microbiota.no

6th National Microbiota Conference

November 19, 2019

Radisson Blu Scandinavia Hotel

UiO : Institute of Clinical Medicine
University of Oslo

Oslo University Hospital

UiO : Life Science
University of Oslo

NOPSC
Norwegian PSC Research Center

Table of Contents

Program

Introduction and Welcome

0900	Registration and coffee	Johannes Hov and Marius Trøseid, OUH/UiO
0930	Introduction and welcome	

Special Topic: Implementing FMT in Clinical Studies and Clinical Practice

0935	Fecal Microbiota Transplantation: Clinical Experience, Regulation and Promises for the Future	Christian Hvaas, Århus
1015	Establishment of a Donor Biobank for Fecal Transplantation: A Real-Life Experience	Peter Holger Johnsen, UNN

Coffee Break 1035-1100

Special Topic Continues

1100	Experiences and Perspectives on Clinical Use of FMT	Michael Bretthauer, OUH/UiO
1120	Bacteriotherapy for Experimental Indications: the Example from Systemic Sclerosis	Anna Hoffmann-Vold, OUH
1140	Discussion	
1155	Update on Clinical Microbiota Research Networks	Johannes Hov and Marius Trøseid, OUH/UiO

Coffee Break 1155-1215

Open Abstract Session

1215	The Lung Microbiome of Stable COPD Is Not Associated with COPD Exacerbations	Elise Leiten, UiB
1230	A Study of the Airway Mycobiome in Obstructive Lung Disease Patients and Controls	Einar Martinsen, UiB
1245	16S V3-V4 MiSeq Sequencing Service at Norwegian Sequencing Centre	Gregor Gilfillan, NSC/OUH

Lunch 1300-1400

Open Abstract Session

1400	Interplay of Gut Microbiota and Immunodeficiency on Excess Metabolic Risk in HIV Infection	Beate Vestad, OUH/UiO
1415	Mucosal Microbiota in Newly Diagnosed Inflammatory Bowel Disease Patients and Its Relation to Treatment Escalation	Jonas Lindstrøm, AHUS
1430	Benchmarking the Novel Innovative Riptide Metagenome Protocol	Even Riiser, Cancer Registry of Norway
1445	A Targeted Approach to Microbiome Analysis	Morten Isaksen, Bio-Me

Coffee Break 1500-1515

Open Abstract Session

1515	Impact of Gut Microbiota on Child Growth – Analysis of a Randomized Nutrition Education Trial among Mother-Child Pairs in Rural Uganda	Prudence Atukunda, UiO
1530	Microbial Diversity and Clinical Outcomes in Recipients of Hematopoietic Stem Cell Transplantation: Results of a Nutritional Intervention Trial	Kristine Skaarud, OUH/UiO
1545	Low Fiber Intake is Associated with Gut Microbiota Alterations in Chronic Heart Failure	Christiane Mayerhofer, OUH/UiO

Closing

1600	Summary	Johannes Hov and Marius Trøseid, OUH/UiO
1610	Abstract award (Tore Midttvedt's Prize) and Closing Remarks	

Abstracts

The Lung Microbiome of Stable COPD Is Not Associated with COPD Exacerbations.

Elise O. Leiten[1], Rune Nielsen[1,2], Harald G. Wiker[1,3], Per S. Bakkea, Einar M. H. Martinsen[1], Christine Drengenes[1,2], Solveig Tangedal[1,2], Gunnar R. Husebø[1,2], Tomas M.L. Eagan[1,2] *[1]Dept. of Clinical Science, UiB, Norway; [2]Dept. of Thoracic Medicine, HUH, Norway; [3]Dept. of Microbiology, HUH, Norway.*

Background: Studies have pointed to an association between the lung microbiome at stable state COPD and frequency of COPD exacerbations, but findings show little consistency.

Materials and methods: We used bronchoscopically collected lung samples (bronchoalveolar lavage and protected specimen brushes) and oral wash from participants with stable COPD. For each subject a negative control sample of the used sample fluid was stored. DNA was extracted, before sequencing of the V3-V4 region of the 16S RNA gene with an Illumina MiSeq. Bioinformatic preprocessing of data in QIIME2 included denoising (DADA2), additional chimera removal (VSEARCH), filtering of low-abundant ASVs, and identification and filtration of contaminants (R package decontam). Taxonomy was assigned using a classifier trained on the HOMD database. ASVs not identified at phylum level were identified using NCBI BLAST. All non-bacterial taxa were filtered out. Fasttree2 was used to build a phylogenetic tree. Sequences were rarefied to sampling depth 1000 for alpha and beta diversity. Differential abundance was analysed with ANCOM and gneiss and with the R package MicrobiomeDDA.

Results: 105 subjects provided sufficient microbiome data and complete exacerbation follow-up for analysis. There were no clear trends of taxonomic distribution differences, no difference in alpha or beta diversity, and no differentially abundant taxa when subjects with and without exacerbations were compared.

Conclusion: In our material there was no clear association between the airway microbiome at baseline, and COPD exacerbations during follow-up.

A Study of the Airway Mycobiome in Obstructive Lung Disease Patients and Controls

Einar Marius H. Martinsen[1], Tomas Mikal L. Eagan[1,2], Elise O. Leitena, Ingvild Haaland[1,2], Gunnar R. Husebø[1,2], Walter Sanseverino[3], Andreu Paytuvi-Gallart[3], and Rune Nielsen[a,b]
[1]Dept. of Clinical Science, UiB, Norway; [2]Dept. of Thoracic Medicine, HUH, Norway: [3]Sequentia Biotech, Spain.

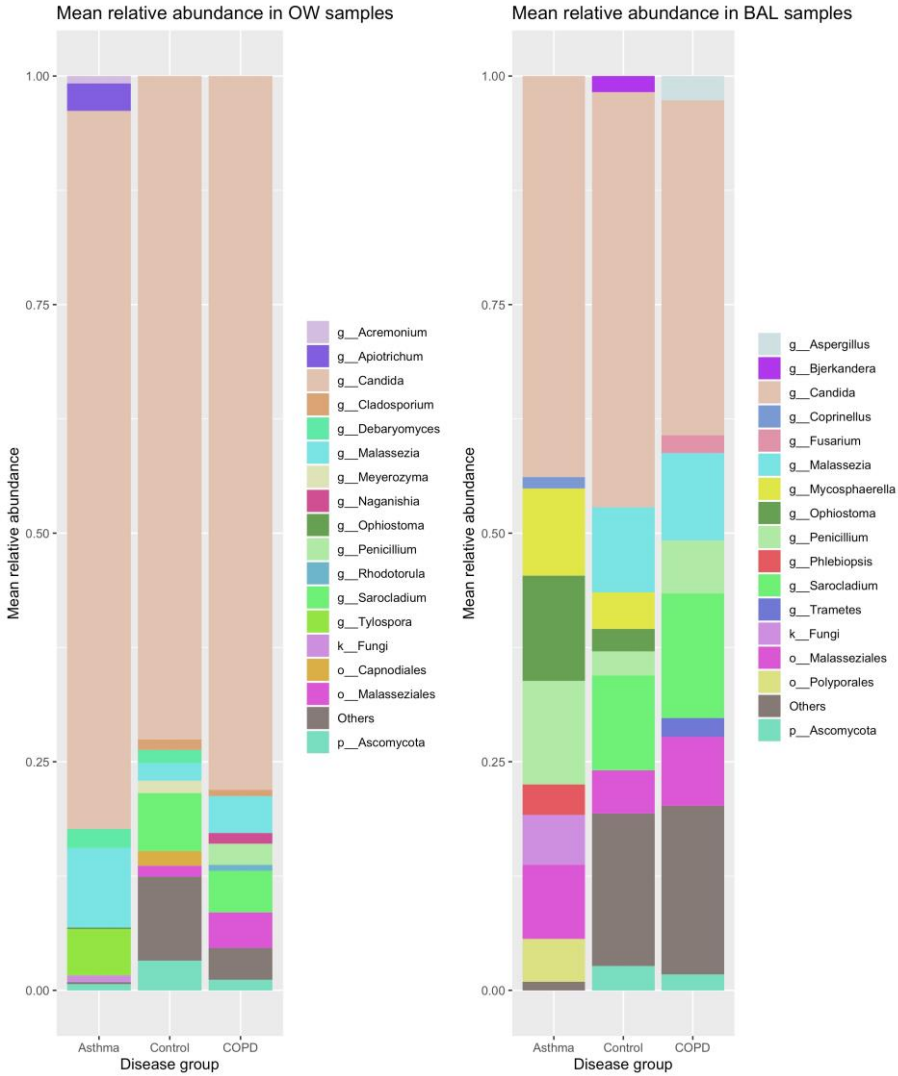
Background: We aimed to explore the role of the airway mycobiome in COPD and asthma patients compared to controls.

Methods: In addition to oral wash (OW) and negative control samples, bronchoalveolar lavage (BAL) was collected through a sterile catheter during bronchoscopies in the Bergen MicroCOPD study. We used QIIME2 and the R package decontam for bioinformatic analyses. UNITE databases were used to assign taxonomy.

Results: We obtained samples from 95 COPD and 13 asthma patients, and 100 healthy controls. 66% were ex-smokers, mean age was 66.3 years, and 60% of COPD patients, and 69% of asthma patients, used inhaled corticosteroids (ICS). After removal of contaminants and low-abundant taxa, 404 samples and 12x106 sequences remained. Candida was the most frequent genus in all groups in both OW and BAL (Fig 1) and differed between OW and BAL in both controls and COPD patients. α diversity differed between OW and BAL samples ($p<0.001$), but not by study group, sex, smoking status, or use of ICS in BAL samples. β diversity differed by sample type ($p=0.001$).

Conclusion: Candida was the most abundant fungal genus in BAL samples. Fungal alpha and beta diversity differed between OW and BAL, possibly indicating the existence of a respiratory mycobiome, but no difference was found between disease groups.

Fig.1:


Mean relative abundance in OW samples
Mean relative abundance
1.00
0.75
0.50
0.25
0.00
Asthma
Control
COPD
Disease group
g__Acremonium
g__Apiotrichum
g__Candida
g__Cladosporium
g__Debaryomyces
g__Malassezia
g__Meyerozyma
g__Naganishia
g__Ophiostoma
g__Penicillium
g__Rhodotorula
g__Sarocladium
g__Tylospora
k__Fungi
o__Capnodiales
o__Malasseziales
Others
p__Ascomycota
Mean relative abundance in BAL samples
Mean relative abundance
1.00
0.75
0.50
0.25
0.00
Asthma
Control
COPD
Disease group
g__Aspergillus
g__Bjerkandera
g__Candida
g__Coprinellus
g__Fusarium
g__Malassezia
g__Mycosphaerella
g__Ophiostoma
g__Penicillium
g__Phlebiopsis
g__Sarocladium
g__Trametes
k__Fungi
o__Malasseziales
o__Polyporales
Others
p__Ascomycota

16S V3-V4 MiSeq sequencing service at Norwegian Sequencing Centre

Teodora Ribarska, Arvind Y.M. Sundaram & Gregor D. Gilfillan

Medical Genetics, Ullevål University Hospital, Oslo

To meet demand, we have launched a 16S amplification and sequencing service. Users of the service need supply only purified DNA, and can choose to receive data in FASTQ, QIIME or MOTHUR analysis formats. We employ the Fadrosh et al[1] procedure, which employs staggered primers to increase sequencing read diversity and gives excellent results with only 10% PhiX blend, thus delivering close to maximum data output. Sequencing is performed on the Illumina MiSeq with 300 bp paired end reads. Positive and negative controls are included in each amplification. Prices for sample batches of ⩽24, ⩽48, ⩽94 and ⩽190 are 30, 35, 40, and 50,000 NOK (including MVA and a MiSeq run). For more information, please contact ous-seq@sequencing.uio.no

[1] An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. Microbiome vol. 2, Article number: 6 (2014)

Interplay of Gut Microbiota and Immunodeficiency on Excess Metabolic Risk in HIV Infection

Beate Vestad*[2,3] & **Marco Gelpi***; Simen Hyll Hansen[2,3,4]; Kristian Holm[2,3,4]; Ninna Drivsholm[1]; Alexandra Goetz[3,4]; Nicolaj Søren Kirkby[5]; Birgitte Lindegaard[6,7]; Anne-Mette Lebech[8]; Hedda Hoel[2,3,9]; Annika E Michelsen[2,3]; Thor Ueland[2,3]; Jan Gerstoft[1]; Jens Lundgren1[,10]; Johannes Roksund Hov[2,3,4,11]; Susanne Dam Nielsen[1]; Marius Trøseid[2,3,12] *These authors have equally contributed to the manuscript

[1]Viro-immunology Research Unit, UIC; [2]Institute of Clinical Medicine, UiO; [3]RIIM, OUH; [4]NoPSC Research Center, OUH; [5]Dept of Microbiology, UIC; [6]Center for Inflammation & Metabolism, UIC; [7]Dept of Pulmonary and Infectious diseases, NH; [8]Dept of Infectious Diseases, CUH; [9]Medical Dept, LDH, Norway; [10]Dept of Infectious Diseases UIC; [11]Section of Gastroenterology OUH; [12]Section of clinical immunology and infectious diseases, OUH.

Background: We aimed to define HIV-associated microbiota alterations, independent of sexual behaviour and demographic confounders, in order to assess a possible impact on excess metabolic risk, an important cause of morbidity and mortality in HIV infection.

Methods: Bacterial 16S rRNA analyses were performed on stool samples from 405 HIV-infected and 111 uninfected participants of the COCOMO study. Individuals were stratified according to sexual behaviour: men who have sex with men (MSM) and non-MSM, a major confounder in previous microbiota studies.

Results: After adjusting for demographic confounders and sexual behaviour, increased abundance of the bacterial clades Gammaproteobacteria and Desulfovibrionaceae and decrease in several Clostridia were identified as HIV-associated traits and defined the HIV-related microbiota index. Elevated HIV-related microbiota index was associated with 2-fold excess risk of metabolic syndrome, driven by increase in Desulfovibrionaceae and decrease in several Clostridia. This association was accentuated (5-fold excess risk) in individuals with previous severe immunodeficiency. Elevated HIV-related microbiota index was associated with 30 cm2 (adjusted β 30.8 [3.1; 58.5]) larger visceral adipose tissue area in individuals with previous severe immunodeficiency, but not in those without (p-interaction 0.013).

Conclusion: The HIV-related microbiota was associated with increased risk of metabolic syndrome and visceral fat accumulation, particularly in individuals with previous severe immunodeficiency, driven by increased Desulfovibrionaceae and lower abundance of several Clostridia. As experimental data have shown that outgrowth of Desulfovibrio and reduction in Clostridia may trigger metabolic alterations in immunodeficient mice, our findings suggest potential interplay between the HIV-related microbiota, immune dysfunction and excess metabolic risk.

Mucosal Microbiota in Newly Diagnosed Inflammatory Bowel Disease Patients and Its Relation to Treatment Escalation

J. C. Lindstrøm[1,2], S. Vatn[1,2], A E. F. Moen[1], T. M. Tannæs[1], P. Ricanek[1], T.E. Detlie[1,2], J. Jahnsen[1,2], M.H. Vatn[1], and the IBD-Character consortium.
[1]Akershus University Hospital, Norway; [2]University of Oslo, Norway.

Background: Inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory diseases with a relapsing course, although some patients experience sustained disease activity. Patients respond differently to the medical treatment given. Predicting response to treatment for patients at time of diagnosis could lower the time span before optimal treatment effect is achieved, hence sparing patients a prolonged period of illness. Development of biomarkers allowing insights into pathogenesis and prognosis is needed. Microbiota signatures could be such a biomarker, predicting disease course and treatment response, and become a tool for personalized medicine.

Methods: Mucosal biopsies were collected during colonoscopy from 118 newly diagnosed IBD patients (63 CD, 55 UC) in the EU IBD-character cohort. The mucosal microbiota composition and potential activity were assessed using 16S DNA and cDNA amplicon sequencing, detecting the total and active microbiota, respectively. Disease course and medical treatment were followed, up to 5 years after inclusion. Time to advanced treatment escalation, defined as need for biological therapies or surgery, was analyzed using cox regression analysis.

Results: 11% (6/55) of the UC and 41% (26/63) of the CD patients escalated. Higher alpha diversity in the total microbiota from both inflamed and non-inflamed mucosa had a significant protective association to escalation in UC patients (HR $\approx$ 0.37, p$\leqslant$ 0.03). This was not found in the active microbiota nor in CD. Associations between the need for escalating treatment and lower abundances of several Firmicutes families, Bifidobacteriaceae and Bacteroidaceae at time of diagnosis were observed for the total and the active microbiota in both UC and CD.

Conclusion: Increased diversity and higher abundance of Firmicutes, Bifidobacteriaceae and Bacteroidacea in the gut mucosal microbiota at time of diagnosis are associated with a less aggressive disease progression.

A Targeted Approach to Microbiome Analysis

Warren G. Flood[1], Jean-Marc Billod[1], Simen Russnes[1], Satyasree Kuraganti[1], Maria Maseng[1], Johannes Hov[2], Martin Kummen[2], Pius Brozska[3], Tibor Füle[3], Camilla Ulekleiv[3], Christian Jonasson[4], Kristian Hveem[4], Morten L. Isaksen[1]

[1]Bio-Me AS, Oslo, Norway, [2]Oslo University Hospital, Oslo, Norway, [3]Thermo Fisher Scientific, USA, [4]NTNU/HUNT Research Center, Norway.

In order to circumvent some or the shortcomings of NGS (speed, costs and resolution), Bio-Me have - in collaboration with Thermo Fisher - developed Precision Microbiome Profiling (PMP™). PMP is a targeted approach to microbiome analysis which provides rapid, detailed, high throughput and accurate quantification of bacterial genomes in the sample.

We have used PMPTM to analyze 1000 samples from a healthy population from the HUNT 4 study. This is the largest study comprising a standardized method of a large cohort and provides important insight into what constitutes a healthy microbiome. Some initial analysis of these results will be shared, together with some basic lessons learnt in setting up a high throughput microbiome analysis platform.

Benchmarking the Novel Innovative Riptide Metagenome Protocol

Even S Riiser[1], Harri Kangas[2], Pekka Ellonen[2], Paula Berstad[1], Trine B Rounge[1,3]
[1]Cancer Registry of Norway; [2]Institute for Molecular Medicine Finland; [3]Dept. of Informatics, UiO, Norway

Metagenomic shotgun sequencing provides high-resolution taxonomic profiles and gene content information, the latter often making it the preferred method in microbiome analysis. Nevertheless, production of metagenomes is costly, largely due to expensive library preparation. In addition, the current generation of Illumina short-read sequencers is prone to index mismatching, partially mitigated by the use of unique dual indices. We have benchmarked the novel innovative library preparation protocol Riptide (iGenomX) versus the well-established Nextera XT (Illumina) protocol for use on long-term stored fecal samples with low DNA input. Riptide includes linear amplification with random primers and dideoxy nucleotide-induced self-termination, avoiding DNA fragmentation. The random primers may also act as unique molecular identifiers. We aimed to determine how accurate the protocols reproduce a community standard, compare taxonomical profiles in the gut samples and characterize protocol-specific differences after sequencing with the Illumina HiSeq sequencing platform. We find that both protocols reproduce the community standard with high accuracy. However, without any option for unique dual indexing, the Riptide protocol is prone to index mismatching, and its random primers are not suitable as unique molecular identifiers in metagenome analysis. In conclusion, Riptide produces equally good metagenomic profiles as Nextera XT at a lower cost, but large-scale application of the protocol is not advised in metagenomic analysis until the index mismatching issue is resolved.

Impact of Gut Microbiota on Child Growth – Analysis of a Randomized Nutrition Education Trial among Mother-Child Pairs in Rural Uganda

Prudence Atukunda[1], Alex Wacoo[2,3], Grace Muhoozi[4], Ane Westerberg[5], Remco Kort[2], Per Ole Iversen[1,6]

1Dept. Nutrition, UiO, Norway; [2]VU Univ Amsterdam, Dept. Molecular Cell Biology, The Netherlands; [3]Dept. Biochemistry, Makerere University, Uganda; [4]Dept. Human Nutrition and Home Economics, Kyambogo Univ, Uganda; [5]Institute of Health Sciences, Kristiania Univ College, Norway; [6]Dept Hematology, OUH, Norway.

Background: In Uganda about 1/3 of children<5 years are stunted (low height-for-age) indicating chronic undernutrition. Studies suggest a role for microbiota in child growth1. Reportedly, aflatoxin (that contaminates agricultural products) causes stunting.2 We examined if microbiota abundance, -composition and -function related to aflatoxin, differed between the intervention- and control children whose mothers participated in an education trial3.

Methods: We performed a randomized education trial regarding nutrition/sanitation/hygiene among mothers of 6-8 months children in Uganda3. Data was available when the children were 20-24 and 36 months. WHO anthropometry assessed growth. Microbiota abundance and composition were determined after 16S rRNA gene amplicon sequencing of feces.

Results: The intervention had no significant effect on growth at 20-24 months, but less growth faltering was noted at 36 months compared with controls. No significant difference between groups was found for microbiota abundance or composition at either time point. Diversity of microbiota increased from 20-24 to 36 months. Transition from autochtonous to allochtonous Lactobacillus species in the microbiota was found. There were no differences ($p>0.05$) between urine aflatoxin levels in stunted compared to non-stunted. Notably, Lactobacillus species, known to degrade aflatoxin, were inversely correlated with height and more abundant in stunted compared to non-stunted at both time points.

Conclusions: The maternal intervention had no impact on microbiota composition or abundance in their children up till 3 years. We found no benefits of Lactobacillus on stunting.

References: 1: Blanton et al. Science 2016; 351. 2: Lombard Ann Nutr Metab 2014; 64: Suppl 2: 42. 3: Muhoozi et al. Matern Child Nutr 2018;14: e12527

Microbial diversity and clinical outcomes in recipients of hematopoietic stem cell transplantation: results of a nutritional intervention trial

K J Skaarud[1,2], J R Hov[3,4,5], M Trøseid[4,6], M Kummen[5], S H Hansen[5], A Bye[7,8], V Paulsen[3], K E A Lundin[3,4,9], G E Tjønnfjord[1,4,10], P O Iversen[1,2]

[1]Department of Hematology, OUH, Norway; [2]Dept. of Nutrition, UiO, Norway; [3]Section of Gastroenterology, OUH, Norway; [4]Institute of Clinical Medicine, UiO, Norway; [5]Norwegian PSC Research Center and Research Institute of Internal Medicine, OUH, Norway; [6]Section of Clinical Immunology and Infectious Diseases, OUH, Norway; [7]European Palliative Care Research Centre, [8]Department of Nursing and Health Promotion, OsloMet, Norway; [9]K.G.Jebsen Centre for Coeliac Disease Research, UiO, Norway; [10]K.G.Jebsen Centre for B cell malignancies, UiO, Norway.

Introduction: Loss of intestinal bacterial diversity has been associated with worsened survival and more acute graft-versus-host disease (aGVHD) among recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Whether nutritional support can modify these associations is not known. We here used data from a nutritional intervention trial to examine the effect on gut microbiota and its association with 1-year mortality and aGVHD.

Method: We included a subset of adult patients undergoing allo-HSCT enrolled in a two-armed RCT with optimized energy and protein intake (n=23) compared to controls (n=24) (1). Stool microbiota profile was determined by sequencing of the V3-V4 region of the 16S rRNA gene.

Results: Stool samples were collected at baseline and 3 weeks after transplantation. The intervention and control groups had similar microbiota profiles at baseline and at 3 weeks and the groups were thus merged for further analysis. There was a major reduction in measures of intra-individual (alpha) diversity from baseline to 3 weeks ($p<0.0001$). Furthermore, at 3 weeks, alpha diversity (observed OTUs and Shannon index) was lower in the patients dying during the first year ($p=0.0075$ and 0.024) than in survivors, while the baseline diversity was not associated with mortality. Loss of the genus Blautia from baseline to 3 weeks ($p=0.047$), as well as low abundance at 3 weeks ($p=0.05$), were associated with 1-year mortality. No associations were observed between aGVHD and microbial diversity.

Conclusion: Reduced microbial diversity and low abundance of Blautia at 3 weeks post-HSCT was associated with 1-year mortality. Nutritional intervention did not appear to influence gut microbiota composition in this patient population.

1. Skaarud et al. Clin Nutr ESPEN. 2018;28:59-66.

Low Fiber Intake is Associated with Gut Microbiota Alterations in Chronic Heart Failure

Cristiane C. K. Mayerhofer*[1,2,3,4], Martin Kummen*[2,4,5], Kristian Holm[2,3,4,5], Kaspar Broch[1], Ayodeji Awoyemi[3,6,7], Beate Vestad[2,3], Christopher Storm-Larsen[2,3,5], Ingebjørg Seljeflot[3,6,7], Thor Ueland[2,3,4], Pavol Bohov[8], Rolf K. Berge[8,9], Asbjørn Svardal[8,9], Lars Gullestad[1,3,7], Arne Yndestad[2,3,4,7], Pål Aukrust[2,3,4,10], Johannes R. Hov†[2,3,4,5,11] and Marius Trøseid†[2,3,10].

*[1]Dept. of Cardiology, OUH; [2]RIIM, OUH Norway; [3]Inst. of Clinical Medicine, UiO; [4]K. G. Jebsen Inflammation Research Centre, UiO; [5]Norwegian PSC Research Center, OUH; [6]Center for Clinical Heart Research, OUH; [7]Center for Heart Failure Research, UiO; [8]Dept. of Clinical Science, UiB; [9]Dept. of Heart Disease, HUH; [10]Section of Clinical Immunology & Infectious diseases, OUH; [11]Section of Gastroenterology, OUH. *†Shared*

Aims: Recent reports have suggested that patients with HF have an altered gut microbiota composition; however, associations with diet remain largely uninvestigated. We aimed to explore differences in the gut microbiota between patients with heart failure (HF) and healthy controls, focusing on associations with diet and disease severity.

Methods and results: The microbiota composition of two independent, cross-sectional cohorts (discovery, n = 40 and validation, n = 44) of patients with systolic HF and healthy controls (n = 266) was characterised by sequencing of the bacterial 16S rRNA gene. The overall microbial community (beta diversity) differed between patients with HF and healthy controls in both cohorts ($P<0.05$). Patients with HF had shifts in the major bacterial phyla, resulting in a lower Firmicutes/Bacteroidetes (F/B)-ratio than controls ($P = 0.005$). Patients reaching a clinical endpoint (heart transplant or death) had lower bacterial richness and lower F/B-ratio than controls ($P<0.01$). Circulating levels of trimethylamine-N-oxide were associated with meat intake ($P = 0.016$), but not with gut microbiota alterations in HF. Low bacterial richness and low abundance of several HF-associated genera in the Firmicutes phylum were associated with low fiber intake.

Conclusions: The gut microbiota composition in chronic HF was characterised by decreased F/B ratio and reduced bacterial diversity associated with clinical outcome. The gut microbiota alterations in HF were partly related to low fiber intake, emphasising the importance of including dietary data as covariates in future studies. Our data, if replicated, could provide rationale for targeting the gut microbiota in HF with high-fiber diet.

Save the date…

7th National Microbiota Conference

November 16, 2020

Radisson Blu Scandinavia Hotel

Questions or comments: post@microbiota.no