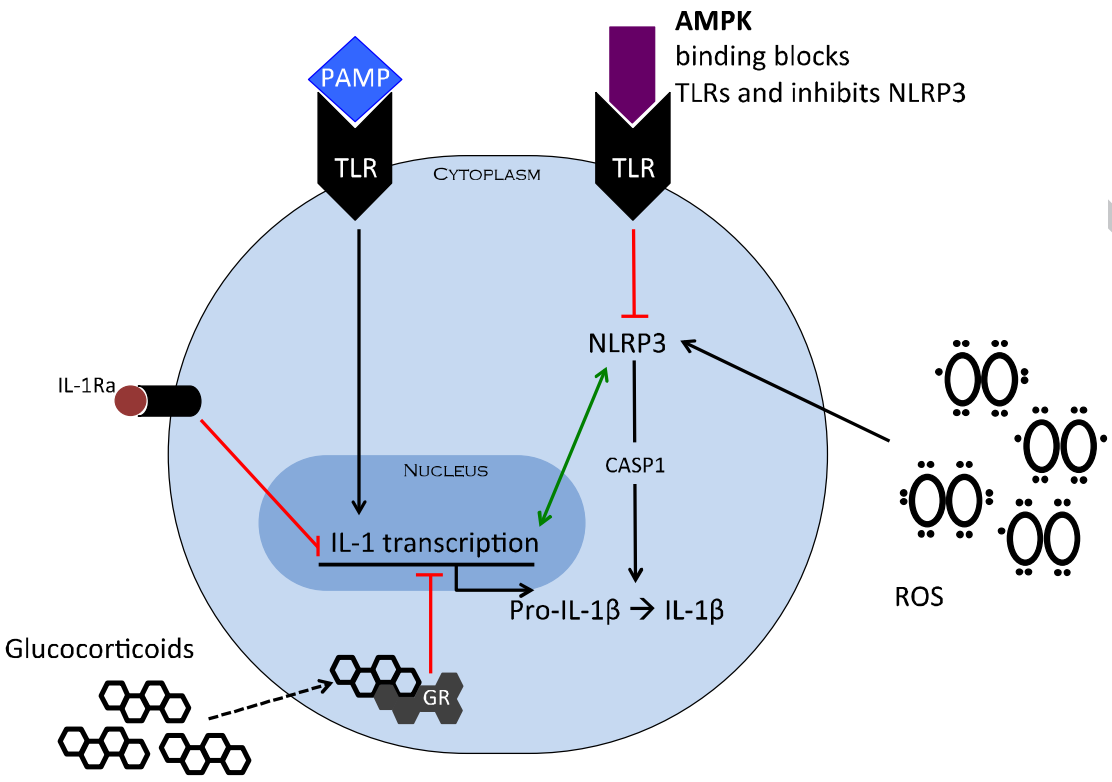
AMPs are produced by macrophages, neutrophils and epithelial cells in response to inflammation, ROS or infection, and suppress the release of inflammatory mediators.[99] Excess ROS activates AMP to AMPK, which suppress NLRP3.[100] AMPs are elevated in tracheal aspirates of newborn ventilated infants with pulmonary infections compared to ventilated infants without infection[101] but it is unclear if these AMPs are active.

Intratracheal LPS administration in mice increases lung endothelial cell permeability and white cell infiltration, in parallel with AMPK inhibition.[102] Pretreatment, but not rescue treatment, of wild-type mice with an AMPK activator reduces LPS-induced inflammation and injury.[102] Infants exposed to prenatal inflammation have similar lung morphology to that observed in mice exposed to intratracheal LPS; thus these infants may have reduced AMPK activity.

AMPK activity is enhanced by resveratrol in obese patients (still in clinical trials),[103] consistent with inhibition of inflammation in LPS-exposed macrophages from mice *in vitro.*[104] The use of resveratrol has not been investigated in lung inflammation, but stimulation of innate AMPK may inhibit inflammation and prevent BPD lung pathology in preterm infants.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED

ACCEPTED MANUSCRIPT

Figure 1. Interplay of factors that may contribute to chronic lung inflammation and the development of BPD in preterm infants. NLRP3 and pathogen-associated molecular patterns (PAMP) both increase transcription of potent pro-inflammatory cytokine IL-1. Imbalances in IL-1 and IL-1 antagonists (i.e. IL-1 receptor antagonist: IL-1Ra) may predispose infants to BPD. IL-1 transcription can be prevented by IL-1Ra. IL-1 production positively feeds back to increase NLRP3 activity (green arrow). Reactive oxygen species (ROS) induced by ventilation also increase NLRP3 activity, inducing caspase-1 (CASP1), and thus IL-1 transcription. Glucocorticoids bind to glucocorticoid receptors (GR) in the cytoplasm and interfere with the transcription of IL-1 in the nucleus. Adenosine monophosphate-activated protein kinase (AMPK) binding to toll-like receptors (TLR) inhibits NLRP3 (red line).

7 Cell therapies for prevention of pulmonary inflammation

7.1 Mesenchymal stem cells

Mesenchymal stem cells (MSCs) with multi-lineage differentiability are usually derived from bone marrow. MSCs home to sites of injury and possess immunomodulatory functions.[105] In culture, MSCs can differentiate into alveolar epithelium.[105, 106] *In vivo,* MSCs engraft in the lung and produce surfactant,[106] but engraftment rates are low,[107, 108] suggesting MSCs work via paracrine effects. Prophylactic administration of MSCs mitigate lung injury in mice but are ineffective in repairing established lung injury.[108]

Meta-analysis of RCTs in adult diseases indicates MSCs are safe;[109] they do not increase rates of infection, death or malignancy,[109] despite previous concerns about tumourogenicity.[110] Transient fever was noted in trials using MSCs[109] but it is unclear whether this is the consequence of an immune reaction to MSCs.

Clinical studies of MSCs in preterm infants are limited. One trial counted MSCs in BAL and another administered MSCs before BPD diagnosis. The presence of endogenous MSCs within BAL of preterm infants was associated with increased risk of developing BPD.[111] However, the source of MSCs in BAL was unclear, and may be a result of injured lung epithelium. A phase I dose-escalation trial examined administration of MSCs intratracheally to preterm infants at risk of developing BPD (23-29 weeks GA) who required continuous ventilator support.[112] Pro-inflammatory cytokines in BAL were lower at day 7 compared to day 3 after MSC transplantation, highlighting the paracrine effects of the cells. Thirty-three percent of preterm infants enrolled developed BPD. This trial targeted infants before BPD developed, who may not have had established lung inflammation. Six patients in the trial developed serious adverse events up to 84 days after MSC transplantation, including pneumothorax, NEC and IVH (< grade 3). The majority of adverse events occurred in infants receiving the highest MSC dose (20 million cells/kg).

The origin or handling of MSCs may influence their therapeutic potential. The yield of cord-blood-derived MSCs is low and MSCs need to be expanded for adequate cell numbers for delivery to a patient, but culturing induces ageing.[113] Cultured, MSCs have weaker immunomodulatory properties *in vitro* compared to primary MSCs,[113] potentially compromising the therapeutic effects of MSCs. Additionally, MSC expansion often requires growth factors (containing animal products),[112] or plating onto a glycoprotein-rich fibronectin matrix.[114] The impact of

ACCEPTED MANUSCRIPT

media, growth factors or matrices used with MSCs, and whether these alter MSC function, must be considered when proposing MSC transplantation in preterm infants.

7.2 Human amnion epithelial cells

The amniotic membrane is the innermost placental membrane surrounding the fetus,[115] made up of a single layer of cuboidal columnar epithelial cells.[115] The amnion predominately provides the developing fetus with protection, but also produces growth factors, cytokines, prostaglandins and erythropoietin.[116] The amniotic epithelium is formed before gastrulation, and thus is pluripotent even at term gestation.[115]

Amniotic membranes were first used over a century ago as biological dressings for skin wounds.[117] This practice continues, highlighting the safety of these cells as a therapeutic for human disease.[118] Unlike MSCs, isolation of human amnion epithelial cells (hAECs) from a single placenta yields enough cells for administration to multiple patients. Primary hAECs may be used immediately after isolation, obviating concerns about cell manipulation.

Human AECs do not express telomerase,[119] and have low tumourigenicity.[120] Rejection does not occur when hAECs are administered to humans[121] or other animals,[119, 122] likely due to low HLA class II expression.[122 123] Thus, hAECs can be applied without concerns of tumorigenesis or rejection.

Human AECs reduce lung collagen and fibrosis in mice,[124, 125] up to 14 days after bleomycin insult.[125] Hyperoxia-exposed mice treated with hAECs have increased expression of vascular endothelial growth factor receptor and angiogenin1 in their lungs and this correlates with normalized pulmonary vasculature.[126] Human AEC administration normalises alveolar structure in hyperoxia-exposed mice,[126] LPS-exposed fetal sheep[127] and in fetal and preterm sheep following injurious ventilation.[128, 129] Thus, hAECs prevent BPD-like lung pathology, independent of the model, and this reduction in BPD-like lung pathology is consistently accompanied by reduced lung inflammation.

The immunomodulatory effects of hAECs are demonstrated by their ability to downregulate pro-inflammatory cytokines, IL-6,[128, 130-132] IL-1α and IL-1β,[131] and upregulate anti-inflammatory cytokine IL-10.[129] However, hAECs increase total immune cell numbers in the lungs of LPS-exposed fetal sheep and mechanically ventilated preterm lambs,[127, 129] demonstrating the ability of hAECs' to augment inflammatory cell recruitment but seemingly without proinflammatory effects. IL-10 has a role in activating M2 pro-reparative macrophages and opposing differentiation of pro-inflammatory M1 macrophages,[133] and is upregulated following hAEC administration.[129, 132] Indeed, lung and liver macrophage activity shifts from a predominately M1 to M2 phenotype after hAEC administration in bleomycin-exposed mice.[134, 135] Similar to MSCs, hAECs modulate the immune system and prevent BPD-like lung pathology, likely via paracrine effects.

The safety of hAECs has been explored in an unpublished phase I trial of hAECs in infants with intractable BPD (ACTRN: 12614000174684). Subsequent trials are required to determine the appropriate dose of hAECs and when to administer hAECs in preterm infants with BPD.

ACCEPTED MANUSCRIPT

8 Final comments and future research

BPD remains a major cause of morbidity and mortality in preterm infants. Historically, therapies like postnatal steroids have been used without appropriate preclinical data regarding safety and long-term outcomes. Pharmacological inhibitors of inflammation, such as IL-1 inhibitors, pentoxifylline and AMP activators, are promising therapies for pulmonary inflammation but are in preclinical stages. Cellular therapies, arguably, have more preclinical evidence surrounding their use for BPD and are approaching RCTs. However, it is unlikely that a one-drug-fix-all approach to BPD will eventuate. Likely, the most beneficial outcomes for infants developing, or who have developed, BPD will be achieved with combined therapies. If an infant is unresponsive to a therapy (e.g. steroids or hAECs) within several days an alternative therapy should be considered. Future studies will need to consider interactions between therapies in preterm infants if tailoring therapies for neonates with BPD is necessary.

Educational aims

The reader will appreciate:

- The incidence of BPD is increasing, despite advancements in clinical care of preterm infants.
- Maturational agents to improve lung architecture, including antenatal glucocorticoids and surfactant therapy, have not improved BPD incidence.
- Anti-inflammatory therapies may be beneficial over maturational agents in preventing BPD incidence.
- Glucocorticoids reduce BPD incidence but have poor neurological outcomes. Glucocorticoid use in the newborn requires significant optimisation.
- Cellular therapies likely modulate lung inflammation associated with BPD, without adverse outcomes.

Future research directions

BPD is a complex disease involving aberrant regulation of lung inflammation. The use of preclinical, translational studies to identify or optimise pre-existing therapies, such as postnatal steroids or IL-1Ra, need to be conducted and compared to less conventional cellular therapies. Future studies should aim to encompass the inflammatory, structural and vascular complications of BPD for the best outcomes in preterm infants.

References

1. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.
2. Stoll BJ, Hansen NI, Bell EF, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. Jama. 2015;314(10):1039-51.
3. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. The Cochrane database of systematic reviews. 2006(3):CD004454.

ACCEPTED MANUSCRIPT

4. Bancalari E, del Moral T. Bronchopulmonary dysplasia and surfactant. Biology of the neonate. 2001;80 Suppl 1:7-13.
5. Morley CJ, Davis PG, Doyle LW, et al. Nasal CPAP or intubation at birth for very preterm infants. The New England journal of medicine. 2008;358(7):700-8.
6. Melville JM, Moss TJ. The immune consequences of preterm birth. Front Neurosci. 2013;7:79.
7. Northway WH, Jr., Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. The New England journal of medicine. 1967;276(7):357-68.
8. Agrons GA, Courtney SE, Stocker JT, et al. From the archives of the AFIP: Lung disease in premature neonates: radiologic-pathologic correlation. Radiographics. 2005;25(4):1047-73.
9. Bhatt AJ, Pryhuber GS, Huyck H, et al. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. American journal of respiratory and critical care medicine. 2001;164(10 Pt 1):1971-80.
10. Vento G, Capoluongo E, Matassa PG, et al. Serum levels of seven cytokines in premature ventilated newborns: correlations with old and new forms of bronchopulmonary dysplasia. Intensive care medicine. 2006;32(5):723-30.
11. Kotecha S, Wilson L, Wangoo A, et al. Increase in interleukin (IL)-1 beta and IL-6 in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. Pediatric research. 1996;40(2):250-6.
12. Munshi UK, Niu JO, Siddiq MM, et al. Elevation of interleukin-8 and interleukin-6 precedes the influx of neutrophils in tracheal aspirates from preterm infants who develop bronchopulmonary dysplasia. Pediatric pulmonology. 1997;24(5):331-6.
13. Husain AN, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. Hum Pathol. 1998;29(7):710-7.
14. Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116(6):1353-60.
15. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. The New England journal of medicine. 2000;342(20):1500-7.
16. Lahra MM, Beeby PJ, Jeffery HE. Maternal versus fetal inflammation and respiratory distress syndrome: a 10-year hospital cohort study. Archives of disease in childhood Fetal and neonatal edition. 2009;94(1):F13-6.
17. Dempsey E, Chen MF, Kokottis T, et al. Outcome of neonates less than 30 weeks gestation with histologic chorioamnionitis. American journal of perinatology. 2005;22(3):155-9.
18. Watterberg KL, Demers LM, Scott SM, et al. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics. 1996;97(2):210-5.
19. Moss TJ, Newnham JP, Willett KE, et al. Early gestational intra-amniotic endotoxin: lung function, surfactant, and morphometry. American journal of respiratory and critical care medicine. 2002;165(6):805-11.
20. Jobe AH, Newnham JP, Willet KE, et al. Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. American journal of respiratory and critical care medicine. 2000;162(5):1656-61.
21. Bachurski CJ, Ross GF, Ikegami M, et al. Intra-amniotic endotoxin increases pulmonary surfactant proteins and induces SP-B processing in fetal sheep. American journal of physiology Lung cellular and molecular physiology. 2001;280(2):L279-85.

ACCEPTED MANUSCRIPT

22. Yum SK, Kim MS, Kwun Y, et al. Impact of histologic chorioamnionitis on pulmonary hypertension and respiratory outcomes in preterm infants. Pulm Circ. 2018;8(2):2045894018760166.
23. Hartling L, Liang Y, Lacaze-Masmonteil T. Chorioamnionitis as a risk factor for bronchopulmonary dysplasia: a systematic review and meta-analysis. Archives of disease in childhood Fetal and neonatal edition. 2012;97(1):F8-F17.
24. Soraisham AS, Singhal N, McMillan DD, et al. A multicenter study on the clinical outcome of chorioamnionitis in preterm infants. American journal of obstetrics and gynecology. 2009;200(4):372 e1-6.
25. Van Marter LJ, Dammann O, Allred EN, et al. Chorioamnionitis, mechanical ventilation, and postnatal sepsis as modulators of chronic lung disease in preterm infants. The Journal of pediatrics. 2002;140(2):171-6.
26. Westover AJ, Moss TJ. Effects of intrauterine infection or inflammation on fetal lung development. Clinical and experimental pharmacology & physiology. 2012;39(9):824-30.
27. Willet KE, Jobe AH, Ikegami M, et al. Antenatal endotoxin and glucocorticoid effects on lung morphometry in preterm lambs. Pediatric research. 2000;48(6):782-8.
28. Galinsky R, Hooper SB, Wallace MJ, et al. Intrauterine inflammation alters cardiopulmonary and cerebral haemodynamics at birth in preterm lambs. J Physiol. 2013;591(8):2127-37.
29. Yoder BA, Coalson JJ, Winter VT, et al. Effects of antenatal colonization with ureaplasma urealyticum on pulmonary disease in the immature baboon. Pediatric research. 2003;54(6):797-807.
30. Polglase GR, Hillman NH, Pillow JJ, et al. Ventilation-mediated injury after preterm delivery of Ureaplasma parvum colonized fetal lambs. Pediatric research. 2010;67(6):630-5.
31. Bhandari V. Postnatal inflammation in the pathogenesis of bronchopulmonary dysplasia. Birth defects research Part A, Clinical and molecular teratology. 2014;100(3):189-201.
32. Koksal N, Kayik B, Cetinkaya M, et al. Value of serum and bronchoalveolar fluid lavage pro- and anti-inflammatory cytokine levels for predicting bronchopulmonary dysplasia in premature infants. Eur Cytokine Netw. 2012;23(2):29-35.
33. Barazzone C, White CW. Mechanisms of cell injury and death in hyperoxia: role of cytokines and Bcl-2 family proteins. American journal of respiratory cell and molecular biology. 2000;22(5):517-9.
34. Kim SR, Kim DI, Kim SH, et al. NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. Cell Death Dis. 2014;5:e1498.
35. Brew N, Hooper SB, Allison BJ, et al. Injury and repair in the very immature lung following brief mechanical ventilation. American journal of physiology Lung cellular and molecular physiology. 2011;301(6):L917-26.
36. Hillman NH, Kallapur SG, Pillow JJ, et al. Airway injury from initiating ventilation in preterm sheep. Pediatric research. 2010;67(1):60-5.
37. Albertine KH, Jones GP, Starcher BC, et al. Chronic lung injury in preterm lambs. Disordered respiratory tract development. American journal of respiratory and critical care medicine. 1999;159(3):945-58.
38. Balinotti JE, Chakr VC, Tiller C, et al. Growth of lung parenchyma in infants and toddlers with chronic lung disease of infancy. American journal of respiratory and critical care medicine. 2010;181(10):1093-7.
39. Korhonen P, Laitinen J, Hyodynmaa E, et al. Respiratory outcome in school-aged, very-low-birth-weight children in the surfactant era. Acta paediatrica. 2004;93(3):316-21.
40. Polglase GR, Nitsos I, Jobe AH, et al. Maternal and intra-amniotic corticosteroid effects on lung morphometry in preterm lambs. Pediatric research. 2007;62(1):32-6.

ACCEPTED MANUSCRIPT

41. Kavelaars A, van der Pompe G, Bakker JM, et al. Altered immune function in human newborns after prenatal administration of betamethasone: enhanced natural killer cell activity and decreased T cell proliferation in cord blood. Pediatric research. 1999;45(3):306-12.
42. Kramer BW, Kallapur SG, Moss TJ, et al. Intra-amniotic LPS modulation of TLR signaling in lung and blood monocytes of fetal sheep. Innate Immun. 2009;15(2):101-7.
43. Newnham JP, Kallapur SG, Kramer BW, et al. Betamethasone effects on chorioamnionitis induced by intra-amniotic endotoxin in sheep. American journal of obstetrics and gynecology. 2003;189(5):1458-66.
44. Moss TJ, Harding R, Newnham JP. Lung function, arterial pressure and growth in sheep during early postnatal life following single and repeated prenatal corticosteroid treatments. Early human development. 2002;66(1):11-24.
45. Nin N, Penuelas O, de Paula M, et al. Ventilation-induced lung injury in rats is associated with organ injury and systemic inflammation that is attenuated by dexamethasone. Critical care medicine. 2006;34(4):1093-8.
46. Held HD, Boettcher S, Hamann L, et al. Ventilation-induced chemokine and cytokine release is associated with activation of nuclear factor-kappaB and is blocked by steroids. American journal of respiratory and critical care medicine. 2001;163(3 Pt 1):711-6.
47. Hillman NH, Pillow JJ, Ball MK, et al. Antenatal and postnatal corticosteroid and resuscitation induced lung injury in preterm sheep. Respiratory research. 2009;10:124.
48. Walsh MC, Yao Q, Horbar JD, et al. Changes in the use of postnatal steroids for bronchopulmonary dysplasia in 3 large neonatal networks. Pediatrics. 2006;118(5):e1328-35.
49. Cummings JJ, D'Eugenio DB, Gross SJ. A controlled trial of dexamethasone in preterm infants at high risk for bronchopulmonary dysplasia. The New England journal of medicine. 1989;320(23):1505-10.
50. Doyle LW, Halliday HL, Ehrenkranz RA, et al. Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease. Pediatrics. 2005;115(3):655-61.
51. Barrington KJ. The adverse neuro-developmental effects of postnatal steroids in the preterm infant: a systematic review of RCTs. BMC pediatrics. 2001;1:1.
52. Doyle LW, Ehrenkranz RA, Halliday HL. Late (> 7 days) postnatal corticosteroids for chronic lung disease in preterm infants. The Cochrane database of systematic reviews. 2014(5):CD001145.
53. Doyle LW, Ehrenkranz RA, Halliday HL. Early (< 8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. The Cochrane database of systematic reviews. 2014(5):CD001146.
54. Claure N, Bello JA, Jain D. Strategies to reduce mechanical ventilation and bronchopulmonary dysplasia in preterm infants. Respir Care. 2013;58(7):1257.
55. Groneck P, Reuss D, Gotze-Speer B, et al. Effects of dexamethasone on chemotactic activity and inflammatory mediators in tracheobronchial aspirates of preterm infants at risk for chronic lung disease. The Journal of pediatrics. 1993;122(6):938-44.
56. Yoder MC, Jr., Chua R, Tepper R. Effect of dexamethasone on pulmonary inflammation and pulmonary function of ventilator-dependent infants with bronchopulmonary dysplasia. The American review of respiratory disease. 1991;143(5 Pt 1):1044-8.
57. Groneck P, Gotze-Speer B, Oppermann M, et al. Association of pulmonary inflammation and increased microvascular permeability during the development of bronchopulmonary dysplasia: a sequential analysis of inflammatory mediators in respiratory fluids of high-risk preterm neonates. Pediatrics. 1994;93(5):712-8.
58. Wilson-Costello D, Walsh MC, Langer JC, et al. Impact of postnatal corticosteroid use on neurodevelopment at 18 to 22 months' adjusted age: effects of dose, timing, and risk of

ACCEPTED MANUSCRIPT

bronchopulmonary dysplasia in extremely low birth weight infants. Pediatrics. 2009;123(3):e430-7.
59. Weichsel ME, Jr. The therapeutic use of glucocorticoid hormones in the perinatal period: potential neurological hazards. Ann Neurol. 1977;2(5):364-6.
60. Howard E. Reductions in size and total DNA of cerebrum and cerebellum in adult mice after corticosterone treatment in infancy. Experimental neurology. 1968;22(2):191-208.
61. Howard E, Benjamins JA. DNA, ganglioside and sulfatide in brains of rats given corticosterone in infancy, with an estimate of cell loss during development. Brain research. 1975;92(1):73-87.
62. Stark AR, Carlo WA, Tyson JE, et al. Adverse effects of early dexamethasone treatment in extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. The New England journal of medicine. 2001;344(2):95-101.
63. Garland JS, Alex CP, Pauly TH, et al. A three-day course of dexamethasone therapy to prevent chronic lung disease in ventilated neonates: a randomized trial. Pediatrics. 1999;104(1 Pt 1):91-9.
64. Yeh TF, Lin YJ, Hsieh WS, et al. Early postnatal dexamethasone therapy for the prevention of chronic lung disease in preterm infants with respiratory distress syndrome: a multicenter clinical trial. Pediatrics. 1997;100(4):E3.
65. Doyle LW, Davis PG, Morley CJ, et al. Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. Pediatrics. 2006;117(1):75-83.
66. Doyle LW, Davis PG, Morley CJ, et al. Outcome at 2 years of age of infants from the DART study: a multicenter, international, randomized, controlled trial of low-dose dexamethasone. Pediatrics. 2007;119(4):716-21.
67. Harris E, Schulzke SM, Patole SK. Pentoxifylline in preterm neonates: a systematic review. Paediatr Drugs. 2010;12(5):301-11.
68. Krause PJ, Kristie J, Wang WP, et al. Pentoxifylline enhancement of defective neutrophil function and host defense in neonatal mice. The American journal of pathology. 1987;129(2):217-22.
69. Lauterbach R, Pawlik D, Kowalczyk D, et al. Effect of the immunomodulating agent, pentoxifylline, in the treatment of sepsis in prematurely delivered infants: a placebo-controlled, double-blind trial. Critical care medicine. 1999;27(4):807-14.
70. Lauterbach R, Szymura-Oleksiak J, Pawlik D, et al. Nebulized pentoxifylline for prevention of bronchopulmonary dysplasia in very low birth weight infants: a pilot clinical study. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2006;19(7):433-8.
71. Aviado DM, Dettelbach HR. Pharmacology of pentoxifylline, a hemorheologic agent for the treatment of intermittent claudication. Angiology. 1984;35(7):407-17.
72. Aviado DM, Porter JM. Pentoxifylline: a new drug for the treatment of intermittent claudication. Mechanism of action, pharmacokinetics, clinical efficacy and adverse effects. Pharmacotherapy. 1984;4(6):297-307.
73. Almario B, Wu S, Peng J, et al. Pentoxifylline and prevention of hyperoxia-induced lung -injury in neonatal rats. Pediatric research. 2012;71(5):583-9.
74. Oliveira-Junior IS, Maganhin CC, Carbonel AA, et al. Effects of pentoxifylline on TNF-alpha and lung histopathology in HCL-induced lung injury. Clinics. 2008;63(1):77-84.
75. Tschopp J, Schroder K. NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? Nature reviews Immunology. 2010;10(3):210-5.
76. He Y, Hara H, Nunez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. Trends Biochem Sci. 2016;41(12):1012-21.

ACCEPTED MANUSCRIPT

77. Baroja-Mazo A, Martin-Sanchez F, Gomez AI, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat Immunol. 2014;15(8):738-48.
78. Kuipers MT, Aslami H, Janczy JR, et al. Ventilator-induced lung injury is mediated by the NLRP3 inflammasome. Anesthesiology. 2012;116(5):1104-15.
79. Busillo JM, Azzam KM, Cidlowski JA. Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome. The Journal of biological chemistry. 2011;286(44):38703-13.
80. Stouch AN, McCoy AM, Greer RM, et al. IL-1beta and Inflammasome Activity Link Inflammation to Abnormal Fetal Airway Development. Journal of immunology. 2016;196(8):3411-20.
81. Liao J, Kapadia VS, Brown LS, et al. The NLRP3 inflammasome is critically involved in the development of bronchopulmonary dysplasia. Nat Commun. 2015;6:8977.
82. Behrashi M, Samimi M, Ghasemi T, et al. Comparison of Glibenclamide and Insulin on Neonatal Outcomes in Pregnant Women with Gestational Diabetes. Int J Prev Med. 2016;7:88.
83. Ilkhanipoor H, Karamizadeh Z. Changing the Treatment of Permanent Neonatal Diabetes Mellitus from Insulin to Glibenclamide in a 4-Month-Old Infant with KCNJ11 Activating Mutation. Int J Prev Med. 2013;4(9):1078-81.
84. Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine and Growth Factor Review. 2002;13:323-40.
85. Dinarello CA, Ikejima T, Warner SJ, et al. Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. Journal of immunology. 1987;139(6):1902-10.
86. Viscardi RM, Muhumuza CK, Rodriguez A, et al. Inflammatory markers in intrauterine and fetal blood and cerebrospinal fluid compartments are associated with adverse pulmonary and neurologic outcomes in preterm infants. Pediatric research. 2004;55(6):1009-17.
87. Rindfleisch MS, Hasday JD, Taciak V, et al. Potential role of interleukin-1 in the development of bronchopulmonary dysplasia. J Interferon Cytokine Res. 1996;16(5):365-73.
88. Kakkera DK, Siddiq MM, Parton LA. Interleukin-1 balance in the lungs of preterm infants who develop bronchopulmonary dysplasia. Biology of the neonate. 2005;87(2):82-90.
89. Johnson BH, Yi M, Masood A, et al. A critical role for the IL-1 receptor in lung injury induced in neonatal rats by 60% O2. Pediatric research. 2009;66(3):260-5.
90. Nold MF, Mangan NE, Rudloff I, et al. Interleukin-1 receptor antagonist prevents murine bronchopulmonary dysplasia induced by perinatal inflammation and hyperoxia. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(35):14384-9.
91. Kallapur SG, Nitsos I, Moss TJ, et al. IL-1 mediates pulmonary and systemic inflammatory responses to chorioamnionitis induced by lipopolysaccharide. American journal of respiratory and critical care medicine. 2009;179(10):955-61.
92. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov. 2012;11(8):633-52.
93. Goldbach-Mansky R, Dailey NJ, Canna SW, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. The New England journal of medicine. 2006;355(6):581-92.
94. Kone-Paut I, Galeotti C. Current treatment recommendations and considerations for cryopyrin-associated periodic syndrome. Expert Rev Clin Immunol. 2015;11(10):1083-92.
95. Neven B, Marvillet I, Terrada C, et al. Long-term efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheum. 2010;62(1):258-67.

ACCEPTED MANUSCRIPT

96. Stenerson M, Dufendach K, Aksentijevich I, et al. The first reported case of compound heterozygous IL1RN mutations causing deficiency of the interleukin-1 receptor antagonist. Arthritis Rheum. 2011;63(12):4018-22.
97. Canakinumab (Ilaris). CADTH Common Drug Reviews. Ottawa (ON)2016.
98. Tarp S, Amarilyo G, Foeldvari I, et al. Efficacy and safety of biological agents for systemic juvenile idiopathic arthritis: a systematic review and meta-analysis of randomized trials. Rheumatology (Oxford). 2016;55(4):669-79.
99. Diamond G, Beckloff N, Weinberg A, et al. The roles of antimicrobial peptides in innate host defense. Curr Pharm Des. 2009;15(21):2377-92.
100. Salminen A, Hyttinen JM, Kaarniranta K. AMP-activated protein kinase inhibits NF-kappaB signaling and inflammation: impact on healthspan and lifespan. J Mol Med (Berl). 2011;89(7):667-76.
101. Schaller-Bals S, Schulze A, Bals R. Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. American journal of respiratory and critical care medicine. 2002;165(7):992-5.
102. Xing J, Wang Q, Coughlan K, et al. Inhibition of AMP-activated protein kinase accentuates lipopolysaccharide-induced lung endothelial barrier dysfunction and lung injury in vivo. The American journal of pathology. 2013;182(3):1021-30.
103. Konings E, Timmers S, Boekschoten MV, et al. The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men. International journal of obesity. 2014;38(3):470-3.
104. Yi CO, Jeon BT, Shin HJ, et al. Resveratrol activates AMPK and suppresses LPS-induced NF-kappaB-dependent COX-2 activation in RAW 264.7 macrophage cells. Anat Cell Biol. 2011;44(3):194-203.
105. Chamberlain G, Fox J, Ashton B, et al. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem cells. 2007;25(11):2739-49.
106. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell. 2001;105(3):369-77.
107. van Haaften T, Byrne R, Bonnet S, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. American journal of respiratory and critical care medicine. 2009;180(11):1131-42.
108. Ortiz LA, Gambelli F, McBride C, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(14):8407-11.
109. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PloS one. 2012;7(10):e47559.
110. Djouad F, Plence P, Bony C, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood. 2003;102(10):3837-44.
111. Popova AP, Bozyk PD, Bentley JK, et al. Isolation of tracheal aspirate mesenchymal stromal cells predicts bronchopulmonary dysplasia. Pediatrics. 2010;126(5):e1127-33.
112. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. The Journal of pediatrics. 2014;164(5):966-72 e6.
113. Stolzing A, Sethe S, Scutt AM. Stressed stem cells: Temperature response in aged mesenchymal stem cells. Stem cells and development. 2006;15(4):478-87.
114. Harting MT, Jimenez F, Xue H, et al. Intravenous mesenchymal stem cell therapy for traumatic brain injury. J Neurosurg. 2009;110(6):1189-97.
115. Ilancheran S, Moodley Y, Manuelpillai U. Human fetal membranes: a source of stem cells for tissue regeneration and repair? Placenta. 2009;30(1):2-10.

ACCEPTED MANUSCRIPT

116. Mamede AC, Carvalho MJ, Abrantes AM, et al. Amniotic membrane: from structure and functions to clinical applications. Cell and tissue research. 2012;349(2):447-58.
117. Davis JS. II. Skin Grafting at the Johns Hopkins Hospital. Annals of surgery. 1909;50(3):542-9.
118. Kesting MR, Wolff KD, Hohlweg-Majert B, et al. The role of allogenic amniotic membrane in burn treatment. Journal of burn care & research : official publication of the American Burn Association. 2008;29(6):907-16.
119. Miki T, Lehmann T, Cai H, et al. Stem cell characteristics of amniotic epithelial cells. Stem cells. 2005;23(10):1549-59.
120. Shay JW, Wright WE. Role of telomeres and telomerase in cancer. Semin Cancer Biol. 2011;21(6):349-53.
121. Akle CA, Adinolfi M, Welsh KI, et al. Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. Lancet. 1981;2(8254):1003-5.
122. Ilancheran S, Michalska A, Peh G, et al. Stem cells derived from human fetal membranes display multilineage differentiation potential. Biology of reproduction. 2007;77(3):577-88.
123. Banas RA, Trumpower C, Bentlejewski C, et al. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. Human immunology. 2008;69(6):321-8.
124. Murphy S, Lim R, Dickinson H, et al. Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. Cell transplantation. 2011;20(6):909-23.
125. Vosdoganes P, Wallace EM, Chan ST, et al. Human amnion epithelial cells repair established lung injury. Cell transplantation. 2013;22(8):1337-49.
126. Zhu D, Muljadi R, Chan ST, et al. Evaluating the Impact of Human Amnion Epithelial Cells on Angiogenesis. Stem Cells Int. 2016;2016:4565612.
127. Vosdoganes P, Hodges RJ, Lim R, et al. Human amnion epithelial cells as a treatment for inflammation-induced fetal lung injury in sheep. American journal of obstetrics and gynecology. 2011;205(2):156 e26-33.
128. Hodges RJ, Jenkin G, Hooper SB, et al. Human amnion epithelial cells reduce ventilation-induced preterm lung injury in fetal sheep. American journal of obstetrics and gynecology. 2012;206(5):448 e8-15.
129. Melville JM, McDonald CA, Bischof RJ, et al. Human amnion epithelial cells modulate the inflammatory response to ventilation in preterm lambs. PloS one. 2017;12(3):e0173572.
130. Murphy SV, Shiyun SC, Tan JL, et al. Human amnion epithelial cells do not abrogate pulmonary fibrosis in mice with impaired macrophage function. Cell transplantation. 2012;21(7):1477-92.
131. Vosdoganes P, Lim R, Koulaeva E, et al. Human amnion epithelial cells modulate hyperoxia-induced neonatal lung injury in mice. Cytotherapy. 2013;15(8):1021-9.
132. Moodley Y, Ilancheran S, Samuel C, et al. Human amnion epithelial cell transplantation abrogates lung fibrosis and augments repair. American journal of respiratory and critical care medicine. 2010;182(5):643-51.
133. Makita N, Hizukuri Y, Yamashiro K, et al. IL-10 enhances the phenotype of M2 macrophages induced by IL-4 and confers the ability to increase eosinophil migration. Int Immunol. 2015;27(3):131-41.
134. Tan JL, Chan ST, Wallace EM, et al. Human amnion epithelial cells mediate lung repair by directly modulating macrophage recruitment and polarization. Cell transplantation. 2014;23(3):319-28.
135. Manuelpillai U, Lourensz D, Vaghjiani V, et al. Human amniotic epithelial cell transplantation induces markers of alternative macrophage activation and reduces established hepatic fibrosis. PloS one. 2012;7(6):e38631.

AUTHOR ACCEPTED MANUSCRIPT

Title: Genetic Variation in *CRHR1* is Associated with Short-Term Respiratory Response to Corticosteroids in Preterm Infants at risk for Bronchopulmonary Dysplasia

Running Title: Steroid Pharmacogenetics in BPD

Authors: Tamorah Lewis*[1]; William Truog[1]; Mike Norberg[1], Philip L. Ballard[2]; Dara Torgerson[2]; TOLSURF Study Group

1 – Children's Mercy Hospital, Department of Pediatrics, University of Missouri Kansas City School of Medicine, Kansas City, MO

2 - Department of Pediatrics, University of California San Francisco, San Francisco, CA

Corresponding Author:

Tamorah Lewis, MD, PhD

Assistant Professor, UMKC School of Medicine

Neonatology | Pediatric Clinical Pharmacology

Children's Mercy Hospital

2401 Gillham Rd., Kansas City, MO 64108

Phone: (816) 234-4295 Fax: 816-234-3590

Email: trlewis@cmh.edu

Financial Support: There was no external financial support for this project.

Disclosure: The authors have no conflicts of interest to disclose.

Article Type: Clinical Research

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR ACCEPTED MANUSCRIPT

Abstract

Background Bronchopulmonary Dysplasia (BPD) is an orphan disease and advances in prevention and treatment are lacking. Clinical efficacy of systemic corticosteroid therapy to reduce the severity of lung disease and BPD is highly variable. Our objective was to assess whether candidate SNPs in corticosteroid metabolism and response genes are associated with short-term phenotypic response to systemic corticosteroids in infants at high risk for BPD.

Methods Pharmacogenetic analysis of data from a large randomized controlled trial (TOLSURF) in infants treated with dexamethasone or hydrocortisone using multivariate linear regression. The primary outcome was change in Respiratory Severity Score (RSS, mean airway pressure x FiO2) at day 7 of corticosteroid treatment.

Results rs7225082 in the intron of *CRHR1* is significantly associated with the magnitude of decrease in RSS 7 days after starting treatment with systemic corticosteroid (meta-analysis $p=2.8x10^{-4}$). Each T allele at rs7225082 is associated with a smaller absolute change in RSS at day 7, i.e. less response to systemic corticosteroids.

Conclusion Genetic variability is associated with corticosteroid responsiveness with regard to respiratory status in preterm infants. Identification of genetic markers of corticosteroid responsiveness may allow for therapeutic individualization, with the goal of optimizing the risk to benefit ratio for an individual child.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Introduction

Bronchopulmonary dysplasia (BPD) is the most frequent complication of extreme prematurity. With increasing survival of the most immature infants, rates of BPD are increasing. Among a large cohort of infants less than 1500 grams at birth, the incidence of BPD increased between 2009 and 2012, up to 55% in infants born at 26 weeks and 40% in infants born at 27 weeks(1). Preventive therapies for BPD are limited, but postnatal treatment with systemic corticosteroids is a common practice grounded in studies that show improved pulmonary outcomes (2, 3). Corticosteroid treatment is highly variable among centers(4), in part due to concern regarding historic associations of high dose dexamethasone with central nervous system injury(5-7). In order for corticosteroid use to be optimized, the risk to benefit ratio must be improved such that a given infant is more likely to be benefited than harmed. Precision therapeutics is a burgeoning field that utilizes genetic information to individualize therapy, and this approach can be used to investigate target populations for optimal corticosteroid benefit. A long-term goal of pharmacogenetic studies in BPD is to preemptively delineate likely responders and non-responders and treat accordingly, and to delineate the biology of differential drug response.

In the original Trial of Surfactant Treatment (TOLSURF) study, many infants received systemic corticosteroids to decrease severity of lung disease and facilitate weaning from mechanical ventilation. In both dexamethasone- and hydrocortisone-treated infants, there is a known large range in phenotypic response to corticosteroid treatment. Many factors can contribute to this variability, including severity of lung disease, variation among centers in management of respiratory support and individual patient co-morbidities such as infections, etc. Genetic variation may also be an important and understudied contributor to differential corticosteroid response.

Genetic variation contributes to the risk of BPD (8, 9). Although yet unstudied in the BPD population, there is a high probability that genetic variability also contributes to variability in drug response. Variant alleles can influence drug metabolism, disposition and drug target sensitivity, and explain some of the heterogeneity in efficacy and toxicity that is observed within a patient population. A recent study (10) links neonatal genetic

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

variation to respiratory phenotype at birth after maternal treatment with antenatal corticosteroids. In asthma, multiple single nucleotide polymorphisms (SNPs) in corticosteroid metabolism and response genes modify clinical response to inhaled corticosteroids(11-17). Though asthma and BPD are separate clinical entities, significant overlap occurs in their pathophysiology (inflammation, pulmonary architecture remodeling, bronchospasm, air trapping). Genes which are implicated in prenatal corticosteroid response and asthma corticosteroid response are potentially relevant to clinical corticosteroid response for BPD.

Given this background, our objective was to identify pharmacogenetic variants from pediatric asthma and perinatal corticosteroid treatment literature, and test these genes and variants for an association with clinical response to systemic corticosteroids in infants at high risk for BPD. Our hypothesis states that variants associated with corticosteroid metabolism and response will correlate with short-term improvement in respiratory phenotype among a clinically homogenous cohort of preterm infants.

Methods

Our investigation was a secondary analysis of existing data collected during a large multi-center, randomized, controlled trial of late surfactant therapy (TOLSURF study)(18, 19). The parent clinical trial was IRB-approved and parental consent was obtained, including collection and study of biospecimens (tracheal aspirate, urine and DNA).The Children's Mercy Hospital IRB deferred review of this secondary study as non-human subjects research (use of de-identified historical data). The source population from the TOLSURF study included preterm infants receiving inhaled nitric oxide, birth weight 701±164 g, and gestational age 25.2±1.2 weeks. There was no differences found in the primary outcome of survival without BPD at 36 weeks between surfactant-treated and placebo groups. Thus, the groups were combined for secondary analyses.

Study Cohort

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

511 infants enrolled in the TOLSURF study were screened for eligibility. 126 infants met the following criteria: received systemic dexamethasone (DEX) or hydrocortisone (HC) at greater than 14 days of life for a minimum of seven days of treatment and on invasive ventilation at corticosteroids start. These criteria were selected to exclude corticosteroid exposure for blood pressure support and brief courses to treat airway edema for extubation. Of these 126, 39 were excluded due to (1) no DNA collected/isolated or failed early sample quality control during genome-wide SNP genotyping (n=26), (2) genotype call rates < 95% (n=3), (3) sibling from a multiple gestation (n=10, only one sibling was included to account for genetic relatedness). Ten were excluded for no primary outcome data; these infants were extubated to low flow NC and mean airway pressure (MAP) was not measureable.

Use of systemic corticosteroids was not prescribed by the study protocol but guidelines for their use were established by trial investigators. Approximately 75% of the source population were treated with postnatal corticosteroids, for various indications, one being BPD. For corticosteroid treatment for BPD, guidelines for use included withholding treatment until at least 2 weeks of age and only for infants with respiratory severity scores (RSS, FIO2 x mean airway pressure) of ≥7. Biologically equivalent doses of either hydrocortisone (15 mg/kg over 9 days) or dexamethasone (0.89 mg/kg over 10 days), based on doses and durations used in prior clinical trials, were recommended per TOLSURF study guidelines (20). BPD at 36 and 40 weeks was defined in the original study with the following criteria: Infants discharged in room air before 36 weeks were designated "No BPD." Infants requiring ventilatory support and any level of supplemental oxygen, or with an effective FiO2 >30% by nasal cannula, were diagnosed with BPD ("severe" BPD by the NIH workshop definition (21)). Infants receiving mechanical ventilation, NCPAP, or >4 L of nasal cannula flow in room air were designated "Yes BPD." Infants at 36 weeks receiving ≤0.3 effective FiO_2 at <2 L flow or on nasal flow <4 L with room air were evaluated for their requirement for respiratory support, determined by an oxygen/flow reduction (room air) challenge test(22).

Corticosteroid Response Phenotyping

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

In order to measure short-term phenotypic response to systemic corticosteroids, the change in RSS was used as a phenotypic marker. In order to evaluate response to corticosteroids, we went back to original TOLSURF data and obtained Mean Airway Pressure (MAP) and FiO2 to calculate RSS at each time point. Then, RSS values for the day were averaged for a 24 hour period and recorded on day 0 (day before treatment), day 4 of treatment and day 7 of corticosteroid treatment. Mode of ventilation was not taken into account in the RSS calculation, but MAP is reliably recorded from conventional ventilators, high frequency ventilators and BiPAP/SiPAP and CPAP modes. Only one infant in the analysis cohort was weaned to HFNC and his average RSS corresponded to a MAP of 2 cm H2O. Absolute change in RSS at day 7 is the primary outcome because this is a clinically meaningful time point and allows time for genetic effects to be assessed with respect to drug response.

Author accepted manuscript

Genotyping and Imputation Methods

DNA was isolated from cells in tracheal aspirates using an AutoGeneprep 965 instrument (Autogen, Holliston, MA) as per the manufacturer's recommended protocols. When protein contamination was evident, DNA was re-precipitated using 3 volumes of 100% ethanol and 3M ammonium acetate at a 3:1 ratio after incubation at -80^{o}C overnight. DNA was quantified by Nanodrop (Thermofisher Scientific, Inc., Waltman MA) and quality was assessed using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). Genotyping was performed on the Affymetrix Axiom LAT1 array (WorldArray 4, >800,000 SNPs). SNPs were filtered based on call rates < 95%, and Hardy-Weinberg equilibrium p-values < 10^{-6} using PLINK(23). Subjects were evaluated for call rates, consistency between genetic and reported sex, autosomal heterozygosity, and cryptic relatedness/genetic identity using IBD/IBS estimates in PLINK(23). In the case of multiples, one individual was selected at random to be included in the study.

Using the complete set of ~800,000 markers, genomic levels of African and European ancestry were evaluated using ADMIXTURE(24) assuming three ancestral populations (K=3). Individuals from the HapMap CEU and YRI were genotyped on the same array and included as reference populations for European and African ancestry, respectively. Windows were offset by a factor of 0.2, the cutoff for linkage was set to 0.1,

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

and a constant recombination rate was set to 10^{-8} $(bp)^{-1}$. Genome-wide SNP genotypes were further used to impute candidate variants in the phase 3 1000 Genomes populations using the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html). Variants were then filtered for imputation quality scores > 0.3.

Candidate SNP Identification

Multiple PubMed queries using search terms "steroid, corticosteroid, pharmacogenetics, asthma, response" were performed. Of the papers that discussed pharmacogenetics of corticosteroids, we chose to only include statistically significant SNPs from papers with an objective and measurable phenotype (e.g. FEV1). Manuscripts reporting genetic associations of prenatal corticosteroid response and neonatal respiratory outcomes were included. The references of pertinent review articles were also reviewed for candidate results.

Statistical Analysis

Continuous variables are presented as means (SDs). Genetic association testing was performed at individual candidate SNPs, and at a collection of pooled SNPs within 50 Kb of candidate genes. Infants of maternal self-reported African-American (AA) and Non-Hispanic White (NHW) race/ethnicity were analyzed separately, then combined in a meta-analysis using Fisher's method. Genetic associations with respiratory phenotypic response were tested at candidate SNPs within each racial/ethnic group using linear regression in PLINK (23), adjusting for gestational age, sex, birth weight, multiple gestation, baseline RSS and African genetic ancestry (for infants of maternal AA race). Gene-based association testing was performed using VEGAS2 v.02 by combining p-values across genotyped variants within each candidate gene, and including those within 50 Kb of the transcription start/stop site. Demographic difference between ethnic groups were assessed using Fisher's exact t-tests. Associations between change in RSS at day 7 and long-term diagnosis of BPD at 36 and 40 weeks were tested with univariate binomial logistic regression. Two

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

post-hoc exploratory analyses were performed, one including infants with missing data at day 7 of treatment (those weaned to low flow nasal cannula), and one with a small for gestational age (SGA) variable instead of birthweight, as birthweight and gestational age are highly correlated. P-values less than 0.05 were considered statistically significant for these comparisons.

Results

Figure 1 depicts the selection of the final pharmacogenetic cohort. The final analysis cohort consisted of 34 AA and 36 NHW infants (Table 1), and the seven Hispanic White infants were excluded from the initial analysis due to lack of sufficient sample size and power for genetic conclusions in this ethnic sub-group. There was a wide phenotypic range in response to systemic corticosteroids (Figure 2). Because the hydrocortisone- and dexamethasone-treated groups were similar in clinical characteristics and clinical response variability, they were combined. Of all Table 1 variables tested for an association with the primary outcome, only baseline RSS (day 0 before steroid treatment) was significant, so this was adjusted for in all subsequent analyses. Day 7 change in RSS was not predictive of BPD status at 36 or 40 weeks gestational age (p-value 0.614 and 0.831 respectively), suggesting that the final diagnosis of BPD is multi-factorial and short-term response to steroids is one of many important contributors.

The list of candidate SNPs tested for an association with phenotypic response are provided in Supplemental Table S1. We examined 21 SNPs in the proximity of 10 genes for associations with the primary outcome. Analyses were run within a racial/ethnic group then combined in a meta-analysis to avoid confounding due to population structure, differences in the distribution of steroid response, and differences in environmental exposures. Because of the small number of Hispanic infants, we limited our primary analysis to the AA and NHW infants. For the primary outcome of absolute change in RSS at day 7, the entire cohort had an average change of -3.07 with standard deviation of 2.76. Genetic associations between candidate SNPs and absolute change in RSS at Day 7 are listed in Table 2.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

We identified a significant association between rs7225082 in the intron of *CRHR1* and absolute change in RSS at Day 7 following Bonferroni correction for 36 tests (meta-analysis $p=2.8x10^{-4}$). In both AA and NHW infants, the T allele at rs7225082 was associated with a smaller absolute change in RSS at day 7, i.e. lower response to systemic corticosteroids (AA: average decrease in absolute change in RSS=1.2, p=0.017; NHW: average decrease in absolute change in RSS =1.74, p=0.016; meta p-value=$2.8x10^{-4}$) (Table 2, Figure 3a,b). Rs7225082 was also significantly associated with % change at Day 7 (meta p-value=$1.8x10^{-4}$), as displayed in Figure 4. Compared to the average change in RSS of -3.07 for the entire cohort, the difference in absolute change in RSS between genetic groups of -1.2 and -1.74 are relatively large. The T allele was at a frequency of 36% in AA infants, and 64% in NHW infants, consistent with observations from African and European continental frequencies from the 1000 Genomes Project (Figure 3c). Results were similar when all infants were pooled, including the 10 Hispanic white infants (total N=87), and adjusted by genomic ancestry (Table 2).

In an exploratory analysis, we included the ten children who were originally excluded because they missed outcome data at day 7 of treatment (Figure 1). We presume that these children were all weaned to low flow NC because they had no Mean Airway Pressure recorded in the TOLSURF dataset. In order to analyze these children, we set the RSS at day 7 to 0.5 for all ten infants, and repeated the regression analysis. The direction of effect of the T allele at rs7225082 was the same (p-values: AA 0.04; NHW 0.006). Since the T allele maintained its association with less corticosteroid response in a larger cohort including ten more extremely good responders, it adds further support that rs7225082 has some influence on variability in corticosteroid response. In the second exploratory analysis, we included a binary SGA variable instead of birthweight. The direction of effect for the T allele at rs7225082 was the same (p-values: AA 0.03; NHW 0.02).

Four infants displayed continued worsening of their lung disease despite systemic corticosteroids, and all were TT homozygotes at rs7225082. When these four outliers whose lung disease continued to worsen despite treatment with corticosteroids (increase in RSS > 1) were excluded, the

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

association with rs7225082 is in the same direction, but does not reach statistical significance. This suggests that the underlying trend is the same direction within infants showing either no change in RSS, or an improvement in RSS over time. The loss of significance could be from smaller sample size or a "pushing over" effect of these four infants, but our goal in this study was to assess the complete range of clinical response to corticosteroids, and this includes those infants who continue to get worse despite therapy. None of the other candidate SNP association tests met Bonferroni corrected significance levels.

A gene based analysis was performed for two sets of candidate genes, those associated with corticosteroid response in asthma (eight genes) and those associated with perinatal corticosteroid response (three genes) (Supplemental Table S1). Within each racial/ethnic group, none of the candidate genes were significantly associated with phenotypic response to corticosteroids following Bonferroni correction for multiple comparisons (Table 3). However, genetic variation in *T* showed an association at p=0.02 in infants of maternal AA race/ethnicity, and variation in *SERPINA6* showed an association at p=0.03 in infants of maternal self-reported NHW race/ethnicity.

Discussion

To our knowledge, this is the first study to assess pharmacogenetic influences on drug response in premature infants at high risk for BPD. We capitalized on published literature to formulate a list of candidate genes and SNPs, and used an existing dataset containing both phenotypic data and genetic data for this proof of principle analysis. Our study is an important first step in bringing concepts of precision therapeutics to a very heterogeneous and difficult to treat population of preterm infants with respiratory failure. It is of paramount importance to develop predictive biomarkers of drug response in order to spare predicted "non-responders" the unwarranted risk of systemic corticosteroid therapy. This is especially important for a medication such as dexamethasone which has the potential to significantly help a sub-set of infants with developing BPD. The risk to benefit ratio of systemic steroids varies with your risk of developing BPD (25), as both "exposures" can lead to brain injury. And while a calculator

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

that estimates BPD risk (26) may be useful in determining which infants to treat, genetic markers of likely responders could strengthen patient selection for steroid treatment.

We identified SNP rs7225082 in *CRHR1* as associated with corticosteroid responsiveness and we also present gene and SNP results that do not meet Bonferroni adjustment for significance. We present all of the results because we also hope that this paper is hypothesis generating, and that these genetic variations may become significant in larger prospective cohorts. Although the effect size of SNP rs7225082 is small, the genetic variation in CRHR1 may be important because of the prevalence of this variant. All ethnic populations have a relatively large proportion of individuals carrying the T allele at rs7225082 (Figure 3c), potentially contributing to partial or non-response to systemic corticosteroid treatment.

The most significant genetic association was between rs7225082 in the intron of *CRHR1* and absolute change in RSS at day 7, whereby individuals that carried the T allele had a smaller improvement in RSS scores. This was true for infants of both maternal Black/AA and NHW race, and given our study design we indeed had the most power to identify genetic associations that are shared among the two racial/ethnic groups. Interestingly, while the variant is common in both African and European populations, the allele associated with greater improvements in RSS scores is at higher frequency in populations with African ancestry. Genotypes were imputed at this variant and passed quality thresholds (Rsq=0.89). However, another variant in *CRHR1* that was directly genotyped and in linkage disequilibrium with the imputed variant at R-squared=0.70 showed a similar trend (rs242941, $p=5.9x10^{-3}$). This suggests our results are not driven by errors in genotype imputation, and strengthens our findings that genetic variation in *CRHR1* contributes to drug response.

The difference in absolute change of RSS between the genetic groups must be interpreted within a clinical context. Absolute change in RSS is not a variable commonly used in clinical practice, so ventilator settings which correspond to this genetic difference are provided as an example of the clinical significance. If an infant starts on a MAP of 11 and FiO2 of 0.5, the baseline RSS is 5.5. An infant with the GG genotype at rs7225082 would

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

have on average a decrease in RSS of 3.1 in response to systemic steroids, corresponding to, for example, a decrease in MAP to 8 and FiO2 to 0.3, a clinically significant improvement in respiratory status. For each copy of the T allele, the change in RSS would likely be less. So a GT genotype infant might have a corresponding decrease in MAP to 10 and FiO2 to 0.4, an RSS of 4 and absolute change of 1.5, a less robust response. A TT genotype infant would respond even less. Depending on the size of the infant, this difference in steroid response may mean ability to wean from High Frequency Ventilation to conventional ventilation, or the decrease in overall toxic oxygen exposure to the developing lung. The variability in response to steroids is multi-factorial, and this SNP in *CRHR1* explains a portion of this variability.

Corticotrophin releasing hormone receptor 1 (*CRHR1*) modulates inflammation through control of ACTH-induced cortisol production. SNPs in this gene may affect baseline endogenous corticosteroid levels and baseline airway inflammation, leading to variation in response to exogenous administration of systemic corticosteroids. The importance of genetic variation in *CRHR1* to corticosteroid response has been described in Chronic Obstructive Pulmonary Disease (COPD)(27) asthma(12) and persistent pulmonary hypertension of the newborn(28). Genetic variation in the ligand for *CRHR1*, corticotropin releasing hormone (CRH), is associated with neonatal respiratory response to maternal prenatal corticosteroid therapy (10). Fetuses who carry the risk allele at rs7225082 are more likely to require CPAP/ventilator postnatally (poor response to maternal corticosteroids) and correspondingly in our study, preterm infants who carry the risk allele have a poorer response to postnatal corticosteroids. Given the importance of this gene in other patient populations, the biologic plausibility, and the same directionality of effect in two patient populations, we feel confident that genetic variation in *CRHR1* is important for corticosteroid response in premature infants.

In the gene based analysis, we identified two genes associated with absolute change in RSS at day 7 at $p<0.05$ that warrant additional investigation – *SERPINA6* that was previously implicated in perinatal corticosteroid response, and the *T* gene that was previously implicated in corticosteroid response in asthma. *SERPINA6* encodes an alpha-globulin protein with corticosteroid-binding properties. This is the major transport

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

protein for endogenous glucocorticoids and progestins in the blood of most vertebrates. *SERPINA6* transcript number and protein function could regulate systemic bioavailability of endogenous glucocorticoids, as the protein sequesters up to 80% of circulating cortisol in an inactive complex (29). Increased levels of SERPINA6, genetically determined, could reduce unbound and biologically active levels of cortisol and influence tissue response to treatment. It is biologically plausible that alterations in SERPINA6 expression could modulate response to corticosteroid therapy in BPD.

The T gene encodes a mesodermal developmental transcription factor and contributes to regulation in lung development. Alterations in the T gene could alter the temporal pattern of lung development, and thus affect lung maturity and responsiveness to corticosteroids. The T gene is expressed in adult lung(30), but the abundance in neonatal lung is not known. The importance of the T gene in corticosteroid response among patients with asthma was first reported in a large GWAS study in which the significant two SNPs were found in a transcription factor located 50 kb downstream of the gene(11). The SNP rs6456042 (intronic) is in tight linkage disequilibrium with three other SNPs with known functional implications: rs3099266 (promoter region), rs1134481 (3' UTR) and rs2305089 (nonsynomous within coding region). Thus, although the top SNP in AA infants in our study is not in a coding region, it is likely a marker of carriage of other tightly linked functionally significant genetic changes.

Use of respiratory severity score (RSS) as the phenotypic biomarker provided an objective measure of pulmonary dysfunction at baseline and served as an index of improvement. The RSS allows latitude among individual treating physician philosophy in choosing the perceived least harmful combination of oxygen and positive airway pressure to achieve the patient's ventilation goals. The RSS has been validated as a close correlate of oxygenation index(31). In addition, RSS is used extensively in studies of preterm infants beyond the first week of life when paO2 is unavailable (32-35). RSS at day of life thirty is predictive of clinically important outcomes of preterm infants needing protracted ventilation (36). While the change in RSS may be exaggerated when an infant weans from High Frequency Ventilation to conventional ventilation because often this includes a drop in the mean airway pressure, we believe that this improved RSS represents a change in lung pathology which is still valid to measure using this

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

phenotypic marker. Our cohort did not have sufficient size to analyze genetic associations among infants only treated with one modality of invasive ventilation.

Many placebo-controlled studies have documented a short term pulmonary response to corticosteroids using endpoints such as the respiratory acuity score (37) peak inspiratory pressure (PIP) and oxygen need (38-40). More recently, McEvoy et al (41) compared short term effects of lower dose dexamethasone and demonstrated improved lung mechanics at 3 and 5 days. Our study adds to the published literature by assessing contributors to variability in clinical response to steroids, moving the field from a population-based approach towards a more personalized medicine approach.

Our study has some weaknesses including small sample size, and no PCR validation of the significant SNP in *CRHR1*. These are common weaknesses among secondary analysis studies which use data collected for alternative scientific purposes. We believe that the question of steroid pharmacogenetics in BPD is important enough that working within these initial limitations is worthwhile. Additionally, we lack a validation cohort for the pharmacogenetic finding. Because of the challenges in making and reproducing gene-disease (or gene-drug response) conclusions in complex disease based on small sample sizes(42), the research team is collaborating in a multi-site consortium and prospectively enrolling preterm infants at risk for BPD who are being treated clinically with systemic corticosteroids in order to recruit a larger patient cohort and further study steroid pharmacogenetics.

Overall our results implicate genetic variation in *CRHR1* in modifying the acute respiratory response to systemic corticosteroids in preterm infants treated for prevention of BPD. Although the identified significant SNP is likely not directly causal, these results add to the body of knowledge that endogenous steroid homeostasis may contribute to variability in response to treatment with exogenous corticosteroids. Additional studies are required to validate these associations and to identify the mechanistic effects of SNPs in *CRHR1* on corticosteroid response.

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

Author accepted manuscript

References

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

1. Stoll BJ, Hansen NI, Bell EF, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. JAMA 314:1039-1051 **(2015)**.
2. Doyle LW, Cheong JL, Ehrenkranz RA, Halliday HL Late (> 7 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants. Cochrane Database Syst Rev 10:CD001145 **(2017)**.
3. Halliday HL, Ehrenkranz RA, Doyle LW Moderately early (7-14 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. Cochrane Database Syst Rev:CD001144 **(2003)**.
4. Yoder BA, Harrison M, Clark RH Time-related changes in steroid use and bronchopulmonary dysplasia in preterm infants. Pediatrics 124:673-679 **(2009)**.
5. Shinwell ES, Karplus M, Reich D, et al. Early postnatal dexamethasone treatment and increased incidence of cerebral palsy. Arch Dis Child Fetal Neonatal Ed 83:F177-181 **(2000)**.
6. Zhang R, Bo T, Shen L, Luo S, Li J Effect of dexamethasone on intelligence and hearing in preterm infants: a meta-analysis. Neural Regen Res 9:637-645 **(2014)**.
7. Powell K, Kerkering KW, Barker G, Rozycki HJ Dexamethasone dosing, mechanical ventilation and the risk of cerebral palsy. J Matern Fetal Neonatal Med 19:43-48 **(2006)**.
8. Bhandari V, Bizzarro MJ, Shetty A, et al. Familial and genetic susceptibility to major neonatal morbidities in preterm twins. Pediatrics 117:1901-1906 **(2006)**.
9. Lavoie PM, Pham C, Jang KL Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. Pediatrics 122:479-485 **(2008)**.
10. Borowski KS, Clark EA, Lai Y, et al. Neonatal Genetic Variation in Steroid Metabolism and Key Respiratory Function Genes and Perinatal Outcomes in Single and Multiple Courses of Corticosteroids. Am J Perinatol 32:1126-1132 **(2015)**.
11. Tantisira KG, Damask A, Szefler SJ, et al. Genome-wide association identifies the T gene as a novel asthma pharmacogenetic locus. Am J Respir Crit Care Med 185:1286-1291 **(2012)**.
12. Tantisira KG, Lake S, Silverman ES, et al. Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. Hum Mol Genet 13:1353-1359 **(2004)**.
13. Stockmann C, Fassl B, Gaedigk R, et al. Fluticasone propionate pharmacogenetics: CYP3A4*22 polymorphism and pediatric asthma control. J Pediatr 162:1222-1227, 1227 e1221-1222 **(2013)**.
14. Park TJ, Park JS, Cheong HS, et al. Genome-wide association study identifies ALLC polymorphisms correlated with FEV(1) change by corticosteroid. Clin Chim Acta 436:20-26 **(2014)**.
15. Tantisira KG, Lasky-Su J, Harada M, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med 365:1173-1183 **(2011)**.
16. Hawkins GA, Lazarus R, Smith RS, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. J Allergy Clin Immunol 123:1376-1383 e1377 **(2009)**.
17. Dijkstra A, Postma DS, Bruinenberg M, et al. SERPINE1 -675 4G/5G polymorphism is associated with asthma severity and inhaled corticosteroid response. Eur Respir J 38:1036-1043 **(2011)**.
18. Ballard RA, Keller RL, Black DM, et al. Randomized Trial of Late Surfactant Treatment in Ventilated Preterm Infants Receiving Inhaled Nitric Oxide. J Pediatr 168:23-29 e24 **(2016)**.
19. Keller RL, Eichenwald EC, Hibbs AM, et al. The Randomized, Controlled Trial of Late Surfactant: Effects on Respiratory Outcomes at 1-Year Corrected Age. J Pediatr 183:19-25 e12 **(2017)**.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

20. Doyle LW, Davis PG, Morley CJ, McPhee A, Carlin JB, Investigators DS Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. Pediatrics 117:75-83 **(2006)**.
21. Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bron chopulmonary dysplasia. Pediatrics 116:1353-1360 **(2005)**.
22. Walsh MC, Wilson-Costello D, Zadell A, Newman N, Fanaroff A Safety, Reliability, and Validity of a Physiologic Definition of Bronchopulmonary Dysplasia. Journal Of Perinatology 23:451 **(2003)**.
23. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-575 **(2007)**.
24. Alexander DH, Novembre J, Lange K Fast model-based estimation of ancestry in unrelated individuals. Genome Res 19:1655-1664 **(2009)**.
25. Doyle LW, Halliday HL, Ehrenkranz RA, Davis PG, Sinclair JC An update on the impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk of bronchopulmonary dysplasia. J Pediatr 165:1258-1260 **(2014)**.
26. Laughon MM, Langer JC, Bose CL, et al. Prediction of bronchopulmonary dysplasia by postnatal age in extremely premature infants. Am J Respir Crit Care Med 183:1715-1722 **(2011)**.
27. Kim WJ, Sheen SS, Kim TH, et al. Association between CRHR1 polymorphism and improved lung function in response to inhaled corticosteroid in p atients with COPD. Respirology 14:260-263 **(2009)**.
28. Byers HM, Dagle JM, Klein JM, et al. Variations in CRHR1 are associated with persistent pulmonary hypertension of the newborn. Pediatr Res 71:162-167 **(2012)**.
29. Moisan MP, Minni AM, Dominguez G, et al. Role of corticosteroid binding globulin in the fast actions of glucocorticoids on th e brain. Steroids 81:109-115 **(2014)**.
30. Park JC, Chae YK, Son CH, et al. Epigenetic silencing of human T (brachyury homologue) gene in non-small-cell lung cancer. Biochem Biophys Res Commun 365:221-226 **(2008)**.
31. Iyer NP, Mhanna MJ Non-invasively derived respiratory severity score and oxygenation index in ventilated newborn infants. Pediatr Pulmonol 48:364-369 **(2013)**.
32. Mercier JC, Hummler H, Durrmeyer X, et al. Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature bab ies (EUNO): a randomised controlled trial. Lancet 376:346-354 **(2010)**.
33. Laughon M, Bose C, Moya F, et al. A pilot randomized, controlled trial of later treatment with a peptide-containing, synthetic surfactant for the prevention of bronchopulmonary dysplasia. Pediatrics 123:89-96 **(2009)**.
34. Ballard RA, Truog WE, Cnaan A, et al. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. N Engl J Med 355:343-353 **(2006)**.
35. Katz LA, Klein JM Repeat surfactant therapy for postsurfactant slump. J Perinatol 26:414-422 **(2006)**.
36. Malkar MB, Gardner WP, Mandy GT, et al. Respiratory severity score on day of life 30 is predictive of mortality and the length of mechanical ven tilation in premature infants with protracted ventilation. Pediatr Pulmonol 50:363-369 **(2015)**.
37. Scott SM, Backstrom C, Bessman S Effect of five days of dexamethasone therapy on ventilator dependence and adrenocorticotropic hormone-stimulated cortisol concentrations. J Perinatol 17:24-28 **(1997)**.
38. Yoder MC, Jr., Chua R, Tepper R Effect of dexamethasone on pulmonary inflammation and pulmonary function of ventilator-dependent infants with bronchopulmonary dysplasia. Am Rev Respir Dis 143:1044-1048 **(1991)**.
39. Durand M, Sardesai S, McEvoy C Effects of early dexamethasone therapy on pulmonary mechanics and chronic lung disease in very low birth weight infants: a randomized, controlled trial. Pediatrics 95:584-590 **(1995)**.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

40. Kari MA, Heinonen K, Ikonen RS, Koivisto M, Raivio KO Dexamethasone treatment in preterm infants at risk for bronchopulmonary dysplasia. Arch Dis Child 68:566-569 **(1993)**.
41. McEvoy C, Bowling S, Williamson K, McGaw P, Durand M Randomized, double-blinded trial of low-dose dexamethasone: II. Functional residual capacity and pulmonary outcome in very low birth weight infants at risk for bronchopulmonary dysplasia. Pediatr Pulmonol 38:55-63 **(2004)**.
42. Ioannidis JP, Trikalinos TA, Khoury MJ Implications of small effect sizes of individual genetic variants on the design and in terpretation of genetic association studies of complex diseases. Am J Epidemiol 164:609-614 **(2006)**.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Author accepted manuscript

Figure Legends

Figure 1: Pharmacogenetic Analysis Cohort Identification

Figure 2: Distribution of short-term phenotypic steroid response at Day 7. Each bar represents an infant treated with systemic dexamethasone (black bars) or hydrocortisone (grey bars). Negative values indicate a decrease in the respiratory severity score (a more positive response to steroids). (a) absolute change in RSS at day 7 of treatment. (b) percent change in RSS at day 7.

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Figure 3: Change in Respiratory Severity Score. Each line represents the change in RSS between the day before treatment and 7 days of treatment. (a) African American (b) Non-Hispanic White. (c) Frequency of alleles in continental populations from the 1000 Genomes Project.

Figure 4: Boxplot of % change in respiratory severity score at day 7, stratified by genotype at rs7225082 (Number of individuals: 19 GG, 38 GT, and 18 GT).

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Table 1 – Clinical characteristics of TOLSURF participants included in genetic studies of corticosteroid response by maternal self-reported race/ethnicity

	African-American (N=34)	Non-Hispanic White (n=36)
Demographics		
Gestational age (weeks)	24.7 ± 1.0	25.6 ± 1.3
Birth weight (g)	667 ± 119	742 ± 169
Birth weight centile	42 ± 25	43 ± 31
Antenatal corticosteroid exposure	30 (78.9)	34 (87.2)
Cesarean delivery	23 (60.5)	29 (74.4)
Male sex	19 (50.0)	27 (69.2)
Product of multiple gestation	8 (21.1)	10 (25.6)
RSS and STEROID Variables		
Postnatal age at start of steroid treatment (days)	29.2 ± 15.8	22.8 ± 9.09
Dexamethasone (N, %)	18 (47)	13 (33)
RSS at TOLSURF Study Entry	4.54 ± 2.52	4.66 ± 2.36
RSS at Day 0	6.85 ± 2.51	7.08 ± 3.12
RSS at Day 4	4.45 ± 2.74	4.97 ± 2.73
RSS at Day 7	3.69 ± 1.62	4.14 ± 2.80
BPD Diagnosis at 36 weeks* N (%)	26 (76.5)	32 (82.1)
BPD Diagnosis at 40 weeks* N (%)	16 (47.1)	23 (58.9)

RSS: Respiratory Severity Score, BPD: Bronchopulmonary Dysplasia

* Among living infants, 4 of 38 AAs died before 36 weeks with no further deaths before 40 weeks. There were no deaths among the 39 NHW infants.

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Table 2 –Candidate SNP Association Testing for absolute change in Respiratory Severity Score (RSS) at Day 7 Linear regression was used to test for an association between copies of the A1 allele and respiratory outcome. Imputed genetic variants have a corresponding quality score (Rsq), otherwise "NA" if the variant was genotyped directly.

			African-Americans (N=34)				Non-Hispanic Whites (N=36)					All, n=87	
Gene	SNP	A1/A2	FreqA1	Difference in absolute change in RSS with 1 variant allele	SE	P	FreqA1	Difference in absolute change in RSS with 1 variant allele	SE	P	Meta-P	Merged-P	Rsq
Perinatal Steroids and Respiratory Outcomes – Candidate Genes and SNPs													
CRH	rs4613981	A/G	0.27	0.15	0.56	0.79	0.85	1.01	0.83	0.23	0.18	0.091	0.98
CRH	rs2446432	C/A	0.33	0.55	0.45	0.23	0.53	0.97	0.80	0.23	0.055	0.027	NA
SERPINA6	rs3748320	A/G	0.06	0.26	1.01	0.80	0.28	1.40	0.74	0.068	0.054	0.041	0.99
CRHR1	**rs7225082**	**T/G**	**0.36**	**1.19**	**0.46**	**0.017**	**0.64**	**1.74**	**0.68**	**0.016**	***2.8x10^{-4}***	**1.5x10^{-3}**	**0.89**
CRHR1	rs173365	G/A	0.33	0.15	0.46	0.74	0.50	0.48	0.54	0.38	0.28	0.32	0.95
Steroid Treatment of Asthma – Candidate Genes and SNPs													
ALLC	rs11123610	A/G	0.53	0.54	0.42	0.21	0.63	0.35	0.58	0.55	0.12	0.19	0.88
T	rs6456042	A/C	0.44	0.32	0.39	0.42	0.46	0.28	0.63	0.66	0.27	0.35	0.97
T	rs3127412	C/T	0.44	0.32	0.39	0.42	0.46	0.28	0.63	0.66	0.27	0.35	0.98
GLCCI1	rs37972	T/C	0.17	0.52	0.58	0.38	0.38	-0.06	0.59	0.92	0.35	0.60	0.91
CYP3A4	rs35599367	A/G	0.02	0.30	1.97	0.88	0.03	-0.12	2.00	0.95	0.84	0.93	0.84
STIP1	rs4980524	C/A	0.31	-0.50	0.60	0.41	0.43	0.12	0.60	0.84	0.34	0.91	0.99
STIP1	rs6591838	G/A	0.22	-0.65	0.68	0.35	0.25	1.15	0.62	0.08	0.026	0.24	0.98
STIP1	rs2236647	C/T	0.38	0.41	0.47	0.39	0.56	-0.07	0.58	0.91	0.36	0.92	0.98
CRHR1	rs242941	C/A	0.30	0.73	0.47	0.14	0.63	1.24	0.59	0.04	5.9x10^{-3}	0.010	NA
CRHR1	rs1876828	T/C	0.08	0.50	0.90	0.58	0.24	0.34	0.69	0.62	0.36	0.48	0.99
TBX21	rs4794067	C/T	0.17	0.92	0.53	0.09	0.32	0.67	0.70	0.35	0.033	0.068	NA
TBX21	rs11650451	A/G	0.05	2.81	0.92	5.5x10^{-3}	0.17	0.54	0.85	0.53	2.9x10^{-3}	0.16	NA
TBX21	rs2240017	G/C	0.00	NA	NA	NA	0.01	-3.08	2.69	0.26	NA	0.14	0.57
TBX21	rs1134481	T/G	0.27	-0.12	0.62	0.85	0.47	0.22	0.64	0.74	0.63	0.62	0.96
TBX21	rs2305089	T/C	0.27	0.53	0.49	0.29	0.43	0.51	0.66	0.45	0.13	0.42	0.94
TBX21	rs3099266	T/C	0.23	0.37	0.53	0.50	0.47	0.11	0.70	0.88	0.43	0.46	0.95

FreqA1 = frequency of the A1 allele; SE = standard error; P=p-value; Rsq = imputation quality score

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

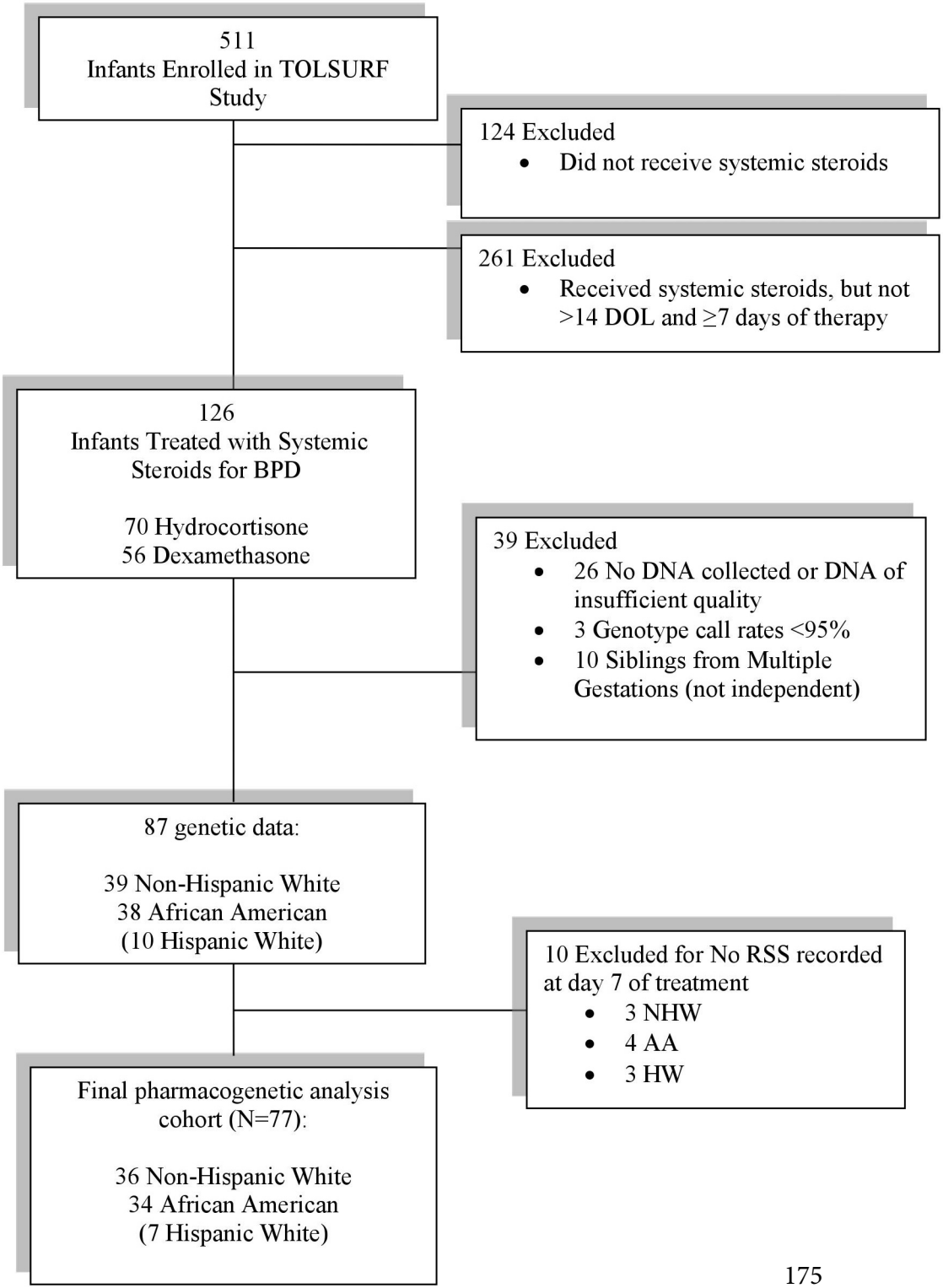
AUTHOR ACCEPTED MANUSCRIPT

Table 3 –Gene-Based Association Testing for absolute change in Respiratory Severity Score (RSS) at Day 7. P-values were combined across all genotyped SNPs within 50 kb of candidate genes using VEGAS2. No individual gene surpassed Bonferroni adjustment for 18 comparisons (9 genes x 2 populations, 0.05/18=0.0028), and no individual SNP within a gene surpassed Bonferroni adjustment for the total number of SNPs within each gene (N SNPs).

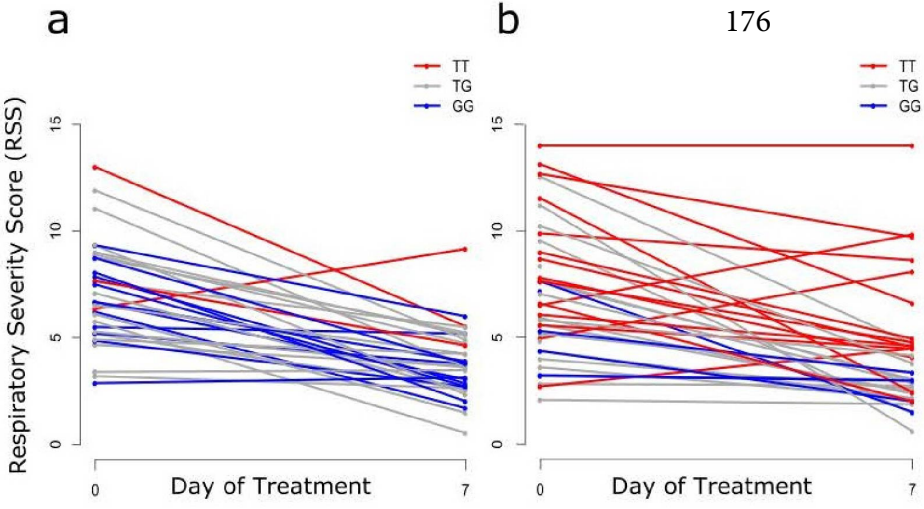
	African-Americans (N=34)					Non-Hispanic Whites (N=36)			
Gene	N SNPs	Test statistic	p-value	Top SNP	Top SNP p-value	N SNPs	Test statistic	p-value	Top SNP
Perinatal Steroids and Respiratory Outcomes									
CRH	33	30.8	0.457	rs7841890	0.028	28	36.65	0.21	rs7835948
CRHR1	15	13.3	0.495	rs114764500	0.067	10	10.69	0.36	rs242941
SERPINA6	56	46.5	0.616	rs56170999	0.002	44	87.70	0.03	rs3827896
Steroid Treatment of Asthma – Candidate Genes									
ALLC	47	46.4	0.435	rs1367275	0.003	53	74.48	0.13	rs1367276
CYP3A4	28	34.0	0.297	rs76994069	0.025	8	2.85	0.77	rs78676479
GLCCI1	58	69.8	0.220	rs17143586	0.002	76	45.47	0.95	rs10268598
SERPINE1	46	52.5	0.288	rs73712100	0.032	35	38.12	0.34	rs10953321
STIP1	16	10.7	0.699	rs2701527	0.113	14	16.47	0.30	rs118154228
T	31	63.5	0.020	rs6456056	0.009	35	30.80	0.53	rs10428822

Author accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.



© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.



© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

Recent advances in antenatal factors predisposing to bronchopulmonary dysplasia

Elizabeth Taglauer[a,1], *Steven H. Abman*[b,2], *and Roberta L. Keller*[c,*,3]

[a]*Harvard Neonatal-Perinatal Fellowship Program, Boston, MA USA*
[b]*Pediatric Heart Lung Center, Department of Pediatrics, University of Colorado Anschutz School of Medicine, Aurora, CO USA*
[c]*Division of Neonatology, Department of Pediatrics, University of California San Francisco, San Francisco, CA USA*

ARTICLE INFO

Keywords:
Alveolarization
Angiogenesis
Placenta
Vasculogenesis
Vascular endothelial growth factor (VEGF)
Respiratory morbidity

ABSTRACT

Bronchopulmonary dysplasia (BPD) remains a major cause of late morbidities and death after preterm birth. BPD is characterized by an arrest of vascular and alveolar growth and high risk for pulmonary hypertension; yet mechanisms contributing to its pathogenesis and early strategies to prevent BPD are poorly understood. Strong epidemiologic studies have shown that the "new BPD" reflects the long-lasting impact of antenatal factors on lung development, partly due to placental dysfunction, as reflected in recent data from animal models. Improved understanding of mechanisms through which antenatal stress alters placental function and contributes to BPD may lead to preventive therapies.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Despite striking improvements in perinatal care and diverse neonatal outcomes related to premature birth, preterm children remain at high risk for significant respiratory morbidities and mortality due to the development of bronchopulmonary dysplasia (BPD),[1] Fig. 1. BPD is the chronic lung disease of prematurity that develops in infants who require respiratory support at birth due to immaturity of the preterm lung.[2] BPD is the most common sequel of prematurity, occurring in roughly 45% of infants born at or less than 29 weeks gestation with birth weights, with approximately 10–15,000 new cases of BPD in the USA alone each year.[1,3,4] Importantly, the incidence of BPD has not changed over the past few decades, likely reflecting improved survival of extremely low gestational age newborns (ELGANS) who are at the highest risk for developing moderate and severe BPD.[5] BPD is also associated with significant neonatal intensive care unit (NICU)-related complications, including the prolonged need for mechanical ventilation, respiratory support and oxygen therapy, longer duration of hospitalization and higher rates of non-respiratory co-morbidities, such as retinopathy of prematurity (ROP) and brain injury.[5,6] Pathogenetic mechanisms

* *Corresponding author at:*
E-mail address: Roberta.Keller@ucsf.edu (R.L. Keller).
[1] Elizabeth Taglauer, Clinical Fellow in Pediatrics, Joint Program in Neonatology, Boston Children's Hospital, 300 Longwood Avenue, Boston MA 02115.
[2] Steven H. Abman, Department of Pediatrics, University of Colorado Denver Anschutz Medical Campus and Children's Hospital Colorado, 13123 E 16th Ave B395, Aurora, CO 80045.
[3] Roberta L. Keller, University of California San Francisco and UCSF Benioff Children's Hospital San Francisco Professor, Department of Pediatrics, 550 16th Street, San Francisco CA 94158, United States.

https://doi.org/10.1053/j.semperi.2018.09.002
0146-0005/© 2018 Elsevier Inc. All rights reserved.

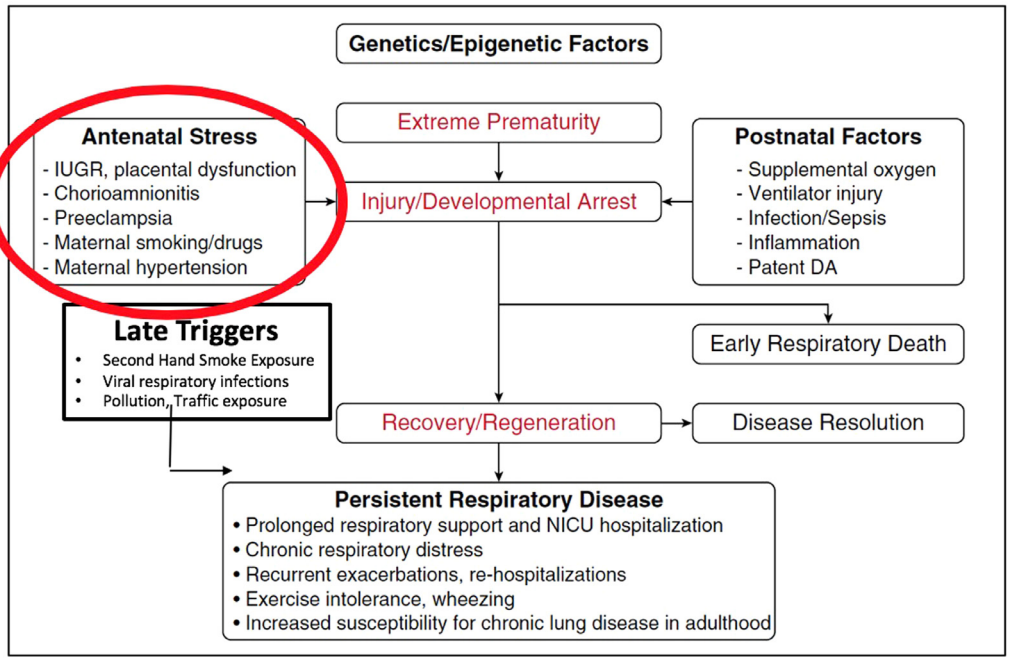


Fig. 1 – Pathogenetic mechanisms underlying the etiology of BPD and persistent late respiratory disease in childhood after preterm birth. In addition to extreme prematurity and postnatal factors that induce lung injury, experimental and clinical data have strongly linked antenatal stress as key determinants of chronic lung disease. (Modified from Abman SH, Bancalari E, Jobe AH. Am J Respir Crit Care Med, 2017).

linking lung, brain, retinal, renal and other systemic complications of preterm birth remain under intense investigation, but these diseases potentially share common origins related to the effects of adverse antenatal factors, as discussed below.

After NICU discharge, infants with BPD often require frequent hospital re-admissions and have high rates of emergency room or physician visits due to recurrent respiratory exacerbations, lower respiratory tract infections, reactive airways disease and pulmonary hypertension. Sustained abnormalities of lung function, poor exercise tolerance and the need for chronic respiratory medications throughout childhood and adolescence are also increased in former preterm infants.[7,8] Past studies have shown that over 50% of preterm infants subsequently require re-hospitalizations or chronic respiratory medications after NICU discharge, including preterm infants without a formal diagnosis of BPD.[8] Controversies persist regarding how to best define BPD and whether bearing this diagnosis at 36 weeks post-menstrual age (PMA) adequately reflects the late risk for lung disease during childhood and into adult life.[9–11]

Although postnatal factors, such as hyperoxia, mechanical ventilation, prolonged patency of the ductus arteriosus, sepsis, inflammation and others, increase the risk for BPD, epidemiologic studies have further identified important roles for antenatal factors as well.[12–19] Adverse antenatal factors, such as chorioamnionitis, preeclampsia (PE), pre-existing hypertensive disorders, gestational diabetes, maternal obesity, and others have been strongly associated with an increased risk for BPD.[18–20]

An NHLBI-sponsored workshop discussed the importance of prenatal and early postnatal influences on lung growth and development on subsequent respiratory function and disease throughout childhood.[11] This workshop further highlighted major gaps in our understanding of how environmental and maternal factors can impact late respiratory outcomes during early childhood, and that the exact relationships between prenatal exposures and early postnatal events on the subsequent development of late respiratory disease during infancy, especially after preterm birth, remain uncertain. As studies have shown that preterm birth alone is associated with late respiratory disease in childhood, links between the diagnosis of BPD at 36 weeks corrected age and persistent chronic lung disease during infancy and beyond remains unclear. This issue of disease definition and identifying critical respiratory outcomes has become especially important in order to better understand the diverse physiology phenotypes of BPD, to enhance clinical research regarding disease pathogenesis, and to define study endpoints to enhance interventional clinical trials.[21]

Importantly, recent prospective cohort studies of very preterm infants have identified antenatal and early neonatal characteristics as strong predictors of both BPD and late respiratory disease during infancy and early childhood,[22–27] suggesting that the fetal environment has a critical influence on the development of persistent lung disease in former preterm newborns. This review provides a brief overview on clinical, epidemiologic and laboratory-based data that support various mechanisms of perturbed lung and vascular development related to this environment, resulting in a neonatal lung with arrested development, altered vulnerability to postnatal injury, and an inadequate capacity for repair and regeneration.

Antenatal factors and BPD susceptibility: epidemiologic studies

Past studies have identified several characteristics that are associated with a higher incidence of BPD, including lower GA, male gender and white race. Postnatal events have long been associated with an increased risk for BPD and poor outcomes, including the severity of acute respiratory distress syndrome (RDS) at birth, prolonged exposure to high inspired oxygen tensions and mechanical ventilator support, lung inflammation, air leaks or pulmonary interstitial emphysema, pulmonary hypertension, systemic and pulmonary infections, prolonged exposure to a patent ductus arteriosus, and either specific or global nutritional deficits.

Although the pathogenesis of BPD and its severity is impacted by the adverse effects of these postnatal exposures, strong experimental and clinical data show that antenatal events are key contributors to BPD risk.[9,11] In fact, prenatal insults may be sufficient to cause sustained disruption of lung development, leading to abnormal lung structure in the absence of additional postnatal stress. Alternatively, interactions between antenatal stress may alter susceptibility to critical postnatal factors, thereby impacting risk for BPD.

One of the strongest clinical markers reflecting the high importance of fetal events in the pathobiology of BPD is demonstrated by studies of the preterm infant with intrauterine growth restriction (IUGR).[22–25] An observational study from the UK demonstrated a roughly 2-fold increase in early (28 days) and late mortality (36 weeks PMA) and the risk for BPD in small for GA (SGA) infants born at or below 32 weeks gestation. Perhaps even more important than the presence or absence of BPD at 36 weeks, preterm infants who were born with IUGR remain at high risk for late respiratory morbidities and lung function at school age.[22–25] When studied at a mean age of 11 years, children who were born prematurely with IUGR had lower lung function, including forced expiratory volume in one second (FEV_1) and diffusion capacity for carbon monoxide (DLCO), suggesting abnormal airway function and decreased lung surface area, when compared with non-IUGR prematurely-born children. Cardio-respiratory abnormalities persisted despite recovery of somatic growth and the impact of IUGR was independent of BPD or prematurity alone.[24]

The outcome of BPD at 36 weeks' PMA has been evaluated in several different cohorts in relationship to fetal growth in very preterm infants (as assessed by birth weight standardized for GA and analyzed by continuous or categorical measures of fetal growth restriction). The ELGAN investigators evaluated maternal, neonatal and placental characteristics, and delivery indication, and showed that decreased fetal growth independently increased the odds of BPD.[22] While lower GA remained an important predictor of BPD, preeclampsia lost its significance in this analysis, although "fetal indications" for delivery remained an independent predictor. After classifying preterm infants into similar categories by delivery indication (preterm labor versus vascular disease), Durrmeyer and colleagues evaluated associations between neonatal characteristics and severity of placental inflammation and BPD.[28] While lower GA was the only factor significantly associated with BPD in multivariate analysis among those infants in the preterm labor group, severe growth restriction was the only factor that remained significant among infants in the vascular disease group. Two other cohorts that analyzed the association of antenatal and birth characteristics and BPD found that lower GA was an important predictor of BPD, but decreasing birth weight z-score, a history of pre-eclampsia and clinical chorioamnionitis were variably associated with increased odds of BPD.[18,27]

In a separate cohort of extremely low GA newborns <29 weeks' GA at birth, Keller and colleagues demonstrated that IUGR (birth weight $<10^{th}$ percentile) was associated with increased odds of persistent respiratory morbidity assessed at 1-year corrected age.[26] Interestingly, each of these studies used different fetal reference growth curves to standardize fetal growth, strengthening the epidemiological relationship between impaired growth due to an adverse fetal environment and poor respiratory outcomes, including BPD.

Two recent prospective multi-center cohort studies further examined the role of antenatal determinants and perinatal factors on the risk for not only developing BPD as defined at 36 weeks PMA but also examined factors associated with the development of late respiratory disease during early childhood.[26,27] Importantly, these studies each noted that perinatal factors identified on the first day of life were strongly associated with BPD risk, especially maternal smoking, SGA status, degree of prematurity and others (Table 1; Fig. 2). In addition, data modeling revealed that these antenatal determinants were at least as strong as the presence of BPD at 36 weeks in predicting late respiratory disease during infancy,[26,27] and that the addition of BPD status to these models did not further strengthen this association.

Abnormal placental structure and function and the risk of BPD

Observations linking antenatal stress with poor respiratory outcomes in preterm infants implicate the critical role of altered placental structure and function in BPD pathobiology. Placental structural abnormalities related to IUGR and PE are strongly associated with fetal growth restriction and examination of placental tissue for vascular lesions after preterm birth may provide an additional approach to predict of the subsequent risk for BPD.[29] Preclinical data from experimental models of severe IUGR that markedly impact placental function and structure can decrease fetal lung airspace and vascular growth in the late gestation fetus,[30] which may persist throughout postnatal life. Recent experimental studies have demonstrated an impaired angiogenic and growth properties of endothelial cells derived from human IUGR placentas, which may relate to decreased aryl hydrocarbon receptor nuclear translocator (ARNT) expression.[31]

Additional studies have strongly linked histologic placental abnormalities that are consistent with patterns of "maternal under-perfusion" with preterm infants with IUGR and high risk for the development of BPD and BPD with pulmonary

Table 1 – Perinatal factors at Day 1 of life and univariate associations with post-prematurity respiratory disease (from Keller RL *et al*, J Pediatr 2017[26]).

Characteristic	Whole cohort $n = 724$	PRD $n = 497$	No PRD $n = 227$	*P* value
Gestational age (weeks)	26.7 ±1.4	26.6 ±1.4	26.9 ±1.3	0.009
Birth weight (g)	918 ± 234	899 ± 232	960 ± 233	0.003
Intrauterine growth restriction	36 (5%)	32 (6%)	4 (2%)	0.02
Multiple gestation	187 (26%)	111 (22%)	76 (3%)	0.006
Smoking during pregnancy	139/723 (19%)	111/496 (22%)	28/227 (12%)	0.004
Stabilization at birth: intubation	564 (78%)	405 (82%)	159 (70%)	0.003
Maternal education				0.007
≤ High school	389 (54%)	283 (57%)	106 (47%)	
Some college	143 (20%)	100 (20%)	43 (19%)	
≥ College graduate	192 (27%)	114 (23%)	78 (34%)	
Public insurance	470/721 (65%)	348/494 (70%)	122/227 (54%)	<0.001
Parent with asthma	173/712 (24%)	129/485 (27%)	44/227 (19%)	0.04

*by trend test.
PRD, post-prematurity respiratory disease.

hypertension[32,33] (Table 2). Cord blood biomarkers, including decreased vascular endothelial growth factor (VEGF) and soluble VEGF antagonist fms-like tyrosine kinase-1 (sFLT-1) levels, were strongly associated with IUGR and predictive of BPD in preterm infants,[34] suggesting that these circulating proteins may serve as effective biomarkers for predicting later neonatal morbidities associated with IUGR, including BPD. In addition, hypertensive disorders of pregnancy, especially PE, have been strongly associated with high risk for BPD after preterm birth.[18,27] In a cohort study of 107 infants born <32 weeks, the risk for BPD was dramatically increased in the presence of PE (OR, 18.7; 95% CI, 2.44–145; p <0.005), even after accounting for IUGR.[18]

The placenta and neonatal disease

The placenta is a major contributor to changes in the intrauterine environment that can lead to fetal and neonatal pathologies. The placenta may also serve as a direct source of early signaling for fetal disease in pregnancy, a concept that has been proposed with placental-derived signals circulating in the maternal serum such as cell free fetal DNA[35] and placental-shed extracellular vesicles.[36] This may be particularly true for the developing fetal lungs, which share some key aspects of structure and function with the placenta (Fig. 3). Insights into placental

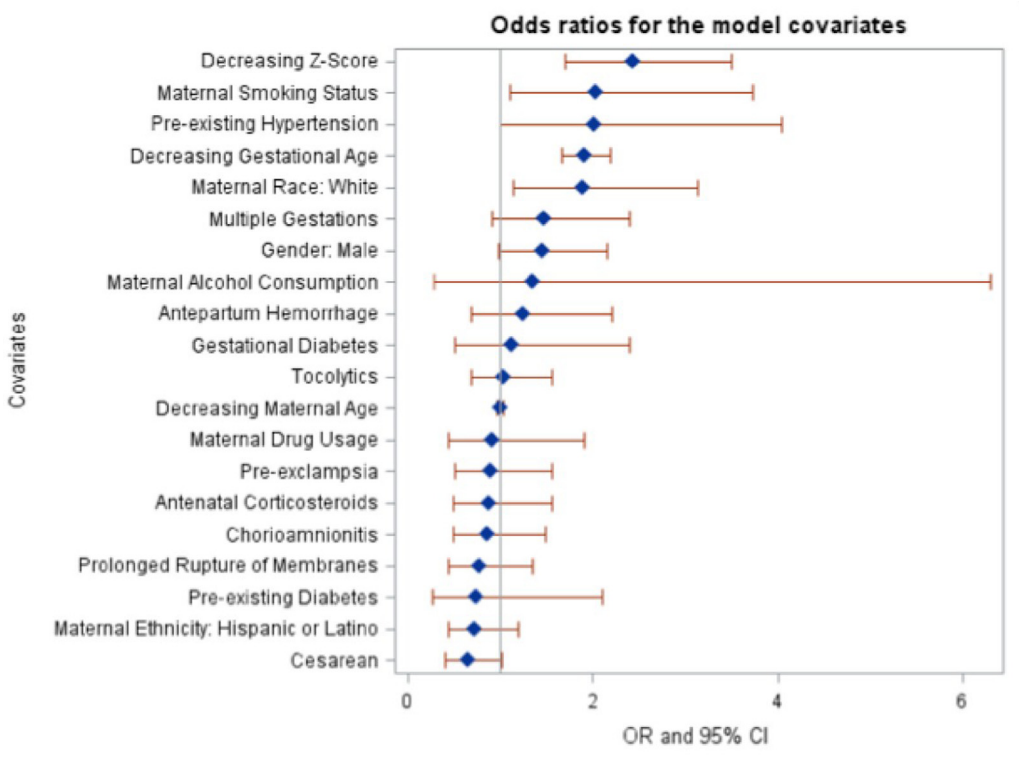


Fig. 2 – Antenatal factors are strongly associated with the risk of developing BPD. (from Morrow L et al. Am J Respir Crit Care Med, 2016).

Table 2 – Abnormal placental histopathology and high risk for bronchopulmonary dysplasia and pulmonary hypertension in preterm infants (from Mestan *et al*, Placenta 2014[29]).

Placental characteristic	No BPD or PH $n = 165$	BPD only $n = 84$	BPD-associated PH $n = 34$	P value
Maternal vascular underperfusion (any)	56 (34%)	43 (51%)*	22 (65%)*	0.001
Severe MVU	6 (4%)	9 (11%)	6 (18%)	0.01
Vessel changes				
FN/AA	6 (4%)	8 (10%)	8 (24%)**	0.001
MBPA	16 (10%)	12 (14%)	10 (29%)*	0.01
MHMA	10 (6%)	9 (11%)	6 (18%)	0.07
Villous changes				
Infarcts	10 (6%)	14 (17%)	4 (12%)	0.02
Increased syncytial knots	53 (32%)	39 (46%)	20 (59%)*	0.01
Villous agglutination	4 (2%)	4 (5%)	2 (6%)	0.36
Increased perivillous fibrin	7 (4%)	2 (2%)	2 (6%)	0.67
DVH/STV	36 (22%)	36 (43%)*	18 (53%)**	< 0.001

PH determined by echocardiogram at 36 weeks' post-menstrual age.
BPD, bronchopulmonary dysplasia; DVH/STV, distal villous hypoplasia/small terminal villi; FN/AA, fibrinoid necrosis/acute atherosis; MBPA, muscularized basal plate arteries; MHMA, mural hypertrophy of membrane arteries; MVU, maternal vascular underperfusion; PH, pulmonary hypertension.

*P < 0.01 versus No BPD or PH.
**P < 0.001 versus No BPD or PH.

development require extensive understanding of specific cell populations in the placenta that mediate intrauterine pathologies, and that these factors have the potential to directly or indirectly affect fetal lung development, especially in the setting of preterm birth. Key anatomical and pathophysiological similarities may exist between placenta and lung that reflect changes in the developing lung, and that the placenta could be a highly important adjunct organ for understanding neonatal lung

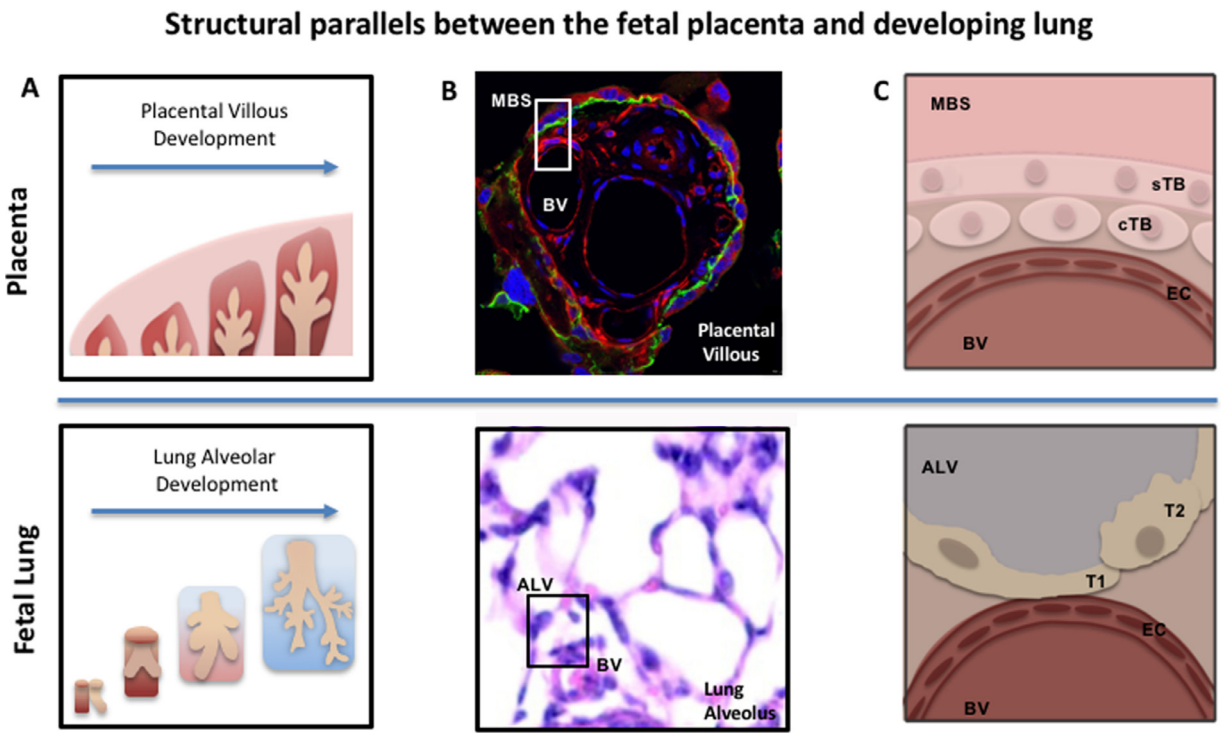


Fig. 3 – Parallel features of placental and lung development: A. Schematics of human placental villous and fetal alveolar networks throughout gestation. B. Histological cross sections of a term human placental villous and neonatal sheep lung alveolus. Boxes outline area of enlarged schematic. C. Exchange interface of maternal blood space with placental villous; alveolar air space with pulmonary blood vessels. MBS: maternal blood space, sTB: syncytiotrophoblast, cTB: cytotrophobalst, ALV: lung alveolus, EC: endothelial cell, BV: blood vessel, T1: type I pneumocyte, T2: type II pneumocyte. Histological images of human placenta and sheep lung courtesy of E. Taglauer and S. Abman, respectively.

biology. For example, cord blood biomarkers from preterm infants that reflect impaired angiogenesis, such as decreased VEGF signaling, are strongly linked with histologic findings of placental vascular lesions and are further associated with a high risk for BPD with pulmonary hypertension, as discussed further below.[32–34]

Parallels between placental and fetal lung disease

The developing fetal lung has many similarities structurally and functionally with the placenta. As described above, the placenta is an organ consisting of budding epithelial structures with an underlying vascular system which creates a branching structure of increasingly smaller functional units which terminate in an exchange interface (Fig. 3). This exchange interface consists of cells of epithelial lineage juxtaposed with endothelial cells interspersed with immune populations. This is a highly similar anatomical and physiologic environment to the developing fetal lung. Based on these similarities, the fetal lung and placenta may have similar responses to alterations in the intrauterine environment during pregnancy. This may be particularly true for responses to hypoxia.

Two reviews have approached this topic. First, van Patot et al proposed that studying hypoxic responses in organs such as the lung, intestine and kidney can inform studies of the placenta, as these organs have some similar underlying vascular responses to hypoxia.[37] In addition, the "hypoxic placenta" may be associated with altered fetal physiology, as suggested by studies that infants from pre-eclamptic pregnancies at high altitude have elevated pulmonary artery pressures. In addition, Byrne suggested that therapies for pulmonary hypertension to lower placental vascular resistance could be trialed for treatments in PE, as based on molecular similarities in hypoxic responses between placental and pulmonary vascular networks.[38] However, neither review expressed the hypothesis that studies of the placenta may provide a novel adjunct research tool for understanding fetal lung disease. Thus, studies of placental physiology in normal and pathological pregnancies may define mechanisms that could lead to new insights into fetal lung disease and links between placenta and lung disease after birth.[39] This concept has been explored in several studies examining placental responses to hypoxia[40] and also summarized in a recent review highlighting parallels in the physiology of pulmonary hypertension and the placental vascular responses to PE.[38] At the molecular level, a vasoconstrictor response to hypoxia may be directed by oxygen sensing potassium channels in vascular smooth muscle cells.[41] A variety of studies have demonstrated that in a hypoxic environment, oxygen sensing potassium channels have decreased activity leading to membrane depolarization, intracellular calcium influx and ultimately vasoconstriction.[42] These channels have been well-characterized in pulmonary vasculature and more recently identified in placental vasculature as well.[43]

There is additional evidence for similarities in the pulmonary and placental vascular networks with regard to endothelial cell responses to hypoxia. The endothelium in pulmonary and placental vascular networks have increased calcium influx in response to hypoxia.[44,45] Increased calcium influx in endothelial cells can then lead to increased microvascular permeability with subsequent localized edema with the potential for altering the surrounding parenchyma.[46] While this response may reflect a systemic response of endothelium to hypoxia (rather than a unique aspect of these organs), it does suggest that the fetal lung and placenta may have additional similar physiological responses during pregnancy.

Thus, the combined study of placental and neonatal lung biology has many mutual benefits. First, the field of neonatal pulmonary research has many well-established *in vitro* and pre-clinical techniques, particularly with regard to the study of hypoxia, which could shed light into placental biology. Concurrently the human placenta is a uniquely accessible source of primary human tissue with still many unexplored aspects, particularly in regard to its influence on the developing fetal lung. We propose that the placenta could be a highly valuable organ in which to explore not only how its pathologies can affect fetal lung biology, but also as a novel adjunct model system for diagnostic and therapeutic targets for neonatal lung disease.

Potential mechanisms of placental disease

Placental structural, cellular and humoral factors have been mechanistically implicated in neonatal lung disorders and postnatal growth, in cohort studies of preterm newborns. The mechanisms primarily implicated include (1) perturbations in vascular development and angiogenesis, and (2) inflammation. The epidemiology of these two mechanisms as the etiology of preterm birth, and the association with adverse outcomes of preterm birth is discussed below. Placental structural and histological differences indicative of hypo-perfusion (maternal vascular under-perfusion, consisting of vascular and villous changes with necrosis and atherosis, increased muscularization and mural hypertrophy, infarction, syncytial knots and villous hypoplasia) have been shown to be more common in extremely preterm infants with BPD compared to those without BPD.[29] Both vessel and villous changes were present more frequently in those infants with BPD complicated by pulmonary hypertension (PH, by echocardiography at 36 weeks' PMA), and villous vascularity was decreased in infants with this late PH, regardless of the diagnosis of BPD.[29,45]

Interestingly, resident villous endothelial cells from pregnancies characterized by abnormal placentation [fetal growth restriction, (FGR) resulting in preterm delivery], demonstrate decreased angiogenesis with impaired signaling in the VEGF pathway.[31] As discussed above, there are parallels in development of the placenta and the lung. However, the direct relationship of placental developmental differences to fetal lung development has not been fully elucidated, although the broader effects on fetal and neonatal growth may be one factor.[30] Regardless, the observations regarding echocardiographic findings of late PH in preterm infants are particularly provocative, given the links between angiogenesis and alveolarization in the developing lung.[46–48]

Additional cellular and humoral effects of this abnormal placentation should also be considered when evaluating these potential mechanisms. The placenta and umbilical cord blood contain, respectively, resident and circulating endothelial progenitor cells.[49] Both cell populations have been shown to have similar proliferative potential in term placentas, although the placental population had greater vasculogenic potential.[49] Decreased numbers of endothelial progenitor cells have been demonstrated in the cord blood of preterm newborns who develop BPD,[50,51] cells which are also decreased in maternal diabetes and preeclampsia.[52,53] Interestingly, cord blood from pregnancies with a placenta that demonstrated severe maternal vascular under-perfusion had lower levels of placental grow factor (PIGF), granulocyte-colony stimulating factor (G-CSF) and VEGF-A.[34] This pattern was consistent in cord blood of those infants who were later diagnosed with BPD complicated by echocardiographic findings of PH, but only the relationship with PIGF and G-CSF held up in a validation cohort. In contrast to these findings, increased placental VEGF levels were found in an analysis of protein expression from placental biopsies at birth from pregnancies affected by PE and extremely preterm delivery.[54] Additional protein expression patterns in the PE cluster (distinct from pregnancies affected by preterm labor or premature rupture of the membranes) include increased levels of P-selectin and transforming growth factor-β (TGF-β), further supporting placental vascular differences in these pregnancies.

Finally, regarding the vulnerability of the lung following pregnancies affected by placental vascular abnormalities and the lung's ability to repair following insult and injury during the neonatal hospitalization, the growth of cord blood endothelial progenitor cells from preterm pregnancies is inhibited by exposure to hyperoxia.[51,55] Hyperoxia decreases nitric oxide (NO) production and endothelial nitric oxide synthase (eNOS) and VEGF receptor-2 (VEGFR-2) protein expression *ex vivo*, an effect that is mimicked by NOS inhibition.[55] The inhibitory growth effect of hyperoxia was mitigated by treatment with NO or VEGF (with increased eNOS expression), and antioxidant therapies.[52,56] Fetal oxidative stress is inversely related to GA (assessed by cord 8-isoprostane levels at birth), resulting in additional vulnerability for the most preterm infants.[56] Later lung function data (3–33 months corrected age) from former preterm newborns without BPD demonstrate that alveolar volume and diffusing capacity are positively related to the pro-angiogenic potential of circulating stem cells from contemporaneous samples, supporting the long-term implications of these antenatal perturbations in angiogenesis and the consequences of neonatal care on these vulnerable newborns. Together, these data suggest that placental vascular abnormalities and the presence and function of endothelial precursor cells derived from the placenta influence both fetal lung development and infant lung and vascular growth during the neonatal hospitalization (manifested as increased susceptibility to BPD and PH), with potential for persistent impact on the dysplastic lungs as these children grow and develop.

Other factors further alter the fetal environment and impact lung development

Inflammation has long been considered key to the pathophysiology of BPD.[1,2] However, antenatal infection and inflammation have been variably demonstrated to be a risk factor for BPD, using clinical chorioamnionitis as the marker for inflammation. Further investigations have evaluated the placenta for histologic chorioamnionitis, acute and chronic inflammation, and the fetal inflammatory response (FIR, assessed as neutrophil infiltration into fetal vessels), and have more convincingly argued that antenatal inflammation (defined by placental histology), plays a role in the later development of BPD.[57] Although placental signs of inflammation are associated with the development of BPD, investigations of early neonatal humoral inflammatory profiles fail to demonstrate consistent relationships with BPD.[58,59] Cord blood levels of interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) have been shown to be significantly elevated in very preterm infants who later developed BPD, although these and other relationships are influenced by the inverse correlation of these inflammatory markers with GA at birth.[60–62] Interestingly, higher C-reactive protein levels were associated with decreased odds of BPD in former preterm newborns < 32 weeks' GA at birth.[62] The co-occurrence of placental inflammation in the setting of maternal vascular under-perfusion also mitigates the strong anti-angiogenic relationships of abnormal placentation.[29,34] Further, histologic chorioamnionitis is associated with lower rates of early lung disease (RDS), as some aspects of the inflammatory response may have maturational effects on the lung.[57] These variable effects of inflammation may explain the inconsistent findings regarding fetal humoral predictors of BPD in human cohort studies.

One factor closely associated with later adverse lung function in term newborns is maternal smoking, which is associated with increased levels of oxidative stress; the effects of *in utero* nicotine exposure may be mitigated by antenatal supplemental vitamin C, initiated at <23 weeks' GA.[63,64] In very preterm infants, maternal smoking during pregnancy is strongly associated with both the development of BPD, and later respiratory morbidity (assessed at 1–2 years corrected age).[26,27] In a small subset of these newborns, maternal smoking during pregnancy was associated with lower levels of Vitamin E isoforms over the first month of life, carrying increased vulnerability to pro-inflammatory and oxidant states.[65] With respect to other environmental influences on lung development, antenatal exposure to particulate matter and other environmental pollutants has been shown to have adverse effects on fetal growth and respiratory outcomes;[66,67] preterm infants may be the most susceptible to the consequences of these exposures.[68]

As suggested, the structural, cellular and humoral aspects of the placenta are closely tied to the primary underlying etiology of preterm birth, providing additional information toward understanding the antenatal underpinnings of BPD and its repercussions. The ELGAN investigators evaluated 8

proposed mutually-exclusive primary etiologies in singleton pregnancies among a prospective cohort of newborns delivered at < 28 weeks' gestation.[69] Through analysis of placental (histological and microbiological), demographic, clinical and neonatal characteristics, the investigators classified these 8 proposed etiologies of preterm birth into two distinct groups, one characterized by placental inflammation and infection and the second characterized by abnormal placentation and the consequences of those abnormalities (maternal PE, fetal growth restriction and other fetal indications). Thus, these preterm deliveries were grouped into either a "spontaneous" delivery, with an inflammatory pathophysiology (e.g., premature rupture of the membranes or preterm labor) or a distinct "indicated" delivery with a pathophysiology of placental dysfunction. Consistent with these findings, high grade maternal and fetal vascular obstructive lesions in the placenta were more likely to be associated with medically indicated preterm delivery and less likely to be associated with spontaneous preterm delivery, in a cohort study by Kelly and colleagues.[70] Similarly, Mestan and colleagues showed that the majority of pregnancies with placentas exhibiting maternal vascular underperfusion were accompanied by fetal growth failure, with 40% co-occurring with a diagnosis of pre-eclampsia.[34] Further data on protein expression from placental biopsies supports these relationships, with placentas from preterm pregnancies complicated by preterm labor or premature rupture of the membranes characterized by one of two inflammatory profiles, whereas those complicated by PE characterized by differences in angiogenic protein expression.[55] In addition, cord blood levels of VEGF were inversely correlated with birth weight standardized for GA in preterm newborns, while sFLT-1 had the opposite relationship.[33] Together, these data provide an important link between the epidemiology of preterm birth and respiratory outcomes among this high-risk population.

Antenatal factors and BPD: experimental models

Experimentally, intra-amniotic exposure to sFlt-1, an endogenous VEGF inhibitor that is markedly elevated in the blood and amniotic fluid of women with PE, is sufficient to cause sustained abnormalities of lung structure in the offspring, including decreased alveolar and vascular growth and pulmonary hypertension, which persists throughout infancy[71,72] (Fig. 4). Importantly, antenatal sFlt-1 exposure was sufficient to impair lung development without additional postnatal injuries, such as exposure to hyperoxia or mechanical ventilation, and may explain in part the sustained incidence of BPD despite marked improvements in respiratory therapies that minimize postnatal lung injury.[73]

Very preterm birth is frequently associated with clinically silent fetal exposures to inflammation/infection as diagnosed by histopathology of the fetal membranes, culture, or pro

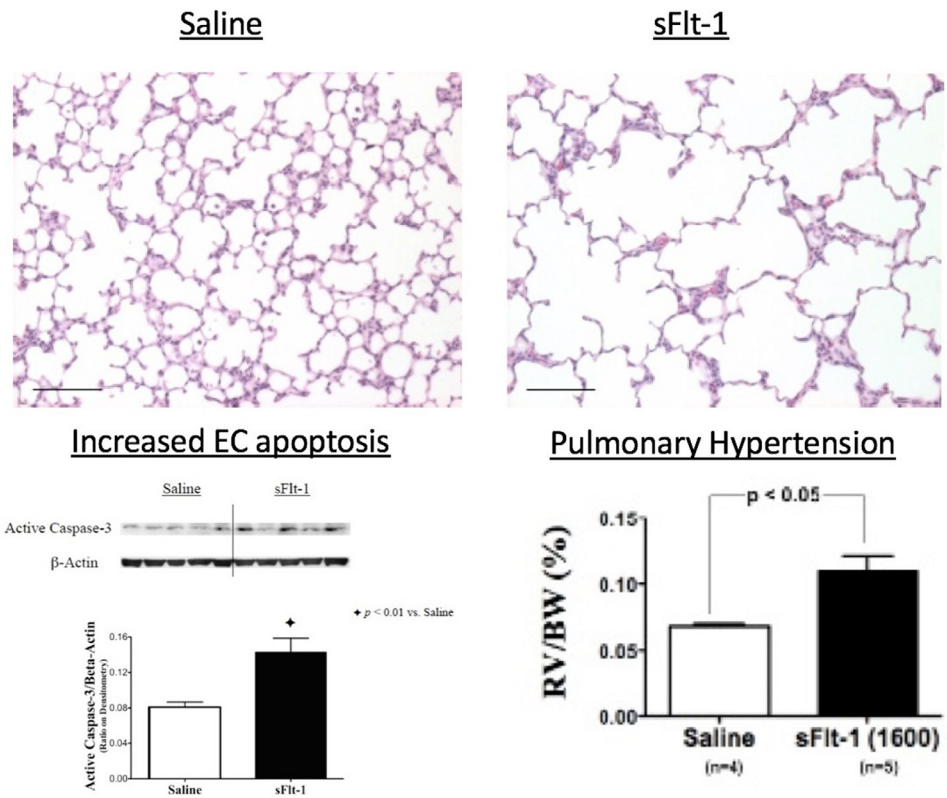


Fig. 4 – Antenatal exposure to sFlt-1, a VEGF receptor decoy, impairs lung alveolar (upper panels) and vascular growth (not shown) and causes lung endothelial cell apoptosis (lower left panel) and the development of pulmonary hypertension (lower right panel). Abbreviations: EC, endothelial cell; RV, right ventricle; BW, body weight). (from Wallace B et al. Am J Respir Crit Care Med, 2018).

inflammatory cytokines in amniotic fluid or cord blood. Other very preterm deliveries are associated with clinical chorioamnionitis, a non-specific diagnosis of limited clinical value. Inflammation associated chorioamnionitis seldom results in positive blood cultures or frank infection in the newborn, but indicators of lung inflammation prior to birth are frequent. While specific infections are seldom identified clinically, the very preterm infant can have lung abnormalities resulting from antenatal exposures that range from severe diffuse pneumonia (indistinguishable from severe RDS) to very mature lungs for GA.

Experimental studies demonstrate this extreme range of effects as well as modulation of fetal and postnatal immune responses. Thus, these antenatal exposures can promote early lung injury by decreasing lung function or decrease the risk of BPD by decreasing the severity of RDS. However, how fetal modulations of immune and inflammatory responses contribute to postnatal lung injury and the development of BPD are poorly understood. Antenatal exposure to endotoxin (e.g., E. Coli lipopolysaccharide, or LPS) is sufficient to cause BPD-type changes in infant rat lungs, even in the absence hyperoxia. mechanical ventilation or other postnatal injuries.[73,74] This provides an additional model to dissect the mechanisms leading to human BPD and the potential for early, preventive interventions.

Early pulmonary vascular disease

Preclinical studies suggest that disruption of angiogenesis due to adverse antenatal factors, such as chorioamnionitis, PE or maternal smoking, and postnatal injury after preterm birth, can cause pulmonary vascular disease (PVD) that not only leads to pulmonary hypertension (PH) but can also impair distal lung growth.[33,34] Laboratory studies have shown that the developing endothelial cell plays a key role in regulation and coordination of epithelial growth and distal airspace structure through the production of critical "angiocrines," such as NO, hepatocyte growth factor, vitamin A, insulin growth factor-1 and others.[11,33] As angiogenesis is necessary for normal alveolarization,[34] it has been suggested that protecting the developing pulmonary vasculature from early injury may not only lower pulmonary vascular resistance as one important goal but such strategies may enhance distal lung growth and improve gas exchange, exercise intolerance and other long-term outcomes.

As noted above, several studies have reported that altered cord blood biomarker levels, including various angiogenic factors and endothelial progenitor cells, are associated with the subsequent risk for BPD.[32] More recently, clinical studies have shown that early echocardiographic findings of PVD after preterm birth are strongly associated with the development and severity of BPD and PH at 36 weeks corrected age.[75–77] Interestingly, these findings were also associated with a worse respiratory course during the initial hospitalization, but also late respiratory outcomes, including respiratory exacerbations, hospitalizations and the need for asthma medications.[77] (Fig. 5). Therapeutic strategies that target enhanced endothelial survival, function and growth may provide novel approaches towards the prevention of BPD after early diagnosis of high risk within preterm populations.

The need for preventive strategies

Thus, the need for preventive strategies persists and remains a major challenge to better improve long-term respiratory outcomes after preterm birth. Despite these remarkable advances, many knowledge barriers and gaps towards enhancing late respiratory outcomes of premature infants continue. The current definition of BPD remains unsatisfying for several reasons, including the concept that BPD at 36 weeks is merely a surrogate of more concerning endpoints,

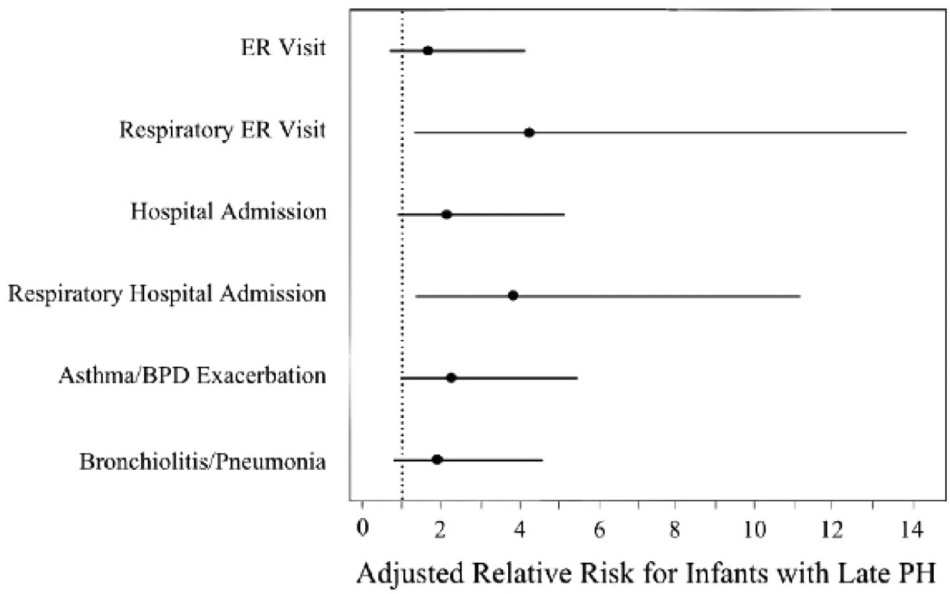


Fig. 5 – Echocardiogram evidence of pulmonary hypertension (PH) is strongly associated with subsequent late respiratory disease during early childhood. (from Mourani PM et al. Am J Respir Crit Care Med. 2017).

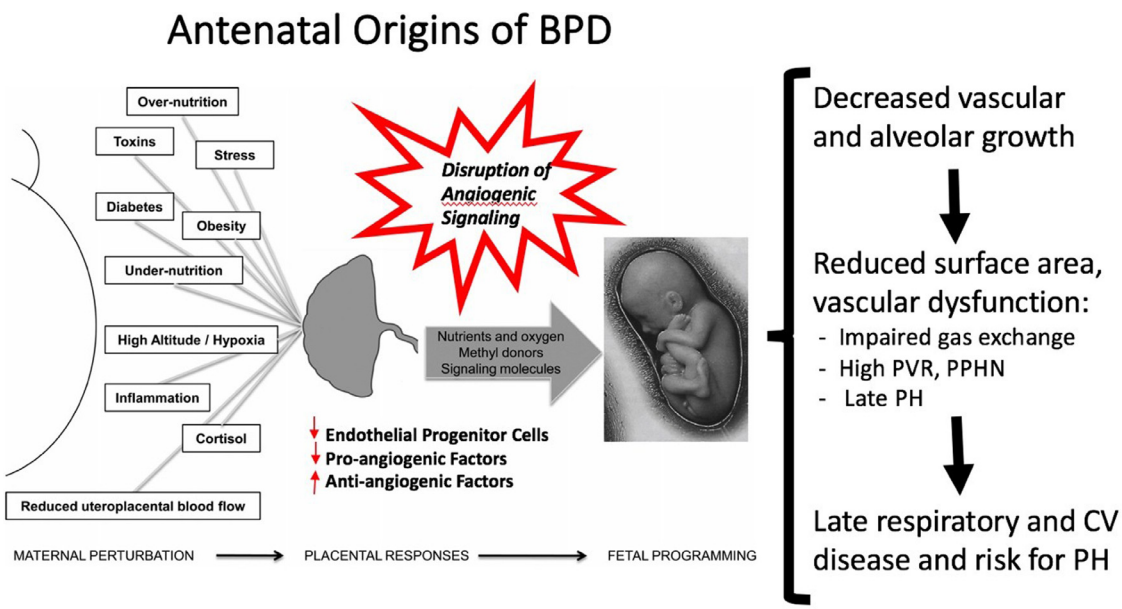


Fig. 6 – Schematic illustrating the hypothesis that antenatal stress may alter placental vascular growth and function through disruption of angiogenesis, leading to decreased angiogenic signaling in the fetus and subsequent complications of prematurity. (modified from Mandell E, Abman SH. J Pediatrics, 2017).

related to late respiratory disease during childhood, such as risks for recurrent respiratory exacerbations, reactive airways disease, re-hospitalizations, exercise intolerance and other problems. In addition, there are several physiologic mechanisms underlying oxygen dependency, including variable contributions of large airways disease, impaired distal lung (airspace) development, pulmonary vascular disease, abnormal ventilator drive, chest wall mechanics and other factors, rendering the current definition imprecise.

An NHLBI workshop further emphasized the importance of preventive strategies, including the need to better understand mechanisms through which antenatal factors and placental dysfunction contribute to BPD risk.[9] (Fig. 6). This report highlighted the importance of developing effective disease predictors, perhaps through such methodologies such as genomics and proteomics in conjunction with clinical features, may help identify early pathologic pathways and therapeutic targets. More basic work is needed to better define interactions between genetic and epigenetic factors, antenatal stress, postnatal factors that contribute to disruption of lung development or alter the response to injury. Further studies using antenatal models of BPD may help better inform the field of novel interventions for disease prevention beyond the use of hyperoxia or mechanical ventilation alone.

Gaps in knowledge/future directions/conclusions

Since its original description, advances to reduce the incidence and severity of BPD have been surprisingly few, while the improved survival of extremely preterm babies has allowed the population of at- risk infants to grow substantially. Keller et al provide several new insights from the NIH-funded "Prematurity Respiratory Outcomes Program" (PROP), a large cohort of extremely preterm babies that were extensively phenotyped through the first year of life for perinatal, early postnatal and late respiratory outcomes. These data report that a perinatal model of risk factors identified on the first day of life - such as smoking in pregnancy, IUGR, public insurance, black race, and others- predicts chronic respiratory morbidity at 1 year, and that further inclusion of the diagnosis of BPD at 36 weeks PMA did not further enhance the strength of association beyond the perinatal model alone. These findings also tell us we need to better understand the importance of the prenatal environmental and other factors before birth in developing high risk for respiratory disease beyond postnatal care alone. Therapies in the neonatal intensive care unit are important but are not sufficient to overcome risk factors present before birth and in the home after discharge. Hopefully, the PROP data will enable better strategies for the early identification of preterm infants at highest risk for late respiratory disease and the development of new strategies for disease prevention.

Disclosure statement

SHA has received provided grant support for laboratory research from United Therapeutics and Shire Pharmaceuticals and support for an educational meeting with funds from Malinckrodt.

REFERENCES

1. Northway WH Jr., Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N *Engl J Med*. 1967;276:357–368.
2. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993-2012. *JAMA*. 2015;314:1039–1051.
3. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723–1729.

4. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics*. 2010;126:443.
5. Schmidt B, Roberts RS, Davis PG, et al. Prediction of late death or disability at age 5 years using a count of 3 neonatal morbidities in very low birth weight infants. *J Pediatr*. 2015;167:982–986: e2.
6. Abman SH. *Bronchopulmonary dysplasia*. NY: Informa; 2010.
7. Islam JY, Keller RL, Aschner JL, Hartert TV, Moore PE. Understanding the short and long-term respiratory outcomes of prematurity and bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2015;192:134–156.
8. Gunville CF, Sontag MK, Stratton K, Ranade DJ, Abman SH, Mourani PM. Scope and impact of early and late preterm infants admitted to PICU with respiratory illness. *J Pediatr*. 2010;157:209–214.
9. McEvoy C, Jain L, Schmidt B, Abman SH, Bancalari E, Aschner J. Bronchopulmonary dysplasia: NHLBI Workshop on the Primary Prevention of Chronic Lung Diseases. *Ann Am Thoracic Soc*. 2014(Suppl 3):S146–S153.
10. Poindexter BB, Feng R, Schmidt B, et al. Comparisons and limitations of current definitions of bronchopulmonary dysplasia for the prematurity and respiratory outcomes program. *Ann Am Thorac Soc*. 2015;12:1822–1830.
11. Manuck TA, Levy PT, Gyamfi-Bannerman C, Jobe AH, Blaisdell CJ. Prenatal and perinatal determinants of lung health and disease in early life. *JAMA Pediatr*. 2016;170:e154577.
12. Farstad T, Bratlid D, Medbø S, Markestad T. Norwegian Extreme Prematurity Study Group. Bronchopulmonary dysplasia – prevalence, severity and predictive factors in a national cohort of extremely premature infants. *Acta Paediatr*. 2011;100:53–58.
13. Laughon MM, Langer JC, Bose CL, et al. Prediction of bronchopulmonary dysplasia by postnatal age in extremely premature infants. *Am J Respir Crit Care Med*. 2011;183:1715–1722.
14. Wai KC, Kohn MA, Ballard RA, et al. Early cumulative supplemental oxygen predicts bronchopulmonary dysplasia in high risk extremely low gestational age newborns. *J Pediatr*. 2016. https://doi.org/10.1016/j.jpeds.2016.06.079. pii: S0022-3476(16)30508-X [Epub ahead of print].
15. Bose C, van Marter LJ, Laughon M, O'Shea TM, Allred EN, Karna P, et al. Fetal growth restriction and chronic lung disease among infants born before the 28th week of gestation. *Pediatrics*. 2009;124:e450–e458.
16. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. *Pediatrics*. 1996;97(2):210–215.
17. Van Marter LJ, Dammann O, Allred EN, et al. Developmental epidemiology network investigators. chorioamnionitis, mechanical ventilation, and postnatal sepsis as modulators of chronic lung disease in preterm infants. *J Pediatr*. 2002;140(2):171–176.
18. Hansen AR, Barnes CM, Folkman J, McElrath TF. Maternal preeclampsia predicts development of BPD. *J Pediatr*. 2010-14;156:532–536.
19. Erikson L, Haglund B, Odlind V, Altman M, Ewald U, Keiler H. Perinatal conditions related to growth restriction and inflammation are associated with an increased risk of BPD. *Acta Paediatrica*. 2015;104:259–263.
20. Jobe AH. Effects of chorioamnionitis on the fetal lung. *Clin Perinatol*. 2012;39:441–457.
21. Steinhorn R, Davis JM, Gopel W, et al. Chronic pulmonary insufficiency of prematurity: developing optimal endpoints for drug development. *J Pediatrics*. 2017;191:15–21.
22. Bose C, van Marter LJ, Laughon M, O'Shea TM, Allred EN, Karna P, et al. Fetal growth restriction and chronic lung disease among infants born before the 28th week of gestation. *Pediatrics*. 2009;124:e450–e458.
23. Lal MK, Manktelow BN, Drpaer ES, Field DJ. Chronic lung disease of prematurity and IUGR: a population-based study. *Pediatrics*. 2003;111:483–487.
24. Ronkainen E, Dunder T, Kaukola T, Marttila R, Hallman M. Intrauterine growth restriction predicts lung function at school age in children born very preterm. *Arch Dis Child Fetal Neonatal Edit*. 2016;101:F412–F417.
25. Greenough A, Yuksel B, Cheeseman P. Effect of in utero growth retardation on lung function at follow-up of prematurely born infants. *Eur Respir J*. 2004;24:731–733.
26. Keller RL, Feng R, DeMauro SB, et al. Prematurity and respiratory outcomes program. Bronchopulmonary dysplasia and perinatal characteristics predict 1-Year respiratory outcomes in newborns born at extremely low gestational age: a prospective cohort study. *J Pediatr*. 2017;187:89–97: e3.
27. Morrow LA, Wagner BD, Ingram DA, et al. Antenatal determinants of bronchopulmonary dysplasia and late respiratory disease in preterm infants. *Am J Respir Crit Care Med*. 2017;196(3):364–374.
28. Durrmeyer X, Kayem G, Sinico M, Dassieu G, Danan C, Decobert F. Perinatal risk factors for bronchopulmonary dysplasia in extremely low gestational age infants: a pregnancy disorder-based approach. *J Pediatr*. 2012;160(4):578–583.e2.
29. Mestan KK, Check J, Minturn L, et al. Placental pathologic changes of maternal vascular under-perfusion in BPD and pulmonary hypertension. *Placenta*. 2014;35:570–574.
30. Rozance PJ, Seedorf GJ, Brown A, et al. IUGR decreased pulmonary alveolar and vessel growth and causes pulmonary artery endothelial cell dysfunction in vitro in fetal sheep. *Am J Physiol. LCMP*. 2011;301:L860–L871.
31. Su E, Xing H, Yin P, et al. Impaired fetoplacental angiogenesis in growth-restricted fetuses with abnormal umbilical artery doppler velocimetry is mediated by ARNT. *J Clin Endocrinol Metab*. 2015;100:E30–E40.
32. Check J, Gotteiner N, Liu X, et al. Fetal growth restriction and pulmonary hypertension in premature infants with BPD. *J Perinatol*. 2013;33:553–557.
33. Voller SB, Chock S, Ernst LM, et al. Cord blood biomarkers of vascular endothelial growth (VEGF adn sFlt-1) and postnatal growth: a preterm birth cohort study. *Early Hum Dev*. 2014;90:195–200.
34. Mestan KK, Gotteiner N, Porta N, et al. Cord blood biomarkers of placental maternal vascular under-perfusion predict bronchopulmonary dysplasia-associated pulmonary hypertension. *J Pediatr*. 2017;185:33–41.
35. Taglauer ES, Wilkins-Haug L, Bianchi DW. Review: cell-free fetal DNA in the maternal circulation as an indication of placental health and disease. *Placenta*. 2014;35:S64–S68.
36. Tannetta D, Collett G, Vatish M, Redman C, Sargent I. Syncytiotrophoblast extracellular vesicles - Circulating biopsies reflecting placental health. *Placenta*. 2017;52:134–138.
37. van Patot MC, Ebensperger G, Gassmann M, Llanos A. The hypoxic placenta. *High Alt Med Biol*. 2012;13(3):176–184.
38. Byrne TJ. A "cure" for preeclampsia: Improving neonatal outcomes by overcoming excess fetal placental vascular resistance. *Med Hypotheses*. 2015 Sep;85(3):311–319.
39. Dunham-Snary KJ, et al. Hypoxic pulmonary vasoconstriction: from molecular mechanisms to medicine. *Chest*. 2017;151(1):181–192.
40. Herrera EA, et al. The placental pursuit for an adequate oxidant balance between the mother and the fetus. *Front Pharmacol*. 2014;5:149.
41. Shimoda LA, Polak J. Hypoxia. 4. Hypoxia and ion channel function. *Am J Physiol Cell Physiol*. 2011;300(5):C951–C967.
42. Wareing M. Oxygen sensitivity, potassium channels, and regulation of placental vascular tone. *Microcirculation*. 2014;21(1):58–66.

43. Tiruppathi C, et al. Role of Ca2+ signaling in the regulation of endothelial permeability. *Vascul Pharmacol*. 2002;39(4-5): 173–185.
44. Aono Y, et al. Human umbilical vein endothelial cells (HUVECs) show Ca(2+) mobilization as well as Ca(2+) influx upon hypoxia. *J Cell Biochem*. 2000;78(3):458–464.
45. Yallapragada SG, Mestan KK, Palac H, et al. Placental villous vascularity is decreased in premature infants with bronchopulmonary dysplasia-associated pulmonary hypertension. *Pediatr Dev Pathol*. 2016;19(2):101–107.
46. Abman S. Bronchopulmonary dysplasia: "a vascular hypothesis". *Am J Respir Crit Care Med*. 2001;164:1755–1756.
47. Jakkula M, Le Cras TD, Gebb S, et al. Inhibition of angiogenesis decreases alveolarization in the developing rat lung. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L600–L609.
48. Le Cras TD Le Cras TD, Markham NE, Tuder RM, Voelkel NF, Abman SH. Brief neonatal treatment with a VEGF receptor inhibitor causes pulmonary hypertension and abnormal lung structure in adult rats. *Am J Phyiol. Lung Cell Mol Biol*. 2002;283: L555–L562.
49. Rapp BM, Saadatzedeh MR, Ofstein RH, et al. Resident endothelial progenitor cells from human placenta have greater vasculogenic potential than circulating endothelial progenitor cells from umbilical cord blood. *Cell Med*. 2011;2(3):85–96.
50. Borghesi A, Massa M, Campanelli R, et al. Circulating endothelial progenitor cells in preterm infants with bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2009;180(6):540–546.
51. Baker CD, Balasubramaniam V, Mourani PM, et al. Cord blood angiogenic progenitor cells are decreased in bronchopulmonary dysplasia. *Eur Respir J*. 2012;40:1516–1522.
52. Acosta JC, Haas DM, Saha CK, Dimeglio LA, Ingram DA, Haneline LS. Gestational diabetes mellitus alters maternal and neonatal circulating endothelial progenitor cell subsets. *Am J Obstet Gynecol*. 2011;204(3):254.: e8-.e15.
53. Gumina DL, Black CP, Balasubramaniam V, Winn VD, Baker CD. Umbilical cord blood circulating progenitor cells and endothelial colony-forming cells are decreased in preeclampsia. *Reprod Sci*. 2017;24(7):1088–1096.
54. Faupel-Badger JM, Fichorova RN, Allred EN, et al. Cluster analysis of placental inflammatory proteins can distinguish preeclampsia from preterm labor and premature membrane rupture in singleton deliveries less than 28 weeks of gestation. *Am J Reprod Immunol*. 2011;66(6):488–494.
55. Fujinaga H, Baker CD, Ryan SL, et al. Hyperoxia disrupts vascular endothelial growth factor-nitric oxide signaling and decreases growth of endothelial colony-forming cells from preterm infants. *Am J Physiol Lung Cell Mol Physiol*. 2009;297(6): L1160–L1169.
56. Mestan K, Matoba N, Arguelles L, et al. Cord blood 8-isoprostane in the preterm infant. *Early Hum Dev*. 2012;88:683–689.
57. Roescher AM, Timmer A, Erwich JJ, Bos AF. Placental pathology, perinatal death, neonatal outcome, and neurological development: a systematic review. *PLoS One*. 2014;9(2): e89419.
58. Ambalavanan N, Carlo WA, D'Angio CT, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Cytokines associated with bronchopulmonary dysplasia or death in extremely low birth weight infants. *Pediatrics*. 2009;123:1132–1141.
59. Bose C, Laughon M, Allred EN. ElGAN Study Investigators. Blood protein concentrations in the first two postnatal weeks that predict bronchopulmonary dysplasia among infants born before the 28th week of gestation. *Pediatr Res*. 2011;69(4):347–353.
60. Matoba N, Yu Y, Mestan K, et al. Differential patterns of 27 cord blood immune biomarkers across gestational age. *Pediatrics*. 2009;123(5):1320–1328.
61. Sorokin Y, Romero R, Mele L, et al. Umbilical cord serum interleukin-6, C-reactive protein, and myeloperoxidase concentrations at birth and association with neonatal morbidities and long-term neurodevelopmental outcomes. *Am J Perinatol*. 2014;31(8):717–726.
62. Otsubo Y, Hashimoto K, Kanbe T, Sumi M, Moriuchi H. Association of cord blood chemokines and other biomarkers with neonatal complications following intrauterine inflammation. *PLoS One*. 2017;12(5):e0175082.
63. McEvoy CT, Schilling D, Clay N, et al. Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: a randomized clinical trial. *JAMA*. 2014;311:2074–2082.
64. McEvoy CT, Milner KF, Scherman AJ, et al. Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP): Rationale, design, and methods of a randomized, controlled trial of vitamin C supplementation in pregnancy for the primary prevention of effects of in utero tobacco smoke exposure on infant lung function and respiratory health. *Contemp Clin Trials*. 2017;58:66–77.
65. Stone C, Qiu Y, Kurland IJ, et al. Effect of maternal smoking on plasma and urinary measures of Vitamin E isoforms in the first month after extreme preterm birth. *J Pediatr*. 2018;197:280–285: e3.
66. Whyatt RM, Perzanowski MS, Just AC, et al. Asthma in inner-city children at 5-11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. *Environ Health Perspect*. 2014;122(10):1141–1146.
67. Michikawa T, Morokuma S, Fukushima K, Kato K, Nitta H, Yamazaki S. Maternal exposure to air pollutants during the first trimester and foetal growth in Japanese term infants. *Environ Pollut*. 2017;230:387–393.
68. Stroustrup A, Teitelbaum SL, Aschner JL. The value of preterm infant environmental health cohorts: the canary in the coal mine. *JAMA Pediatr*. 2017;171(12):1139–1140.
69. McElrath TF, Hecht JL, Dammann O. ELGAN Study Investigators. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. *Am J Epidemiol*. 2008;168(9):980–989.
70. Kelly R, Holzman C, Senagore P, et al. Placental vascular pathology findings and pathways to preterm delivery. *Am J Epidemiol*. 2009;170(2):148–158.
71. Tang JR, Karumanchi SA, Seedorf G, Markham N, Abman SH. Excess soluble vascular endothelial growth factor receptor-1 in amniotic fluid impairs lung growth in rats: linking preeclampsia with bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2012;302:L36–L46.
72. Wallace B, Peisl A, Seedorf G, et al. Anti-sFlt-1 monoclonal antibody therapy preserves lung alveolar and vascular growth in antenatal models of BPD. *Am J Respir Crit Care Med*. 2018;197:776–787.
73. Tang JR, Seedorf G, Muehlethaler V, et al. Moderate hyperoxia accelerates lung growth and attenuates pulmonary hypertension in infant rats after exposure to intra-amniotic endotoxin. *Am J Physiol: Lung*. 2010;299:L735–L748.
74. Tang JR, Michaelis KA, Nozik-Grayck E, et al. NF-kB regulates the responses of fetal pulmonary artery endothelial response to antenatal inflammation: differential roles of IkBa and IkB. *J Immunol*. 2013;190:2913–2923.
75. Mourani PM, Abman SH. Pulmonary hypertension and vascular abnormalities in BPD. *Clin Perinatol*. 2015;42:839–855.
76. Mirza H, Ziegler J, Ford S, Padbury J, Tucker R, Laptook A. Pulmonary hypertension in preterm infants: prevalence and association with BPD. *J Pediatr*. 2014;165:909–914.
77. Mourani PM, Sontag MK, Younoszai A, et al. Early pulmonary vascular disease in preterm infants at risk for BPD. *Am J Respir Crit Care Med*. 2015;191:87–95.

Seminars in Perinatology 42 (2018)

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

An update on pulmonary and neurodevelopmental outcomes of bronchopulmonary dysplasia☆

Jeanie L.Y. Cheong[a,b,c,*], **and Lex W. Doyle**[a,b,c,d]

[a]Neonatal Services, Royal Women's Hospital, Melbourne, Australia
[b]Department of Obstetrics and Gynaecology, University of Melbourne, Australia
[c]Clinical Sciences, Murdoch Children's Research Institute, Melbourne, Australia
[d]Department of Paediatrics, University of Melbourne, Melbourne, Australia

ARTICLE INFO

Keywords:
Bronchopulmonary dysplasia
preterm
neurodevelopment
long-term outcomes

ABSTRACT

Bronchopulmonary dysplasia (BPD) is a common complication of extreme prematurity, and its rate is not improving, despite advances in perinatal intensive care. Children with BPD diagnosed in the neonatal period have higher risks for hospitalizations for respiratory problems over the first few years of life, and they have more asthma in later childhood. Neonates diagnosed with BPD have substantial airway obstruction on lung function testing in later childhood and early adulthood, and many are destined to develop adult chronic obstructive pulmonary disease. Survivors with neonatal BPD have more adverse motor function, worse cognitive development and poorer academic progress than those without BPD. Long-term outcomes for children born extremely preterm will improve if the rate of BPD can be substantially reduced.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Bronchopulmonary dysplasia (BPD) was first described in the 1960s in preterm infants who had been exposed to invasive mechanical ventilation and high concentrations of inspired oxygen[1]; the mean birth weight (2158 g) and mean gestational age (34 weeks) of the children in that study reflected the fact that very few extremely low birth weight (ELBW; <1000 g) or extremely preterm (EP; <28 weeks' gestational age) infants survived in the 1960s. Today, most infants born ELBW or EP survive. Although there are many possible solutions to preventing BPD,[2] rates in ELBW or EP infants have not improved, and indeed may be rising.[3,4] Not only are rates of BPD not improving, but long-term cognitive,[5] academic[5] and motor[6,7] performance, and executive function[7] are also not improving in children born ELBW or EP in recent eras; improvements in long-term function might depend in part on solving the problem of how to reduce rates of BPD in these children.

Long-term pulmonary and neurodevelopmental outcomes of children who had BPD in the newborn period have been reviewed several times in the past.[8–10] In this article we update the associations of BPD with long term pulmonary and neurodevelopmental outcomes of children born preterm, focusing on more recent data on the topic, where available.

☆ Supported by grants from the National Health and Medical Research Council of Australia (Centre of Research Excellence #1060733; Career Development Fellowship #1141354 to JC), and the Victorian Government's Operational Infrastructure Support Program.
* Corresponding author at: Level 7, Newborn Research, Royal Women's Hospital, 200 Flemington Road, Parkville, VIC 3052, Australia.
E-mail address: jeanie.cheong@thewomens.org.au (J.L.Y. Cheong).

https://doi.org/10.1053/j.semperi.2018.09.013
0146-0005/© 2018 Elsevier Inc. All rights reserved.

Pulmonary outcomes

Respiratory health

Children with BPD have higher rates of rehospitalization in the first few years after discharge home, most commonly because of respiratory illness, and they spend more days in hospital compared with children without BPD.[11,12] Rehospitalization rates exceeded 50% for those with BPD in the first year of life in one study.[11] Fortunately, rates of rehospitalization diminish as children with BPD grow older.[12–14]

Children with BPD diagnosed in the neonatal period have higher rates of wheezing or asthma compared with preterm survivors without BPD, are treated with more bronchodilators up to the age of 2 years, and have more persistent wheezing between 2 and 5 years.[12,15] Wheezing rates are typically up to twice as likely in children with neonatal BPD compared with preterm children without BPD.[12,15] In a study from Alberta of 1030 infants <1250 g birthweight admitted to one neonatal intensive care unit (NICU) between 1995–2007 who survived and were followed up to 3 years, those who had severe BPD and required oxygen after discharge home had higher rates of requiring respiratory medications in the previous 12 months than survivors with less severe BPD and survivors without BPD.[16]

With increasing age, the overall rates of wheezing decrease but the risk remains higher in children with BPD than those without. In one study, children at a mean age of 9.5 years who had BPD were 4.5 times more likely to have respiratory symptoms than matched preterm controls without BPD, including wheezing and chronic cough, and 21% were receiving asthma medication compared with 0% in controls.[17] In a study of 11-year-olds born <26 weeks' gestation in the United Kingdom, those with a history of BPD had higher rates of asthma than those without a history of BPD (28% vs. 19%, respectively).[18]

Higher rates of cough, wheezing and dyspnea have been reported in some studies of adult survivors who had BPD compared with term born controls.[19] However, not all studies have reported substantial differences between adult BPD survivors and non-BPD controls.[20,21] Doyle et al reported similar rates of asthma, defined as requiring bronchodilator therapy for recurring wheezing in the preceding 12 months, in 19 year-old very low birthweight (VLBW; <1500 g) survivors with BPD (24.2%) and those without BPD (21.9%).[21]

Studies of the quality of life of older survivors with neonatal BPD have reported conflicting outcomes. In one study of 11-19 year-olds those with neonatal BPD reported similar quality of life compared with controls, despite having poorer lung function.[22] However, in another study those with neonatal BPD reported a poorer quality of life when they were in their mid-20s compared with term-born controls and with non-BPD subjects.[23]

Children with BPD diagnosed as newborns have persisting structural abnormalities in their lungs. In a study of 21 schoolchildren with neonatal BPD, abnormalities on high resolution computed tomography of the lung were detected on 81%, with most abnormalities in the children who had the most severe BPD.[24] Higher abnormality scores on imaging were associated with more airway obstruction on lung function testing. In another study from the era before exogenous surfactant was available, all 21 survivors of moderate or severe BPD at ages ranging from 17–33 years had abnormalities on computed tomography, most commonly having features consistent with emphysema, with worse emphysema associated with more airway obstruction on lung function testing.[25]

Lung function

There are mixed reports of the effects of BPD on lung function in infancy. In a study of 55 preterm children <31 weeks' gestation born between 2006 and 2008, those who had BPD in the newborn period had reduced respiratory system compliance compared with normative data at 6 and 18 months, but other measures of lung function were similar between groups.[26] The findings were considered to be consistent with impaired alveolarization.[26] In a study of 43 children with neonatal BPD compared with 32 children without BPD at 6 months' corrected age and again 12 months later, forced expiratory flows at 0.5 s, at 75% of forced vital capacity, and between 25–75% of forced vital capacity were up to two-thirds of a standard deviation (SD) lower in those without BPD.[27]

By mid-childhood, airflow limitation in children with neonatal BPD has been consistently reported.[18,21,28–30] A cohort study of 68 VLBW children born in the early 1990s found that children with neonatal BPD had a decrease in their forced expired volume in 1 s (FEV_1), a higher ratio of residual volume to total lung capacity, and higher airway resistance than children without BPD and term controls at 7 years of age.[28] In a recent study of children born EP in the post-surfactant era and aged between 8 and 12 years, there was deterioration in airway growth trajectory and lung function in children in the BPD subgroup, which was not evident in the non-BPD children born EP.[29] In the 4-year period between assessments, the mean z-scores for expiratory flows dropped in those who had BPD by 0.3 SD for the FEV_1, by 0.7 SD for the ratio of FEV_1 to the forced vital capacity (FVC; FEV_1/FVC), and by 0.6 SD for forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25-75\%}$).[29]

Late adolescents or young adults with a history of BPD have more airflow obstruction, as shown by a lower FEV_1 compared with those without BPD[23,31–38] (Fig. 1); the mean reduction is −0.75 SD in those with BPD compared with their peers. Although there is some variability between studies, overall there is little difference in the reduction of FEV_1 regardless of cohort demographics i.e. hospital or regional cohorts, and whether they were born prior to or in the era when surfactant has been available (Fig. 1).

The reduction in FEV_1 is worse with increasing severity of BPD. Halvorsen et al.[20] reported that the % predicted value for FEV_1 decreased from a mean of 108.1% in controls, to 101.8%, 96.1% and 87.8%, respectively, in participants born preterm with no, mild (oxygen requirements at 28 days) and moderate/severe (oxygen at 36 weeks or discharged home on oxygen) BPD. Not only are there reductions in the mean FEV_1, but more survivors with neonatal BPD had reductions in clinically important ranges. In a study from Belfast, 40% of BPD survivors had an FEV_1 <80% predicted compared with only 6% in term controls.[23] Some adult survivors with BPD diagnosed in

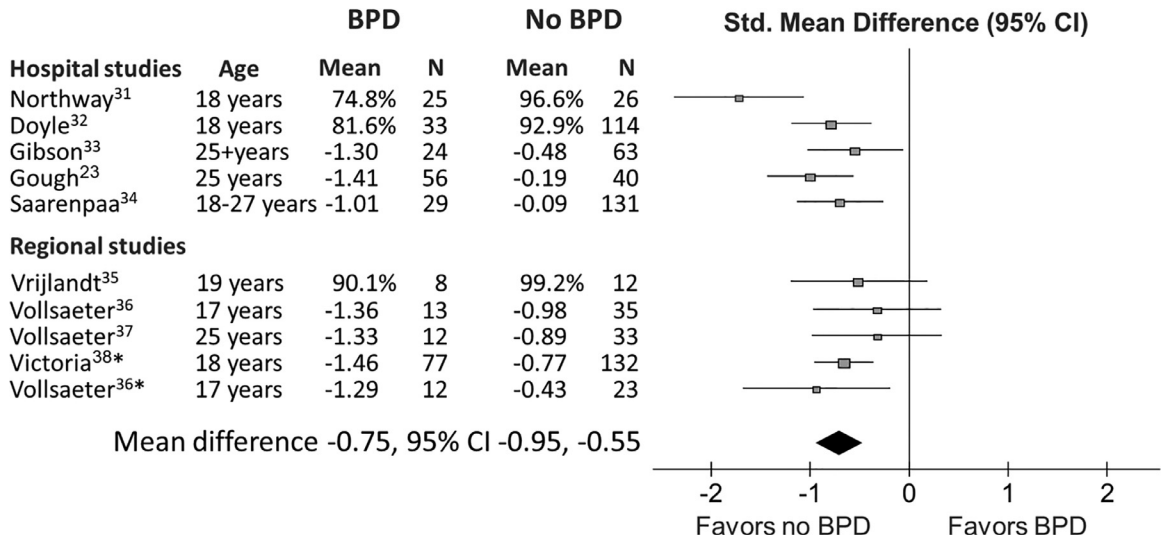


Fig. 1 – Mean values for forced expired volume in one second (either % predicted or as z-scores) and sample sizes compared between those with and without BPD in late adolescence or early adulthood, for either hospital or regional cohort studies. Differences between groups shown by standardized mean differences and 95% confidence intervals (CIs). *participants born when surfactant was available. Superscripts relate to reference numbers.

the neonatal period also had fixed airway obstruction, occurring in 25% of survivors who had BPD compared with none in the controls.[39] In the same study, impaired gas transfer was detected in both BPD and non-BPD adults born preterm compared with controls.[39] In contrast, in another study from Norway of 25-year-olds, although diffusion capacity in preterm subjects was lower than term controls, BPD was not related to the findings.[37]

Kotecha et al.[40] reviewed results from studies of lung function performed in the pre- and post-surfactant era, in participants with age ranging from 5 to 23 years. FEV_1 as % predicted was decreased in preterm survivors with BPD compared with term controls, with the size of the reduction related to the severity of BPD. Participants born preterm but without BPD had a reduction in their FEV_1 of approximately −7.2%, while those with mild BPD (supplemental oxygen at 28 days only) and severe BPD (oxygen at 36 weeks) had greater deficits in FEV_1 of −16.2% and −18.9%, respectively.[40] One interesting finding from their review was that the mean FEV_1 for survivors with neonatal BPD had improved over time from those born in the late 1960s through to those born in the early 1990s, whereas it was unchanged for controls born at term over the same period, suggesting that lung injury in the newborn period might be less severe with advances in perinatal and neonatal intensive care. However, in a subsequent study comparing all survivors born <28 weeks' gestational age in the state of Victoria, Australia, in three eras, 1991–92, 1997 and 2005, at 7,8 years of age those born in 2005 had higher rates of BPD and more airway obstruction than those born in the 1990s.[4]

Changes in lung function between 8 and 18 years have been reported from a regional cohort of ELBW/EP survivors born in the post-surfactant era.[41] Those with BPD had lower spirometry values compared with those without BPD at both ages, but most concerningly those with neonatal BPD deteriorated more over time in most spirometry values; for example the reduction in the z-score for FEV_1/FVC between 8 and 18 years was almost 0.5 SD greater in the participants born preterm with neonatal BPD compared with those who did not. However, some other studies have reported stable lung function, as survivors of BPD become older. In one study of 45 survivors <1000 g BW or <29 weeks gestational age born in 1982–85 in Norway, lung function between 18 and 25 years was similar regardless of gestation at birth and BPD severity.[37]

Exercise capacity is reduced in BPD survivors. Children with neonatal BPD walk shorter distances on the 6-minute walk test than do children without BPD.[15,42] Using a cycle ergometer to measure exercise capacity in children born preterm, those with neonatal BPD had lower % predicted oxygen uptake compared with children without BPD.[30] In a recent study of young adults at a mean age 24 years, those who had BPD had impaired exercise capacity compared with term controls, although weekly activity levels were similar between groups.[39]

Neurodevelopmental outcomes

BPD is strongly associated with neurodevelopmental deficits in children born preterm.[43,44] Preterm infants with BPD in the newborn period have recurrent episodes of hypoxia, hypercapnia, and respiratory acidosis,[45] which may predispose them to hypoxic-related brain injury. In addition, postnatal corticosteroids in the newborn period to prevent or treat BPD are a risk factor for neurodevelopmental deficits in preterm children.[46]

Cerebral palsy and other motor impairments

The rate of cerebral palsy (CP) is approximately 10–14% in survivors born <28 weeks' gestation,[5,47] and 14% in those born <26 weeks' gestation.[48] CP occurs more frequently in

survivors with neonatal BPD; in one study of 839 infants <25 weeks' gestation the odds ratio (OR) for CP in those with neonatal BPD was 1.66 (95% confidence interval [CI] 1.01, 2.74), after adjustment for major intraventricular hemorrhage and cystic periventricular leukomalacia.[49]

The risk of CP may be related to the severity of BPD. In the ELGAN study of more than 1000 infants born <28 weeks, the risk of CP in children with the most severe BPD (receiving mechanical ventilation at 36 weeks' postmenstrual age) was 6-fold higher for quadriplegic CP, and 4-fold higher for diplegic CP compared with preterm children without BPD.[50] Those with less severe BPD (not receiving mechanical ventilation at 36 weeks' postmenstrual age) still had increased risks of quadriplegic and diplegic CP compared with preterm children without BPD, but the size of the risk was lower.

In a study from the NICHD Neonatal Research Network of ELBW infants born between 2006-2007, BPD was defined as either a) any form of assisted ventilation or continuous positive airway pressure or supplemental oxygen with an effective fraction of inspired oxygen (FiO_2) >0.3 at 36 weeks' postmenstrual age, or b) supplemental oxygen via nasal cannula or hood with effective FiO_2 <0.3 and failed the stepwise oxygen reduction challenge in the 36th postmenstrual week i.e. "physiological BPD".[51] At 18-22 months, children with "physiological BPD" compared with non-BPD children had higher rates of all CP phenotypes, of moderate to severe CP (Gross Motor Function Classification System level 2 or greater) (7.0% vs 2.1%), of spastic diplegia (7.8% vs. 4.1%) and of quadriplegia (3.9% vs. 0.9%).

Other non-CP motor impairments also occur more frequently in children who had BPD. Several studies of children aged 5–10 years have reported poorer motor performance in BPD survivors compared with non-BPD and term-born controls.[52–54] In a study of 8-year-olds both gross and fine motor skills were lower in children with neonatal BPD (42nd centile) compared with the non-BPD children (58th centile) and term controls (70th centile).[55] In another study gross and fine motor scores were approximately 1 SD lower, and upper extremity postural stability was worse in BPD children than in preterm controls assessed at 10 years of age.[44] Consequently, more survivors who had BPD have been reported to access intervention services, such as occupational therapy (71% vs 44%), and physical therapy (71% vs 41%), than do preterm children who did not have BPD.[53]

Some studies have assessed postnatal corticosteroid therapy, to treat or prevent BPD, as a risk factor for motor dysfunction in preterm children. In the EPIPAGE study of births <33 weeks' gestation born in France, postnatal corticosteroid therapy was associated with more minor neuromotor dysfunction (OR 1.8; 95% CI 1.3, 2.6), and with more moderate neuromotor dysfunction (OR 2.7; 95% CI 1.2, 6.1) on multivariable analysis adjusting for other perinatal variables that affect neurodevelopment.[52] Davis et al.[54] assessed the perinatal associations with Developmental Coordination Disorder, or minor motor dysfunction, in a geographic cohort of EP or ELBW children at age 8-9 years. With both variables included in a multivariable analysis, BPD, defined as oxygen requirement at 36 weeks' corrected gestation, was associated with Developmental Coordination Disorder (p=0.06), but the association with postnatal corticosteroids was stronger (p=0.03).

Neurosensory impairments and disabilities

BPD is also an independent risk factor for major neurosensory problems overall. The OR (95% CI) for any of CP, blindness, deafness, or cognitive delay at 18 months was 2.4 (1.8, 3.2) in the follow-up of a randomized controlled trial of indomethacin prophylaxis in infants <1000 g birthweight.[43] In a similar study, but this time from a randomized controlled trial of caffeine in the newborn period in infants with BW <1251 g, those alive at 36 weeks' postmenstrual age had a higher chance of death or disability in one or more area (motor impairment, cognitive impairment, behavior problems, poor general health, deafness, or blindness) at 5 years than did those who did not have BPD (OR 2.3; 95% CI 1.8, 3.0). In a study of survivors born <28 weeks' gestation between 2000–2008 in Switzerland, BPD was an independent risk factor for a composite adverse outcome of moderate/severe neurosensory disability or death (OR 1.92; 95% CI 1.24, 2.99).[56]

Cognitive delay and academic performance

There are several studies that have reported persistence of cognitive deficits from early childhood to school age. In one study of VLBW children and term controls, between 8 months to 3 years of age VLBW children with BPD diagnosed in the neonatal period had persistently lower scores on the Mental Developmental Index of the Bayley Scales than did VLBW children without BPD, who in turn were always lower than the term controls.[57] When the children were reassessed at age 8 years, performance on general intelligence, reading, mathematics, motor performance, memory and attention followed a similar pattern; children with neonatal BPD performed the worst, followed by children without BPD, with the best performance in term controls.[53] Children who had more severe BPD compared with children who had mild/moderate BPD performed worse in Performance IQ (75 vs 86) and Perceptual Organization (76 vs 86), representing clinically important differences of approximately 0.6 SD.[58] The differences persisted despite adjusting for BW and brain injury, suggesting that BPD is an independent risk factor for later cognitive development. In a more recent study from the state of Victoria investigating the associations between several biologic and social variables and cognitive and academic performance through childhood and into adolescence among survivors born either ELBW or EP, postnatal corticosteroids were independently associated with lower IQ and academic performance into adolescence, whereas BPD not treated with corticosteroids was not independently associated with those outcomes.[46] These findings are consistent with the possibility that more severe BPD is related to adverse neurocognitive outcomes, but part of the association might be explained by the need to prevent or treat BPD with postnatal corticosteroids, and it was infants with more severe lung disease who were treated with corticosteroids in that study.

Differences in cognitive development between BPD and non-BPD children persisted from early to mid-childhood in a study from Cleveland, Ohio, of very low birth weight children born 1989–1991.[55,57] Not only were the absolute scores on developmental testing lower, but the proportions of children who were significantly delayed i.e. performing at <–2 SD below the

mean, were increased in the group with BPD. At 3 years, the mean Bayley scores for those with BPD, without BPD and term controls were 84 vs 90 vs 96 for the Mental Developmental Index, and 84 vs 98 vs 103 for the Psychomotor Developmental Index. The percentages of children across the 3 groups who had cognitive delay was 21% vs 11% vs 4% and motor delay was 20% vs 9% vs 1%, respectively.[57] The rates of cognitive delay at 3 years of age were much higher than those reported in a more recent study using a physiological definition for BPD, where only 12.8% of the cohort had cognitive scores <70 at 18-22 months.[51] However, the latter study used the Bayley-III to determine delay, and it has been widely reported that the Bayley-III underestimates rates of delay in clinical populations.[59] When the same cohort was reassessed at 8 years, similar deficits were recorded across the 3 groups for Full Scale IQ (85 vs 92 vs 102, respectively), as well as Verbal and Performance IQ.[55]

Language delay

Language development has been reported to be delayed in some studies of children with BPD. In a study of extremely low birth weight children aged 18–22 months, significant language delay (<−2 SD below the mean) was reported in 24.2% of those who had BPD compared with only 12.3% in those who did not have BPD ($p<0.001$).[51] In 3-year-old VLBW preschool children, comparing those with BPD and those without, Singer et al reported lower scores in several domains of language, including receptive, expressive and total communicative competence scores.[60] The proportion of children with receptive and expressive language delay in that study was also higher in BPD infants than term controls (49% vs 35% for receptive language, and 44% vs 25% for expressive language). When the findings were adjusted for IQ, only receptive language remained independently affected. When the same cohort were reassessed at 8 years of age, stepwise decreases in scores in both expressive and receptive language were noted in VLBW children with BPD, compared with VLBW children who did not have BPD, and term-born controls, who had the highest scores.[55] As was found at the 3-year assessment, the differences were most marked in receptive language. In addition, children with BPD had reduced articulation skills compared with the other two groups. As might have been expected, enrolment in speech therapy was highest in the BPD group (48%) compared with 21% in the non-BPD group and 9% in term controls.[55] Other studies have also documented language deficits in children with neonatal BPD at school age, with differences 0.5–0.8 SD lower than in preterm controls without BPD.[58,61]

Visuospatial problems

Visuospatial perception is also reported to be impaired in children with BPD, although it must be remembered that deficits in fine motor control may affect the results, and low scores on tests of visuospatial perception or visuomotor integration may not necessarily indicate defects in visuoperceptual skills. Several studies of VLBW children born in the mid to late 1980s reported poorer visuomotor integration in 8–10-year-old children with BPD compared with non-BPD children and term children.[44,62] Data from a more recent cohort reported around 30% of BPD children performing below age expectations on visuomotor integration testing.[63] In another study a longer duration of oxygen therapy to treat chronic lung disease has been reported to be associated with difficulties in perceptual motor tasks in VLBW children aged 7 years,[64] and again at 16 years.[65]

Conclusions

Children with BPD have more respiratory ill-health in later life, manifest in more hospital readmissions in the first few years after discharge home, particularly for respiratory illnesses, and exhibit impaired lung function and exercise tolerance. As a group they also have more neurosensory problems, particularly motor dysfunction, and poorer performance on cognitive, academic, language and visuoperceptual function. As rates of BPD are not decreasing with advances in perinatal and neonatal care,[66] yet survival rates have increased, there are many more children surviving with BPD diagnosed as newborns than have ever occurred in the past. Consequently, clinicians need to be aware of the risks of adverse problems to which these children are predisposed. The challenges are to reduce the rates of BPD, and to prevent the adverse sequelae for those with BPD who survive, not only through childhood, but also into adulthood.

Disclosures

Neither author has any financial or personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

REFERENCES

1. Northway WH Jr., Rosan RC, Porter DY. Pulmonary disease following respiratory therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med*. 1967;276:357–368.
2. Poets CF, Lorenz L. Prevention of bronchopulmonary dysplasia in extremely low gestational age neonates: current evidence. *Arch Dis Child Fetal Neonat Ed*. 2018;103:F285–F291.
3. Rysavy MA, Li L, Bell EF, et al. Between-hospital variation in treatment and outcomes in extremely preterm infants. *N Engl J Med*. 2015;372:1801–1811.
4. Doyle LW, Carse E, Adams AM, et al. Ventilation in extremely preterm infants and respiratory function at 8 years. *N Engl J Med*. 2017;377:329–337.
5. Cheong JLY, Anderson PJ, Burnett AC, et al. Changing neurodevelopment at 8 years in children born extremely preterm since the 1990s. *Pediatrics*. 2017;139.
6. Spittle AJ, Cameron K, Doyle LW, Cheong JL. Victorian Infant Collaborative Study Group. Motor impairment trends in extremely preterm children: 1991-2005. *Pediatrics*. 2018;141: pii: e20173410.
7. Burnett AC, Anderson PJ, Lee KJ, et al. Trends in executive functioning in extremely preterm children across three birth eras. *Pediatrics*. 2018;141: pii: e20173102.
8. Doyle LW. Cardiopulmonary outcomes of extreme prematurity. *Semin Perinatol*. 2008;32:28–34.
9. Doyle LW, Anderson PJ. Long-term outcomes of bronchopulmonary dysplasia. *Semin Fetal Neonatal Med*. 2009;14:391–395.

10. Doyle LW, Anderson PJ. Pulmonary and neurological follow-up of extremely preterm infants. *Neonatology*. 2010;97:388–394.
11. Ralser E, Mueller W, Haberland C, et al. Rehospitalization in the first 2 years of life in children born preterm. *Acta Paediatr*. 2012;101:e1–e5.
12. Skromme K, Leversen KT, Eide GE, Markestad T, Halvorsen T. Respiratory illness contributed significantly to morbidity in children born extremely premature or with extremely low birthweights in 1999-2000. *Acta Paediatr*. 2015;104:1189–1198.
13. McCormick MC, Workman-Daniels K, Brooks-Gunn J, Peckham GJ. Hospitalization of very low birth weight children at school age. *J Pediatr*. 1993;122:360–365.
14. Doyle LW, Cheung MM, Ford GW, Olinsky A, Davis NM, Callanan C. Birth weight <1501 g and respiratory health at age 14. *Arch Dis Child*. 2001;84:40–44.
15. Praprotnik M, Stucin Gantar I, Lucovnik M, Avcin T, Krivec U. Respiratory morbidity, lung function and fitness assessment after bronchopulmonary dysplasia. *J Perinatol*. 2015;35:1037–1042.
16. Lodha A, Ediger K, Rabi Y, et al. Does chronic oxygen dependency in preterm infants with bronchopulmonary dysplasia at NICU discharge predict respiratory outcomes at 3 years of age? *J Perinatol*. 2015;35:530–536.
17. Vom Hove M, Prenzel F, Uhlig HH, Robel-Tillig E. Pulmonary outcome in former preterm, very low birth weight children with bronchopulmonary dysplasia: a case-control follow-up at school age. *J Pediatr*. 2014;164:40–45: e4.
18. Fawke J, Lum S, Kirkby J, et al. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Resp Crit Care Med*. 2010;182:237–245.
19. Gough A, Spence D, Linden M, Halliday HL, McGarvey LP. General and respiratory health outcomes in adult survivors of bronchopulmonary dysplasia: a systematic review. *Chest*. 2012;141:1554–1567.
20. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Carlsen KH, Bakke P. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. *Acta Paediatr*. 2004;93:1294–1300.
21. Doyle LW. and the Victorian Infant Collaborative Study Group. Respiratory function at age 8-9 years in extremely low birthweight/very preterm children born in Victoria in 1991-92. *Pediat Pulmonol*. 2006;41:570–576.
22. Bozzetto S, Carraro S, Tomasi L, Berardi M, Zanconato S, Baraldi E. Health-related quality of life in adolescent survivors of bronchopulmonary dysplasia. *Respirology*. 2016;21:1113–1117.
23. Gough A, Linden M, Spence D, Patterson CC, Halliday HL, McGarvey LP. Impaired lung function and health status in adult survivors of bronchopulmonary dysplasia. *Eur Resp J*. 2014;43:808–816.
24. Ronkainen E, Perhomaa M, Mattila L, Hallman M, Dunder T. Structural pulmonary abnormalities still evident in school-children with new bronchopulmonary dysplasia. *Neonatology*. 2018;113:122–130.
25. Wong PM, Lees AN, Louw J, et al. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. *Eur Resp J*. 2008;32:321–328.
26. Thunqvist P, Gustafsson P, Norman M, Wickman M, Hallberg J. Lung function at 6 and 18 months after preterm birth in relation to severity of bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2015;50:978–986.
27. Sanchez-Solis M, Garcia-Marcos L, Bosch-Gimenez V, Perez-Fernandez V, Pastor-Vivero MD, Mondejar-Lopez P. Lung function among infants born preterm, with or without bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2012;47:674–681.
28. Korhonen P, Laitinen J, Hyodynmaa E, Tammela O. Respiratory outcome in school-aged, very-low-birth-weight children in the surfactant era. *Acta Paediatr*. 2004;93:316–321.
29. Fortuna M, Carraro S, Temporin E, et al. Mid-childhood lung function in a cohort of children with "new bronchopulmonary dysplasia". *Pediatr Pulmonol*. 2016;51:1057–1064.
30. MacLean JE, DeHaan K, Fuhr D, et al. Altered breathing mechanics and ventilatory response during exercise in children born extremely preterm. *Thorax*. 2016;71:1012–1019.
31. Northway WH Jr., Moss RB, Carlisle KB, et al. Late pulmonary sequelae of bronchopulmonary dysplasia. *N Engl J Med*. 1990;323:1793–1799.
32. Doyle LW, Faber B, Callanan C, Freezer N, Ford GW, Davis NM. Bronchopulmonary dysplasia in very low birth weight subjects and lung function in late adolescence. *Pediatrics*. 2006;118:108–113.
33. Gibson AM, Reddington C, McBride L, Callanan C, Robertson C, Doyle LW. Lung function in adult survivors of very low birth weight, with and without bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2015;50:987–994.
34. Saarenpaa HK, Tikanmaki M, Sipola-Leppanen M, et al. Lung function in very low birth weight adults. *Pediatrics*. 2015;136:642–650.
35. Vrijlandt EJ, Gerritsen J, Boezen HM, Grevink RG, Duiverman EJ. Lung function and exercise capacity in young adults born prematurely. *Am J Resp Crit Care Med*. 2006;173:890–896.
36. Vollsaeter M, Roksund OD, Eide GE, Markestad T, Halvorsen T. Lung function after preterm birth: development from mid-childhood to adulthood. *Thorax*. 2013;68:767–776.
37. Vollsaeter M, Clemm HH, Satrell E, et al. Adult respiratory outcomes of extreme preterm birth. A regional cohort study. *Ann Am Thorac Soc*. 2015;12:313–322.
38. Doyle LW, Adams AM, Robertson C, et al. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. *Thorax*. 2017;72:712–719.
39. Caskey S, Gough A, Rowan S, et al. Structural and functional lung impairment in adult survivors of bronchopulmonary dysplasia. *Ann Am Thorac Soc*. 2016;13:1262–1270.
40. Kotecha SJ, Edwards MO, Watkins WJ, et al. Effect of preterm birth on later FEV1: a systematic review and meta-analysis. *Thorax*. 2013;68:760–766.
41. Doyle LW, Adams AM, Robertson C, et al. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. *Thorax*. 2017;72:712–719.
42. Vardar-Yagli N, Inal-Ince D, Saglam M, et al. Pulmonary and extrapulmonary features in bronchopulmonary dysplasia: a comparison with healthy children. *J Phys Ther Sci*. 2015;27:1761–1765.
43. Schmidt B, Asztalos EV, Roberts RS, Robertson CM, Sauve RS, Whitfield MF. Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months: results from the trial of indomethacin prophylaxis in preterms. *JAMA*. 2003;289:1124–1129.
44. Majnemer A, Riley P, Shevell M, Birnbaum R, Greenstone H, Coates AL. Severe bronchopulmonary dysplasia increases risk for later neurological and motor sequelae in preterm survivors. *Dev Med Child Neurol*. 2000;42:53–60.
45. Sekar KC, Duke JC. Sleep apnea and hypoxemia in recently weaned premature infants with and without bronchopulmonary dysplasia. *Pediatr Pulmonol*. 1991;10:112–116.
46. Doyle LW, Cheong JL, Burnett A, et al. Biological and social influences on outcomes of extreme-preterm/low-birth weight adolescents. *Pediatrics*. 2015;136:e1513–e1520.
47. Serenius F, Ewald U, Farooqi A, et al. Neurodevelopmental outcomes among extremely pretermiInfants 6.5 years after active perinatal care in Sweden. *JAMA Pediatr*. 2016;170:954–963.

48. Moore T, Hennessy EM, Myles J, et al. Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the EPICure studies. *Br Med J*. 2012;345:e7961.
49. Hintz SR, Kendrick DE, Vohr BR, Poole WK, Higgins RD. Changes in neurodevelopmental outcomes at 18 to 22 months' corrected age among infants of less than 25 weeks' gestational age born in 1993-1999. *Pediatrics*. 2005;115:1645–1651.
50. Van Marter LJ, Kuban KC, Allred E, et al. Does bronchopulmonary dysplasia contribute to the occurrence of cerebral palsy among infants born before 28 weeks of gestation? *Arch Dis Child Fetal Neonat Ed*. 2011;96:F20–F29.
51. Natarajan G, Pappas A, Shankaran S, et al. Outcomes of extremely low birth weight infants with bronchopulmonary dysplasia: impact of the physiologic definition. *Early Hum Dev*. 2012;88:509–515.
52. Arnaud C, Daubisse-Marliac L, White-Koning M, et al. Prevalence and associated factors of minor neuromotor dysfunctions at age 5 years in prematurely born children: the EPIPAGE study. *Arch Pediatr Adolesc Med*. 2007;161:1053–1061.
53. Short EJ, Klein NK, Lewis BA, Fulton S, Eisengart S, Kercsmar C, et al. Cognitive and academic consequences of bronchopulmonary dysplasia and very low birth weight: 8-year-old outcomes. *Pediatrics*. 2003;112:e359.
54. Davis NM, Ford GW, Anderson PJ, Doyle LW. Developmental coordination disorder at 8 years of age in a regional cohort of extremely-low-birthweight or very preterm infants. *Dev Med Child Neurol*. 2007;49:325–330.
55. Lewis BA, Singer LT, Fulton S, et al. Speech and language outcomes of children with bronchopulmonary dysplasia. *J Commun Disord*. 2002;35:393–406.
56. Schlapbach LJ, Adams M, Proietti E, et al. Outcome at two years of age in a Swiss national cohort of extremely preterm infants born between 2000 and 2008. *BMC Pediatr*. 2012;12:198.
57. Singer L, Yamashita T, Lilien L, Collin M, Baley J. A longitudinal study of developmental outcome of infants with bronchopulmonary dysplasia and very low birth weight. *Pediatrics*. 1997;100:987–993.
58. Short EJ, Kirchner HL, Asaad GR, et al. Developmental sequelae in preterm infants having a diagnosis of bronchopulmonary dysplasia: analysis using a severity-based classification system. *Arch Pediatr Adolesc Med*. 2007;161:1082–1087.
59. Anderson PJ, De Luca CR, Hutchinson E, Roberts G, Doyle LW. Underestimation of developmental delay by the new Bayley-III Scale. *Arch Pediatr Adolesc Med*. 2010;164:352–356.
60. Singer LT, Siegel AC, Lewis B, Hawkins S, Yamashita T, Baley J. Preschool language outcomes of children with history of bronchopulmonary dysplasia and very low birth weight. *J Dev Behav Pediatr*. 2001;22:19–26.
61. Robertson CM, Etches PC, Goldson E, Kyle JM. Eight-year school performance, neurodevelopmental, and growth outcome of neonates with bronchopulmonary dysplasia: a comparative study. *Pediatrics*. 1992;89:365–372.
62. Hughes CA, O'Gorman LA, Shyr Y, Schork MA, Bozynski ME, McCormick MC. Cognitive performance at school age of very low birth weight infants with bronchopulmonary dysplasia. *J Dev Behav Pediatr*. 1999;20:1–8.
63. Gray PH, O'Callaghan MJ, Rogers YM. Psychoeducational outcome at school age of preterm infants with bronchopulmonary dysplasia. *J Paediatr Child Health*. 2004;40:114–120.
64. Taylor HG, Klein N, Schatschneider C, Hack M. Predictors of early school age outcomes in very low birth weight children. *J Dev Behav Pediatr*. 1998;19:235–243.
65. Taylor HG, Minich N, Bangert B, Filipek PA, Hack M. Long-term neuropsychological outcomes of very low birth weight: associations with early risks for periventricular brain insults. *J Int Neuropsychol Soc*. 2004;10:987–1004.
66. Doyle LW, Ranganathan S, Cheong JLY. Ventilation in preterm infants and lung function at 8 years. *N Engl J Med*. 2017;377:1601–1602.

Seminars in Perinatology 42 (2018)

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

An update on the post-NICU discharge management of bronchopulmonary dysplasia

Anita Bhandari*, and Howard Panitch

Division of Pulmonary Medicine, Department of Pediatrics, Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, 11th floor Colket Building, 3501 Civic Center Boulevard, Philadelphia, PA 19446, United States

ARTICLE INFO

Keywords:
outpatient care
chronic lung disease
diuretics
inhaled steroids

ABSTRACT

Bronchopulmonary dysplasia (BPD) is a chronic lung disease which develops as a result of neonatal/perinatal lung injury. It is the commonest cause of chronic lung disease in infancy and the most frequent morbidity associated with prematurity. The incidence of BPD has continued to rise despite many advances in neonatal care and this increase has been attributed to the increased survival of younger and more premature babies. There have been many advances in the care of patients with early and evolving BPD, yet there is a paucity of data regarding outpatient management of patients with established BPD. There are limited adequately-powered high-quality studies/randomized controlled trials which assess commonly used therapies such as supplemental oxygen, bronchodilators, steroids and diuretics in patients with BPD, beyond short-term effects. Further research is needed to improve our understanding of the role of currently used treatments on the long-term outcomes of patients with established BPD, post-discharge from the neonatal intensive care unit.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease, which develops as a result of neonatal/ perinatal lung injury.[1] It is the commonest cause of chronic lung disease in infancy and the most frequent morbidity associated with prematurity.[2] Athough many definitions of BPD exist, the most widely used is that suggested by the national institutes of health (NIH) consensus statement, where BPD is defined as a need for oxygen supplementation for 28 days and/or 36 weeks post menstrual age (PMA).[3]

They further suggested criteria for assigning BPD severity. Mild BPD was defined as a need for supplemental oxygen for 28 days or greater but not at 36 weeks PMA or at the time of discharge. Moderate BPD was defined as a need for supplemental oxygen for 28 days or greater and treatment with supplemental oxygen not greater than 30% at 36 weeks PMA or at the time of discharge. Severe BPD was defined as a need for supplemental oxygen for 28 days or greater and treatment with supplemental oxygen greater than 30% and/or need for positive pressure at 36 weeks PMA or at the time of discharge. This consensus definition has been found to identify risk for adverse pulmonary outcomes in early infancy more accurately than others.[4] A further subclassification of severe BPD into Type 1 which includes patients using high flow nasal cannula (HFNC) or continuous positive airway pressure (CPAP) and Type 2 which includes those who require mechanical ventilation, has been suggested.[5]

*Corresponding author.
E-mail address: bhandaria@email.chop.edu (A. Bhandari).

https://doi.org/10.1053/j.semperi.2018.09.011
0146-0005/© 2018 Elsevier Inc. All rights reserved.

The incidence of BPD from 2009–2012 has increased in preterm babies born between 26 and 27 weeks of gestation from 51% to 55% despite many advances in neonatal care.[6] This increase is attributed to increased survival of younger and more immature babies. BPD occurs most commonly in babies who are less than 1000 g and less than 28 weeks of gestation, and its incidence is inversely proportional to gestational age or its surrogate, birth weight (BW). The incidence of BPD has been reported to be 42% (BW 501–750 g), 25% (751–1000 g), 11% (1001–1250), and 5% (1251–1500 g).[7,8]

BPD is often divided into early, evolving and established BPD. Early BPD refers to the time from birth to the first week of life. Evolving BPD refers to the period between 1 week of life and 1 month of life and established BPD is considered to occur after 36 weeks PMA.[9] For the purpose of this review, we will focus on therapies utilized for outpatient management of patients with established BPD. While many newer therapies and modes of ventilation have been proposed and studied for management of early BPD, the management of established BPD has changed little in the last two decades.

Diuretics

Diuretics are the most commonly used medications in the management of established BPD. Infants with BPD are prone to develop interstitial pulmonary edema from increased capillary permeability caused by lung injury from volutrauma or barotaruma, heart failure and generalized fluid overload. The degree of free lung water has been correlated with the severity of BPD.[10] These considerations form the basis for using diuretics in BPD infants.

Furosemide is a loop diuretic. Its diuretic action is a result of inhibition of the $Na^{+-}K^{+-}2Cl^{-}$ cotransporter located on the luminal surface of the ascending loop of Henle. In addition, vascular effects of furosemide result in fluid shifting out of the alveoli into the venous system, which can lead to improved oxygenation and pulmonary mechanics.[11]

In older infants with BPD, furosemide has been shown to improve oxygenation and decrease PCO_2. This improvement in ventilation does not corelate well with diuresis suggesting a different mechanism of action. One such mechanism may be improved oxygenation via release of prostaglandins from the renal or vascular endothelium. It is this vascular effect that has also been implicated in increasing the risk of persistent patent ductus areteriosus (PDA) that has been reported with furosemide use in the neonatal intensive care unit (NICU) in patients with early BPD.

Furosemide is the single most frequently used diuretic in the NICU.[12,13] Furosemide has been shown to decrease airway resistance and increase dynamic airway compliance acutely and sub-acutely in spontaneously breathing patients with BPD.[14,15] Others have shown improvement in lung mechanics using nebulized furosemide, suggesting a direct effect of furosemide on airway smooth muscle.[16,17]

Thiazides (chlorothiazide, hydrochlorothiazide) and spironolactone together comprise the most commonly used diuretic combination therapy in patients with established BPD.[18,19,20] Thiazides and spironolactone both act on the distal tubule. Thiazides act by binding to the chloride site of electroneutral sodium chloride channel and spironolactone acts by competitive binding of receptors at the aldosterone dependent sodium–potassium exchange site in the distal tubule. Thiazaides and spironolactone are less likely to cause electrolyte abnormalities as compared to furesomide, hence they are preferred for chronic use.

In a randomized double blind crossover trial with sequential analysis, oral diuretics improved pulmonary mechanics in 10 infants with BPD.[21] This was based on measurement of pulmonary mechanics at the beginning and the end of the week after therapy with chlorothiazide and spirinolcatone combination or placebo.[21]

The long term use of this combination therapy for oxygen-dependent BPD patients was also studied in a randomized double blind, placebo-controlled study, where patients who received spironolactone and chlorthiazide had a significant improvement in respiratory scores and lung mechanics and a decrease in fraction of inspired oxygen (FiO_2) used, as compared to those who received placebo. The therapy did not, however, result in a significant decrease in the duration of oxygen supplementation.[22] A large meta-analysis showed that in preterm infants greater than 3 weeks of age with chronic lung disease, acute and chronic administration of diuretics acting on distal segments of the renal tubule (distal diuretics) improved pulmonary mechanics. The authors, however, suggested that the positive effects should be interpreted with caution given the small number of patients studied in a few randomized controlled trials (RCTs).[23] Of note, RCTs looking at efficacy of diuretic use are from the pre-surfactant era which may limit their applicability in current times. Furthermore, most studies evaluate short-term effects of therapy. There are no trials that demonstrate a reduction in NICU length of stay, duration of mechanical ventilation, or duration of supplemental oxygen use resulting from chronic diuretic therapy among infants with established BPD.

Recent data from a retrospective cohort study in patients with evolving BPD conducted among 35 hospitals showed that use of diuretics is common in US hospitals and that the diuretic regimens and the frequency of diuretic use vary markedly between institutions.[24] The prescribed use of diuretics among patients with established BPD is quite inconsistent as well. In a single center study the duration of outpatient diuretic therapy was significantly variable; patients treated with diuretics who required supplemental oxygen were more likely to have a longer course of diuretic therapy compared to patients receiving diuretics alone.[25] In contrast, a multicenter survey regarding oxygen weaning practices of pediatric pulmonologists in North America disclosed that 58% of the respondents weaned diuretics before weaning supplemental oxygen; these two studies highlight the inconsistency in approach towards prescribing diuretics in patients with established BPD.[26]

Long term diuretic therapy after discharge is often used in infants with severe BPD requiring high levels of supplemental oxygen, positive pressure ventilation, and those with underlying cardiac issues. Diuretic therapy may confer a benefit in these unique situations, although this has not been systematically studied.

Chronic diuretic therapy has been associated with adverse effects including electrolyte disturbances, calciuria,

nephrocalcinosis, ototoxicity and delayed closure of the ductus arteriosus. All these adverse effects are more common with use of loop diuretics, but still exist with chronic use of spironolactone, hydrochlorothiazide and chlorothiazide.

Currently, there are no evidence-based guidelines regarding duration of diuretic therapy in patients with established BPD. Additionally, there are no data to support whether diuretics should be weaned, rather than simply discontinued, in patients with established BPD.

Corticosteroids

Inhaled corticosteroids

Inflammation plays a critical role in the pathogenesis of BPD. Steroids are potent anti-inflammatory agents and when administered systemically in patients with early BPD they produce a rapid improvement in pulmonary function and gas exchange and facilitate weaning from mechanical ventilation. In animal models steroids have been shown to enhance surfactant production, decrease airway edema, stabilize capillary leakage, augment beta receptor activity and decrease overall lung fibrosis.[27]

Given this background, steroids have been used in preterm babies both to prevent development of BPD and to treat BPD. Systemic steroids have many adverse effects including hyperglycemia, systemic hypertension, increased risk of infection, intestinal perforation, steroid induced cardiomyopathy, growth retardation and most importantly, adverse neurological outcomes including cerebral palsy. Given the increased incidence of cerebral palsy in preterm infants with use of systemic steroids, it is now recommended that systemic steroids be used judiciously in preterm infants.[28]

Intratracheal administration of budesonide along with surfactant when used in very low birthweight infants with severe respiratory distress syndrome (RDS) significantly decreased the incidence of BPD or death as compared to administration of surfactant alone although larger studies are needed to confirm these findings.[29,30]

Inhaled steroids have been used in preterm infants with RDS to try to prevent progression to BPD, because they have both anti-inflammatory properties and a lower potential for causing adverse effects as compared to high dose systemic steroids.[31] Inhaled steroids can be systemically absorbed, but the majority of data from their use in early and evolving BPD report no side effects. Among surviving preterm infants who received courses of inhaled budesonide beginning within 12 h of birth, there was no significant difference in the rate of neurodevelopmental disability on follow-up at 2 years of age compared with those who did not.[32] Suppression of endogenous cortisol production with no difference in somatic growth has been reported in preterms with BPD who received budesonide for 4 weeks as compared to age and disease matched controls,[33] whereas others reported no evidence of adrenal suppression in response to cosyntropin stimulation in preterms with BPD during beclomethasone therapy.[34] Nevertheless, the possibility of systemic effects from inhaled steroids exists and caution should be used when using inhaled steroids long term.[35]

Although inhaled steroids are commonly used in patients with BPD as part of the clinical asthma paradigm, there are data to suggest that air flow limitation seen in patients with BPD is not caused by the same factors as are present in those with asthma. Nitric oxide (NO) plays a critical role in regulation of lung vasculature and airway tone. Exhaled NO in breath condensate (fraction of exhaled NO or FeNO) has been used as a marker of eosinophilic inflammation in asthma and is typically elevated in patients with asthma. Baraldi et al. reported lower FeNO concentrations in patients with BPD with obstructive lung disease when compared with those with asthma and obstructive lung disease suggesting different underlying pathophysiology in the disease processes.[36] Others have also shown that the effect of atopy and familial asthma is less in BPD survivors as compared to those born fullterm, suggesting that obstructive disease seen in BPD survivors is different from asthma, thus questioning the role of routine inhaled corticosteroid treatment in infants with established BPD.[37]

Once discharged from the hospital, infants with established BPD are more likely to experience respiratory illnesses and episodes of wheezing, require hospitalization in the first 2 years of life, and to be prescribed respiratory medications including inhaled steroids versus preterms with no BPD.[38,39] In a randomized placebo controlled trial, Beresford et al studied 30 infants with chronic lung disease who received fluticasone or a placebo via a metered dose inhaler and spacer starting at 36 weeks PMA for a duration of one year. They reported no difference in respiratory symptoms, duration of supplemental oxygen use, respiratory illnesses or need for hospitalization.[40] The small number of patients involved in the study, however, may have contributed to the lack of differences noted. In a double blind placebo controlled trial Yuskel et al. showed improvement in respiratory symptoms in premature infants (with or without chronic lung disease/BPD) and an increase in functional residual capacity (FRC) as measured by helium dilution in the cohort who received beclomethasone, when compared to those who received placebo. They attributed the increase in FRC to a decrease in gas trapping with beclomethasone use.[41] This study too had a small sample size and did not compare the effects of beclomethasone on respiratory symptoms and lung function of patients born prematurely without lung disease to those who were born prematurely with chronic lung disease or BPD.

Despite data suggesting that the underlying pathophysiology of obstructive disease in BPD survivors is likely different from that of asthma and that inhaled steroids confer little or modest benefit, inhaled steroids continue to be widely prescribed in BPD survivors during their lifetime.

Systemic steroids

There is no clear indication for use of systemic/enteral steroids in patients with established BPD. Although their use has not been studied, in clinical practice providers often use a short course of oral steroids during to decrease work of breathing or a lingering supplemental oxygen requirement especially during an intercurrent viral illness. A single center study examining the effect of oral prednisolone on weaning oxygen in patients with established BPD showed that 63% of

Table 1 – Suggested post-NICU therapy in patients with BPD.

Drug	Initial/maintenance dosing	Weaning	Comments
Diuretics			
Furosemide	PO/IV, 1–2 mg/kg/24 h or every other day	Consider wean to every other day therapy if on daily therapy	Prefer to discontinue before weaning chlorothiazide and spironolactone given side effects with chronic therapy
Chlorothiazide	PO/IV, 20–40 mg/kg/24 h, alone or with spironolactone	May wean by 25% at each visit if at higher end of the dosing range or discontinue if at the lower end of the range.	Wean if respiratory status is stable. Prefer to wean after patient is off oxygen
Spironolactone	PO, 2–4 mg/kg/24 h	May wean by 25% at each visit if at higher end of the dosing range or discontinue if at the lower end of the range.	Wean if respiratory status is stable. Prefer to wean after patient is off oxygen
Corticosteroids			
Systemic	PO, 2 mg/kg/24 h × 5 days, then 1 mg/kg/24 h × 3 days, then 1 mg/kg/24 h every other day for 3 doses) PO, 2 mg/kg/24 h × 5 days	May help wean off supplemental oxygen May use as therapy to decrease work of breathing with intercurrent viral illness	
Inhaled	Budesonide 500 mcg, 1 vial 1–2 times a day Fluticasone propionate 110 mcg, 1–2 puff 2 times a day	Consider if patient has wheezing or documented response to albuterol or oral steroids and has a strong family history of asthma	Other inhaled steroids such as Beclomethasone and Mometasone can be used. Dose based on severity of symptoms
Bronchodilators	Albuterol 1.25–2.5 mg given via nebulizer or 2 puffs [180 mcg] given via MDI with spacer device, every 3–4 h as needed Ipratropium bromide 250–500 mcg via nebulizer or 18 mcg/puff via MDI with spacer device, every 6–8 h as needed		Use for patients with wheezing or past history reversible bronchospasm. May be a useful adjunctive therapy especially in patients who are not responsive to albuterol alone. It may be better tolerated than albuterol in patients with significant tracheomalacia.
Oxygen	Majority of patients are discharged on up to 1LPM of oxygen via nasal cannula	Initiate weaning during the day time first and then wean night time oxygen. Monitor work of breathing, oxygen saturations and weight gain during weaning	Wean in consultation with cardiology when patient has pulmonary hypertension.

PO: per os (oral); IV: intravenous; MDI: metered dose inhaler; LPM: liters per minute.

patients were successfully weaned off supplemental oxygen prior to hospital discharge. Patients who were successfully weaned off supplemental oxygen after they received oral prednisolone were able to do so after one course of oral steroids; no additional benefit was conferred with multiple courses.[42]

Bronchodilators

There are insufficient data to support the role of beta agonists in the prevention of chronic lung disease; yet, inhaled bronchodilators are frequently used in US hospitals in patients with evolving BPD, and a longer duration of mechanical ventilation increases the odds of receiving a bronchodilator.[43] Furthermore, there are no published data that address the role of beta agonists on duration of mechanical ventilation, frequency of rehospitalization, or mortality in infants and children with BPD.[44]

BPD survivors are more likely to have respiratory symptoms such as recurrent wheezing and cough, and are more likely to have a diagnosis of asthma.[45] Small airway dysfunction and decreased expiratory flows are common lung abnormalities found in infants.[46] Significantly higher rates of airway reactivity in very low birth weight (VLBW) infants have been reported.[47] Data using the forced oscillation technique show that 3–5 year old children with a history of BPD had higher resonant frequency and lower mean reactance compared to children without BPD, suggesting that the children with BPD had a decreased peripheral airway patency.[48] These airway abnormalities have been shown to persist in children and young adults with a history of BPD[49,50,51] and bronchodilator responsiveness is common in children with established BPD.[52]

Although there are higher rates of airway reactivity in BPD survivors, Kim et al reported that pre-school aged BPD survivors showed bronchial hyperresponsiveness to methacholine, which stimulates airway smooth muscle directly, but no response when challenged with adenosine 5-monophosphate (AMP).[53] The latter drug causes airway smooth muscle contraction through release of mast cell contents, suggesting that the BPD patients do not have an inflammatory airway response as seen in patients with asthma. This is in tandem with low exhaled NO levels seen in patients with BPD and obstructive lung disease, in contrast to patients with asthma and a similar degree of obstruction. Taken together, there is strong evidence against eosinophilic inflammation as the basis of obstructive lung disease in BPD.[36]

Structural abnormalities in the airways along with other factors including reduced small airway caliber and reduced elastic recoil increase the propensity for airway narrowing with smooth muscle contraction in BPD survivors. Together these structural changes are thought to be the cause of persistent obstruction seen on spirometry in BPD patients. A recent study reported improvement in infant lung function tests in VLBW babies with evolving BPD.[54] In clinical practice, BPD survivors who exhibit wheezing are often prescribed and will respond to albuterol even when they do not fit the asthma paradigm.

Other airway abnormalities such as tracheomalacia and bronchomalacia are more common in patients with BPD.[55] In BPD infants with tracheobronchomalacia, use of beta agonists can worsen wheezing by causing airway smooth muscle relaxation in an already floppy airway.[56]

There are no randomized trials that evaluate the effect of anticholinergic medications such as ipratropium bromide in patients with BPD. They have been shown to be effective in BPD patients in small studies and case reports.[20] Ipratropium bromide in low dose stiffens the central airway in animal models via inhibition of presynaptic regulatory M2 receptors; so it is occasionally used in BPD infants with tracheomalacia to improve airway mechanics.

Oxygen supplementation

Infants with BPD are more likely to have intermittent hypoxemia (oxygen saturations less than 92%) during feeds and during sleep. Intermittent hypoxemia has been shown to be associated with poor growth.[57] Over 30% of infants with BPD require supplemental oxygen at the time of discharge although most outgrow the need for supplemental oxygen by 2 years of life.[38,39,58] Despite a third of patients with BPD being discharged home with supplemental oxygen, there are no evidence-based guidelines or consensus statements regarding oxygen weaning in BPD patients. Palm et al. found a wide variation in practice among pediatric pulmonologists regarding the minimum acceptable peripheral blood oxygen saturation (SpO_2), initial amount of supplemental oxygen being delivered from which the weaning process should begin, signs considered to be important in determining readiness to wean, or the methods used to wean patients with BPD from supplemental oxygen.[26] In their study, the majority of pediatric pulmonologists reported using nocturnal saturations as the primary indication for weaning oxygen while only 8% reported using standardized protocols. Minimum oxygen saturation in room air required to discontinue supplemental oxygen varied from 90% to 95% and many respondents elected to wean flow rate of oxygen delivered prior to weaning the duration of time that the patient received oxygen supplementation. Although there are no guidelines regarding oxygen weaning, experts recommend weaning oxygen during the daytime first, followed by weaning oxygen at nighttime.[18] Most pediatric pulmonolgists use clinical criteria including growth and clinical examination to guide weaning oxygen supplementation in addition to monitoring oxygen saturations. Room air challenge tests have been used to guide oxygen weaning by some[59] while others recommend obtaining a polysomnogram to rule out nocturnal hypoxemia prior to oxygen weaning.[60,61] Although weaning is typically guided by pediatric pulmonologists or neonataologists this not always the case; 30% of infants followed in a single center underwent discontinuation of supplemental oxygen by their parents without guidance of a physician.[58]

BPD is the most common cause of pulmonary arterial hypertension (PAH) in pediatric patients, with an incidence of up to 25% of BPD infants. Furthermore, the presence of PAH correlates with the severity of BPD.[62,63] Thus, it is more common in patients with severe BPD, who are also more likely to

require supplemental oxygen and positive pressure support. It can occur late, so a normal screening echocardiogram at 36 weeks PMA does not preclude the subsequent development of PAH.[64] Treatment of PAH includes use of supplemental oxygen to minimize hypoxemic events and to encourage growth[64,65] in addition to pharmacotherapy. The presence of PAH should be assessed when planning an oxygen wean in this patient population.

Conclusions

There have been many advances in care of patients with early and evolving BPD. There is a paucity of data regarding outpatient management of patients with established BPD and limited studies that assess such interventions beyond short-term effects. A suggested dosing/weaning schedule of the common medications used in out-patient therapy of patients with established BPD have been shown in Table 1. Further research is needed to improve our understanding of the role of currently used treatments on outcomes in a stable patient with established BPD.

REFERENCES

1. Baraldi E, Carraro S, Filippone M. Bronchopulmonary dysplasia: definitions and long-term respiratory outcome. *Early Hum Dev*. 2009;85:S1–S3.
2. McEvoy CT, Jain L, Schmidt B, Abman S, Bancalari E, Aschner JL. Bronchopulmonary dysplasia: NHLBI workshop on the primary prevention of chronic lung diseases. *Ann Am Thorac Soc*. 2014;11:S146–S153.
3. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723–1729.
4. Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics*. 2005;116:1353–1360.
5. Abman SH, Collaco JM, Shepherd EG, et al. Interdisciplinary care of children with severe bronchopulmonary dysplasia. *J Pediatr*. 2017;181:12–28.
6. Stoll BJ, Hansen NI, Bell EF, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA*. 2015;314:1039–1051.
7. Fanaroff AA, Stoll BJ, Wright LL, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. *Am J Obstet Gynecol*. 2007;196(147):e141–e148.
8. Van Marter LJ. Epidemiology of bronchopulmonary dysplasia. *Semin Fetal Neonatal Med*. 2009;14:358–366.
9. Bhandari A, Bhandari V. Pitfalls, problems, and progress in bronchopulmonary dysplasia. *Pediatrics*. 2009;123:1562–1573.
10. Adams EW, Harrison MC, Counsell SJ, et al. Increased lung water and tissue damage in bronchopulmonary dysplasia. *J Pediatr*. 2004;145:503–507.
11. Cotton R, Suarez S, Reese J. Unexpected extra-renal effects of loop diuretics in the preterm neonate. *Acta Paediatr*. 2012;101:835–845.
12. Slaughter JL, Stenger MR, Reagan PB. Variation in the use of diuretic therapy for infants with bronchopulmonary dysplasia. *Pediatrics*. 2013;131:716–723.
13. Blaisdell CJ, Troendle J, Zajice A. Acute responses to diuretic herapy in extremely low gestational age newborns: results from the prematurity and respiratory outcomes program cohort study. *J Pediatr*. 2018;197:42–47.
14. Kao LC, Warburton D, Sargent CW, Platzker AC, Keens TG. Furosemide acutely decreases airways resistance in chronic bronchopulmonary dysplasia. *J Pediatr*. 1983;103:624–629.
15. Engelhardt B, Elliott S, Hazinski TA. Short- and long-term effects of furosemide on lung function in infants with bronchopulmonary dysplasia. *J Pediatr*. 1986;109:1034–1039.
16. Rastogi A, Luayon M, Ajayi OA, Pildes RS. Nebulized furosemide in infants with bronchopulmonary dysplasia. *J Pediatr*. 1994;125:976–979.
17. Prabhu VG, Keszler M, Dhanireddy R. Dose-dependent evaluation of the effects of nebulized furosemide on pulmonary function in ventilated preterm infants. *J Perinatol*. 1998;18:357–360.
18. Bancalari E, Wilson-Costello D, Iben SC. Management of infants with bronchopulmonary dysplasia in North America. *Early Hum Dev*. 2005;81:171–179.
19. Christou H, Brodsky D. Lung injury and bronchopulmonary dysplasia in newborn infants. *J Intensive Care Med*. 2005;20:76–87.
20. Tin W, Wiswell T E. Adjunctive therapies in chronic lung disease: examining the evidence. *Semin Fetal Neonatal Med*. 2008;13:44–52.
21. Kao LC, Warburton D, Cheng MH, Cedeño C, Platzker AC, Keens TG. Effect of oral diuretics on pulmonary mechanics in infants with chronic bronchopulmonary dysplasia: results of a double-blind crossover sequential trial. *Pediatrics*. 1984;74:37–44.
22. Kao LC, Durand DJ, McCrea RC, Birch M, Powers RJ, Nickerson BG. Randomized trial of long term diuretic therapy for infants with oxygen dependent bronchopulmonary dysplasia. *J Pediatr*. 1994;124:772–781.
23. Stewart A, Brion LP, Ambrosio-Perez I. Diuretics acting on the distal renal tubule for preterm infants with (or developing) chronic lung disease. *Cochrane Datab Syst Rev*. 2011;9:CD001817.
24. Slaughter JL, Stenger MR, Reagan PB. Variation in the use of diuretic therapy for infants with bronchopulmonary dysplasia. *Pediatrics*. 2013;131:716–723.
25. Bhandari A, Chow U, Hagadorn JI. Variability in duration of outpatient diuretic therapy in bronchopulmonary dysplasia: a clinical experience. *Am J Perinatol*. 2010;27:529–535.
26. Palm K, Simoneau T, Sawicki G, Rhein L. Assessment of current strategies for weaning premature infants from supplemental oxygen in the outpatient setting. *Adv Neonatal Care*. 2011;11:349–356.
27. Baveja R, Christou H. Pharmacological strategies in the prevention and management of bronchopulmonary dysplasia. *Semin Perinatol*. 2006;30:209–218.
28. American Academy of Pediatrics. Committee on fetus and newborn and Canadian paedtric society, fetus and newborn committee. Post natal corticosteroids to treat or prevent chronic lung disease in preterm infants. *Pediatrics*. 2002;109:330–338.
29. Yeh TF, Chen CM, Wu SY, et al. Intratracheal administration of budesonide/surfactant to prevent bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2016;193:86–95.
30. Venkataraman R, Kamaluddeen M, Hasan SU, Robertson HL, Lodha A. Intratracheal administration of budesonide-surfactant in prevention of bronchopulmonary dysplasia in very low birth weight infants: a systematic review and meta-analysis. *Pediatr Pulmonol*. 2017;52:968–975.
31. Bassler D, Plavka R, Shinwell ES, et al. Early inhaled budesonide for the prevention of bronchopulmonary dysplasia. *N Engl J Med*. 2015;373:1497–1506.

32. Bassler D, Shinwell ES, Hallman M, et al. Long-term effects of inhaled budesonide for bronchopulmonary dysplasia. *N Engl J Med*. 2018;378:148–157.
33. Bauer J, Teufel U, Maser-Gluth C, Doege C. Effects of budesonide inhalation on energy expenditure, somatic growth and salivary cortisol levels in preterm infants with chronic lung disease. *Horm Res*. 2009;72:146–152.
34. Cole CH, Shah B, Abbasi S, et al. Adrenal function in premature infants during inhaled beclomethasone therapy. *J Pediatr*. 1999;135:65–70.
35. Nelin LD, Logan JW. The use of inhaled corticosteroids in chronically ventilated preterm infants. *Semin Fetal Neonatal Med*. 2017;22:296–301.
36. Baraldi E, Bonetto G, Zacchello F, Filippone M. Low exhaled nitric oxide in school-age children with bronchopulmonary dysplasia and airflow limitation. *Am J Respir Crit Care Med*. 2005;171:68–72.
37. Halvorsen T, Skadberg BT, Eide GE, Røksund O, Aksnes L, Øymar K. Charachteristics of asthma and airway hyperresponsiveness after premature birth. *Pediatr Allergy Immunol*. 2005;16:487–494.
38. Hennessy EM, Bracewell MA, Wood N, Wolke D, Costeloe K, Gibson A, Marlow N. EPI Cure Study Group. Respiratory health in pre-school and school age children following extremely preterm birth. *Arch Dis Child*. 2008;93:1037–1043.
39. Greenough A. Long term pulmonary outcomes of premature birth at less than 32 weeks of gestation. *Early Hum Dev*. 2013;89:S25–S27.
40. Beresford MW, Primhak R, Subhedar NV, Shaw NJ. Randomised double blind placebo controlled trial of inhaled fluticasone propionate in infants with chronic lung disease. *Arch Dis Child Fetal Neonatal Ed*. 2002;87:F62–F63.
41. Yuskel B, Greenough A. Randomised trial of inhaled steroids in preterm infants with respiratory symptoms at follow up. *Thorax*. 1992;47:910–913E.
42. Bhandari A, Schramm CS, Kimble C, Pappagallo M, Hussain N. Effect of a short course of prednisolone in infants with oxygen-dependent bronchopulmonary dysplasia. *Pediatrics*. 2008;121:e344–e349.
43. Slaughter JL, Stenger MR, Reagan PB, Jadcherla SR. Inhaled bronchodilator use for infants with bronchopulmonary dysplasia. *J Perinatol*. 2015;35:61–66.
44. Ng G, da Silva O, Ohlsson A. Bronchodilators for the prevention and treatment of chronic lung disease in preterm infants. *Cochrane Database of Syst Rev*. 2016;12:CD003214.
45. Fawke J, Lum S, Kirkby J, et al. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Respir Crit Care Med*. 2010;182:237–245.
46. Filbrun AG, Popova AP, Linn MJ, McIntosh NA, Hershenson MB. Longitudinal measures of lung function in infants with bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2011;46:369–375.
47. Korhonen P, Laitinen J, Hyodynmaa E, Tammela O. Respiratory outcome in school-aged, very-low-birth-weight children in the surfactant era. *Acta Paediatr*. 2004;93:316–321.
48. Vrijlandt EJ, Boezen HM, Gerritsen J, Stremmelaar EF, Duiverman EJ. Respiratory health in prematurely born preschool children with and without bronchopulmonary dysplasia. *J Pediatr*. 2007;150:256–261.
49. Lum S, Kirkby J, Welsh L, Marlow N, Hennessy E, Stocks J. Nature and severity of lung function abnormalities in extremely pre-term children at 11 years of age. *Eur Respir J*. 2011;37:1199–1207.
50. Gibson AM, Reddington C, McBride L, Callanan C, Robertson C, Doyle LW. Lung function in adult survivors of very low birth weight, with and without bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2015;50:987–994.
51. Landry JS, Chan T, Lands L, Menzies D. Long-term impact of bronchopulmonary dysplasia on pulmonary function. *Can Respir J*. 2011;18:265–270.
52. Fawke J, Lum S, Kirkby J, et al. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Respir Crit Care Med*. 2010;182:237–245.
53. Kim DK, Choi SH, Yu J, Yoo Y, Kim B, Koh Y. Bronchial responsiveness to methacholine and adenosine 5'monophosphate in preschool children with bronchopulomnoay dysplasia. *Pediatr Pulmonol*. 2006;41:538–548.
54. Morrow DK, Schilling D, McEvoy CT. Response to bronchodilators in very preterm infants with evolving bronchopulmonary dysplasia. *Res Rep Neonatol*. 2015;5:113–117.
55. Hysinger EB. Laryngomalacia, tracheomalacia and bronchomalacia. *Curr Probl Pediatr Adolesc Health Care*. 2018;48:113–118.
56. Panitch HB, Keklikian EN, Motley RA, Wolfson MR, Schidlow DV. Effect of altering smooth muscle tone on maximal expiratory flows in patients with tracheomalacia. *Pediatr Pulmonol*. 1990;9:170–176.
57. Moyer-Mileur LJ, Nielson DW, Pfeffer KD, Witte MK, Chapman DL. Eliminating sleep-associated hypoxemia improves growth in infants with bronchopulmonary dysplasia. *Pediatrics*. 1996;98:779–783.
58. Yeh J, McGrath-Morrow SA, Collaco JM. Oxygen weaning after hospital discharge in children with bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2016;51:1206–1211.
59. Trzaski JM, Hagadorn JI, Hussain N, Schwenn J, Wittenzellner C. Predictors of successful discontinuation of supplemental oxygen in very low-birth-weight infants with bronchopulmonary dysplasia approaching neonatal intensive care unit discharge. *Am J Perinatol*. 2012;29:79–86.
60. Saletti A, Stick S, Doherty D, Simmer K. Home oxygen therapy after preterm birth in Western Australia. *J Paediatr Child Health*. 2004;40:519–523.
61. Khetan R, Hurley M, Spencer S, Bhatt JM. Bronchopulmonary dysplasia within and beyond the Neonatal Unit. *Adv Neonatal Care*. 2016;16:17–25: quiz E1.
62. Mourani PM, Abman SH. Pulmonary hypertension and vascular abnormalities in bronchopulmonary dysplasia. *Clin Perinatol*. 2015;42:839–855.
63. Al-Ghanem G, Shah P, Thomas S, et al. Bronchopulmonary dysplasia and pulmonary hypertension: a meta-analysis. *J Perinatol*. 2017;37:414–419.
64. Abman SH, Hansmann G, Archer SL, et al. Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. *Circulation*. 2015;132:2037–2099.
65. Papoff C, Cerasaro C, Caresta E, Barbara C, Midulla F, Moretti C. Current strategies for treating infants with severe bronchopulmonary dysplasia. *J Matern Fetal Neonatal Med*. 2012;25:15–20.

XVIII SEMINARIO INTERNACIONAL

Avances en Pediatría Neonatal

7, 8, 9 y 10 de mayo 2019 | Centro de Extensión UC

7 mayo CURSOS PRE SEMINARIO
SIMPOSIO DE **REANIMACIÓN NEONATAL**
SIMPOSIO DE **HEMODINAMIA**

Información General

Organiza:
Departamento de Neonatología
División de Pediatría
Escuela de Medicina
Pontificia Universidad Católica de Chile

Directores:
Dra. Paulina Toso
Dr. José Luis Tapia
Dr. Javier Kattan
Dr. Jorge Fabres
Dr. Álvaro González

Comité Científico:
Dr. Alberto Estay
Dr. Matías Luco
Dra. Patricia Mena
Dr. Ricardo Uauy
Dra. Soledad Urzúa

Contenidos

- Problemas respiratorios del recién nacido.
- Evaluación y manejo hemodinámico.
- Reanimación neonatal.
- Nutrición
- Neurología Neonatal
- MBE en Neonatología
- Redes Neonatales
- Enfermería Neonatal
- otros

Expositores Internacionales

Dr. Eduardo Bancalari
University of Miami, Miami, **EEUU**

Dr. Peter Davis
University of Melbourne, Victoria, **Australia**

Dra. Camilia Martin
Harvard University, Boston, **EEUU**

Dr. Patrick McNamara
University of Iowa, **EEUU**

Dr. Steven Miller
University of Toronto, **Canadá**

Dr. Roger Soll
Vermont University, **EEUU**