L-ISSN 0539-6115
ISSN 1665-1146

Boletín Médico del Hospital Infantil de México

Hospital Infantil de México Federico Gómez
Instituto Nacional de Salud

www.bmhim.com bolmedhospinfantmex@gmail.com
Vol. 78 • Núm. 3 • Mayo – Junio 2021

Permanyer México
www.permanyer.com

Nestlé Nutrition Institute

LÍDER MUNDIAL EN EDUCACIÓN EN NUTRICIÓN BASADA EN CIENCIA

Regístrese en:

NNILATAM.ORG

Y OBTENGA ACCESO A CONTENIDO ACTUALIZADO QUE LO APOYARÁ EN SU PRÁCTICA DIARIA.

#NutriciónParaExpertos

Síganos en Facebook

/NestleNutritionInstituteLATAM

Material exclusivo para el profesional de la salud.
La Lactancia Materna es lo mejor para los bebés y deberá de continuarse el mayor tiempo posible.

Nestlé Baby & me Medical

PRÓXIMAMENTE

Material exclusivo para el profesional de la salud.
La Lactancia Materna es lo mejor para los bebés y deberá de continuarse el mayor tiempo posible.

Boletín Médico del Hospital Infantil de México

L-ISSN: 0539-6115
ISSN: 1665-1146

Incluida en/Indexed in:
PubMed/Medline, Emerging Source Citation Index (ESCI), Scielo, Scopus, Latindex, Embase, EBSCO y DOAJ

www.bmhim.com

Vol. 78 • Número 3 • Mayo-Junio 2021

Hospital Infantil de México Federico Gómez
Instituto Nacional de Salud

Permanyer México
www.permanyer.com

PERMANYER
www.permanyer.com

Permanyer
Mallorca, 310 – Barcelona (Cataluña), España – permanyer@permanyer.com

Permanyer México
Temístocles, 315
Col. Polanco, Del. Miguel Hidalgo – 11560 Ciudad de México
Tel.: (044) 55 2728 5183 – mexico@permanyer.com

www.permanyer.com

TCF Impreso en papel totalmente libre de cloro

Este papel cumple los requisitos de ANSI/NISO Z39.48-1992 (R 1997) (Papel Permanente)

Edición impresa en México
ISSN: 0539-6115
Ref.: 6273AX203

Boletín Médico del Hospital Infantil de México

Vol. 78 • Núm. 3 • Mayo-Junio 2021 www.bmhim.com *Indexada en Scopus y MEDLINE*

Contenido

Artículos de revisión

Artículos de investigación

Casos clínicos

Boletín Médico del Hospital Infantil de México

Vol. 78 • No. 3 • May-June 2021 www.bmhim.com *Indexed in Scopus and MEDLINE*

Contents

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

REVIEW ARTICLE

Molecular and cellular markers for measurable residual disease in acute lymphoblastic leukemia

Gerardo Juárez-Avendaño[1#], Nereida Méndez-Ramírez[2#], Nuria C. Luna-Silva[3], Victor A. Cruz-Hernández[4], David Gómez-Almaguer[2], Rosana Pelayo[5], and Juan C. Balandrán[1,6]**

[1]Laboratorio Juárez, Medicina de Laboratorio Clínico de Alta Especialidad, Biología Molecular e Investigación Clínica, Oaxaca de Juárez, Oaxaca, Mexico; [2]Hospital Universitario de la Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico; [3]Servicio de Hematología, Hospital de la Niñez Oaxaqueña Doctor Guillermo Zárate Mijangos, Secretaría de Salud, Oaxaca de Juárez, Oaxaca, Mexico; [4]Servicio de Hematología, Hospital Dr. Aurelio Valdivieso, Secretaría de Salud, Oaxaca de Juárez, Oaxaca, Mexico; [5]Centro de Investigación Biomédica de Oriente, Instituto Mexicano del Seguro Social Delegación Puebla, Metepec, Puebla, Mexico; [6]Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Department of Cell Biology, Albert Einstein College of Medicine, New York, USA

 # Equal contribution

Abstract

Acute leukemia is the leading cause of death in children worldwide, particularly in developing countries where the growing number of cases with unfavorable prognosis and high risk of early relapse have positioned pediatric cancer as a priority. The late and imprecise diagnosis, malnutrition and unfavorable environmental conditions, and toxicity-associated therapy are some of the factors that compromise the success of the treatment and affect survival rates in vulnerable regions. An early and exhaustive classification of malignant neoplasms at the clinical debut and the proper follow-up of treatment's response constitute one of the most powerful prognostic factors. Remarkably, the ultrasensitive detection of residual and relapse clones that determine the minimal/measurable residual disease (MRD) has been a milestone in the comprehensive management of hematologic malignancies that favorably improve the complete remission cases. In this review, we discuss the scientific and technological advances applied to laboratory diagnosis in MRD determination: from the multiparametric immunophenotyping to next-generation sequencing and cytomics. As a result of multidisciplinary research in the main concentration oncology centers and laboratories, residual leukemia detection strategies that combine molecular analysis and cellular markers are recommended as the most valuable tools, making them the paradigm for stratification campaigns in vulnerable regions.

Keywords: *Acute leukemia. Minimal/measurable residual disease. Flow cytometry. Polymerase chain reaction. Next-generation sequencing. Bone marrow.*

Marcadores celulares y moleculares para la enfermedad residual medible en la leucemia linfoblástica aguda

Resumen

La leucemia aguda es la principal causa de muerte por enfermedad en la población infantil mundial, en particular en los países con economías en desarrollo, donde el creciente número de casos con pronóstico desfavorable y riesgo de recaídas tempranas ha posicionado a esta enfermedad como una prioridad de salud. El diagnóstico tardío y de baja precisión, la

Correspondence:
*Juan Carlos Balandrán
E-mail: jcbalandran@gmail.com
*Rosana Pelayo
E-mail: rosana.pelayo@imss.gob.mx

Date of reception: 13-06-2020
Date of acceptance: 20-07-2020
DOI: 10.24875/BMHIM.20000155

Available online: 08-06-2021
Bol Med Hosp Infant Mex. 2021;78(3):159-170
www.bmhim.com

ausencia de condiciones favorables de alimentación y entorno ambiental, así como la toxicidad asociada a la terapia, son algunos de los factores que condicionan el éxito del tratamiento y afectan las tasas de supervivencia en las regiones más vulnerables. La clasificación temprana y exhaustiva del tumor maligno en la presentación clínica y durante el seguimiento de respuesta al tratamiento es uno de los más poderosos factores pronósticos. En especial, la detección ultrasensible de clonas residuales y reemergentes que determinan la enfermedad residual mínima medible ha sido un hito en el manejo integral de las neoplasias hematológicas y ha impactado favorablemente en las cifras de remisión completa. En esta revisión se comentan los avances científicos y tecnológicos aplicados al diagnóstico de laboratorio y a la determinación de la enfermedad residual mínima: desde la inmunofenotipificación multiparamétrica hasta la secuenciación y la citómica de última generación. Como resultado de las investigaciones multidisciplinarias en los principales centros oncológicos de concentración y los laboratorios de clase mundial, las estrategias de detección de la leucemia residual que combinan análisis moleculares y marcadores celulares han sido recomendadas como las de mayor utilidad, por lo que son el paradigma para las campañas de estratificación en las regiones vulnerables.

Palabras clave: *Leucemia aguda. Enfermedad mínima/medible residual. Citometría de flujo. Reacción en cadena de la polimerasa. Secuenciación masiva de siguiente generación. Médula ósea.*

Introduction

Acute leukemia (AL) is the leading cause of death among children in Mexico and the rest of the world[1]. A new epidemiological health metric reflecting global tumor burden through the years lost due to disease or disability, and considering the life expectancy of each region, has placed Latin America as one of the regions where children lose more years of life due to leukemia[2]. This type of neoplasm starts and progresses in the bone marrow, the tissue where hematopoiesis occurs, with the resulting imbalance in the formation of all blood cell types due to tumor growth[3]. Pediatric AL can occur in the lymphoid hematopoietic lineage, where B-cell precursor acute lymphoblastic leukemia (B-ALL) predominates, followed by the less frequent but very high-risk types T-cell precursor acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML) of myeloid origin. Thanks to multi-institutional research programs, new conditions have been discovered recently, such as leukemia of ambiguous lineage, early T-cell precursor (ETP) leukemia, and Philadelphia chromosome-like (+) leukemia (Ph-like ALL). The knowledge of their complete identity results from the morphological analysis, karyotyping, immunophenotyping, chromosomal aberrations, transcriptomic analysis, and detection of mutations. Moreover, several clinical subgroups are now recognizable due to proteomic analysis of the surface of leukemic blasts and genomic studies that allow identifying the main differences between malignant clones and normal cell populations, revealing the high intra- and inter-tumor heterogeneity that characterizes the pathobiology of this group of diseases[4,5].

In this review, we will focus on the leading cellular and molecular markers of utility as a guide for the diagnosis and tracking of minimal/measurable residual disease (MRD) by the latest generation of multiparametric flow cytometry (more than eight colors) and by molecular techniques such as quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR), digital PCR (dPCR), massive next-generation sequencing (NGS), as well as their prognostic value in clinical practice.

Etiopathology of acute leukemia

The uncontrolled proliferation of oligoclonal precursors (lymphoid or myeloid) is the common feature of these diseases. According to Lapidot et al., a cell subpopulation with stemness properties—cancer stem cells—in adult myeloid leukemia gives rise to and maintains the tumor load when transplanted into immunodeficient mice[6]. In contrast, Ie Visuer et al. reported that this does not appear to be the case in lymphoblastic leukemia, as stemness has no obvious phenotype[7], but the existence of functional leukemia-initiating cells (LICs) has been extensively documented[8,9]. Although the etiology of the disease is still uncertain and variable, the existence of cells that acquire stemness properties to establish and maintain the disease is undoubted. In investigating the origin, the differentiation pathways involved, and clinical diagnosis, flow cytometry has been fundamental for determining the compromised lineage, identifying the aberrant expression of specific markers and tracing them throughout the disease.

Moreover, hematopoietic differentiation maps have been constructed through this tool and functional assays *in vitro* and *in vivo*, and new markers defining the function of progenitor cells in health and disease

have been discovered. Thus, the compartment that enriches stem and progenitor cell populations is $CD34^{+}CD38^{-}$ with the absence of mature lineage markers (Lin-)[3]. The high frequency of $CD34^{+}CD38^{-}$ cells at diagnosis has been associated with unfavorable outcomes and increased risk of relapse[10]. Under leukemic conditions, the normal progenitor compartment is numerically and functionally reduced, causing severe pancytopenia that clinically includes anemia, recurrent infections, and petechiae[3,11]. However, it is still a challenge to distinguish LICs from healthy hematopoietic stem cells phenotypically.

The competition between leukemic growth and normal cell development is under intense investigation. Among the most important findings is the tumor microenvironment and the remodeling of normal niches, that is, the sites where stem cells and progenitors inhabit[12]. It is suggested that leukemic clones produce cytokines, inflammatory factors[13], and exosomal microvesicles[14] containing micro RNAs and other products. These cell products drive the "conditioning" of normal niches and form hostile sites for normal clones favoring their escape or depletion while creating optimal niches for the proliferation of malignant clones[12].

Clinical diagnosis and laboratory evidence

The oncologic treatment depends on the diagnosis, which should be comprehensive and include morphologic criteria, karyotyping, immunophenotyping, and the presence of confirmed genetic alterations. According to the World Health Organization (WHO), bone marrow aspirates should be used for leukemia diagnosis (Fig. 1). However, on certain occasions, the diagnosis can be implemented in peripheral blood as well[15]. Other criteria such as age, leukocyte count, evidence of infiltration to other organs, and early response to therapy help identify standard risk from high-risk patients.

The lineage suspicion is clarified by immunophenotyping, which should be initiated with a robust strategy to detect at least whether the affected lineage is lymphoid (B or T) or myeloid. If morphology yields more data on the myeloid precursor type, subsequent antibody combination or characterization panels should be based on these aspects to avoid performing lymphoid panels on clearly myeloid leukemia and vice versa[16]. The most commonly used antigens for the identification of B-cell lymphoid leukemia are CD19 or cytoplasmic CD79a (cyCD79a), while for T-lineage leukemia are surface CD3 (smCD3), cytoplasmic (cyCD3), and CD7, as well as the absence of antigens associated with the opposite lineage. On screening panels, myeloid leukemia is identified by the absence of the previously mentioned lymphoid antigens and the presence of myeloperoxidase (MPO) or some myeloid membrane antigens, including CD33, CD14, or CD13. In normal hematopoiesis, the common leukocyte antigen CD45 is acquired with maturation; thus, its low expression or absence is associated with more than 90% of AL cases and allows a clear identification of immature populations. The CD34 antigen, associated with stem cells and hematopoietic progenitors, is widely used in various diagnostic panels and denotes the level of (in) differentiation of leukemic blasts. In parallel, the search for translocations is usually performed routinely through commercial kits that can detect up to 28 leukemia-associated translocations: del1 (p32) (STIL-TAL1), t(1;11) (p32;q23) (MLL-EPS15), t(1;11) (q21;q23) (MLL-MLLT11), t(1;19) (q23;p13) (TCF3-PBX1), t(3;5) (q25;q34) (NPM1-MLF1), t(3;21) (q26;q22) (RUNX1-MECOM), t(4;11) (q21;q23) (MLL-AFF1), t(5;12) (q33;p13) (ETV6-PDGFRB), t(5;17) (q35;q21) (NPM1-RARA), t(6;9) (p23;q34) (DEK-NUP214), t(6;11) (q27;q23) (MLL-MLLT4), t(8;21) (q22;q22) (RUNX1-RUNX1T1), t(9;9) (q34;q34) (SET-NUP214), t(9;11) (p22;q23) (MLL-MLLT3), t(9;12) (q34;p13) (ETV6-ABL1), t(9,22) (q34;q11) (BCR-ABL1), t(10;11) (p12;q23) (MLL-MLLT10), t(11;17) (q23;q21) (MLL-MLLT6), t(11;17) (q23;q21) (ZBTB16-RARA), t(11;19) (q23;p13.1) (MLL-ELL), t(11;19) (q23;p13.3) (MLL-MLLT1), t(12;21) (p13;q22) (ETV6-RUNX1), t(12;22) (p13;q11) (ETV6-MN1), t(15;17) (q24;q21) (PML-RARA), inv(16) (p13;q22) (CBFB-MYH11), t(16;21) (p11;q22) (FUS-ERG), t(17;19) (q22;p13) (TCF3-HLF), and t(X;11) (q13;q23) (MLL-FOXO4)[17].

Only 20% of B-lineage leukemia and 60% of myeloid leukemia have common translocations[18]. However, more than 200 aberrations associated with this group of diseases are being explored through massive sequencing, real-time quantitative reverse transcription PCR (RT-qPCR), and digital PCR strategies, although they are only available for research. Notably, no other prognostic factor has been described with such a high significance as the ultrasensitive detection of MRD for AL through high-resolution technological systems. In addition, sensitivity is a highly relevant subject that involves technological aspects, reproducible protocols, systematized analysis strategies, and high technical resolution power. We describe the main benefits of several technologies applied to the diagnosis and evaluation of residual leukemia.

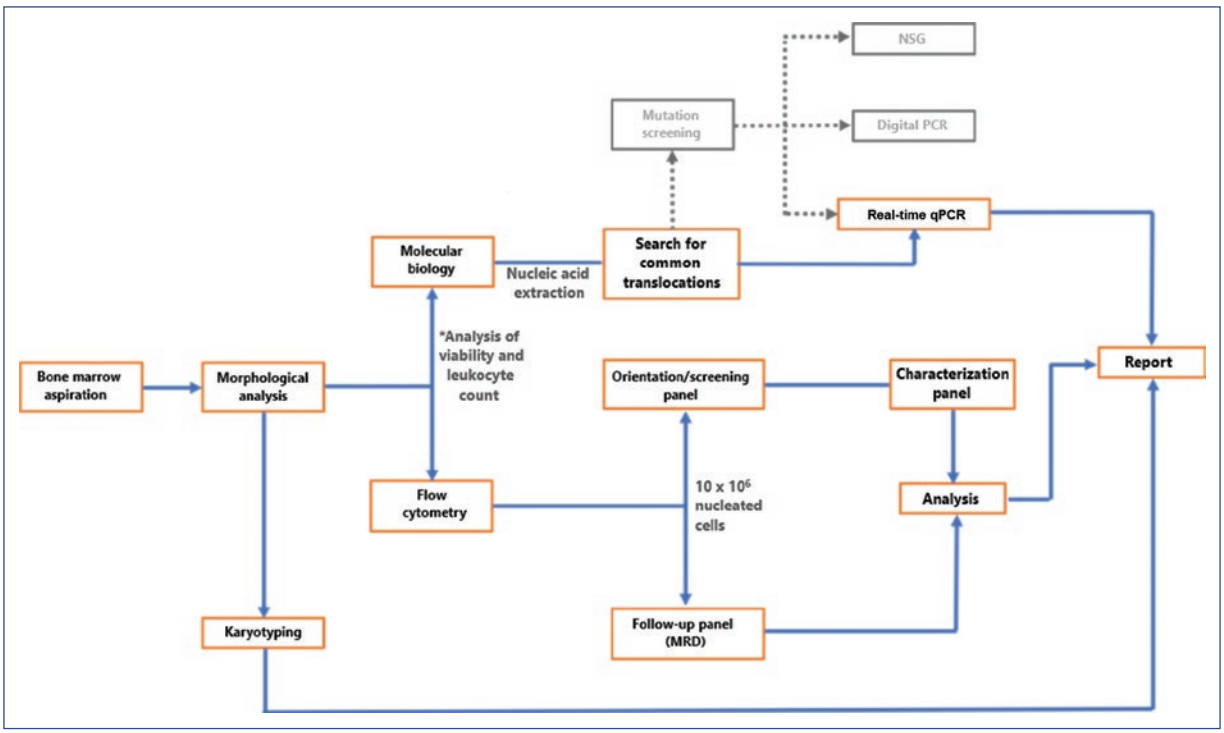

Figure 1. Workflow diagram for the comprehensive diagnosis of acute leukemia. According to the World Health Organization recommendations, leukemia diagnosis should be made from bone marrow aspirates, although peripheral blood smears can be used in particular situations. Collectively, morphological analysis, karyotyping, immunophenotyping, and molecular testing for the presence of genetic aberrations outline the comprehensive diagnosis.

MRD and its prognostic value

Once the diagnosis is established, the next step is to monitor the disease and the effectiveness through the detection of MRD. Several studies have emphasized the usefulness of monitoring tumor burden throughout treatment. Depending on the protocol, MRD can be determined on days 14, 21, 28, 33, or up to 78 days after treatment. The first attempts to monitor the reduction of malignant cells were carried out by observing histology slides and conventional staining. The introduction of terminal deoxynucleotidyl transferase (TdT) detection by fluorescence microscopy was of limited utility compared to first-generation flow cytometry (with two or three colors and high instrumental speed). The next challenge was distinguishing abnormal from normal developing clones after chemotherapy (hematogones), so more colors/markers were needed. The advent of modern cytometers allows gathering up to 6-8 markers in single staining, increasing the resolution power, and studying the co-expression of several molecules. Although classical microscopic techniques are still performed, MRD by molecular or cytometry techniques can redefine risk groups in complete morphological remissions[19].

High sensitive molecular techniques have changed clinical outcomes, as documented by several controlled clinical trials demonstrating the superior prognostic value of MRD compared with leukocyte count, age, genotype, and early steroid response[20]. The purpose of MRD detection is to distinguish patients who respond well to therapy from those who require re-intensification, thus reducing high doses of chemotherapy in those patients at low risk, and recognizing patients who presumably have a low risk of relapse[21]. As previously mentioned, the prognostic value of MRD (currently determined by cytometry or RT-qPCR) is accepted as the most important factor in the clinical management of the disease due to a high correlation with the risk of early relapse[22,23]. Immunophenotyping at diagnosis is desirable but not necessary when following MRD. Innovative approaches used for tracking clones in B-ALL will be further discussed. However, such panels are not helpful for T-ALL and less so for myeloid origin leukemia, in which the recognition of the affected lineage at disease onset is essential for the appropriate

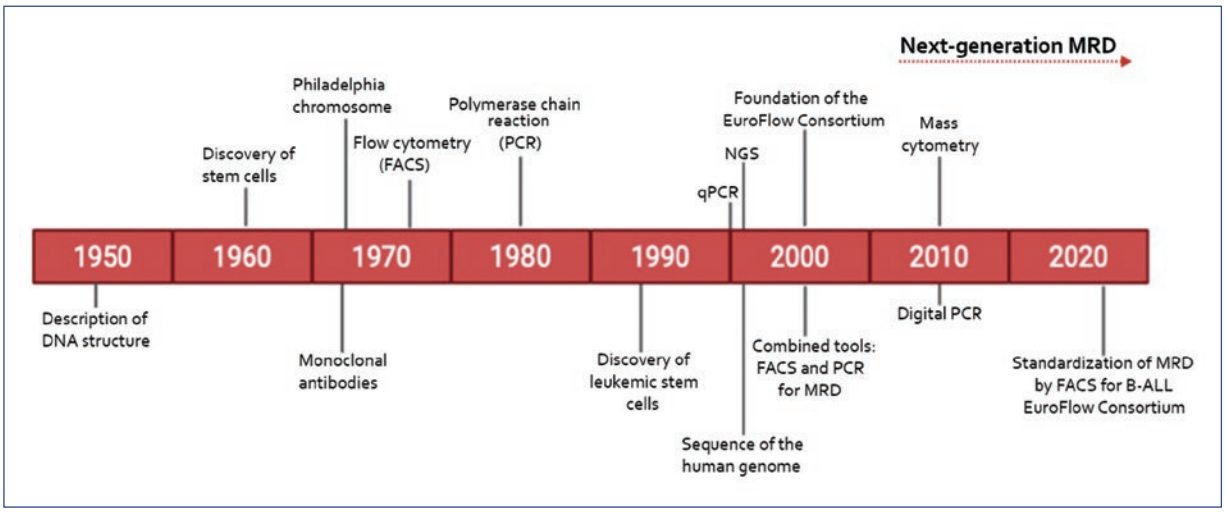

Figure 2. Timeline of relevant discoveries for the biological characterization of leukemia. Today's technology has been tangible thanks to fundamental discoveries that allowed the birth of modern molecular biology and the consideration of leukemic stem cells as the functional origin of these diseases and, therefore, as a target for treatment.

choice of MRD markers. Some case reports describe leukemic reemergence with lineage changes, possibly due to leukemic subclone's selection or the emergence of new clones during relapse[24]. Therefore, cytometry strategies should be sufficiently sensitive and phenotypically broad to identify re-emerging clones not associated with the lineage of origin.

Interestingly, MRD is also crucial in the prognosis of patients undergoing stem cell and hematopoietic progenitor transplantation, in whom MRD+ is often associated with unfavorable outcomes[25].

Unfortunately, not all laboratories can determine MRD, as this does not depend on infrastructure but analysis and interpretation based on intensive training and in-depth knowledge of genotypic and phenotypic changes due to chemotherapy and pathways of differentiation of normal and malignant clones, among other biological aspects of the disease.

Technology applied to the diagnosis and monitoring of hematological neoplasms

The description of the double helix chain in the 1950s based on crystallographic studies by Rosalind Franklin was undoubtedly one of the most important discoveries for modern molecular biology. However, in the early 1960s, James Till and Ernest McCulloch demonstrated for the first time that bone marrow contained cells capable of regenerating blood in mice subjected to lethal doses of radiation. The hematopoietic system has been a gateway to several paradigms in medicine, as it has allowed various discoveries such as the description of the first cancer-associated translocation, the Philadelphia chromosome (by Janet Rowley). Later, this translocation led to discovering the first targeted therapy using inhibitors of the tyrosine kinase activity of the protein resulting from the fusion BCR-ABL and, of course, the discovery of cancer stem cells.

Undoubtedly, the development of monoclonal antibodies by Milstein and Köhler (1975) and the invention of flow cytometry made it possible to profile the immunophenotype of primitive cell populations and purify them through cell sorting. The development of these new tools prompted John Dick, who described a population parallel to that of normal stem cells capable of initiating leukemia. Research has expanded the knowledge of normal and pathological protein expression at the cellular level. The creation of consortia such as EuroFlow has allowed establishing the AL cell classification and detecting residual clones after chemotherapy.

The ability to amplify a specific region of the genome through PCR in the mid-1980s and, later, the ability to quantify the number of copies in real-time made it possible to measure, for the first time, the success of chemotherapy with molecular platforms (Fig. 2). Since then, and thanks to the human genome project, molecular tools such as massive NGS have made it possible to identify specific regions associated with cancer and new mutations, particularly those associated with leukemia. However, a combination of cellular and

molecular strategies is recommended for the management of MRD[18]. Future technologies such as mass cytometry and other cytomics and molecular tools will allow highly sensitive detection of potentially MRD relapse-causing populations in the next generation.

Multiparametric flow cytometry

In daily practice, morphologic observations are usually confirmed by immunophenotyping. Although lymphoid blasts are usually distinguished from myeloid blasts, it is impossible to identify B or T lymphocyte blasts by simple colorimetric staining. Immunophenotyping is a highly specialized technique that uses flow cytometry to detect different fluorescent signals chemically coupled to antibodies that bind antigens with high specificity. As these antigens are usually membrane or cytoplasmic molecules, they can be detected simultaneously, if present in cells, according to the flow cytometer's optical configuration. At present, immunophenotyping is based on panels of 6-8 colors (or more), translating into the detection of multiple fluorescences associated with different cell markers. At least in commercial panels, this information enables identifying the presence/absence of several key parameters for the classification of the most relevant hematopoietic lineages. Validated *in vitro* diagnostic (IVD) antibody panels are available commercially to confirm the clinical suspicion of AL through a cytometry approach. The EuroFlow™ Consortium has worked multicentrically to develop an intelligent combination screening tool that searches for eight specific markers of the B, T, and myeloid lineages, as well as molecules of hematological immaturity (Table 1). A correct analysis of such a guidance tube allows identifying AL of B, T, and myeloid lineage. Details on the aberrant expression of other molecules, identification of mixed phenotypes, and other markers of prognostic value are explored by adding other tubes with new antibody combinations.

The detection sensitivity for most commercially available flow cytometers is 0.01-0.001% (1×10^{-4}-1×10^{-5}), although the latter is not usually achieved. As the number of cells per sample is not always abundant, the acquisition of multiple tubes reduces the number of cells available for analysis. Therefore, the lower tubes acquired, the higher the number of markers involved (depending on the number of detectors of the equipment), and the higher the number of cells acquired, the higher the resolution capacity. Analysis software such as Infinicyt™ allows the fusion of tubes from four markers—known as the backbone—and automatically calculates cell populations[26]. Achieving a high number of cells in samples obtained after chemotherapy is challenging because the collection of mononuclear cells may compromise the recovery of leukemic clones to the point of loss. Therefore, staining of the entire sample before bulk lysis is strongly recommended[19].

Table 1. Antigens of the Euroflow™ panel ALOT (acute lymphoblastic orientation tube)

Antigen	Cells expressing the antigen
CD45	Leukocytes
CD34	Stem cells and hematopoietic progenitors
CD19	B Lineage
CyCD79a	
smCD3	T Lineage
CyCD3	
CD7	
CyMPO	Myeloid

Next-generation flow cytometry

Undoubtedly, one of the fundamental laboratory challenges for leukemia is the detection of MRD. Some protocols define MRD prognostic value as early as day 15, while others at day 28 or 33. The real challenge lies in finding residual clones with alterations in the normal maturation pattern or the presence of aberrant markers. Either scenario requires training in detecting normal cell maturation to identify pathological clones and markers to discern between developing cells and those that have survived chemotherapy.

As mentioned previously, flow cytometry can reach the detection limit of 1×10^{-5}. However, to achieve maximum reliability, it is necessary to acquire an optimal number of cells (at least 5 million). There is no consensus on the minimum number of cells to define a cluster as a cell population; however, it is suggested to be between 10 and 50 events[27]. Another recommendation is to use specialized software for analysis, especially in MRD studies, where many nucleated cells (up to 10 million) have been acquired to search for residual clones.

Another challenge is the markers' stability during treatment, as some disappear or are induced after

Table 2. Patients with positive blast populations for CD73, CD86, CD44, and CD304 antigens in various international studies

Antigen	Percentage (%) of patients in each study				
	Meyerson et al.[30]	Coustan-Smith et al.[4]	Solly et al.[31]	Sędek et al.[28]	Nagant et al.[44]
CD73	76.7	54.5	—	66	—
CD81	—	—	—	—	100
CD86	56.7	46.7	—	58	—
CD44	50	53.5	—	—	—
CD24	20	11.5	—	—	—
CD304	—	71	48	59	—
CD123	—	—	—	—	34
CD58	—	—	—	—	90

rounds of chemotherapy[28]. Several studies have aimed to find the best combination of markers for disease tracing, especially in B-lymphoid origin leukemia, for which panels include, at least, the detection of CD19, CD79a, CD34, CD45, and CD3 antigens. Some options for monitoring MRD are further discussed (Table 2).

CD73

CD73 is an ectonucleotidase that produces adenosine (ADO) from extracellular ADO triphosphate (ATP). Under physiological conditions, it is expressed on the surface of some B cells and subpopulations of T and NK cells[29]. The role of CD73 is to create a suppressive environment after hydrolysis of extracellular ATP (damage signal) and reduce inflammation by generating ADO, a molecule with immunosuppressive potential. In a multicenter study, CD73 was shown to be aberrantly highly expressed on B-lineage blasts compared to its normal counterpart in 66% of the enrolled patients and was stable 15 days after initiation of treatment[28]. More recent reports have documented that CD73 has a greater tendency to increase significantly after treatment[29].

CD304

CD304 (also called neuropilin 1) functions as a co-receptor for the vascular endothelial growth factor and semaphorin. It is expressed on plasmacytoid dendritic cells and some monocyte populations under physiological conditions, although normal pre-B populations usually express discrete levels[30]. Based on expression data and subsequently corroborating the findings by flow cytometry, Coustan-Smith et al. demonstrated the role of CD304 as a valuable molecule for monitoring MRD by comparing purified leukemic blasts with their normal counterpart[4]. B-ALL leukemic blasts significantly overexpress CD304. Its expression correlates with the presence of t(12;21) *ETV6-RUNX1*, which originates the TEL/AM1 fusion, and is inversely related to rearrangements in the MLL gene[4,28,31]. In combination with CD9, it could be helpful for the prediction of t(12;21) translocation[32].

Interestingly, CD73 showed a positive correlation with CD304 expression, for which their combination results in a powerful strategy to identify residual clones during B-ALL (Fig. 3).

CD86

Under physiological conditions, B cells express basal levels of CD86. However, after activation, its expression increases considerably. Besides, plasma cells maintain abundant expression. CD86 may be expressed on the surface of leukemic blasts in some patients with B-ALL, but there is no apparent correlation with any particular immunophenotype (pro-B or pre-B) or genetic subgroup. Rearrangements of the MLL gene and the BCR-ABL1 fusion protein have been associated with positivity to the marker. However, due to the low frequency of these subgroups, it is challenging to generate significant statistics[28]. The trend has been confirmed in at least half of the patients with B-ALL in whom CD86 expression has been explored, but few studies have addressed its potential use for MRD follow-up[4,28].

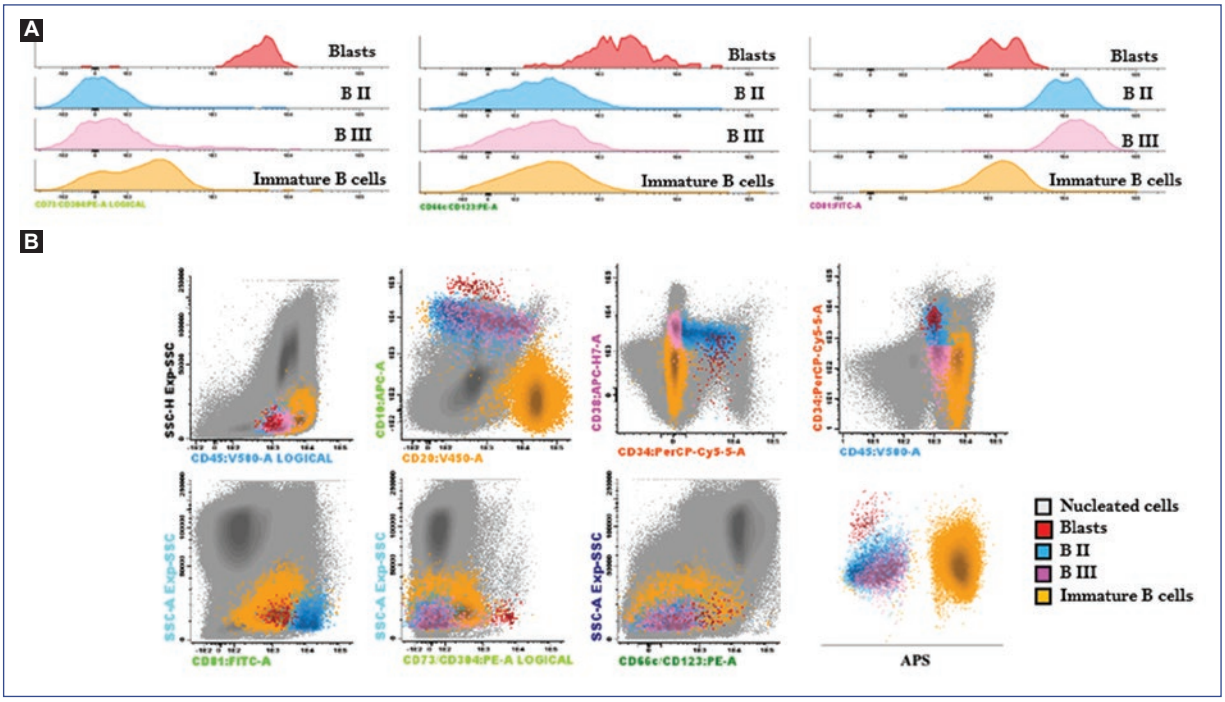

Figure 3. Differential expression of some markers useful for monitoring minimal/measurable residual disease. **A.** Differential expression of CD73/CD304 (left), CD66c/CD123 (center), and CD81 (right) in populations of residual B lymphoid blasts (CD45low CD34+ CD10+ CD20low), BII precursors (CD45low CD34+ CD38+ CD10+ CD20low/int), BIII (CD34- CD38+ CD10+ CD20low/int) and immature B cells (CD45hi CD34- CD38- CD10- CD20+). **B.** Dot plots of the different populations described above, the blasts population represents 0.0018% of the nucleated cells. APS: automatic population separator. Analysis performed in Infinicyt 2.0, Nereida Méndez.

CD58

Lymphocyte function-associated antigen 3 (LFA-3) or CD58 is expressed on the surface of antigen-presenting cells, especially macrophages. In most cases (> 90%), it is present in leukemic blasts and is stable in the treatment phase, making it a good candidate for monitoring MRD[33]. In a study that included 69 patients, CD58 helped detect ten patients with MRD (+) confirmed by PCR[34]. Despite being identified as one of the most outstanding molecules as MRD markers, other studies have shown that its implementation generates limited information for detecting remnant clones[27,34].

CD66c

This glycoprotein participates in cell adhesion and is expressed on the surface of cells of myeloid origin under normal conditions; however, it is absent in non-pathologic lymphocyte populations. In B-ALL, this molecule is aberrantly expressed on the surface of blasts, especially in cases with the t(9;22) translocation that originates the BCR-ABL1 fusion protein[35,36]. Molecular subgroups with hyperdiploidy frequently express the CD66c antigen[28,37]. Recently, the EuroFlow™ Consortium observed that the combination of CD66c with CD123 provided valuable information (Fig. 3A and B) by the separation obtained of malignant populations[27].

CD123

Myeloid progenitors, plasmacytoid dendritic cells, and basophils express the alpha chain of the IL-3 receptor (IL-3), CD123, whose signaling is involved in differentiation, proliferation, and survival processes. Experimental data confirm that CD123 is overexpressed on the surface of B-ALL and AML leukemic blasts; however, it is underrepresented in T-type leukemias[38]. Interestingly, the hyperdiploid genotype is associated with high CD123 expression. Therefore, in combination with CD86 and CD200, it could potentially identify this molecular subgroup[4,39]. In addition to being expressed in lymphoid cells, this molecule is currently used as a therapeutic target for immunological therapy since it is

also explicitly expressed in leukemic stem cells of AML[40].

CD44

CD44 is expressed in the cell membrane of various tissues and participates in cell adhesion and migration. During the early development of T cells, CD44 plays an essential role during the arrival of progenitors from the bone marrow to the thymus. Although its role seems more significant for T-ALL, B-lineage blasts express CD44 in almost 53.5% of cases[4,30]. Furthermore, its expression has been confirmed in AML blasts[41]. CD44 is one of the most studied tumor markers; its combination with CD24 has helped identify tumor stem cells in various solid tumors. Notably, BCR-ABL patients directly correlate with CD44 expression[42].

CD81

CD81 belongs to a family of proteins known as tetraspanins. Under physiological conditions, CD81 is expressed in the surface of mature B and T cells, for which it is considered a molecule associated with lymphocyte maturation. Therefore, CD81 may be detectable from the early stages of lymphopoiesis, and its density increases as populations mature[43-45]. The absence of CD81 expression and positivity to other markers, such as CD58, marks suspiciously malignant populations[44]. Thus, the combination allows identifying residual blastic clones from developing normal progenitors (hematogones) (Fig. 3).

Mass cytometry

This technology merges two well-known approaches: flow cytometry and mass spectrometry. This combination generates proteomic analyses at a single-cell level. In practical terms, mass cytometry or cytometry by time-of-flight requires a suspension of cells "labeled" with antibodies coupled to pure elements (initially metals) instead of fluorochromes as in traditional flow cytometry. In this technique, cells are nebulized, and metals are associated with the cells and detected by mass spectrometry due to specific binding with the antibodies. Therefore, more than 40 proteins can be analyzed simultaneously without the need for compensation since each element is unique and identifiable by its atomic mass. Although this tool is mainly used in research, the number of parameters exceeds those achieved by the most equipped traditional cytometer. In the future, this technology could be applied to detect MRD with a much higher resolution[46,47]. However, one of the disadvantages is that the sample has to be disintegrated, so cells cannot be recovered for further studies. With the recent emergence of spectral flow cytometry, it is possible to investigate 48 colors/markers by analyzing the entire emission spectrum of each fluorochrome that is part of the panel rather than the peak emission, as occurs with conventional flow cytometry. Moreover, the development of cell purification kits based on this detection strategy is underway, as well as the detection of messenger RNAs useful for the most common genetic abnormalities (in combination with classical immunophenotyping). In the coming years, the implementation of specific oligonucleotide-conjugated antibodies will provide the possibility of single-cell transcriptomics based on traditional flow cytometry[48].

Molecular biology in MRD

Before cytometry, MRD was traditionally measured through RT-PCR, detecting fusion products generated by translocations, although limited. With the development of digital PCR, a high precision quantification is possible now. Furthermore, the measurement of V(D)J rearrangement products allows identifying the immunological reconstitution of patients. However, as only one technique has been mainly used historically, few studies were initially consistent when combining techniques, possibly due to the lack of standardization[49]. Because of their predictive value, numerous technological and scientific efforts are underway to improve methods and innovate to identify clones that resist traditional therapy. Thus, results on massive sequencing techniques for MRD determination suggest a higher predictive accuracy for predicting relapse compared with other strategies[50].

Recently, Waanders et al. demonstrated that relapse clones originated from subclones existing at diagnosis with variable abundance and identified some candidate genes that may undergo additional mutations such as *NCOR2*, *USH2A*, and *NT5C2*, evidenced by digital PCR before clinical relapse[51]. Undoubtedly, MRD detection is aided by cutting-edge technology but requires rapid and standardized techniques[52]. For low- and middle-income countries, the cost of such assays should be affordable for patients or public health services.

MRD in myeloid leukemia

Detection of gene fusion products by RT-qPCR is one of the most commonly used strategies in AML alongside flow cytometry. Since approximately 60% of myeloid leukemia belong to a molecular group, their traceability is facilitated by this method during treatment. At present, NGS's advantage is that it allows parallel information on the associated mutations and the detection of fusion products for these disorders[53]. Because of the high phenotypic heterogeneity within the same disease, there has been a significant delay in developing guidelines addressing all the characteristics. ALL protocols have been adapted to search residual cells through flow cytometry, but the results have differed considerably[54]. One of the biggest challenges for the cytometrist is distinguishing malignant clones from normal myeloid reconstitution. Despite the description of leukemic stem cells, only a few studies have followed these cells during MRD. However, given that the immunophenotype of CTLs can be variable between patients, proposing a panel that allows generalization is complex[10]. The search for stemness in AML during MRD and LICs in acute lymphoblastic leukemia would be handy.

MRD in the cerebrospinal fluid

Only about 30% of relapses occur in the central nervous system (CNS), and the detection of leukemic clones in the cerebrospinal fluid (CSF) at diagnosis occurs in < 15% of patients. These results are associated with a worse prognosis due to an increased risk of relapse[55]. The mechanisms underlying CNS colonization are not well understood, but experimental investigations indicate that blasts can cross the blood-brain barrier. Alternatively, potential "seeding" of blasts in the CNS during sample collection or prophylaxis protocols has been suggested[56]. Furthermore, the use of immunodeficient animal models has highlighted the determinant role of the antitumor surveillance system and the migratory capacity of leukemic populations in relapsing leukemia, distinguishable by a high expression of cortactin[57].

Malignant blasts in the CSF are traditionally detected by cytology after concentrating the sample (cytospin). Moreover, blasts can be evaluated by flow cytometry using strategic combinations, including CD45, CD19, CD20, CD4, CD8, CD56, Ig lambda, Ig kappa, CD3, CD14, and CD38 antigens[16], to identify the infiltrate's cellular composition. A study of 673 patients showed that the relapse incidence at 4 years was higher in those where leukemic blasts were detected in the CSF by flow cytometry. Despite the superior advantages of flow cytometry over cytology, there is no consensus for leukemia diagnosis in the CSF, although several algorithms have already been thoroughly reviewed[58]. Other studies rely on molecular techniques for the detection of MRD in the CSF through PCR[59]. Regardless of the above, the biggest challenge is the correct preservation of the cellular material from the CSF, possibly implementing cell stabilizers for flow cytometry or nucleic acids. However, the low-volume sample cutting-edge techniques discussed here are also being explored for such frontier approaches[60].

Future perspectives and challenges

MRD detection represents a challenge from several perspectives, including low cell number in aspirates, lack of smart marker configuration, need for cytometers with more than five colors, the involvement of highly trained analysts, and specialized software, among others. Sometimes, diagnostic immunophenotyping is not available, or the laboratories' lack of systematization makes it challenging to follow-up residual clones. An advantage of hematological disorders with common molecular signatures is that such translocations can be monitored with high sensitivity by RT-qPCR. Moreover, mass cytometry or massive sequencing proposals will be explored in the coming years and addressed in pilot studies before being transferred to the clinic as routine protocols in our country. However, with the advancement of technology and the collaboration of different reference centers, most MRD measurement protocols are being standardized by conventional flow cytometry, which will provide greater accuracy and robustness. In Mexico, several initiatives for the consensus of markers and protocols for cytometry diagnosis will reduce the number of cases inaccurately diagnosed and evaluate treatment response and the increase in the overall survival of pediatric patients.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflict of interest.

Funding

The research of the Laboratorio de Oncoinmunología CIBIOR is supported by the Programa Nacional Estratégico de Salud del Consejo Nacional de Ciencia y Tecnología CONACYT (PRONAII Leucemia Infantil 302994) and by the Coordinación de Investigación en Salud del IMSS (FIS/IMSS/PROT/G18/1828).

Acknowledgments

The authors acknowledge the collaboration of the members of the Laboratorio Juárez S.A. de C.V. and the Hospital Universitario, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico.

References

1. Gómez-Almaguer D, Marcos-Ramírez ER, Montaño-Figueroa EH, Ruiz-Argüelles GJ, Best-Aguilera CR, López-Sánchez MDC, et al. Acute leukemia characteristics are different around the world: the Mexican perspective. Clin Lymphoma Myeloma Leuk. 2017;17:46-51.
2. Force, LM, Abdollahpour I, Advani SM, Agius D, Ahmadian E, Alahdab F, et al. The global burden of childhood and adolescent cancer in 2017: an analysis of the Global Burden of Disease Study 2017. Lancet Oncol. 2019;20:1211-25.
3. Balandrán, JC, Vadillo E, Dozal D, Reyes-López A, Sandoval-Cabrera A, Laffont-Ortiz MD, et al. Analysis of normal hematopoietic stem and progenitor cell contents in childhood acute leukemia bone marrow. Arch Med Res. 2016;47:629-43.
4. Coustan-Smith E, Song G, Clark C, Key L, Liu P, Mehrpooya M, et al. New markers for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2011;117:6267-76.
5. Mirkowska P, Hofmann A, Sedek L, Slamova L, Mejstrikova E, Szczepanski T, et al. Leukemia surfaceome analysis reveals new disease-associated features. Blood. 2013;121:e149-59.
6. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367:645-8.
7. le Viseur C, Hotfilder M, Bomken S, Wilson K, Röttgers S, Schrauder A, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell. 2008;14:47-58.
8. Ebinger S, Özdemir EZ, Ziegenhain C, Tiedt S, Castro Alves C, Grunert M, et al. Characterization of rare, dormant, and therapy-resistant cells in acute lymphoblastic leukemia. Cancer Cell. 2016;30:849-62.
9. Elder A, Bomken S, Wilson I, Blair HJ, Cockell S, Ponthan F, et al. Abundant and equipotent founder cells establish and maintain acute lymphoblastic leukaemia. Leukemia. 2017;31:2577-86.
10. Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS One 2014;9:e107587.
11. Purizaca J, Contreras-Quiroz A, Dorantes-Acosta E, Vadillo E, Arriaga-Pizano L, Fuentes-Figueroa S, et al. Lymphoid progenitor cells from childhood acute lymphoblastic leukemia are functionally deficient and express high levels of the transcriptional repressor Gfi-1. Clin Dev Immunol. 2013;2013:349067.
12. Balandrán JC, Purizaca J, Enciso J, DozaL D, Sandoval A, Jiménez-Hernández E, et al. Pro-inflammatory-related loss of CXCL12 niche promotes acute lymphoblastic leukemic progression at the expense of normal lymphopoiesis. Front Immunol. 2016;7:666.
13. Vilchis-Ordoñez A, Contreras-Quiroz A, Vadillo E, Dorantes-Acosta E, Reyes-López A, Quintela-Nuñez del Prado HM, et al. Bone marrow cells in acute lymphoblastic leukemia create a proinflammatory microenvironment influencing normal hematopoietic differentiation fates. Biomed Res. Int 2015;2015:386165.
14. Kumar B, Garcia M, Weng L, Jung X, Murakami JL, Hu X, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. Leukemia. 2018;32:575-87.
15. Arriaga-Pizano L, Ramírez-Ramírez D, Prieto-Chávez J, Pelayo R, Ruiz-Argüelles A. Reporte de la Primera Reunión Nacional de Consenso para la Inmunofenotipificación de Leucemias Agudas. Gac Med Mex. 2019;155:20-9.
16. Van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26:1908-75.
17. Meyer-Monard S, Parlier V, Passweg J, Mühlematter D, Hess U, Bargetzi M, et al. Combination of broad molecular screening and cytogenetic analysis for genetic risk assignment and diagnosis in patients with acute leukemia. Leukemia. 2006;20:247-53.
18. Neale, GAM, Coustan-Smith E, Stow P, Pan Q, Chen X, Pui CH, et al. Comparative analysis of flow cytometry and polymerase chain reaction for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia. 2014;18:934-8.
19. Paietta E. Assessing minimal residual disease (MRD) in leukemia: a changing definition and concept? Bone Marrow Transplant. 2002;29:459-65.
20. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFMALL 2000 study. Blood. 2010;115:3206-14.
21. Vora A, Goulden N, Wade R, Mitchell C, Hancock J, Hough R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013;14:199-209.
22. Starza ID, Chiaretti S, De Propris MS, Elia L, Cavalli M, De Novi LA, et al. Minimal residual disease in acute lymphoblastic leukemia: technical and clinical advances. Front Oncol. 2019;9:726.
23. Pui Ch, Pei D, Raimondi SC, Coustan-Smith E, Jeha S, Cheng C, et al. Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with response-adapted therapy. Leukemia. 2017;31:333-9.
24. Dorantes-Acosta E, Pelayo R. Lineage switching in acute leukemias: a consequence of stem cell plasticity? Bone Marrow Res. 2012;2012:406796.
25. Sramkova L, Muzikova K, Fronkova E, Krejci O, Sedlacek P, Formankova R, et al. Detectable minimal residual disease before allogeneic hematopoietic stem cell transplantation predicts extremely poor prognosis in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2007;48:93-100.
26. Pedreira CE, Costa ES, Lecrevisse Q, van Dongen JJM, Orfao A, EuroFlow Consortium. Overview of clinical flow cytometry data analysis: recent advances and future challenges. Trends Biotechnol. 2013;31:415-25.
27. Theunissen P, Mejstrikova E, Sedek L, van der Sluijs-Gelling AJ, Gaipa G, Bartels M, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood 2017;129:347-57.
28. Sędek Ł, Theunissen P, Sobral da Costa E, van der Sluijs-Gelling A, Mejstrikova E, Gaipa G, et al. Differential expression of CD73, CD86, and CD304 in normal vs. leukemic B-cell precursors and their utility as stable minimal residual disease markers in childhood B-cell precursor acute lymphoblastic leukemia. J Immunol Methods. 2019;475:112429.
29. Allard B, Longhi MS, Robson SC, Stagg J. The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets. Immunol Rev. 2017;276:121-44.
30. Meyerson HJ, Blidaru G, Edinger A, Osei E, Schweitzer K, Fu P, et al. NRP-1/CD304 expression in acute leukemia: a potential marker for minimal residual disease detection in precursor B-cell acute lymphoblastic leukemia. Am J Clin Pathol. 2012;137:39-50.
31. Solly F, Angelot F, Garand R, Ferrand C, Seillès E, Schillinger F, et al. CD304 is preferentially expressed on a subset of B-lineage acute lymphoblastic leukemia and represents a novel marker for minimal residual disease detection by flow cytometry. Cytometry A. 2012;81:17-24.
32. Gandemer V, Aubry M, Roussel M, Rio AG, de Tayrac M, Vallee A, et al. CD9 expression can be used to predict childhood TEL/AML1-positive acute lymphoblastic leukemia: proposal for an accelerated diagnostic flowchart. Leuk Res. 2010;34:430-7.
33. Veltroni M, De Zen L, Sanzari MC, Maglia O, Dworzak MN, Ratei R, et al. Expression of CD58 in normal, regenerating and leukemic bone marrow B cells: implications for the detection of minimal residual disease in acute lymphocytic leukemia. Haematologica. 2003;88:1245-52.
34. Shaver AC, Greig BW, Mosse CA, Seegmiller AC. B-ALL minimal residual disease flow cytometry: an application of a novel method for optimization of a single-tube model. Am J Clin Pathol. 2015;143:716-24.

35. Kiyokawa N, Iijima K, Tomita O, Miharu M, Hasegawa D, Kobayashi K, et al. Significance of CD66c expression in childhood acute lymphoblastic leukemia. Leuk Res. 2014;38:42-8.
36. Kalina T, Vaskova M, Mejstrikova E, Madzo J, Trka J, Stary J, et al. Myeloid antigens in childhood lymphoblastic leukemia: clinical data point to regulation of CD66c distinct from other myeloid antigens. BMC Cancer. 2005;5:38.
37. Cruz Rubio S, Lancheros A, Márquez Benitez Y, Mosquera Heredia M, Oliveros Barros J. Caracterización biológica del marcador CD66c y su importancia clínica en la leucemia linfoide aguda. Rev Cubana Hematol Inmunol Hemoter. 2018;34:1-12.
38. Bras AE, de Haas V, van Stigt A, Jongen-Lavrencic M, Beverloo HB, Te Marvelde JG, et al. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. Cytometry B Clin Cytom. 2019;96:134-42.
39. Djokic M, Björklund E, Blennow E, Mazur J, Söderhäll S, Porwit A. Overexpression of CD123 correlates with the hyperdiploid genotype in acute lymphoblastic leukemia. Haematologica. 2009;94:1016-9.
40. Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stems cells. Leukemia. 2000;14:1777-84.
41. Codeco Marques LVC, Pereira Noronha E, Gomes Andrade F, Dos Santos-Bueno FV, Mansur MB, Terra-Granado E, et al. CD44 expression profile varies according to maturational subtypes and molecular profiles of pediatric T-cell lymphoblastic leukemia. Front Oncol. 2018;8:488.
42. Tembhare PR, Ghogale S, Ghatwai N, Badrinath Y, Kunder N, Patkar NV, et al. Evaluation of new markers for minimal residual disease monitoring in B-cell precursor acute lymphoblastic leukemia: CD73 and CD86 are the most relevant new markers to increase the efficacy of MRD 2016; 00B: 000-000. Cytometry B Clin Cytom. 2018;94:100-11.
43. Tsitsikov E, Harris MH, Silverman LB, Sallan SE, Weinberg OK. Role of CD81 and CD58 in minimal residual disease detection in pediatric B lymphoblastic leukemia. Int J Lab Hematol. 2018;40:343-51.
44. Nagant C, Casula D, Janssens A, Nguyen VTP, Cantinieaux B. Easy discrimination of hematogones from lymphoblasts in B-cell progenitor acute lymphoblastic leukemia patients using CD81/CD58 expression ratio. Int J Lab Hematol. 2018;40:734-9.
45. Chernysheva O, Grivtsova LY, Popa A, Tupitsyn N. B-Cell Precursors: Immunophenotypic Features in the Detection of Minimal Residual Disease in Acute Leukemia. In: Aribi M, editor. Normal and Malignant B-Cell. London: IntechOpen; 2019. Available from: https://www.intechopen.com/books/normal-and-malignant-b-cell/b-cell-precursors-immunophenotypic-features-in-the-detection-of-minimal-residual-disease-in-acute-le.
46. Han L, Qiu P, Zeng Z, Jorgensen JL, Mak DH, Burks JK, et al. Single-cell mass cytometry reveals intracellular survival/proliferative signaling in FLT3-ITD-mutated AML stem/progenitor cells. Cytometry A 2015;87:346-56.
47. Behbehani GK, Samusik N, Bjornson ZB, Fantl WJ, Medeiros BC, Nolan GP. Mass cytometric functional profiling of acute myeloid leukemia defines cell-cycle and immunophenotypic properties that correlate with known responses to therapy. Cancer Discov. 2015;5:988-1003.
48. Martin J, Fan HC, Shum E. Oligo-conjugated antibodies (Ab-seq) and massively parallel single-cell sequencing reveal the high parameter correlation of protein and mRNA expression in individual immune cells. J Immunol. 2018;200 Suppl 1:120.33.
49. Ryan J, Quinn F, Meunier A, Boublikova L, Crampe M, Tewari P, et al. Minimal residual disease detection in childhood acute lymphoblastic leukaemia patients at multiple time-points reveals high levels of concordance between molecular and immunophenotypic approaches. Br J Haematol. 2009;144:107-15.
50. Kotrova M, Muzikova K, Mejstrikova E, Novakova M, Bakardjieva-Mihaylova V, Fiser K, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. Blood. 2015;126:1045-7.
51. Waanders E, Gu Z, Dobson SM, Antić Z, Crawford JC, Ma X, et al. Mutational landscape and patterns of clonal evolution in relapsed pediatric acute lymphoblastic leukemia. Blood Cancer Discov. 2020;1:1-16.
52. Van Dongen JJ, Van Der Velden VH, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood. 2015;125:3996-4009.
53. Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. Blood. 2016;127:29-41.
54. Coustan-Smith E, Ribeiro RC, Rubnitz JE, Razzouk BI, Pui CH, Pounds S, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. Br J Haematol. 2003;123:243-52.
55. Thastrup M, Marquart HV, Levinsen M, Grell K, Abrahamsson J, Klug Albertsen B, et al. Flow cytometric detection of leukemic blasts in cerebrospinal fluid predicts risk of relapse in childhood acute lymphoblastic leukemia: a nordic society of pediatric hematology and oncology study. Leukemia. 2020;34:336-46.
56. Williams MT, Yousafzai YM, Elder A, Rehe K, Bomken S, Frishman-Levy L, et al. The ability to cross the blood-cerebrospinal fluid barrier is a generic property of acute lymphoblastic leukemia blasts. Blood. 2016;127:1998-2006.
57. Velázquez-Ávila M, Balandrán JC, Ramírez-Ramírez D, Velázquez-Ávila M, Sandoval A, Felipe-López A, et al. High cortactin expression in B-cell acute lymphoblastic leukemia is associated with increased transendothelial migration and bone marrow relapse. Leukemia. 2018;33:1337-48.
58. Crespo-Solis E, López-Karpovitch X, Higuera J, Vega-Ramos B. Diagnosis of acute leukemia in cerebrospinal fluid (CSF-Acute Leukemia). Curr Oncol Rep. 2012;14:369-78.
59. Biojone E, Queiróz RD, Valera ET, Odashima NS, Takayanagui OM, Viana MB, et al. Minimal residual disease in cerebrospinal fluid at diagnosis: a more intensive treatment protocol was able to eliminate the adverse prognosis in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2012;53:89-95.
60. Bartram J, Goulden N, Wright G, Adams S, Brooks T, Edwards D, et al. High throughput sequencing in acute lymphoblastic leukemia reveals clonal architecture of central nervous system and bone marrow compartments. Haematologica. 2018;103:e110-4.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

REVIEW ARTICLE

Analytical recommendations for SARS-CoV-2 identification by RT-PCR in pediatric patients

*Israel Parra-Ortega[1], Armando Vilchis-Ordoñez[1], Briceida López-Martínez[2], and Tania Angeles-Floriano[1]**

[1]Departamento de Laboratorio Clínico; [2]Subdirección de Servicios Auxiliares de Diagnóstico. Hospital Infantil de México Federico Gómez, Mexico City, Mexico

Abstract

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2) and is currently listed as a global public health emergency. Timely identification and protocol implementations for molecular detection of this virus are vital for medical decision-making. Identification of SARS-CoV-2 infection cases is based on detection of the virus RNA by molecular tests, particularly real-time reverse transcription-polymerase chain reaction (RT-PCR). Technical and operational details specific to each center must be considered to perform the molecular diagnosis of SARS-CoV-2 in pediatric patients. The term "qualified laboratories" involves laboratories in which all users, analysts, and anyone reporting results are trained to develop and interpret results through a procedure implemented previously by an instructor. Such knowledge is essential in detecting and identifying errors during each of its phases: pre-analytical, analytical, and post-analytical, which allow the establishment of continuous improvement policies to ensure the quality of the results, but above all, the physical integrity of health workers.

Key words: *COVID-19. SARS-CoV-2. RT-PCR.*

Recomendaciones analíticas para la identificación de SARS-CoV-2 por RT-PCR en pacientes pediátricos

Resumen

La enfermedad por coronavirus de 2019 (COVID-19), causada por el coronavirus del síndrome respiratorio agudo grave 2 (SARS-CoV-2), está catalogada actualmente como una emergencia de salud pública mundial. La oportuna identificación y la implementación de protocolos para la detección molecular de este virus son de vital importancia para la toma de decisiones médicas. La identificación de los casos de infección por SARS-CoV-2 se basa en la detección de ARN del virus mediante pruebas moleculares, específicamente la reacción en cadena de la polimerasa de transcripción inversa (RT-PCR) en tiempo real. Existen detalles particulares de cada centro, tanto técnicos como operacionales, que deben considerarse para llevar a cabo el diagnóstico molecular de SARS-CoV-2 en pacientes pediátricos. El término «laboratorios calificados» se refiere a laboratorios en los cuales todos los usuarios, los analistas y cualquier persona que reporta resultados están capacitados para el desarrollo y la interpretación de estos a través de un procedimiento previo implementado por un instructor. Dichos conocimientos son indispensables para la detección y la identificación de errores durante el proceso en cada

Correspondence:
*Tania Angeles Floriano
E-mail: tania.angeles@yahoo.com.mx

Date of reception: 07-07-2020
Date of acceptance: 14-08-2020
DOI: 10.24875/BMHIM.20000210

Available online: 03-05-2021
Bol Med Hosp Infant Mex. 2021;78(3):171-180
www.bmhim.com

una de sus fases: preanalítica, analítica y posanalítica. Además, permiten establecer políticas de mejora continua que aseguran la calidad de los resultados, pero sobre todo la integridad física de los trabajadores de la salud.

Palabras clave: *COVID-19. SARS-CoV-2. RT-PCR.*

Introduction

In December 2019, a series of atypical pneumonia cases occurred in Wuhan, Hubei province, China, caused by a new β-coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV)[1-3].

Coronaviruses are highly diverse RNA viruses of the *Coronaviridae* family divided into four genera: alpha, beta, gamma, and delta. They are widely distributed in humans and other mammals. These viruses cause different illnesses: from common flu in its less aggressive form to pneumonia; in some cases, they cause severe acute respiratory syndrome (SARS) that requires ventilatory or mechanical support, and in the most severe cases, they cause death[2-5].

The World Health Organization (WHO) declared a global pandemic on March 11, 2020, as part of a global effort to coordinate the management of the impact caused by the SARS-CoV-2 virus. This pandemic has caused 462,684 confirmed cases (81,968 cases in China, including 11,977 severe cases) and 20,834 deaths (3293 deaths in China), figures up to March 26, 2020. Some studies have suggested that it could take more than a decade for the world to recover socially and economically[2,3].

According to the WHO, the implementation of systems to diagnose cases, timely identification of suspected cases, collection and delivery of samples to reference laboratories, and the implementation of molecular detection protocols for SARS-CoV-2, depending on the capacity of each laboratory, are among the priority strategies to be followed for pandemic management. Therefore, the coordination and collaboration of the clinical laboratory to support diagnosis based on molecular tests is of significant importance for the timely care of patients and decision-making in the healthcare sector[2-5].

Clinical and epidemiological studies have shown that patients with COVID-19 present symptoms ranging from mild to severe, including fever, fatigue, severe pneumonia, acute respiratory distress syndrome (ARDS), severe inflammatory response syndrome (SIRS), target organ damage, and multiple organ failure (MOF). According to different studies, SARS-CoV-2 infection is associated with significant morbidity and mortality ranging from 4.3% to 15%, especially in patients with chronic medical conditions. At least one in five cases requires intervention in intensive care units, which are limited in developing countries[1-5].

Recently, some laboratory parameters useful to predict the course of the disease have been described: some clinical features include peripheral blood leukocyte count (TCD4+ and TCD8+ cells) decrease, and the increase of proinflammatory cytokines in serum such as interleukin 6 (IL-6) and, interferon-gamma (IFN-γ). Moreover, acute-phase proteins (C-reactive protein, fibrinogen, ferritin) and cardiac biomarkers (troponin and BNP) quantification, the assessment of thrombosis risk, and liver and renal function evaluation tests are essential elements for the clinician to establish therapeutic and prophylactic measures when treating patients with COVID-19[5-7].

Several studies have reported that child infection represents from 1-5% of the total cases. This population often shows milder symptoms than the adult population, and deaths are extremely rare[5-7].

This review aimed to provide information regarding the implementation of molecular tests to detect SARS-CoV-2 in the Hospital Infantil de México Federico Gómez (HIMFG) clinical laboratory. These procedures are based on the molecular diagnostic protocols established by the WHO "Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases."

Molecular diagnosis of COVID-19

SARS-CoV-2 is a single-stranded RNA virus with a 29,903 nucleotides genome, making it the second-largest known RNA genome. It consists of two untranslated regions (UTR) at the 5' and 3' ends and 11 open reading frames (ORFs), which encode 27 proteins[6-10].

SARS-CoV-2 confirmation is based on detecting its RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) assays. In general, SARS-CoV-2 identification is carried out to detect three genes: *N*, *E*, and *RdRp* genes (when using the Berlin protocol). Both the *E* and *N* genes serve as screening genes to detect any β-coronavirus associated with bats, while

the *RdRp* gene is specific for SARS and SARS-like coronaviruses (including 2019-nCoV)[10-18].

Tests for SARS-CoV-2 identification should be considered for those patients who meet the case definition. If possible, it is recommended to use the laboratory algorithm for influenza detection suggested by PAHO for influenza surveillance.

Additionally, sequencing platforms can be used for virus identification in laboratories with both Sanger and Next-Generation sequencing technology[12-16].

Although serological methods based on the detection of IgM and IgG immunoglobulin antibodies exist in the market, they are not recommended, mainly because the dynamics between the response and production of antibodies during the infection are not entirely established; also, cross-reactivity with other coronaviruses makes the interpretation of the results difficult. Molecular detection of SARS-CoV-2 by RT-PCR has higher specificity and sensitivity (95-97%, confidence interval (CI): 83-97%); therefore, a positive result confirms the detection of the virus[6-14].

The efficiency of RT-PCR in microbiology has been described in multiple diagnostic algorithms, and now it is widely used in diagnostic virology. As a first step, the reverse transcriptase enzyme is used to synthesize complementary DNA (cDNA) from RNA obtained from SARS-CoV-2 present in nasopharyngeal swab samples. Subsequently, the region of interest of the cDNA is amplified with the use of specific primers.

Early and timely detection of SARS-CoV-2 cases allows the implementation of isolation measures to reduce and delay the pandemic peak, allowing greater responsiveness for the healthcare sector. Molecular detection tests for SARS-CoV-2 are critical for diagnosing the disease, understanding its epidemiology, and managing the cases, leading to a decrease in infections[8-10].

In our country, the Epidemiological Diagnostic and Reference Institute (InDRE, for its Spanish acronym) has developed a protocol for SARS-CoV-2 molecular identification, which has been transferred to the national network of public health laboratories, national health institutes, and other institutions that generate information for epidemiological surveillance.

It is essential to follow the general laboratory guidelines established by the InDRE and those documented in the Norma Oficial Mexicana *(*Mexican Official Standard*)* NOM-017-SSA2-2012 for epidemiological surveillance.

Currently, in our country, commercial tests must be subjected to an evaluation process by the InDRE for the accreditation of their analytical performance (Table 1). The complete list is available at the following link: https://www.gob.mx/cms/uploads/attachment/file/561223/Listado_de_estuches_comerciales_utiles_para_el_diagn_stico_de_SARS-CoV-2.pdf

Pre-analytical phase

The pre-analytical phase is considered the first stage of process development in the clinical laboratory. Deviations or omissions in this stage impact adequate performance, generating failures in the tests' performance or even affecting the quality of the results. In the case of SARS-CoV-2 detection, this phase is essential for identifying the virus, diagnosing the disease, and the care of healthcare personnel exposed to such processes.

It is crucial to be aware of biosafety measures during all stages of the analytical process considering all the risks involved in handling biological samples of infectious diseases. The minimum essential personal protective equipment (PPE) is described in the Biosafety and Biosecurity Protocol issued by the InDRE to handle patients during sample collection. Further details can be found in the following link: https://www.gob.mx/salud/documentos/lineamientos-vigentes-red-nacional-de-laboratorios-de-salud-publica?state=published) (Table 2).

During the development of molecular tests, errors to identify SARS-CoV-2 in the pre-analytical phase are similar to those in other diagnostic areas. An example is the type of specimen recommended for these tests; the primary specimen should come from the respiratory tract of the individual with suspected disease. Nasopharyngeal (NP) and oropharyngeal (OP) swabs should be collected and transported in the same tube containing viral transport medium or sterile saline. Primary samples should be kept refrigerated (4-8 °C) and processed within 24-72 h after sampling.

Both the process and the timing of sample collection are critical to the outcome. By the process, we mean the swabbing technique and the region where it will be performed, while the timing of sample collection refers to the time elapsed after exposure to the virus. The estimated incubation period of this new virus ranges from 2 to 14 days; however, incubation periods of 21, 24, or 27 days have been observed in some cases. Therefore, sampling at the early stages of exposure may generate false negative or indeterminate results in most cases[19-25].

Table 1. Description of molecular tests for the diagnosis of SARS-CoV-2 during the COVID-19 contingency approved by the InDRE in Mexico

Name of the test	Catalog number	Manufacturer	Analytical sensitivity (detection limit)	Analytical specificity (absence of cross-reactivity)	Genes detected	Number of reactions per sample
Berlin reference test performed at InDRE and the National Network of Public Health Laboratories (Red Nacional De Laboratorios de Salud Pública)	Not available commercially	Institutional implementation (InDRE)	10 copies/reaction	100%	E, RdRp, RNAse P*	3
Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)	S3102E	Sansure Biotech Inc.	4 copies/reaction	100%	ORF1ab, N, RNAse P*	1
MOLgen SARS-CoV-2 Real Time RT-PCR Kit	MESARS-CoV-2	Adaltis S. r. l.	100 copies/reaction	100%	RdRp, N, E	1
QuantiVirusTM SARS-CoV-2 Test Kit	DC-11-008	DiaCarta, Inc.	50 copies/reaction	100%	ORF1ab, N, E, RNAse P*	4
SARS-CoV-2 Nucleic Acid Detection Kit (PCR-Fluorescent Probe Method)	CoV2-32	Zybio Inc.	100 copies/reaction	100%	ORF1ab, N	1
BioFire® COVID-19 Test	423744	BioFire Defense, LLC	**	100%	ORF1ab, ORF8	1-Direct sample
Novel Coronavirus (2019-nCoV) RT-PCR	DNK-1418-1	Dynamiker Biotechnology (Tianjin) Co., Ltd.	10 copies/reaction	100%	ORF1ab, N, Actin*	1
PhoenixDx® SARS-CoV-2 IVD	PCCSKU15261	Procomcure Biotech GmbH	50 copies/reaction	100%	RdRp, E	3
U-TOPTM COVID-19 Detection Kit	SS-9930	SEASUN BIOMATERIALS	10 copies/reaction	100%	ORF1ab, N, RNAse P*	1
NeoPlex™ COVID-19 Detection Kit	NR05A	GeneMatrix, Inc.	50 copies/reaction	100%	RdRp y N	1
iAMP® COVID-19 Detection Kit	iAMP-100	Atila BioSystems	72 copies/reaction	100%	ORF1ab, N	1
genesig® Coronavirus COVID-19 Real-Time PCR Assay	Z-Path-COVID-19-CE	Primerdesign™ Ltd.	5 copies/reaction	100%	RdRp	1
DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit	SQD52-K100	SolGent Co., Ltd.	10 copies/reaction	100%	ORF1ab, N	1
RIDA ® GENE SARS-CoV-2 RUO	PG6815RUO	R-Biopharm AG	10 copies/reaction	100%	E	1
COVID-19 Real-Time PCR Kit	HBRT-COVID-19	Chaozhou Hybribio Biochemistry Ltd.	250 copies/reaction	100%	ORF1ab, N	1

(Continues)

Table 1. Description of molecular tests for the diagnosis of SARS-CoV-2 during the COVID-19 contingency approved by the InDRE in Mexico *(Continued)*

Name of the test	Catalog number	Manufacturer	Analytical sensitivity (detection limit)	Analytical specificity (absence of cross-reactivity)	Genes detected	Number of reactions per sample
Abbott Real Time SARS-CoV	09N77-090, 09N77-080	Abbott Molecular Inc.	**	100%	RdRp, N	1- Direct sample
CDC 2019-Novel Coronavirus (2019-nCoV) (CDC)	10006606, 10006625, 10006626, A6121	Integrated DNA Technologies-Promega Corporation	5 copies/reaction	100%	N1 and N2, RNAse P*	3
AccuPower SARS-CoV-2 Real-Time RT-PCR Kit	SCV-2122	BIONEER CORPORATION	10 copies/reaction	100%	E, RdRp	2
SARS-CoV-2 Real Time PCR Kit	RTPCR001	Vircell microbiologists	10 copies/reaction	100%	E, N, RNAsa P*	2
Allplex™ 2019-nCoV Assay	RP10244Y	Seegene Inc	10 copies/reaction	100%	RdRp, N, E	1
GeneFinder ™ COVID 19 PLUS RealAmp Kit	IFMR-45	OSANG HEALTHCARE LTD	10 copies/reaction	100%	RdRp, N, E, RNAse P*	1
Logix Smart (COVID-19)	COVID-K-001	CO-DIAGNOSTICS, INC	10 copies/reaction	100%	RdRp, human gene*	1
Commercial cases LightMix® Modular SarbecoV E-gene EAV, SARS-CoV (COVID19) N-gene, and SARS-CoV-2 (COVID-19) RdRP	40-0776-96, 53-0775-96, and 53-0777-96, respectively	TIB MOLBIOL, LLC	10 copies/reaction	100%	RdRp, N, E	3
Xpert® Xpress SARS-CoV-2	XPRSARS-COV2-10	CEPHEID	**	100%	N2, E	1-Direct sample
cobas®SARS-CoV-2 Test	9175431190	Roche Molecular Systems	**	100%	ORF1, E	1-Direct sample
Detection kit for 2019 Novel Coronavirus (2019- nCoV) RNA (PCR-Fluorescence Probing)	#DA-930	Daan Gene Co., Ltd. Sun Yat-Sen University	10 copies/reaction	100%	ORF1ab, N	1
VIASURE SARS-CoV-2	VS-NCO206L	CerTest Biotec, S.L.	10 copies/reaction	100%	ORF1ab, N	1
WoV19 Kit	G2L-WoV19-SP- 100E	GENES2LIFE SAPI DE CV	250 copies/reaction	100%	E, RdRp, RNAse P*	3
DeCoV19 Kit	G2L-DeCoV19-SP	GENES2LIFE SAPI DE CV	250 copies/reaction	100%	N1, N2, N4, RNAse P*	3
DeCoV19 Kit Triplex	G2L-DeCoV19-MP	GENES2LIFE SAPI DE CV	250 copies/reaction	100%	N1, N2, N3, RNAse P*	2
TaqMan 2019-nCoV Assay Kit v1	A47532	Applied Life Technologies Corporation, ThermoFisher	10 copies/reaction	100%	S, ORF1ab, N, RNAse P*	3

Obtained from the following electronic address (constantly updated) (date of consultation 01-07-2020): https://www.gob.mx/cms/uploads/attachment/file/561223/Listado_de_estuches_comerciales_utiles_para_el_diagn_stico_de_SARS-CoV-2.pdf
*RNAse P and actin genes are human genes that assess the quality of the sample.
**Closed platforms with higher sensitivity than conventional tests.

Table 2. Minimum essential personal protective equipment to handle patients during sample collection

Description of the personal protective equipment	Quantity needed for each sample collection
Disposable, single-use, waterproof gown with reinforced sleeves and chest, length exceeding the height of the boots	2
Long-sleeved nitrile gloves or as recommended by the WHO 280 mm (kit or pair)	2
Hair cover with elastic	2
Shoe covers (kit or pair)	2
Surgical gown (optional)	2
NIOSH N95 or N100 respirator or disposable surgical mask	2
3.5 cm wide microporous tape	1
Safety glasses, preferably with silicone seals	2
Rigid closed-toe shoe (white rubber sanitary boot when available) (kit or pair)	2
Supplies for an incident	1
Product description	1
Autoclave sterilized paper roll	1
Red translucent plastic bag for biohazardous and infectious waste, 200 caliber that fulfills NOM-087-SEMARNAT-SSA1-2002	1
Gel alcohol 60-80%	1
Sodium hypochlorite solution 0.05%	1
Ethanol 70% solution	1
Triple packaging system Category B	1
Hermetic container for the transfer of packaged waste to the sterilization facility	1

*Taken from the Biosafety and Biosecurity Protocol issued by the Epidemiological Diagnostic and Reference Institute (InDRE).
NIOSH, National Institute for Occupational Safety and Health; PPE, personal protective equipment; WHO, World Health Organization.

Kucirka et al. used a model to estimate the false-negative rate and found that sampling suspected individuals before the onset of symptoms show up to a 100% chance of a false-negative result. This value decreases with the time of symptoms onset. For example, on day 1 of symptoms onset, the estimate reaches 67% (95%CI 27-94%), and on day 4, the mean false negative rate is 38% (95%CI 18-65%). This rate decreased to 20% (95%CI 12-30%) on day 8, and by day 21, the false-negative rate increased again[26].

Therefore, the sampling moment should be considered decisive since the incubation cycle of the virus and the manifestations of the disease are variable in each patient and may affect the interpretation of the results (Figure 1).

Another critical variable is the difficulty of sampling in newborns and infants due to the characteristics of the material used for sampling. Although small or low-caliber swabs are available for these patients, the sample collected is usually not representative. Therefore, the personnel in charge of sample collection must be trained in patient-management and sampling material.

For upper respiratory tract sampling, including NP and OP swabs, the following recommendations should be considered:

1) Synthetic fiber (flocked dacron or polyester) swabs, preferably with a flexible wire handle, should be used. As calcium alginate or wood-based swabs may contain substances that affect some viruses' viability and inhibit RT-PCR, their use is not recommended. Swabs should be immediately placed in sterile tubes containing viral transport medium (commercial or prepared in the laboratory; e.g., Hank's salts, depending on the diagnostic protocol established or used)[10-17]. The swab should be cut at the height of the tube to facilitate handling the primary sample during the analytical phase.
2) For NP sampling, it is recommended to insert a synthetic fiber swab with a flexible wire handle through the nares parallel to the palate (not upward) until resistance is encountered or the distance is equivalent to that from the patient's ear to the nose, indicating contact with the nasopharynx. The swab should reach a depth equal to the distance from the nostrils to the external ear. Once inside, it is essential to gently rub and rotate the swab for a moment (3 to 5 seconds) to absorb the secretions. Then the removal should be done slowly with a gentle twist. Finally, a visual check should be made to ensure that the swab is moist with the mucosal sample[10-17].
3) In OP sampling, the swab should be inserted into the posterior pharyngeal and tonsillar areas, swabbing while the swab is rotated over the tonsillar pillars and posterior oropharynx, avoiding touching the tongue, teeth, and gums. As with NP sampling, it is important to visually verify that the swab is moist with the mucosal sample[10-17].

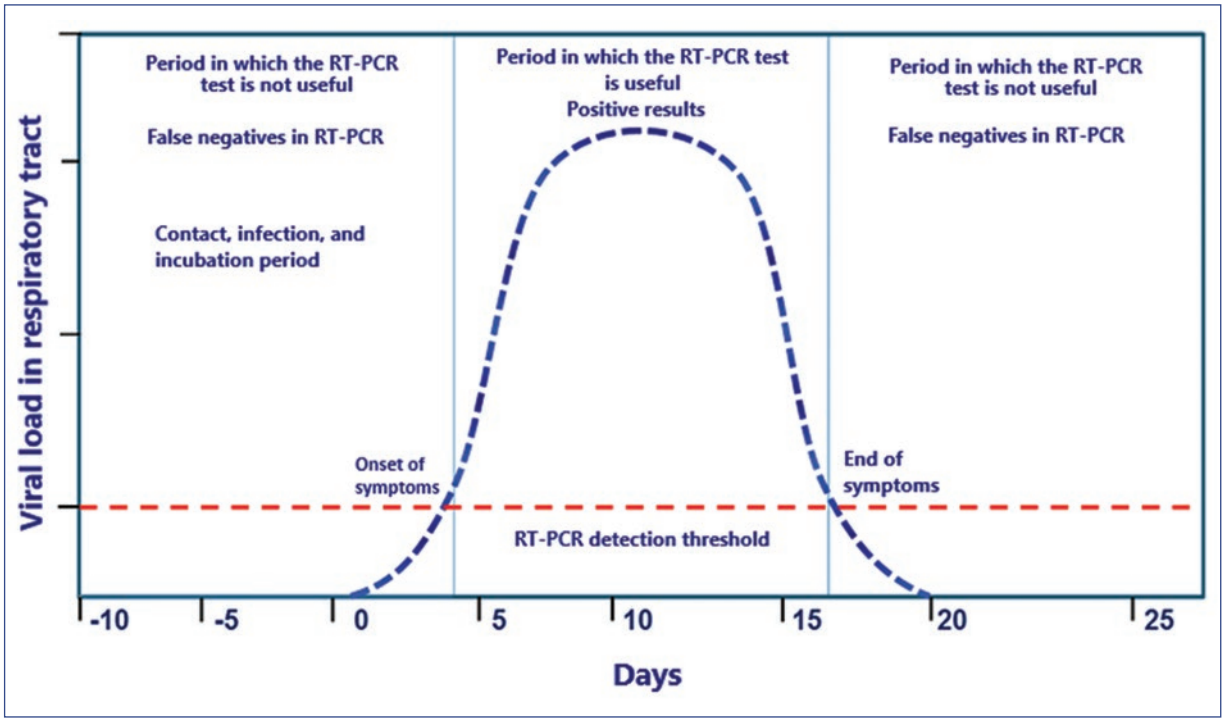

Figure 1. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection clinical course concerning the reverse transcription-polymerase chain reaction (RT-PCR) test positivity.

4) It is necessary to be trained in obtaining samples such as sputum, saliva, NP aspirate, or nasal aspirate, and have experience in the handling of vials, containers, and adapters to reduce the risk of contamination, leakage, and generation of aerosols[10-17].
5) Personnel must be trained in the fitting and removal of PPE, considering that all material and equipment are disposable (except for safety glasses) and must undergo a sterilization process before disposal.
6) It is recommended to work in pairs, as this facilitates the handling of pediatric patients and manipulating the material.
7) The site for outpatient sampling should be different from the site routinely used for blood and microbiological sampling. Due to the pathogenic characteristics of SARS-CoV-2, an exclusive site for sample collection is essential, considering infrastructure, lighting, ventilation, biosafety equipment, contamination control equipment, and the identification of the bio-hazardous waste-collection route.
8) Samples must be transported with triple packaging specifications. A trackable control of the route, temperature, transport times, and the contacts during the whole process must be recorded.

Analytical phase

For molecular biology tests, it is crucial to recognize two stages within the analytical phase. The first phase involves obtaining the genetic material, which will be the primary source of the process, while the second stage corresponds to the RT-PCR. Before implementing the tests, the following elements are required: the qualification of the facilities and the verification of the chosen analytical platform, in addition to a specific documented operating procedure.

When performing these analytical procedures, the PPE used should be the same as in the pre-analytical phase, and the analytical process should be performed in a biosafety level 2 (BSL-II) laboratory at least[10-28].

As previously described, identification of SARS-CoV-2 in the upper and lower respiratory tract is performed by RNA amplification via real-time RT-PCR during the virus infection cycle that allows qualitative detection *in vitro*. Positive results indicate an active viral infection; however, bacterial infection or co-infections with other viruses are not excluded. Therefore, negative results should be combined with clinical observations, patient history, and epidemiological information to guide treatment and other medical decisions.

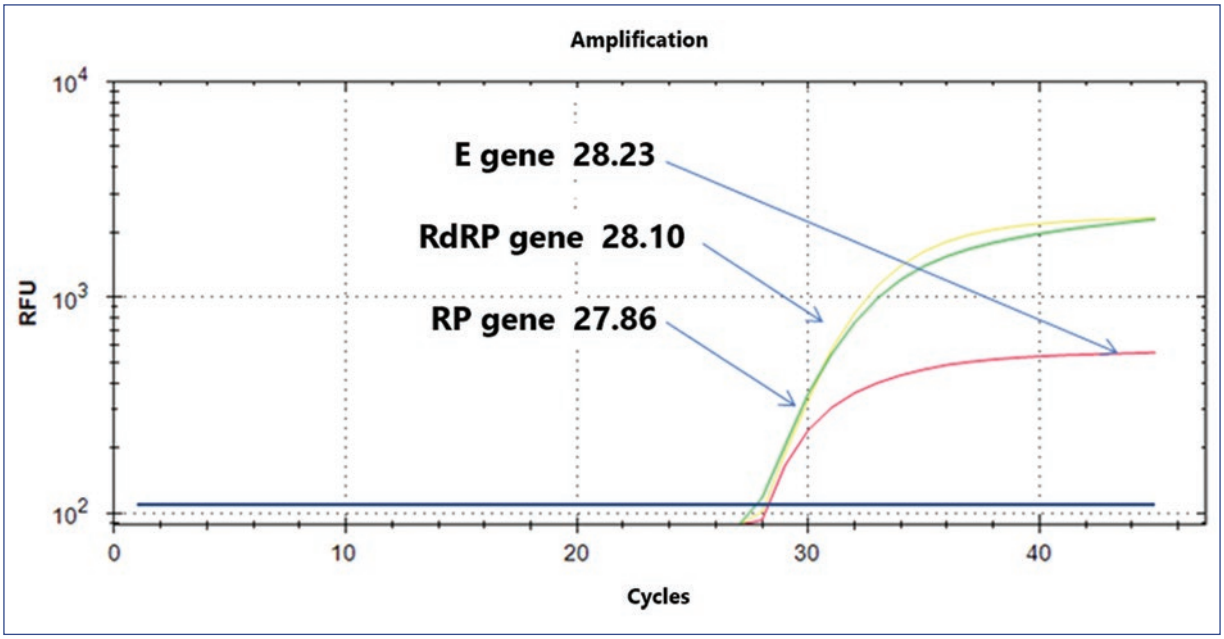

Figure 2. Detection of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) using the Berlin protocol in a positive case (amplification cycles of the three genes). E gene, envelope protein gene; RdRP gene, RNA-dependent RNA polymerase gene; RP, RNase P gene.

We suggest the following recommendations for handling the primary sample and obtaining viral RNA:

1) The sample matrix's handling, previously identified, should be carried out in a BSL-II laminar flow hood; cabinets should not be used in this procedure. All the material with direct contact with the sample should be kept inside the hood and should not be removed until it has been correctly decontaminated with UV light for at least one hour. In addition to the PPE previously described, double pair of gloves is recommended during this procedure.
2) Automated equipment is recommended to obtain viral RNA and reduce the analyst's contact with the sample, thus decreasing contagion risk. Also, the concentration and purity of the viral RNA obtained should be registered.

The viral RNA obtained is not infectious; however, it should be kept refrigerated (4-8°C) before the RT-PCR test (which should be performed within 2-3 h after obtaining the RNA) and separate from the reagents used for the test. Once used, this biological material can be stored at -80ºC.

For RT-PCR performance, the PPE equipment should be new. It is crucial to consider the following recommendations:

1) The reaction mixture's preparation should be carried out in a different location from where the genetic material and the negative and positive controls will be added. The reaction mixture can be prepared either in a cabinet or in a laminar flow hood.
2) The reagents used in the reaction mixture should be stored separately from the genetic material used as positive and negative controls. The positive and negative controls are synthetic genetic material without infectious capacity and are designed and determined by the choice protocol. In our protocol, we use the ribonuclease p gene (*RP gene*) as an endogenous gene, while for the detection of SARS-CoV-2, we use the *RdRP* and *E* genes (Figure 2).
3) It is preferable to make aliquots of the reagents, considering the number of samples processed routinely, to avoid the continuous freezing and thawing of reagents and samples.
4) Sanitization and decontamination of both work surfaces and equipment (micropipettes, centrifuges, among others) should be performed before and after use. Among the agents that can be used for this purpose are 10% bleach, 70% ethanol, and commercial RNA inhibitors to reduce contamination risk.

An essential part of the analytical process is diagnostic accuracy. In the tests for SARS-CoV-2 identification, there are different methodological proposals or analytical designs. The most widely used at the international level are the following:

1) The protocol designed by the CDC and the protocol developed by the Institute of Virology of Berlin. Both have been compared in several publications, making evident the complexity of homogenizing the various protocols to have similar analytical precision. For this reason, the minimum operating conditions of each protocol should be evaluated[15,16].
2) Xie et al. described that 3% of patients with imaging evidence of COVID-19 (chest CT scan with COVID-19 related damage, ground-glass imaging, or mixed ground-glass imaging with consolidation) initially showed a negative RT-PCR test result for SARS-CoV-2 identification. In this group of patients, test results were positive for COVID-19 after some days in the hospital[17].
3) In another series of patients, Ai et al. reported that 88% (888/1014 patients) showed chest CT scans with data of SARS-CoV-2 infection but only 59% (601/1014) with RT-PCR positivity, adding to the patients' biological variability[18]. Despite the assay or test variation, we should clearly understand our platform's analytical performance based on the tools that each laboratory should consider within the internal quality control program.

Post-analytical phase

The recommendations during this phase are the following:

1) Each gene's amplification cycles in each sample should be considered concerning the amplification cycle of the positive and negative controls. If possible, generate cut-off points according to the population evaluated.
2) Good transcription of the results to the reporting system and matching patient data (name, age, diagnosis, room, among others) must be verified.

In the case of the graphs or the experiments performed to identify SARS-CoV-2, interpretation criteria should consider each gene's characteristics and, if necessary, the presence, absence, or combinations that can be obtained. For example, the Berlin protocol seeks to identify three genes (a constitutive gene, a generic viral gene, and a specific viral gene) (Table 3).

Currently, no quantitative method can detect the number of virus copies in a sample; however, the number of amplification cycles of a specific gene detected in RT-PCR can provide information on the concentration of viral load (Table 4). It is important to be aware of some considerations to extrapolate these data: a) RNA concentration obtained during the extraction should be homogenized for all tests. Therefore, RNA should be quantified before the RT-PCR test and dilutions should be performed if necessary; b) the gene used for this determination should always be the same; 3) the interpretation will depend on the protocol implemented, the equipment used for the determination, and the brand of reagents selected.

In the case of interpretation errors, the final report delivered to the physician should be clear and concrete. In the case of positive results for COVID-19, unnecessary information (e.g., primers' sequence, enzymes used, and other methodological details) should not be included in the reports.

Finally, laboratories from public and private institutions that perform SARS-CoV-2 identification tests must generate a report of positive cases to the federal government's epidemiological surveillance network for the

Table 3. Table of interpretation of reverse transcription-polymerase chain reaction (RT-PCR) results

E gene	RdRp gene (discriminatory)	RNase P	Results
+	+	+	Positive for SARS-CoV-2
+	-	+	Probable Sarbecovirus
-	-	+	Negative for Sarbecovirus and SARS-CoV-2
-	-	-	Not adequate assay
-	+	+	Repeat/Send to InDRE for advice

InDRE (Spanish acronym), Epidemiological Diagnostic and Reference Institute; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4. Relative estimation of viral load in positive results, using a specific gene (RdRp gene) of SARS-CoV-2 by reverse transcription-polymerase chain reaction

Amplification cycle	Relative estimate of viral load
< 20	Very high viral load
20-25	Medium/high viral load
25-30	Intermediate viral load
> 30	Very low viral load

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

follow-up of the pandemic in the country. Each healthcare center must report the results of the SARS-CoV-2 tests to each country's competent health authorities. In the HIMFG Laboratory, we generate weekly reports following the current strategies, and we are part of the epidemiological surveillance support laboratories.

Molecular detection of SARS-CoV-2 is critical for taking action against the pandemic. However, it is essential to perform these procedures using protocols that promote patients' and healthcare personnel's safety. In Mexico, the Official Standards (NOM, for its Spanish acronym) for the organization and operation of clinical laboratories (NOM-007-SSA3-2011) and for the management of bio-hazardous waste (NOM-087-ECOL-SSA1-2002), among others, allow the establishment of clinical diagnostic tests.

Considering the heterogeneity of detection protocols, we should promote inter-laboratory networks for the appropriate exchange of information and relevant actions, besides following the guidelines issued by the InDRE to strengthen the timely detection of SARS-CoV-2 in Mexico. Furthermore, laboratories should continue to use the influenza laboratory algorithm recommended by PAHO and WHO for routine surveillance, considering that testing for COVID-19 should be performed only for patients who meet the case definition following epidemiological surveillance strategies.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflict of interest.

Funding

None.

References

1. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-9 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8:420-2.
2. Colaneri M, Sacchi P, Zuccaro V, Biscarini S, Sachs M, Roda S, et al. Clinical characteristics of coronavirus disease (COVID-19) early findings from a teaching hospital in Pavia, North Italy, 21 to 28 February 2020. Euro Surveill. 2020;25:2000460.
3. Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J Clin Med. 2020;9:1225.
4. Cagliani R, Forni D, Clerici M, Sironi M. Computational inference of selection underlying the evolution of the novel coronavirus, SARS-CoV-2. J Virol. 2020;94:e00411-20.
5. Xu H, Zhong L, Deng J, Peng J, Dan H, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12:8.
6. Djalante R, Lassa J, Seiamarga D, Sudjatma A, Indrawan M, Haryanto B, et al. Review and analysis of current responses to COVID-19 in Indonesia: period of January to March 2020. Pog Dis Sci. 2020;6:100091.
7. Mustafa NM, Selim LA. Characterization of COVID-19 pandemic in paediatric age group: a systematic review and meta-analysis. J Clin Virol. 2020;128:104395.
8. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell. 2020;181:894-904.
9. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Ribera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis. 2020;34:101623.
10. Lippi G, Plebani M. The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. Clin Chem Lab Med. 2020;58:1063-9.
11. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020;58:1131-4.
12. Lippi G, Plebani M. Laboratory medicine resilience during coronavirus disease 2019 (COVID-19) pandemic. J Bras Patol Med Lab. 2020;56:e3202020.
13. Triggle CR, Bansal D, Farag EABA, Ding H, Sultan AA. COVID-19: learning from lessons to guide treatment and prevention interventions. mSphere. 2020;5:e00317-20.
14. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25:2000045.
15. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Geneva: World Health Organization; 2020. Accessed: 27-04-2020. Available from: https://apps.who.int/iris/rest/bitstreams/1271387/retrieve
16. Centers for Disease Control and Prevention. CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel. Atlanta: Centers for Disease Control and Prevention, Division of Viral Diseases; 2020. Accessed: 27-04-2020. Available from: https://www.fda.gov/media/134922/download
17. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing. Radiology. 2020;296:E41-5.
18. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020;296:E32-40.
19. Tufan ZK, Kayaaslan B. Crushing the curve, the role of national and international institutions and policymakers in COVID-19 pandemic. Turk J Med Sci. 2020;50:495-508.
20. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. Euro Surveill. 2020;25: 2000062.
21. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1061-9.
22. Young BE, Ong SW, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA. 2020;323:1488-94.
23. Lippi G, Simundic AM, Plebani M. Potential pre-analytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med. 2020;58:1070-6.
24. Plebani, M, Lippi, G. Improving the post-analytical phase. Clin Chem Lab Med. 2020:48:435-6.
25. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020;55:102763.
26. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in the false-negative rate of reverse transcriptase-polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med. 2020;173:262-7.
27. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12:8.
28. Lippi G, Henry BM, Bovo C, Sanchis-Gomar F. Health risks and potential remedies during prolonged lockdowns for coronavirus disease 2019 (COVID-19). Diagnosis (Berl). 2020;7:85-90.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

REVIEW ARTICLE

Pediatric acute respiratory distress syndrome: How to protect the lungs during mechanical ventilation?

Álvaro Coronado-Muñoz[1,2] and Raffo Escalante-Kanashiro[3,4]*

[1]Department of Pediatrics, University of Texas Health Science Center, Houston, Texas, USA; [2]Pediatric Critical Care, Children's Memorial Hermann Hospital, Houston, Texas, USA; [3]Departamento de Emergencias y Áreas Críticas, Unidad de Cuidados Intensivos, Instituto Nacional de Salud del Niño, Lima, Peru; [4]Escuela de Medicina, Universidad Peruana de Ciencias Aplicadas, Lima, Peru

Abstract

Pediatric acute respiratory distress syndrome (PARDS) is a frequent diagnosis in critical care. This inflammatory process has different stages characterized by mild-to-severe hypoxia, and the management will vary according to the severity. New definitions for pediatric patients were published in 2015; new epidemiological evidence revising those definitions has helped understand the mortality associated with PARDS and the impact on ventilation. The strategies to protect the lungs during mechanical ventilation have been successful in reducing mortality and complications. In clinical situations where high levels of critical support are limited, other therapies with a lower level of evidence can be attempted to gain time without worsening the ongoing pulmonary injury. We offer a complete narrative revision of this syndrome, with the critical management of these patients as a priority.

Key words: *Artificial respiration/methods. Respiratory distress syndrome. Adult. Prone position. Acute lung injury. Pediatrics.*

Síndrome de dificultad respiratoria aguda pediátrica: ¿cómo proteger los pulmones durante la ventilación mecánica?

Resumen

El síndrome de dificultad respiratoria aguda pediátrica (SDRAP) es un diagnóstico frecuente en cuidados intensivos. Este proceso inflamatorio se caracteriza por diferentes grados de hipoxia, de leve a grave, y el manejo varía de acuerdo con la gravedad. En 2015 se publicaron nuevas definiciones para pacientes pediátricos, así como nueva evidencia epidemiológica, que toma como punto de partida dichas definiciones, lo cual ha ayudado a entender la mortalidad asociada y el impacto del manejo ventilatorio con respecto a la morbilidad en este síndrome. Las estrategias que protegen los pulmones durante la ventilación mecánica han sido exitosas en reducir la mortalidad y las complicaciones subsecuentes. En situaciones en las que existen limitaciones que impiden suministrar altos niveles de soporte crítico se pueden implementar otras medidas de menor evidencia para ganar tiempo e impedir que se extiendan las lesiones pulmonares. A continuación, se ofrece una revisión narrativa completa de este síndrome, con un enfoque que prioriza el manejo crítico de estos pacientes.

Palabras clave: *Respiración artificial, métodos. Síndrome de dificultad respiratoria aguda. Adulto. Posición prono. Lesión pulmonar aguda. Pediatría.*

Correspondence:
*Álvaro Coronado-Muñoz
E-mail: Alvaro.j.coronadomunoz@uth.tmc.edu

Date of reception: 08-06-2020
Date of acceptance: 27-08-2020
DOI: 10.24875/BMHIM.20000148

Available online: 08-06-2021
Bol Med Hosp Infant Mex. 2021;78(3):181-190
www.bmhim.com

Introduction

Pediatric acute respiratory distress syndrome (PARDS) is one of the most severe problems managed in pediatric critical care units. The presence of pulmonary inflammation with compromised oxygenation is an unmistakable sign of this syndrome, which requires comprehensive and multidisciplinary management. The present article displays a narrative review, emphasizing the management of this condition (Fig. 1).

The literature search has been exhaustive in different search engines, including PubMed, EMBASE, and SciELO. Articles in English, Spanish, French, and Portuguese were reviewed and included. The age criteria of the population were determined according to the type of publication. For high-quality evidence articles (meta-analyses, systematic reviews, randomized studies, and cohorts), pediatric studies (age < 21 years) were selected. For other observational studies (excluding cohorts), consensuses, and reviews, priority was given to pediatric studies, but adult literature was not excluded if no pediatric literature was found. Studies published from 2005 onward were included. Since this is a narrative review, the assessment of the included articles considered the authors' experience. A method of evidence assessment was not included in the review.

Epidemiological aspects

The most recent PARDS definition was published by the Pediatric Acute Lung Injury Consensus Conference (PALICC) in 2015[1]. That definition was validated by an international, multicenter, epidemiological study called PARDIE, published in 2019. To date, this study is the most comprehensive in terms of epidemiological data on PARDS[2]. One hundred and forty-five centers in 27 countries participated for 10 non-consecutive weeks. A total of 744 patients with PARDS were identified; 708 were included. The study reported 3.2% of new cases of PARDS, with a mortality of 16%. Patients with PARDS comprised 6.1% of all patients on mechanical ventilation. Mortality was 32.7% for patients with severe PARDS versus mild (12.4%) and moderate (10.3%) PARDS. Patients with severe PARDS had twice as many days with mechanical ventilation as those with mild or moderate PARDS.

Comorbidities are associated with some risk factors. Barreira et al.[3] studied patients with chronic diseases, including respiratory pathologies (26%), prematurity (19.3%), gastrointestinal disorders (12.3%), and genetic diseases (10.5%). In this study, risk factors associated with mortality were multiple organ dysfunction syndrome, hypoxemia, and refractory shock. PARDS risk was high in such cases, with elevated scores on the PRISM pediatric mortality risk scale. These factors were analyzed in the study of mortality prediction models performed with data from the PARDIE study[4].

Etiology

PARDS develops due to pulmonary (primary) or systemic (secondary) damage. According to the PARDIE study, the most common causes in pediatrics are pneumonia (mortality 12%) and sepsis (mortality 30%); trauma is a common cause in adolescents. Cases of immersion and non-septic shock represented 1% of the cases but high mortality (67% and 60%, respectively). In nosocomial etiologies, post-transfusion pulmonary reactions are common causes. Lung injury induced by uncontrolled mechanical ventilation is common. Therefore the understanding of its pathophysiology and management is of primary importance to avoid worsening lung injury.

Pathophysiology

PARDS is characterized by a succession of sequential inflammatory events that ultimately disrupt the alveolocapillary unit. The pathophysiology is complex and involves the stimulus nature, host response, and side effects of management[5,6].

Three histopathological stages have been described: exudative phase, proliferative phase, and fibrotic phase. The time of onset and duration of each stage is variable, and there is evidence of phase overlap. In the exudative phase, the lung's response to the occurrence of a lesion is initiated by the alveolar macrophage. The M1 macrophage is activated by NF-κB and secretes potent inflammatory mediators, including tumor necrosis factor, various interleukins (IL), and chemotactic cytokines (CCL), generating destruction and apoptosis of pneumocytes 1 and 2, and the subsequent release of epithelial mediators. The alveoli fill with protein-rich edema, inactivating the surfactant. The endothelial barrier disintegrates, and migration of neutrophils and monocytes initiates. Neutrophils promote the destruction of the alveolar epithelium and also induce hyaline membrane formation. Disruption of the endothelial-alveolar membrane generates interstitial edema. Endothelial damage in capillaries promotes intravascular coagulation and microthrombosis formation.

The proliferative phase is mediated by M2 macrophages, which release pulmonary repair mediators, reestablish the pulmonary epithelium's cellular

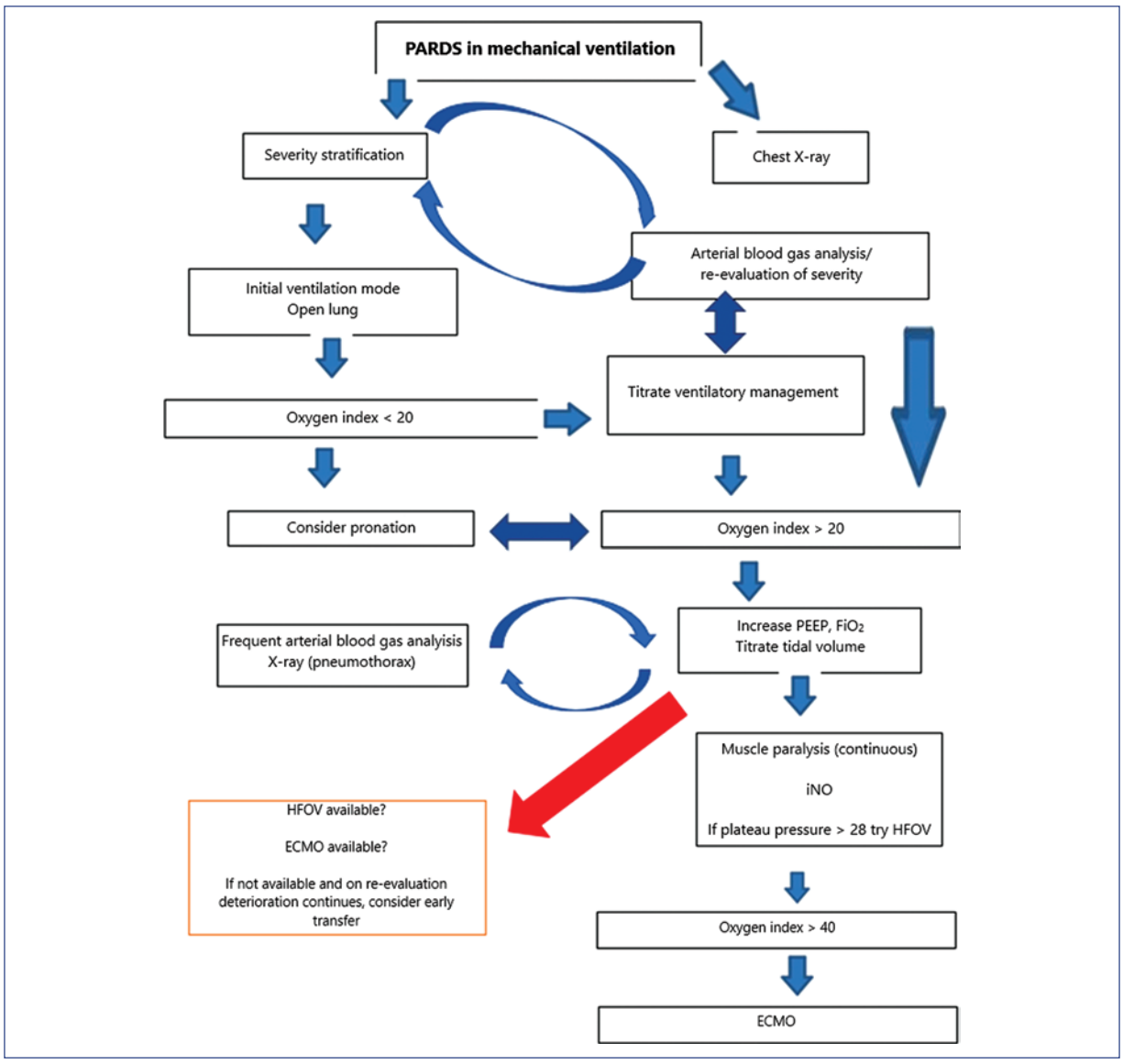

Figure 1. Management of pediatric acute respiratory distress syndrome (PARDS) in invasive mechanical ventilation (IMV). PEEP: positive end-expiratory pressure; FiO2: inspired fraction of oxygen; iNO: inhaled nitric oxide; HFOV: high-frequency oscillatory ventilation; ECMO: extracorporeal membrane oxygenation.

junctions, phagocyte apoptotic neutrophils, and facilitate the migration of bronchial progenitors and type 2 pneumocytes, which differentiate from type 1 pneumocytes and generate new surfactant. New membrane pumps help to drain the edema. The endothelial barrier is reestablished, and fibroblast proliferation favors the formation of a provisional matrix.

The fibrotic phase is the most destructive phase associated with mechanical ventilation and is characterized by permanent damage to the basement membrane. These changes do not allow alveolar epithelialization and result in persistent edema, intra-alveolar coagulation, collapse, and uncontrolled expression of M2 macrophages. The extracellular matrix develops interstitial and intra-alveolar fibrosis. The endothelium then undergoes a process of capillary obliteration and proliferation of fibroblasts and myofibroblasts.

Lung tissue damage is heterogeneous and leaves collapsed areas and alveolar units that preserve or lose gas exchange capacity. The “baby lung” theory states that in adult patients with ARDS, the lung portions that continued to have healthy tissue and where the gas exchange still occurs are equivalent to the volume of the lungs of pediatric patients aged 6-7 years. This

theory was based on the portions of the lung that remained air filled and appeared normal on tomographic images. These intact lung spaces constitute a volume less than the corresponding for the patient's weight and size. This concept is fundamental when considering the tidal volumes to be used in PARDS. It is more relevant the tidal volume/"baby lung" ratio than the traditional tidal volume/kilogram of weight[7]. The process of mechanical ventilation-associated or mechanical ventilation-induced lung injury is part of the pathophysiological process of this disease. The physiological principle of this concept has been called *mechanical power*. This concept encompasses driving pressure, tidal volume, airflow, positive end-expiratory pressure (PEEP), and respiratory rate. This concept facilitates understanding the contribution of mechanical power to ventilator-associated lung injury[8]. Understanding these concepts help to avoid perpetuating lung injury and facilitate lung recovery[9-11].

Definition and diagnosis of PARDS

The current definition of PARDS includes clinical criteria[1]. PARDS should be part of the diagnostic suspicion in patients presenting acute hypoxemic respiratory failure. Patients who require oxygen support are at risk of developing PARDS. The causes can be diverse, and the common manifestations are signs and symptoms caused by hypoxemia.

Age of patients

For the first time, the definition includes an age criterion for pediatric patients, unlike the Berlin definition. Patients with perinatal lung disease should be excluded. However, neonates who are hospitalized with respiratory failure unrelated to perinatal disease may present PARDS. Perinatal lung disease includes lung disease related to prematurity, perinatal lung injury (meconium aspiration, pneumonia, or neonatal sepsis), or other congenital abnormalities (such as a congenital diaphragmatic hernia or alveolar capillary dysplasia).

- Origin of edema. Respiratory failure cannot be fully explained by cardiac failure or fluid overload.
- Chronological criteria. Patients must present hypoxia, with radiographic changes, to be diagnosed with PARDS within 7 days of presenting a known clinical lesion. It is important to remember that this is an acute syndrome.
- Chest imaging studies. In chest imaging studies, the presence of new infiltrates is consistent with acute parenchymal lung disease. There is no need for the infiltrates to be bilateral, unlike previous definitions.
- Oxygenation criteria. The definition is based on oxygenation criteria (Table 1). The PALICC definition proposes stratifying the severity of PARDS based on oxygenation index (OI) or oxygen saturation index (OSI). The purpose of using oxygen saturation (SpO_2) is to avoid the exclusion of patients with PARDS who do not have an arterial blood gas report, which facilitates diagnosis and stratification in pediatric patients. OI is defined as the ratio of the mean airway pressure (P$\bar{a}$w, mmHg) multiplied by the inspired fraction of oxygen (FiO_2), multiplied by 100, and divided by the partial pressure of oxygen in arterial blood (PaO_2): $OI = P\bar{a}w \times FiO_2 \times 100/PaO_2$. OSI is calculated by dividing by the arterial oxygen saturation (SaO_2): $OSI = P\bar{a}w \times FiO_2 \times 100/SaO_2$. The inspired fraction of oxygen must be titrated to obtain saturations ≤ 97% to use OSI, while in patients in whom arterial blood gases are available, the OI should be used (Table 1).

Furthermore, the oxygenation criteria include definitions for patients on non-invasive mechanical ventilation with continuous positive airway pressure (CPAP) ≥ 5 cmH_2O, in whom PARDS is defined with PaO_2/FiO_2 ≤ 300 or SaO_2/FiO_2 ≤ 264.

Table 1. Oxygenation criteria according to PALICC definition

Meet age and chronological requirements, the origin of edema, and chest image			
Non-invasive mechanical ventilation Face mask, BiPAP or CPAP ≥ 5 cmH2O	Invasive mechanical ventilation		
	Mild	Moderate	Severe
PaO2/FiO_2≤ 300; SaO2/FiO_2 ≤ 264	4 ≤ OI < 8 5 ≤ OSI < 7.5	8 ≤ OI < 16 7.5 ≤ OSI < 12.3	OI ≥ 16 OSI ≥ 12.3

$OI = P\bar{a}w \times FiO_2 \times 100/PaO_2$; $OSI = P\bar{a}w \times FiO_2 \times 100/SaO_2$.
The OI is defined as the ratio of the mean airway pressure (Pāw, mmHg) multiplied by the inspired fraction of oxygen (FiO2) multiplied by 100 and divided by the partial pressure of oxygen in arterial blood (PaO2). It should be divided by oxygen saturation (SaO_2) to calculate OSI. OI should be used if arterial blood gases are available. Titrate FiO2 to obtain saturations ≤ 97% to use OSI.
BiPAP: bilevel positive airway pressure; CPAP: continuous positive airway pressure; OI: oxygen index; OSI: oxygen saturation index; PALICC: Pediatric Acute Lung Injury Consensus Conference.

Special populations

Cases of children with the left ventricular cardiac dysfunction, who meet the other PARDS criteria, should

be diagnosed as such if hypoxemia is acute and new chest imaging changes cannot be explained by acute left ventricular heart failure or fluid overload[3]. In cyanotic heart disease cases, the same criteria are applied, as long as they have acute oxygenation impairment not explained by the heart disease.

In patients with chronic lung disease, the same criteria are used. The radiograph should show new infiltrates compared to the baseline radiograph and deterioration of oxygenation above the patient's standard requirement and meeting the oxygenation parameters described below.

Patients at risk for PARDS

One of the updates presented in the PALICC definitions was the inclusion of the diagnosis of patients at risk for PARDS. The criteria for age, chronology, edema origin, and chest imaging are the same as those already described in the primary definition; the difference occurs in the oxygenation criteria. The criterion for non-invasive mechanical ventilation patients on CPAP (continuous positive airway pressure) or bilevel positive airway pressure with a nasal mask is that $FiO_2 \geq 40\%$ must maintain saturations of 88-97%. For patients with an oxygen mask, nasal cannula, and high-flow nasal cannula, a minimum oxygen flow should be provided to maintain 88-97% oxygen saturation. The minimum flow is defined by age: 2 L/min for < 1-year-old patients, 4 L/min for patients aged 1-5 years, 6 L/min for patients aged 5-10 years, and 8 L/min for > 10-year-old patients.

For invasive mechanical ventilation, the criterion for patients at risk is oxygen supplementation necessary to maintain oxygen saturations > 88%, but with OI < 4 and OSI < 5.

Differential diagnosis

Several conditions associated with hypoxia are part of the differential diagnosis. These conditions require critical and urgent management and may include the following conditions:

- Interstitial or eosinophilic pneumonia.
- Obliterans bronchiolitis.
- Alveolar hemorrhage.
- Pneumonitis.
- Neurogenic, reperfusion, or high-altitude pulmonary edema.
- Pulmonary embolism.
- Neoplasm.

Complementary tests

- Arterial blood gas analysis.
- Inflammatory markers (routine use is not suggested).
- Chest X-ray. The definition does not require the use of an X-ray (it can be any chest image). It is fast, available in many places, can be portable, is inexpensive, has less radiation than CT scans, is easy to interpret, and does not require sedation[12].
- Computed axial tomography, nuclear magnetic resonance, and ultrasound should be used when there is suspicion of an abscess, pleural effusions, persistent pneumothorax, pulmonary embolism, or vascular malformations.
- Echocardiogram (not routinely recommended). We strongly suggest considering it only to exclude pulmonary hypertension in patients with a history of prematurity[13].
- Bronchoscopy and alveolar lavage. Not recommended unless fungal or pneumocystis pneumonia is suspected. Inflammatory markers in alveolar lavage are not routinely recommended[14].
- Lung biopsy. Not recommended as a routine procedure[15].

Principles of PARDS management

The challenge is the identification of the population at risk and its immediate stratification. No specific treatment for the inflammatory process and alveolar-epithelial damage has been identified. The key to managing patients with PARDS is to treat the cause while lung tissue recovery occurs[16].

Understanding these patients' ventilatory management has allowed intensivists to improve the survival rates among patients affected by this syndrome[9]. The key to management lies in not iatrogenically damage the lungs[17,18]. This concept is the most critical in the current management of PARDS. Mechanical ventilation is associated with different lung injuries, and parameters must be established to avoid these injuries' persistence and allow alveolar recovery[10,11].

- Barotrauma: The pulmonary lesion is generated by elevated ventilatory pressures with peak and plateau pressures > 35 cmH_2O, which generates disruption of the alveolar wall. These patients are at high risk of pneumothorax and emphysematous lesions, which reduce the gas exchange surface. Patients with air leak syndrome secondary to pneumothorax are very complex.

– Volutrauma: It is the pulmonary lesion generated by high tidal volumes. The overdistension of the alveoli, according to the pulmonary area, would generate inflammation. The force it exerts on the different histological layers of the lungs generates an inflammatory cascade similar to that occurring in PARDS.
– Atelectrauma: Patients who have a low PEEP will lose alveolar recruitment. These patients will have alveoli that repeatedly collapse and distend, and this generates a loss of surfactant, which causes further collapse. As in volutrauma, the forced opening of collapsed alveoli will generate more inflammation.

Mechanical ventilation

The strategy and mode of ventilation can be chosen according to the medical team's expertise and ventilators' generation. The synchronized intermittent mandatory ventilation (SIMV) mode, with pressure control, offers certain advantages; however, no studies recommend only one mode[19,20].

In any of the chosen modes, parameters should be used to maintain the concept of open lung ventilation[11]. It is suggested to use the open lung and permissive hypercapnia strategy to avoid overdistension of the lung, causing further damage[9,10]. A pH of 7.15-7.30 can be used as a target, except in cases of elevated intracranial pressure, pulmonary hypertension, congenital heart disease, hemodynamic instability, and ventricular dysfunction. The principle of mechanical ventilation in PARDS is not to perpetuate lung damage but provide a lung-protective strategy (Fig. 1)[21].

a) Endotracheal tube and ventilator circuit

An endotracheal tube cuff is recommended to avoid air leakage during invasive mechanical ventilation. Modern ventilators have compensation for air leakage. The cuff should be inflated to maintain < 10% air leakage and, if possible, with a manometer to avoid tracheal damage. It is suggested to perform recruitment maneuvers after each suction through the endotracheal tube in tidal volume loss. Also, to use closed suction circuits in PARDS.

b) Tidal volume and pressures

It is recommended to use an alveolar protective tidal volume (4-8 mL/kg based on ideal body weight prediction)[22]. For patients with pressure-controlled ventilation (pressure control, PC), it is essential to adjust the pressure and obtain a tidal volume between 4 and 8 mL/kg to avoid barotrauma and volutrauma. Lung compliance or lung distensibility deteriorates in patients with PARDS. As controlled ventilatory parameters are increased, the risk of ventilator-induced lung injury (VILI) increases. It is suggested that the pressure difference (driving pressure = plateau pressure - PEEP) should preferably not be > 15 cmH_2O in pressure-controlled or pressure-limited volume-controlled ventilation. It is recommended to limit the plateau pressure to 28-32 cmH_2O. The ventilator must be in the volumetric mode to calculate the plateau pressure. In patients with plateau pressure > 28 cmH_2O and moderate or severe PARDS, the use of high-frequency oscillatory ventilation (HFOV) should be considered. Also, pH and pCO_2 should be monitored.

Tidal volume can be maintained between 6 and 8/kg, although it can be lowered to 4 and 5/kg if there are no ventilatory problems to avoid volutrauma. The volume/pressure curve should be carefully observed to determine the lower and upper inflection points and minimize alveolar recruitment loss. The objective of using this curve (V/P) is to keep it in the maximum slope segment (low pressure for large volumes) and not at the extremes, between the lower inflection point (onset of recruitment) and the upper inflection point (deflection, the onset of overdistension). This part of the curve is where the lowest pressure difference (driving pressure) will be needed (Fig. 2).

c) PEEP and oxygen

The FiO_2 should be adjusted according to the patient's needs and targets established according to the case's severity[23]. Oxygen supplementation should be adjusted to maintain SaO_2 between 92% and 97%, as long as PEEP is < 10 cmH_2O. In severe PARDS, if PEEP is > 10 cmH_2O, the goal is to maintain SaO_2 between 88% and 92%. This goal should be achieved with the lowest FiO_2 possible since high values cause O_2 toxicity. Regarding PaO_2, we could consider acceptable values between 55 and 80 mmHg, as long as hemodynamic stability is maintained and hemoglobin > 7 g/dl.

Regarding PEEP, a range of 5-15 cmH_2O is recommended. According to the severity of PARDS, PEEP can increase, and the possibility of pulmonary overdistension and pneumothorax should be monitored, and the hemodynamic status of the patient as well. Regarding the lower inflection point on the volume/pressure curve used as a reference, the PEEP value should be at or above that point to avoid atelectasis and atelectrauma (Fig. 2).

d) Time and inspiratory cycle

Increasing inspiratory time has a minor impact on the elevation of mean airway pressure. A ratio of 1:1 between inspiratory and expiratory times (I:E) can be maintained. The normal ratio is 1:2. I:E ratio of 1:1 does not appear to improve survival, although it may improve oxygenation. Reversing the I:E ratio is not recommended.

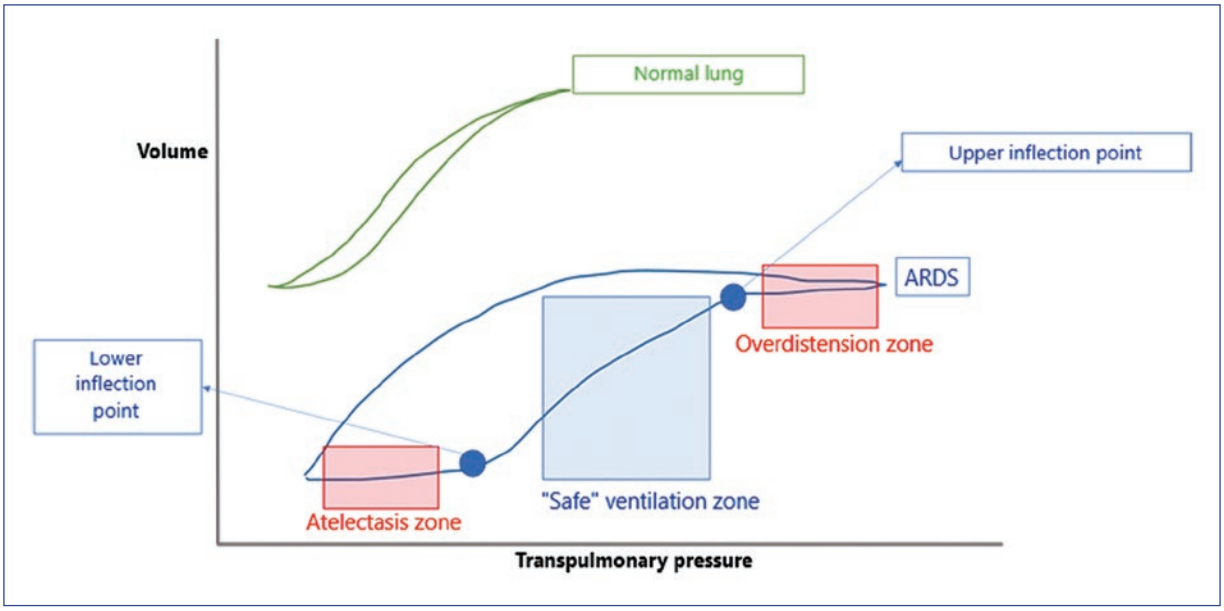

Figure 2. Volume/pressure curves in normal lung and acute respiratory distress syndrome (ARDS) lung. The safe ventilation zone is where no significant damage to the lungs is caused.

e) Recruitment maneuvers with hemodynamic status monitoring

It is recommended to maintain the patient's hemodynamic status at all times and avoid a shunt phenomenon. Preferably use a progressive recruitment strategy and assess the patient's condition comprehensively.

It is important to remember that these maneuvers are transitory and that studies have shown that they do not improve survival. They should only be used in patients who do not respond to other recruitment strategies and only if the response is favorable, without hemodynamic decompensation.

f) HFOV

A plateau pressure > 28 cmH_2O is recommended as a parameter to consider HFOV. Remember the negative impact on the right heart, which is one of the causes of significant morbidity and mortality in HFOV[24,25]. The usefulness of HFOV is unclear, but it is a rescue mode in severe cases, especially when the highest level of ventilation available is required. In a secondary analysis, HFOV is related to a longer time on ventilation in patients in whom it was initiated early. Clinically, one of the drawbacks of this mode of ventilation is the need for muscle paralysis.

g) Bilevel or airway pressure release ventilation (APRV)

Data regarding this mode of ventilation are limited. The evidence has been based on patient series, and there are medical groups that favor this ventilation method[26]. A randomized study of APRV versus low tidal volume mode was discontinued after enrolling 52 patients, as it was associated with increased mortality. This method could be applied during the transition between conventional ventilation and HFOV or extracorporeal membrane oxygenation (ECMO), but its routine use is not recommended.

Ventilation in a prone position

The principal advantage of this method is its easy application in children[27]. The principle is that ventilation in prone position patients improves oxygenation; however, pediatric clinical studies do not demonstrate its usefulness when studying mortality as an outcome. Moreover, a Cochrane review published in 2012 concluded that the prone position improves oxygen saturation and arterial oxygen pressure, reduces hypoxemia events, and improves thoracoabdominal synchrony compared to the supine position[28]. These findings are similar to those reported in adults, in which whom its early use is recommended[29,30]. By improving recruitment in dependent areas, volutrauma and barotrauma could be minimized and hence the decrease of FiO_2[31].

Establishing patient care measures using a prone positioning strategy and verifying the endotracheal tube position to avoid unplanned extubation is essential. Also, to have a rotation protocol to avoid pressure ulcers, especially on the face.

Since prone ventilation is an unproblematic measure, it can be used quickly to gain time and improve oxygenation. An early implementation may avoid escalation to other more invasive or unavailable therapies. During the coronavirus pandemic, adult intensivist teams recommended prone ventilation in non-intubated patients with high oxygen requirements to avoid early intubation[32,33].

ECMO

This strategy may improve survival in PARDS patients with severe manifestations but high costs and severe possible side effects. The moment at which the patient should start this approach has not yet been determined. It is suggested to consider ECMO in patients in whom the open lung strategy fails[34,35] and is recommended in patients with severe PARDS in which the cause is considered reversible. The introduction of percutaneous venous ECMO makes it easier to consider its use in pediatrics. It is postulated that a very high OI value (> 40) is one of the criteria for considering ECMO[36]; another criterion is the recurrence of pneumothorax.

Other therapeutic approaches

There is no specific pharmacological treatment for PARDS. Some therapeutic strategies are used even with unfavorable evidence. The management, especially in severe cases, includes all necessary measures to gain time. Considering that these therapies have a low level of evidence, they can be initiated if they have no major risks for the patient[29,37,38].

a) Negative cumulative balance

Negative water balance is ideal in PARDS, as long as hemodynamic stability is preserved. The aim should be to achieve a negative balance between 48 and 72 h. The internal environment and acid-base status management should not be forgotten; hypovolemia and poor perfusion should be avoided. This measure has low evidence[39].

b) Sedation/analgesia and muscle relaxants

It is necessary to limit muscle relaxants to the minimum number of days possible. The harmful effect of the association of corticoids and muscle relaxants on the appearance of myopathy in the critical patient should always be remembered, which can make extubation more difficult[40]. Relaxation is important and necessary at the onset of ventilation to assess lung compliance and minimize any restrictive pattern. In patients in whom HFOV is used, muscle paralysis will be necessary. The time of opioid sedation and analgesia should always be minimized to avoid adverse effects such as tolerance, withdrawal, and delirium (high level of evidence)[41]. Likewise, the paralysis time should be minimized to reduce the risk of myopathy (high level of evidence)[42].

c) Inhaled nitric oxide (iNO)

iNO has produced short-term physiological improvements in ventilation-perfusion adaptation and intrapulmonary shunt[43,44]. According to current evidence, it can be used in patients with severe PARDS. It is helpful in cases of pulmonary hypertension, but its routine use is not recommended for PARDS.

d) Corticosteroids

The routine use of corticosteroids is not recommended since there are no studies in pediatric patients, except for one pilot study. Data in adults are controversial. According to the protocol described by Meduri et al., some centers use it regularly; inhaled corticosteroids have been investigated in a pilot study in adults (2016), which showed improved oxygenation following their administration[45,46]. A pilot study on applying this therapy in pediatric patients is currently underway at the University of Texas in Houston.

e) Surfactant

Regarding exogenous surfactant, the evidence is insufficient to recommend its use. The principal study to demonstrate its use was interrupted due to futility criteria[47].

Extubation and weaning from invasive mechanical ventilation

Extubation should be initiated on reversal of the causes that precipitated the need for invasive mechanical ventilation (IMV) and the PARDS resolution. The PICU work plan and protocol for weaning from IMV should be followed.

Prognosis

As demonstrated in the PARDIE study, the mortality of severe cases is 32.7%. Surviving patients have prolonged intensive care stays, especially patients with severe PARDS. The impact on long-term respiratory and pulmonary function has not been measured in large-scale prospective studies. The available information has been obtained from small series. These studies have followed patients for up to 12 years after discharge from hospitalization. Studies with larger populations are needed. The age of the patients limits

pulmonary function tests. Patients requiring sedation and prolonged paralysis are at risk for withdrawal syndrome, delirium, and myositis requiring critical care. The impact on neurodevelopment in these patients has not been studied in detail. However, information on cognitive, emotional, social, and behavioral deficits from intensive care hospitalization is not specific for PARDS[48].

As PARDS is complex, physicians treating pediatric patients must be aware of management's new definitions and principles.

The primary approach to the management of PARDS is based on mechanical ventilation, with lung protective strategies. This mode of ventilation improves patient survival. The PARDS criteria and concepts are essential for any physician treating patients in pediatric critical care units.

It is necessary to know the equipment available in each center and recognize the transfer processes to advanced centers since the severe form of PARDS has a high mortality. Oxygenation compensation measures, such as prone ventilation, may help the patient before advanced critical care therapies.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

None.

References

1. Khemani RG, Smith LS, Zimmerman JJ, Erickson S, Pediatric Acute Lung Injury Consensus Conference G. Pediatric acute respiratory distress syndrome: definition, incidence, and epidemiology: proceedings from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S23-40.
2. Khemani RG, Smith L, Lopez-Fernandez YM, Kwok J, Morzov R, Klein MJ, et al. Paediatric acute respiratory distress syndrome incidence and epidemiology (PARDIE): an international, observational study. Lancet Respir Med. 2019;7:115-28.
3. Barreira ER, Munoz GO, Cavalheiro PO, Suzuki AS, Degaspare NV, Shieh HH, et al. Epidemiology and outcomes of acute respiratory distress syndrome in children according to the Berlin definition: a multicenter prospective study. Crit Care Med. 2015;43:947-53.
4. Yehya N, Harhay MO, Klein MJ, Shein SL, Pineres-Olave BE, Izquierdo L, et al. Predicting mortality in children with pediatric acute respiratory distress syndrome: a pediatric acute respiratory distress syndrome incidence and epidemiology study. Crit Care Med. 2020;48:e514-22.
5. Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. N Engl J Med. 2017;377:562-72.
6. Sapru A, Flori H, Quasney MW, Dahmer MK, Pediatric Acute Lung Injury Consensus Conference G. Pathobiology of acute respiratory distress syndrome. Pediatr Crit Care Med. 2015;16:S6-22.
7. Gattinoni L, Pesenti A. The concept of "baby lung". Intensive Care Med. 2005;31:776-84.
8. Gattinoni L, Tonetti T, Cressoni M, Cadringher P, Herrmann P, Moerer O, et al. Ventilator-related causes of lung injury: the mechanical power. Intensive Care Med. 2016;42:1567-75.
9. Biehl M, Kashiouris MG, Gajic O. Ventilator-induced lung injury: minimizing its impact in patients with or at risk for ARDS. Respir Care. 2013; 58:927-34.
10. Curley GF, Laffey JG, Zhang H, Slutsky AS. Biotrauma and ventilator-induced lung injury: clinical implications. Chest. 2016;150:1109-17.
11. Amado-Rodríguez L, del Busto C, García-Prieto E, Albaiceta GM. Mechanical ventilation in acute respiratory distress syndrome: the open lung revisited. Med Intensiva. 2017;41:550-8.
12. Cressoni M, Cadringher P, Chiurazzi C, Amini M, Gallazzi E, Marino A, et al. Lung inhomogeneity in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med. 2014;189:149-58.
13. Hopper RK, Abman SH, Ivy DD. Persistent challenges in pediatric pulmonary hypertension. Chest. 2016;150:226-36.
14. Grunwell JR, Giacalone VD, Stephenson S, Margaroli C, Dobosh BS, Brown MR, et al. Neutrophil dysfunction in the airways of children with acute respiratory failure due to lower respiratory tract viral and bacterial coinfections. Sci Rep. 2019;9:1-13.
15. Forel JM, Guervilly C, Hraiech S, Voillet F, Thomas G, Somma C, et al. Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation. Intensive Care Med. 2015;41:1-11.
16. Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimarães HP, et al. Effect of lung recruitment and titrated positive end-expiratory pressure (PEEP) vs. low peep on mortality in patients with acute respiratory distress syndrome: a randomized clinical trial. JAMA. 2017;318:1335-45.
17. Walkey AJ, Goligher EC, Del Sorbo L, Hodgson CL, Adhikari NK, Wunsch H, et al. Low tidal volume versus non-volume-limited strategies for patients with acute respiratory distress syndrome. A systematic review and meta-analysis. Ann Am Thorac Soc. 2017;14:S271-9.
18. Walkey AJ, Del Sorbo L, Hodgson CL, Adhikari NK, Wunsch H, Meade MO, et al. Higher PEEP versus lower PEEP strategies for patients with acute respiratory distress syndrome. A systematic review and meta-analysis. Ann Am Thorac Soc. 2017;14:S297-303.
19. Essouri S, Carroll C, Pediatric Acute Lung Injury Consensus Conference G. Non-invasive support and ventilation for pediatric acute respiratory distress syndrome: proceedings from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S102-10.
20. Farias JA, Fernandez A, Monteverde E, Flores JC, Baltodano A, Menchaca A, et al. Mechanical ventilation in pediatric intensive care units during the season for acute lower respiratory infection: a multicenter study. Pediatr Crit Care Med. 2012;13:158-64.
21. Kneyber MC, de Luca D, Calderini E, Jarreau PH, Javouhey E, Lopez-Herce J, et al. Recommendations for mechanical ventilation of critically ill children from the Paediatric Mechanical Ventilation Consensus Conference (PEMVECC). Intensive Care Med. 2017;43:1764-80.
22. Rimensberger PC, Cheifetz IM, Pediatric Acute Lung Injury Consensus Conference G. Ventilatory support in children with pediatric acute respiratory distress syndrome: proceedings from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S51-60.
23. Britos M, Smoot E, Liu KD, Thompson BT, Checkley W, Brower RG, et al. The value of positive end-expiratory pressure and FiO_2 criteria in the definition of the acute respiratory distress syndrome. Crit Care Med. 2011;39:2025-30.
24. Bateman ST, Borasino S, Asaro LA, Cheifetz IM, Diane S, Wypij D, et al. Early high-frequency oscillatory ventilation in pediatric acute respiratory failure. A propensity score analysis. Am J Respir Crit Care Med. 2016;193:495-503.
25. Lall R, Hamilton P, Young D, Hulme C, Hall P, Shah S, et al. A randomised controlled trial and cost-effectiveness analysis of high-frequency oscillatory ventilation against conventional artificial ventilation for adults with acute respiratory distress syndrome. The OSCAR (OSCillation in ARDS) study. Health Technol Assess. 2015;19:1-177.
26. Ganesan SL, Jayashree M, Singhi SC, Bansal A. Airway pressure release ventilation in pediatric acute respiratory distress syndrome. A randomized controlled trial. Am J Respir Crit Care Med. 2018;198:1199-207.

27. Fineman LD, LaBrecque MA, Shih MC, Curley MA. Prone positioning can be safely performed in critically ill infants and children. Pediatr Crit Care Med. 2006;7:413-22.
28. Gillies D, Wells D, Bhandari AP. Positioning for acute respiratory distress in hospitalised infants and children. Cochrane Database Syst Rev. 2012;7:1-68.
29. Tamburro RF, Kneyber MC, Pediatric Acute Lung Injury Consensus Conference G. Pulmonary specific ancillary treatment for pediatric acute respiratory distress syndrome: proceedings from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S61-72.
30. Curley MA, Hibberd PL, Fineman LD, Wypij D, Shih MC, Thompson JE, et al. Effect of prone positioning on clinical outcomes in children with acute lung injury randomized controlled trial. JAMA. 2005;294:229-37.
31. Sud S, Friedrich JO, Taccone P, Polli F, Adhikari NK, Latini R, et al. Prone ventilation reduces mortality in patients with acute respiratory failure and severe hypoxemia: systematic review and meta-analysis. Intensive Care Med. 2010;36:585-99.
32. Meng L, Qiu H, Wan L, Ai Y, Xue Z, Guo Q, et al. Intubation and ventilation amid the COVID-19 outbreak: Wuhan's experience. Anesthesiology. 2020;132:1317-32.
33. Sorbello M, El-Boghdadly K, Di Giacinto I, Cataldo R, Esposito C, Falcetta S, et al. The Italian coronavirus disease 2019 outbreak: recommendations from clinical practice. Anaesthesia. 2020;75:724-32.
34. Barrett KA, Hawkins N, Fan E. Economic evaluation of venovenous extracorporeal membrane oxygenation for severe acute respiratory distress syndrome. Crit Care Med. 2019;47:186-93.
35. Crow S, Fischer AC, Schears RM. Extracorporeal life support: utilization, cost, controversy, and ethics of trying to save lives. Semin Cardiothorac Vasc Anesth. 2009;13:183-91.
36. Dalton HJ, Macrae DJ, Pediatric Acute Lung Injury Consensus Conference G. Extracorporeal support in children with pediatric acute respiratory distress syndrome: proceedings from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S111-7.
37. Rowan CM, Klein MJ, Hsing DD, Dahmer MK, Spinella PC, Emeriaud G, et al. Early use of adjunctive therapies for pediatric acute respiratory distress syndrome: a PARDIE study. Am J Respir Crit Care Med. 2020;201:1389-97.
38. Valentine SL, Nadkarni VM, Curley MA, Pediatric Acute Lung Injury Consensus Conference G. Nonpulmonary treatments for pediatric acute respiratory distress syndrome: Proceedings From The Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S73-85.
39. Flori HR, Church G, Liu KD, Gildengorin G, Matthay MA. Positive fluid balance is associated with higher mortality and prolonged mechanical ventilation in pediatric patients with acute lung injury. Crit Care Res Pract. 2011;2011:1-5.
40. Chan KS, Mourtzakis M, Friedman LA, Dinglas VD, Hough CL, Ely EW, et al. Evaluating muscle mass in survivors of acute respiratory distress syndrome: a 1-year multicenter longitudinal study. Crit Care Med. 2018;46:1238-46.
41. National Heart, Lung, and Blood Institute PETAL Clinical Trials Network; Moss M, Huang DT, Brower RG, Ferguson ND, et al. Early neuromuscular blockade in the acute respiratory distress syndrome. N Engl J Med. 2019;380:1997-2008.
42. Needham CJ, Brindley PG. Best evidence in critical care medicine: the role of neuromuscular blocking drugs in early severe acute respiratory distress syndrome. Can J Anaesth. 2012;59:105-8.
43. Gebistorf F, Karam O, Wetterslev J, Afshari A. Inhaled nitric oxide for acute respiratory distress syndrome (ARDS) in children and adults. Cochrane Database Syst Rev. 2018;12:1-77.
44. Ruan SY, Huang TM, Wu HY, Wu HD, Yu CJ, Lai MS. Inhaled nitric oxide therapy and risk of renal dysfunction: a systematic review and meta-analysis of randomized trials. Crit Care. 2015;19:1-10.
45. Drago BB, Kimura D, Rovnaghi CR, Schwingshackl A, Rayburn M, Meduri GU, et al. Double-blind, placebo-controlled pilot randomized trial of methylprednisolone infusion in pediatric acute respiratory distress syndrome. Pediatr Crit Care Med. 2015;16:e74-81.
46. Meduri GU, Bridges L, Shih MC, Marik PE, Siemieniuk RA, Kocak M. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. Intensive Care Med. 2016;42:829-40.
47. Willson DF, Thomas NJ, Markovitz BP, Bauman LA, DiCarlo JV, Pon S, et al. Effect of exogenous surfactant (calfactant) in pediatric acute lung injury: a randomized controlled trial. JAMA. 2005;293:470-6.
48. Quasney MW, Lopez-Fernandez YM, Santschi M, Watson RS, Pediatric Acute Lung Injury Consensus Conference G. The outcomes of children with pediatric acute respiratory distress syndrome: Proceedings From The Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015;16:S118-31.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

Respiratory viral infections in pediatric patients with hematopoietic stem cell transplantation

*José F. Gaytán-Morales[1], Iván Castorena-Villa[1], Dolores C. Cortés-Flores[1], Martha J. Avilés-Robles[2], José L. Sánchez-Huerta[3], Vianney Ortiz-Navarrete[4], Irlanda Olvera-Gómez[5,6], Briceida López-Martínez[7], and Israel Parra-Ortega[3]**

[1]Servicio de Trasplante de Médula Ósea, Hospital Infantil de México Federico Gómez, Mexico City; [2]Departamento de Infectología Pediátrica, Hospital Infantil de México Federico Gómez, Mexico City; [3]Departamento de Laboratorio Clínico, Hospital Infantil de México Federico Gómez, Mexico City; [4]Centro de Investigación y Estudios Avanzados, Instituto Politécnico Nacional, Mexico City; [5]Hospitales Federales de Referencia, Hospital Nacional Homeopático, Mexico City; [6]Universidad Anáhuac Norte, Naucalpan de Juárez, State of Mexico; [7]Subdirección de Servicios Auxiliares de Diagnóstico, Mexico City. Mexico

Abstract

Background: *Viral respiratory infections in pediatric patients with hematopoietic stem cell transplantation (HSCT) significantly impact morbidity and mortality. It is necessary to determine the viral agents and their frequency of presentation to understand their impact on transplantation patients' evolution.* **Methods:** *From January 2017 to December 2019, we conducted a cross-sectional, descriptive, and observational study of patients who underwent HSCT with a viral respiratory infection. Viral identification was performed using multiplex polymerase chain reaction for nine respiratory viruses. Descriptive statistics were performed with a report of central tendency measures and percentages.* **Results:** *Of the 54 pediatric patients who underwent HSCT, 59.2% presented an airway infection; in turn, at least one viral agent was identified in 59.3% of these patients. The most frequent viral agents were influenza (25.9%), human rhinovirus (18.5%), and respiratory syncytial virus (18.5%). Viral co-infections occurred in 36.8% of the cases. The reported complications were supplemental oxygen requirement (73.6%), support with mechanical ventilation (21%), admission to the pediatric intensive care unit (15.7%), and mortality associated with a viral respiratory infection (10.5%).* **Conclusions:** *Viral respiratory infections are frequent in pediatric patients with HSCT; influenza A/B virus was the most frequent agent. As morbidity and mortality increase due to these infections in patients with HSCT, strategies are necessary for its prevention and timely treatment after transplantation.*

Key words: *Stem cell transplantation. Respiratory tract infections. Respiratory virus.*

Infecciones respiratorias virales en pacientes pediátricos con trasplante de células progenitoras hematopoyéticas

Resumen

Introducción: *Las infecciones respiratorias virales en los pacientes pediátricos con trasplante de células progenitoras hematopoyéticas (TCPH) impactan significativamente la morbilidad y la mortalidad. Para comprender su impacto en la evolución de los pacientes receptores de trasplantes es necesario conocer la frecuencia de presentación y los agentes virales.*

Correspondence:
*Israel Parra Ortega
E-mail: i_parra29@hotmail.com

Date of reception: 08-05-2020
Date of acceptance: 03-09-2020
DOI: 10.24875/BMHIM.20000126

Available online: 08-06-2021
Bol Med Hosp Infant Mex. 2021;78(3):191-199
www.bmhim.com

Métodos: *De enero de 2017 a diciembre de 2019 se llevó a cabo un estudio transversal, descriptivo y observacional de los pacientes sometidos a TCPH que tuvieron una infección viral de vías respiratorias. La identificación de los virus se realizó por medio de la prueba de reacción en cadena de la polimerasa multiplex para nueve virus respiratorios. Se realizó estadística descriptiva con reporte de medidas de tendencia central y porcentajes.* **Resultados:** *De los 54 pacientes incluidos, el 59.2% presentaron una infección de vías respiratorias y se identificó al menos un agente viral en el 59.3% de estos casos. Los virus más frecuentes fueron influenza (25.9%), rinovirus humano (18.5%) y virus sincitial respiratorio (18.5%). En el 36.8% de los casos se detectaron coinfecciones virales. Se presentaron las siguientes complicaciones: requerimiento de oxígeno suplementario (73.6%), soporte con ventilación mecánica (21%), ingreso a la unidad de cuidados intensivos pediátricos (15.7%) y muerte asociada a infección por virus respiratorios (10.5%).* **Conclusiones:** *Las infecciones respiratorias virales en los pacientes pediátricos con TCPH son frecuentes; el virus influenza A/B es el agente más habitual. Debido a que estas infecciones se asocian con mayor morbimortalidad en los pacientes con TCPH, son estrategias necesarias para su prevención y tratamiento oportuno posterior al trasplante.*

Palabras clave: *Trasplante de médula ósea. Infecciones de vías respiratorias. Virus respiratorio.*

Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for multiple hematologic and non-hematologic pathologies in pediatric patients. One of HSCT's objectives is reconstituting the hematopoietic system, including the immune system. Post-transplant immune reconstitution begins with the recovery of the innate immune response (natural killer [NK] cells, neutrophils, monocytes, dendritic cells, and T cells) from 15 days to 2 months after transplant. The adaptive immune response (CD4+ T cells and B cells) has a later recovery, occurring up to 24 months after transplantation[1]. During viral infections, macrophages and NK cells belong to the innate immune system and are part of the first defense barrier. Toll-like receptors recognize pathogen ligands, such as nucleic acids, and lead to the production of type I interferons (IFN-α and IFN-β), which will limit the infection of other cells[2]. Furthermore, NK cells produce IFN-γ synergizing with other cells, such as dendritic cells. After the onset of the antiviral immune response, T cells and B cells begin their participation. In their CD8+ subpopulation, T cells contribute directly to eliminating infected cells through their TNF-tumor necrosis factor and CD95-CD95R perforin-granzyme B mediated cytotoxic activity[3]. Simultaneously, CD4+ T cells are critical participants for B cell activation, producing a cytokine pro-inflammatory environment and antibody isotype switching[4]. These responses contribute to the control of future viral infections. The absence of normal cellular or effector capacity influences the control of infections and reinfections.

The post-transplant immune response can be affected by multiple factors (the type of transplant, cell source, T cell depletion, graft-versus-host disease [GVHD], immunosuppressive drugs, and associated infections), which favor a state of immunosuppression predisposing post-transplant patients to infections[5,6].

Viral respiratory infections in pediatric patients with HSCT impact overall survival and are associated with increased morbidity and mortality. With a prevalence between 1 and 55% in pediatric patients with HSCT, viral respiratory infections are usually present with a seasonal distribution similar to that of the pediatric population, prevailing during the autumn/winter season[7-9]. Most viral respiratory infections in these patients are usually acquired in the community, with the most frequently reported agents being human rhinovirus, influenza, respiratory syncytial virus (RSV), parainfluenza, adenovirus, metapneumovirus, and coronavirus, among others. However, up to 48% of respiratory viral infections may be associated with nosocomial transmission[10,11]. Clinical manifestations range from mild upper airway symptoms (rhinorrhea, odynophagia, coughing, sneezing, or epiphora) to severe pneumonia with respiratory failure. The incidence of the upper respiratory tract infections (URTIs) in pediatric patients with HSCT varies depending on the viral agent: influenza A/B virus and human rhinovirus show the highest incidence (1-50%). Lower respiratory tract infections (LRTIs) also depend on the viral agent; however, they can present an incidence from 0 to 70% at diagnosis. Between 20 and 30% of URTIs progress to LRTIs, depending on the viral agent causing the infection[7,12].

Risk factors for URTI progression to LRTI in pediatric patients with HSCT are the following: GVHD, steroid use, a myeloablative conditioning regimen, the timing of infection relative to the transplant period,

lymphopenia (< 200 lymphocytes/μl), and neutropenia (< 500 neutrophils/μl)[13,14].

In patients undergoing HSCT, viral respiratory infections are associated with prognosis and survival. Mortality associated with viral respiratory infections is approximately 20%, although some studies report up to 40%[15-17]. Other complications associated with viral respiratory infections are decreased total lung capacity (67%), alloimmune pulmonary syndromes, idiopathic interstitial pneumonia, bronchiolitis obliterans (10.9%), and bronchiolitis obliterans with organizing pneumonia (16.4%). Case series where the viral respiratory infection was associated with delayed engraftment or, in the most severe cases, secondary long-term graft loss have been reported[7,18,19].

In the HSCT patient, the immune system does not generate a response similar to that of the healthy pediatric patient during a viral infection since there is a depletion of innate and adaptive immunity, together with the loss of barrier mechanisms (mucosa) secondary to the transplantation process (chemotherapy and radiotherapy). In addition to the previously mentioned factors, these alterations generate a greater predisposition to complications and death in the transplanted patient[20-22]. To know the frequency and evolution of viral respiratory infections and the viral agents found in patients who underwent HSCT, we reviewed the cases at the Hospital Infantil de México Federico Gómez (HIMFG).

Methods

From January 2017 to December 2019, we conducted a cross-sectional, descriptive, observational study in HSCT patients (aged 0 to 18 years) with a viral respiratory tract infection (RTIs), and viral agent isolation at the HIMFG.

Patients

We included inpatients and outpatients with HSCT presenting to the emergency department with respiratory symptoms and a positive respiratory panel, patients in the immediate post-transplant period, and patients under surveillance with a survival of more than 2 years. Furthermore, patients with clinically GVHD data and under treatment with immunosuppressive drugs were considered. We excluded patients with incomplete data on the record and no isolated viral agent in the respiratory virus panel.

We collected the following data: underlying disease, type of transplant, GVHD, immunosuppressive treatment, other treatments applied, and patient evolution. Chemotherapy and radiotherapy were used in all patients according to the underlying pathology and the indicated conditioning scheme. Furthermore, patients who had an allogeneic transplant received prophylactic treatment for GVHD. As a protocol and following international guidelines, antiviral (herpes family virus), antifungal, and antibacterial prophylaxis were used in all patients. In patients whose report was positive for a viral agent in a sample obtained from the nasopharyngeal swab, no isolation of bacterial pathogens was evidenced by other laboratory means.

Definitions

Airway infection was defined in a patient who presented respiratory symptoms during hospitalization or as an outpatient with rhinorrhea, cough, odynophagia, and nasal congestion. Viral airway infection was considered in a patient with respiratory symptoms in whom a viral agent was identified by polymerase chain reaction (PCR) test. URTI was defined in cases with respiratory symptoms and no hypoxemia or changes in chest X-ray, while LRTI in cases with respiratory symptoms and hypoxemia and changes in chest X-ray. Reinfection was classified in any patient who presented a repeated clinical event of respiratory infection, with the isolation of the viral agent. GVHD was defined and classified according to the literature[23]. Neutropenia was defined as neutrophils < 500 cells/μl and lymphopenia as lymphocytes < 200 cells/μl.

Complications assessed were the requirement for supplemental oxygen (non-rebreather mask, face tent, or nasal cannula), non-invasive mechanical ventilation, invasive mechanical ventilation, admission to the pediatric intensive care unit, and death related to RTIs.

Respiratory virus panel

Multiplex PCR was performed using low-density microarrays, employing the CLART® PneumoVir platform (Genomica), following the manufacturer's instructions for the detection of the following viruses: adenovirus, human rhinovirus, bocavirus, coronavirus, and enterovirus (echovirus), influenza A (human H3N2, human H1N1, and H1N1/2009 subtypes), influenza B, influenza C, human metapneumovirus (subtypes A and B), parainfluenza (1, 2, 3, and 4), and RSV type A and B. Subsequently, on March 2019, 21 respiratory

tract infectious agents were determined simultaneously by identifying nucleic acids of respiratory viruses and bacteria with labeled microspheres. The NxTAG® Respiratory Pathogen Panel (qualitative test) was used. The reaction was amplified through reverse transcription and PCR in a single step, and the resulting product was subjected to microsphere hybridization within the same reaction tube. The labeled and hybridized microspheres were then classified and read using the MAGPIX® instrument, and the signals were analyzed using SYNCT™ software. Agents detected were influenza A, influenza A H1, influenza A H3, influenza B, RSV (A and B), coronavirus 229E, coronavirus OC43, coronavirus NL63, coronavirus HKU1, human metapneumovirus, human rhinovirus/human enterovirus, adenovirus, parainfluenza (1, 2, 3, and 4), human bocavirus, *Chlamydophila pneumoniae*, *Mycoplasma pneumonia*, and *Legionella pneumophila*.

Statistical analysis

Descriptive statistics were performed reporting measures of central tendency and percentages.

Results

Fifty-four patients with HSCT were reported during the study, from which 66.6% (36/54) presented a clinical picture of respiratory infection in the post-transplant stage (from day 0 to 1140). Four events were excluded from the analysis because of missing data on the clinical record; thus, 32 clinical events of RTIs were reviewed. Patients with positive virus results by PCR were analyzed to avoid bias due to other infectious agents. In 62.5% (20/32) of these clinical events, the respiratory virus panel confirmed a viral etiology.

The patients' mean age was 8 years 11 months (2 years 8 months-15 years 4 months), and 65% (13/20) were female. The diseases present were acute lymphoblastic leukemia (10/20), acute myeloid leukemia (4/20), benign hematologic diseases (2/20), solid tumors (2/20), and osteopetrosis (1/20). A total of 7/20 haploidentical transplants (35%), 2/20 autologous transplants (10%), and 18/20 allogeneic transplants (90%) were performed. The following conditioning regimens were used: myeloablative in 16/20 (80%) and reduced intensity in 4/20 (20%). The characteristics of the population are described in table 1.

Patients with RTIs were at different post-transplant stages (day 0-day 1140). There were 7/20 (35%) RTI

Table 1. Characteristics of patients with hematopoietic stem cell transplantation who developed a viral respiratory infection

	Patients with HSCT and respiratory infection of viral etiology (n = 20)
Mean age	8 years 11 months
Gender	
Male	7
Female	13
Underlying disease	
ALL	10
AML	4
SAA	2
PRCA	1
Neuroblastoma	1
Retinoblastoma	1
Osteopetrosis	1
Type of transplant	
Allogeneic	11
DR 100%	7
DR 90%	1
DR 75%	2
Not related 100%	1
Haploidentical	7
Autologous	2
Cell source	
Bone marrow	2
Peripheral blood	18
Conditioning regimen	
Myeloablative	16
Reduced-intensity	4
Type of infection	
URTI	30%
LRTI	70%
Progression	10%
Symptoms	
Rhinorrhea	25%
Cough	40.6%
Crackles	18.7%
Respiratory distress	50%
Fever	31.2%
Low oxygen saturation or hypoxemia	46.8%
Odynophagia	15.6%
Changes in imaging studies	46.8%
Results of blood analysis	
Lymphopenia (< 200 cells/µl)	6.2%
Neutropenia (< 500 cells/µl)	9.3%
Complications	
Supplemental oxygen requirement	65.6%
Mechanical ventilation	18.7%
Intensive care unit	12.5%
Death	6.2%

(Continues)

Table 1. Characteristics of patients with hematopoietic stem cell transplantation who developed a viral respiratory infection *(Continued)*

	Patients with HSCT and respiratory infection of viral etiology (n = 20)
Treatment	
Antibiotic	80%
Antiviral	15%
Symptomatic/surveillance	5%

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HSCT: hematopoietic stem-cell transplantation; LRTI: lower respiratory tract infection; PRCA: pure red cell aplasia; SAA: severe aplastic anemia; URTI: upper respiratory tract infection.

Table 2. Respiratory viruses detected in patients with respiratory symptomatology

Virus isolated	n (%)
Adenovirus	4 (14.8)
Influenza	7 (25.9)
Influenza A	2 (7.4)
Influenza A H1N1	1 (3.7)
Influenza A H3N2	2 (7.4)
Influenza B	2 (7.4)
Metapneumovirus B	4 (14.8)
Parainfluenza 3	2 (7.4)
Rhinovirus	5 (18.5)
Respiratory syncytial virus	5 (18.5)
Respiratory syncytial virus A	4 (14.8)
Respiratory syncytial virus B	1 (3.7)
Total viruses isolated	27 (100)

Table 3. Respiratory viruses isolated according to the clinical presentation

	Isolated agents n (%)
URTI	8 (29.6)
Adenovirus	2
Parainfluenza	1
Influenza	2
Influenza A	1
Influenza B	1
Metapneumovirus B	2
Human rhinovirus/enterovirus	1
LRTI	19 (70.3)
Adenovirus	2
Parainfluenza	1
Influenza	5
Influenza A	1
Influenza A H3N2	2
Influenza A H1N1	1
Influenza B	1
RSV	5
RSV A	4
RSV B	1
Metapneumovirus B	2
Human rhinovirus	4
Total isolated agents	27 (100%)

LRTI: lower respiratory tract infection; RSV: respiratory syncytial virus; URTI: upper respiratory tract infection.

events during the first 100 days post-transplant, 3/20 (15%) between 100 and 365 days post-transplant, and 9/20 (45%) after 365 days post-transplant. Only 2/20 (10%) of RTIs occurred in the first 30 days post-HSCT.

A total of 27 viruses were identified (Table 2). In URTI, the most frequent viruses were adenovirus, influenza A/B, and metapneumovirus, while in LRTI, influenza, RSV, and human rhinovirus (Table 3). Viral co-infections were identified in 36.8% of the viral infections (7/19), the most frequent being influenza A-metapneumovirus B in two cases. Other co-infections were influenza A-human rhinovirus, influenza B-metapneumovirus B, influenza A H3N2-metapneumovirus B, human rhinovirus-metapneumovirus B, RSV B-adenovirus, and one case of three viral agents' co-infection: RSV A-human rhinovirus-adenovirus. A significant seasonal distribution was observed in the fall/winter season (15/19 events; 78.9%) in comparison to spring/summer (4/19 events; 21%) (Fig. 1).

Clinically, 31% of patients (6/20) showed URTI symptoms, and 15% (3/20) progressed to LRTI. A complication was observed in 83.3% of patients (5/6) in the URTI group. Furthermore, 65% of patients (13/20) showed LRTI symptoms, and all had at least one complication. In 52.6% of the chest X-rays, variable images ranging from mild interstitial infiltrate to consolidation were identified.

Regarding treatment, 84% of the patients were administered with empirical broad-spectrum antibiotics (cefepime, meropenem, and vancomycin) before identifying the viral agent. Management with an antiviral (oseltamivir) was initiated in 21% of the patients, and only supportive and symptomatic management in 5%.

Regarding complications, supplemental oxygen was the most frequently used in 70% of the events (nasal

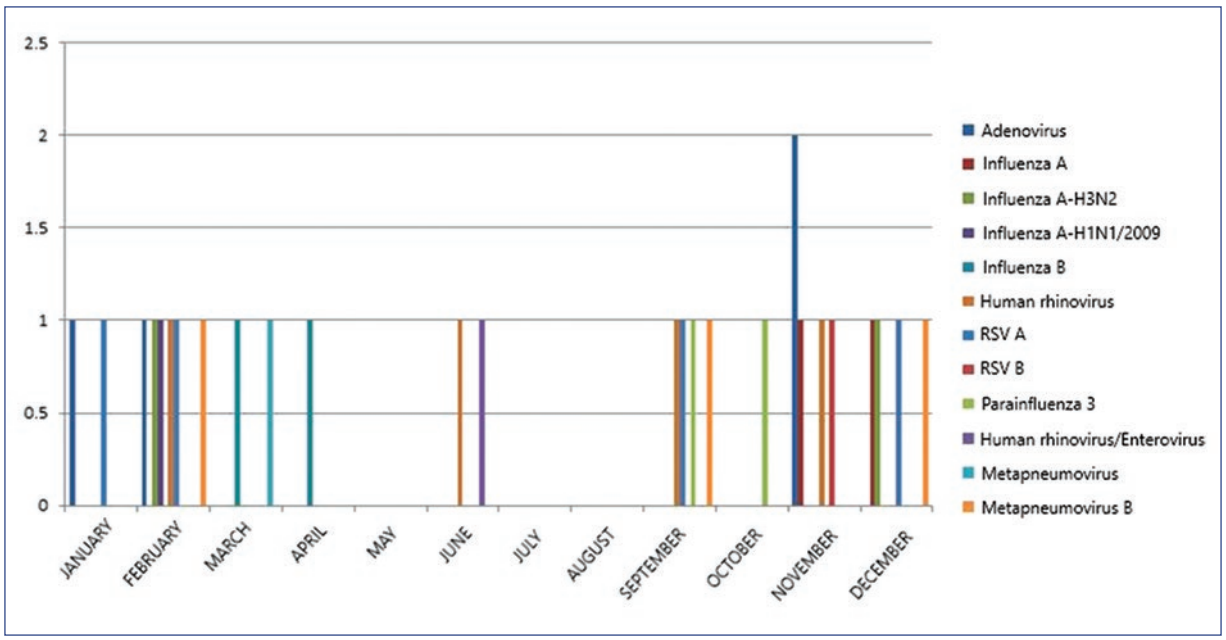

Figure 1. Seasonal distribution of viruses in hematopoietic stem cell transplant patients. RSV: respiratory syncytial virus.

prongs [2/20] or non-rebreathing mask [12/20]), followed by mechanical ventilation support (non-invasive [2/20] or invasive [2/20]), which was required in 20% of the events. Furthermore, 15.7% of the events required management in a pediatric intensive care unit. Mortality of 10.5% was associated with RTIs, isolating RSV A in one patient and influenza A- metapneumovirus B in another patient.

Discussion

According to our results, community-acquired viral respiratory infections are frequent infectious events in post-transplant patients. Literature has reported a predominance of viral respiratory infections in non-transplanted patients < 5 years old; however, we did not observe an age-related predominance of viral respiratory infections in this group of patients. It is necessary to consider that HSCT favors the immunosuppressive state of patients at any age[12,24].

Furthermore, we detected a viral RTIs in 25.9% of the HSCT patients (14/54) during the post-transplant stage, and 52% of the events occurred in the first year post-transplant. Different studies have shown higher frequencies of infection and risk of complications in the first year post-transplant[25-27]. Our sample of HSCT patients behaved similarly to other literature reports since respiratory viral infections can occur in any HSCT patient, even with different underlying diagnoses (oncologic or hematologic, and benign or malignant), different conditioning regimens, types of transplant, and cell sources.

Notably, only two patients showed respiratory infections during the period in which they were in the HSCT unit (first 30 days after transplantation). During this period, they have more extraordinary protection measures. Health-care personnel (medical, nursing, cleaning, and maintenance personnel) at the hospital and family members accompanying the patient should be considered a source of infection. Respiratory infections after 100 days post-transplant occurred in a significant percentage of patients (63%). Considering that they are under ambulatory follow-up and in contact with community agents, it is necessary to be aware of multiple risk factors for viral respiratory infections, such as myeloablative conditioning regimen, immune reconstitution, GVHD activity, use of immunosuppressive treatment, and preventive measures (vaccines)[27-29].

A highly variable frequency of respiratory viral infections (5-46%) has been described in the literature, depending on the respiratory virus isolated. The frequency of presentation in the population included in this study is in line with other reports, suggesting that viruses are an important part of the etiological agents of respiratory infections in post-transplant patients[7,29,30]. The seasonal distribution of viral agents found in

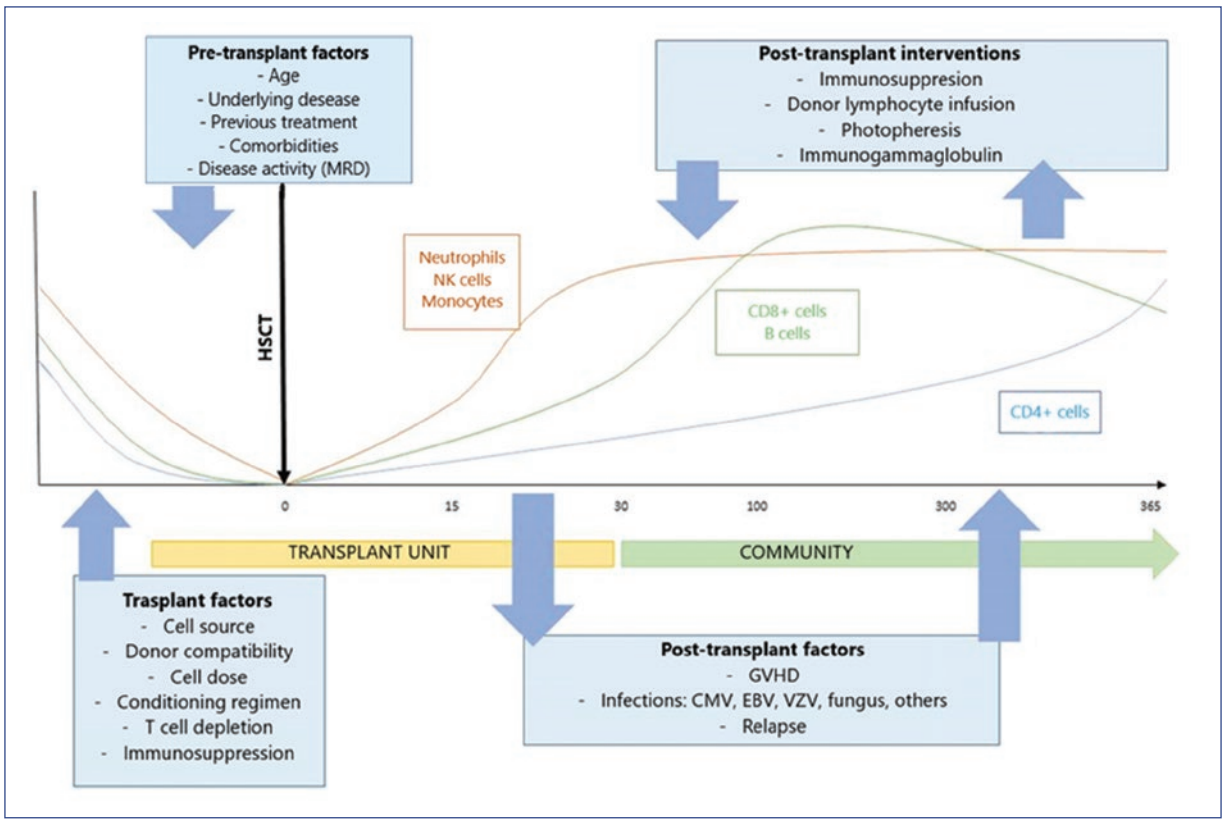

Figure 2. Factors involved and expected immune reconstitution in the hematopoietic stem cell transplant patient. CMV: cytomegalovirus; EBV: Epstein Barr virus; GVHD: graft-versus-host disease; MRD: minimal residual disease; NK: natural killer cells; VZV: varicella-zoster virus.

patients who underwent HSCT was similar to the seasonal distribution of viruses in other pediatric patients at the Hospital Infantil de México Federico Gómez. In a study conducted by Ison and Hirsch in 2019, it is mentioned that the most frequently identified viral agents in patients with HSCT were human rhinovirus, RSV, and influenza, which present a similar seasonality to that of the community, being more frequent in autumn and winter[31].

Soudani et al. conducted a study of the prevalence of acute respiratory infections in patients with cancer and HSCT, demonstrating a higher incidence of respiratory infections, in addition to co-infections with multiple viruses[9]. In our patients, we found a high frequency (36.8%) of viral co-infections; additionally, three patients had recurrent reinfections. This high frequency observed in our study may be due to multiple factors such as GVHD (present in 14/20) or immunosuppressant use (15/20), among others. When dealing with patients with HSCT, it is necessary to consider that respiratory infections with community viral agents are facilitated. Due to a compromised immune system, these patients may present reinfections with the same viral agent and, occasionally, co-infections.

One of the limitations of this study is that we did not correlate risk factors with the presentation of RTIs.

More LRTI than URTI were found (70% vs. 30%, respectively), with a progression from upper to lower clinical presentation. In Korea, in 2013, Choi et al. studied 175 pediatric transplant patients who had 89 clinical episodes of viral respiratory infections: about 61.8% of the infections were in upper airways, 38.2% in lower airways, and mortality was associated with viral infections in 3.3% (3/89)[32]. We consider that our patients' immunosuppression state favored the presentation of severe clinical pictures and a tendency to complications.

Since these patients were immunocompromised empirical antibiotic coverage was initiated in 80% of the patients as part of the initial management of respiratory infections. However, after identifying the viral agent responsible for the respiratory symptoms, directed

treatment was given in 20% of the patients. Management with neuraminidase inhibitors was initiated in patients in whom influenza A/B was detected; 5% of the patients received treatment for general symptoms. In our patients, treatment with antiviral agents was only given to those who presented influenza. However, it has been described that the use of inhaled ribavirin as a treatment for RSV infections in patients with HSCT reduced viral load, invasive mechanical ventilation, and hospital stay[15,27].

Complications occurred in 90% of patients, the most frequent being the requirement of supplemental oxygen (70%). Two patients died in association with the viral respiratory infection: the first patient required HSCT due to pure red cell aplasia and had three clinical events of respiratory infections 100 days post-transplant, with different viral agents isolated in each infection. In the last infectious event, the patient presented a co-infection with influenza A and metapneumovirus B, which contributed to respiratory failure, requiring support with assisted mechanical ventilation, and progressing to severe pulmonary damage. The second patient required an HSCT for acute myeloid leukemia and died secondary to a respiratory failure event with the isolation of influenza A H1N1; besides, she presented septic shock data. Although no other pathogenic agent was isolated as the septic shock etiology, we cannot rule out its participation. Mortality associated with viral respiratory infections in pediatric patients with HSCT is highly variable. Some studies reported that it is 3% and as high as 70% in other studies. This frequency of presentation is associated with multiple characteristics: the identified viral agent, time of presentation of the infection regarding the transplant, type of transplant performed, conditioning regimen used, presence of GVHD, immunosuppressants (steroids), and chronic complications[25,28]. We consider that one explanation for this response is the late immune reconstitution of hematopoietic progenitor cell transplantation (Fig. 2). In the study conducted by Parra et al., it was reported that patients with HSCT achieve a late immune reconstitution, having an adequate cellular number up to 2 years after transplantation; however, no association with a more significant number of infections was observed[33].

Viral respiratory infections are frequent in pediatric patients with HSCT (62.5%) and occur throughout the year. The most frequent viruses were human rhinovirus, RSV, and influenza. As these infections have an essential impact on morbidity and mortality, efforts should be made to establish strategies for their prevention, identification, and timely supportive management.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare no conflict of interest.

Funding

None.

References

1. de Koning C, Plantinga M, Besseling P, Boelens JJ, Nierkens S. Immune reconstitution after allogeneic hematopoietic cell transplantation in children. Biol Blood Marrow Transplant. 2016;22:195-206.
2. Honda K, Yanai H, Takaoka A, Taniguchi T. Regulation of the Type I IFN induction: a current view. Int Immunol. 2005;17:1367-78.
3. Kulinski JM, Tarakanova VL, Verbsky J. Regulation of antiviral CD8 T-cell responses. Crit Rev Immunol. 2013;33:477-88.
4. Chen CC, Koenig A, Saison C, Dahdal S, Rigault G, Barba T, et al. CD4+ T cell help is mandatory for naive and memory donor-specific antibody responses: impact of therapeutic immunosuppression. Front Immunol. 2018;9:1-14.
5. van der Maas NG, Berghuis D, van der Burg M, Lankester AC. B Cell reconstitution and influencing factors after hematopoietic stem cell transplantation in children. Front Immunol. 2019;10:1-8.
6. Bae KW, Kim BE, Koh KN, Im HJ, Seo JJ. Factors influencing lymphocyte reconstitution after allogeneic hematopoietic stem cell transplantation in children. Korean J Hematol. 2012;47:44-52.
7. Versluys AB, Boelens JJ. Morbidity and mortality associated with respiratory virus infections in allogeneic hematopoietic cell transplant: too little defense or harmful immunity? Front Microbiol. 2018;9:1-7.
8. Vliora C, Papadakis V, Doganis D, Tourkantoni N, Paisiou A, Kottaridi C, et al. A prospective study on the epidemiology and clinical significance of viral respiratory infections among pediatric oncology patients. Pediatr Hematol Oncol. 2019;36:173-186.
9. Soudani N, Caniza MA, Assaf-Casals A, Shaker R, Lteif M, Su Y, et al. Prevalence and characteristics of acute respiratory virus infections in pediatric cancer patients. J Med Virol. 2019;91:1191-1201.
10. Abbas S, Raybould JE, Sastry S, de la Cruz O. Respiratory viruses in transplant recipients: more than just a cold. Clinical syndromes and infection prevention principles. Int J Infect Dis. 2017;62:86-93.
11. Lo MS, Lee GM, Gunawardane N, Burchett SK, Lachenauer CS, Lehmann LE. The impact of RSV, adenovirus, influenza, and parainfluenza infection in pediatric patients receiving stem cell transplant, solid organ transplant, or cancer chemotherapy. Pediatr Transplant. 2013;17:133-43.
12. Pochon C, Voigt S. Respiratory virus infections in hematopoietic cell transplant recipients. Front Microbiol. 2019;9:1-17.
13. Hijano DR, Maron G, Hayden RT. Respiratory viral infections in patients with cancer or undergoing hematopoietic cell transplant. Front Microbiol. 2018;9:1-19.
14. Shah DP, Ghantoji SS, Ariza-Heredia EJ, Shah JN, El Taoum KK, Shah PK, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. Blood. 2014;123:3263-8.
15. Khawaja F, Chemaly RF. Respiratory syncytial virus in hematopoietic cell transplant recipients and patients with hematologic malignancies. Haematologica. 2019;104:1322-31.

16. Ljungman P. Respiratory virus infections in stem cell transplant patients: the European experience. Biol Blood Marrow Transplant. 2001;7:5S-7S.
17. Rivadeneyra P. Factores Asociados a Complicaciones Infecciosas en Pacientes Pediátricos con Trasplante de Células Progenitoras Hematopoyéticas. Ciudad de México: Hospital Infantil de México Federico Gómez-UNAM, México. Tesis de Posgrado; 2019.
18. Versluys AB, Rossen JW, van Ewijk B, Schuurman R, Bierings MB, Boelens JJ. Strong association between respiratory viral infection early after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic alloimmune lung syndromes. Biol Blood Marrow Transplant. 2010;16:782-91.
19. McCarthy AJ, Kingman HM, Kelly C, Taylor GS, Caul EO, Grier D, et al. The outcome of 26 patients with respiratory syncytial virus infection following allogeneic stem cell transplantation. Bone Marrow Transplant. 1999;24:1315-22.
20. Lewin SR, Heller G, Zhang L, Rodrigues E, Skulsky E, van de Brink MR, et al. Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. Blood. 2002;100:2235-42.
21. Novitzky N, Davison GM. Immune reconstitution following hematopoietic stem-cell transplantation. Cytotherapy. 2001;3:211-20.
22. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Wingard JR, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15:1143-238.
23. Ferrara J, Antin J. The pathophysiology of graft-versus-host disease. In: Forman S, Negrin R, editors. Thomas' Hematopoietic Cell Transplantation: stem Cell Transplantation. West Sussex: Wiley Blackwell; 2015. p. 146-55.
24. Weigt SS, Gregson AL, Deng JC, Lynch JP 3rd, Belperio JA. Respiratory viral infections in hematopoietic stem cell and solid organ transplant recipients. Semin Respir Crit Care Med. 2011;32:471-93.
25. Hutspardol S, Essa M, Richardson S, Schechter T, Ali M, Krueger J, et al. Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21:1802-7.
26. Fontana L, Strasfeld L. Respiratory virus infections of the stem cell transplant recipient and the hematologic malignancy patient. Infect Dis Clin North Am. 2019;33:523-44.
27. Piñana JL, Pérez A, Montoro J, Hernani R, Lorenzo I, Giménez E, et al. The effect of timing on community-acquired respiratory virus infection mortality during the first year after allogeneic hematopoietic stem cell transplantation: a prospective epidemiological survey. Bone Marrow Transplant. 2020;55:431-40.
28. Renaud C, Xie H, Seo S, Kuypers J, Cent A, Corey L, et al. Mortality rates of human metapneumovirus and respiratory syncytial virus lower respiratory tract infections in hematopoietic cell transplantation recipients. Biol Blood Marrow Transplant. 2013;19:1220-6.
29. Paulsen GC, Danziger-Isakov L. Respiratory viral infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med. 2017;38:707-26.
30. García-Castillo C, Olaya-Vargas A, Pérez-García M, Melchor-Vidal YB, Cardoso-Yah G, Torres-Alarcón CG. Procesos infecciosos en el trasplante de células progenitoras hematopoyéticas. Rev Hematol Mex. 2020; 21:41-50.
31. Ison MG, Hirsch HH. Community-acquired respiratory viruses in transplant patients: diversity, impact, unmet clinical needs. Clin Microbiol Rev. 2019;32:1-32.
32. Choi JH, Choi EH, Kang HJ, Park KD, Park SS, Shin HY, et al. Respiratory viral infections after hematopoietic stem cell transplantation in children. J Korean Med Sci. 2013;28:36-41.
33. Parra-Ortega I, Nájera-Martínez N, Gaytán-Morales F, Castorena-Villa I, Cortés-Flores C, López-Martínez B, et al. Reconstitución de células NK posterior al trasplante de células progenitoras hematopoyéticas (TCPH) en pacientes pediátricos. Gac Med Mex. 2020;156:188-94.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

Placental *SLC38A4* gene polymorphisms 1304 G > A and 292 C > T, and their association with glucose > 95 mg/dL in normal weight full-term healthy newborns

Carlos Galaviz-Hernández[1], *Martha Sosa-Macías*[1], *Martha Rodríguez-Morán*[2], *Gerardo Martínez-Aguilar*[2], *Fernando Guerrero-Romero*[2], *and Siblie M. González-Rentería*[3]*

[1]*Academia de Genómica, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Durango, Instituto Politécnico Nacional;* [2]*Unidad de Investigación Biomédica, Instituto Mexicano del Seguro Social;* [3]*Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango. Durango, Mexico*

Abstract

Background: *The SLC38A4 gene encodes for the SNAT4 protein, which has been related to glucose metabolic alterations in human newborns. This study aimed to determine whether the 1304 G > A and 292 C > T polymorphisms of the SLC38A4 gene are associated with the presence of glucose levels > 95 mg/dL in normal weight full-term healthy newborns.* **Methods:** *We conducted a case–control study and analyzed 50 normal weight full-term healthy newborns. Groups were defined based on glucose levels: > 95 mg/dL (cases; n = 13) and < 95 mg/dL (controls; n = 37). The 1304 G > A and 292 C > T polymorphisms of the SLC38A4 gene were determined through quantitative polymerase chain reaction using placental DNA. The association between polymorphism and glucose levels > 95 mg/dL was established using multivariate logistic regression analysis.* **Results:** *No significant differences were observed either for gestational age or body weight at birth between groups. In the case group, newborns showed significantly higher homeostatic model assessment for insulin resistance than those in the control group (p < 0.0005). The odds ratio (OR) between the SLC38A4 gene 292 C > T single-nucleotide polymorphism (SNP) and glucose levels > 95 mg/dL was 7.78 (p = 0.024), whereas no significant association was found for the 1304 G > A SNP (OR 1.46; p = 0.77).* **Conclusions:** *Our results suggest that the SLC38A4 gene 292 C > T SNP is associated with glucose levels > 95 mg/dL in normal weight full-term healthy newborns.*

Key words: *SLC38A4. Polymorphisms. Glucose. Newborns.*

Polimorfismos 1304 G > A y 292 C > T del gen placentario SLC38A4 y su asociación con glucosa > 95 mg/dL en recién nacidos a término, sanos y con peso normal al nacimiento

Resumen

Introducción: *El gen SLC38A4 codifica la proteína SNAT4, que se ha relacionado con alteraciones en el metabolismo de la glucosa en los humanos. El objetivo de este estudio fue determinar si los polimorfismos 1304 G > A y 292 C > T del gen SLC38A4 se asocian con concentraciones de glucosa > 95 mg/dL en recién nacidos a término.* **Métodos:** *Se llevó a cabo*

Correspondence:
*Siblie M. González-Rentería
E-mail: siblie2@hotmail.com

Date of reception: 16-04-2020
Date of acceptance: 03-09-2020
DOI: 10.24875/BMHIM.20000108

Available online: 03-05-2021
Bol Med Hosp Infant Mex. 2021;78(3):200-206
www.bmhim.com

un estudio de casos y controles con 50 recién nacidos a término, sanos, con peso normal al nacimiento. Los grupos se definieron de acuerdo con las concentraciones de glucosa: > 95 mg/dL (casos; n = 13) y < 95 mg/dL (controles; n = 37). Los polimorfismos 1304 G > A y 292 C > T del gen SLC38A4 se genotipificaron por qPCR utilizando ADN de la placenta. La asociación entre los polimorfismos y la concentración de glucosa > 95 mg/dL se estableció mediante la estimación de la razón de momios (RM) en un análisis múltiple de regresión logística. **Resultados:** *No se observaron diferencias estadísticamente significativas para la edad gestacional y el peso al nacer entre los grupos de estudio. El modelo homeostático para evaluar la resistencia a la insulina (HOMA-IR) fue significativamente más alto en los recién nacidos del grupo de casos que en el grupo control (p < 0.0005). La RM mostró asociación significativa entre el polimorfismo de nucleótido único (SNP) 292 C > T del gen SLC38A4 y la concentración de glucosa > 95 mg/dL (RM: 7.78; p = 0.024); el SNP 1304 G > A no mostró asociación significativa (RM: 1.46; p = 0.77).* **Conclusiones:** *Los resultados de este estudio sugieren que el SNP 292 C > T del gen SLC38A4 se asocia con concentraciones de glucosa > 95 mg/dL en recién nacidos a término.*

Palabras clave: *SLC38A4. Polimorfismos. Glucosa. Recién nacidos.*

Introduction

In the early postnatal period, newborn infants maintain glucose homeostasis through glycogenolysis and gluconeogenesis, both essential processes for the central nervous system after birth. Neonatal glucose imbalance is among the most common metabolic abnormalities in preterm newborns and is inversely related to gestational age and birth weight[1]. Furthermore, conditions such as perinatal asphyxia, respiratory distress, chronic stress, and some mothers' characteristics such as a history of gestational diabetes, high blood pressure, obesity, and aging are risk factors for neonatal glucose imbalance[2,3].

Normal glucose levels vary between 60 and 80 mg/dL in newborns, while values ≥ 126 mg/dL are considered as hyperglycemia[1].

The genetic/environment interaction during pregnancy results in fetal programming, a process that may alter the structure and function of tissues, predisposing to the presence of adulthood diseases[4]. Furthermore, several genes expressed in the placenta play an essential role in resource utilization. Thus, single-nucleotide polymorphisms (SNPs) in these genes may affect the growth and development of the fetus or placental function, thereby determining the susceptibility to certain diseases[5].

Human solute carrier family 38 member 4 (*SLC38A4*) gene, located in 12q13.11, is subjected to genomic imprinting in the human placenta and highly expressed at all stages of the development[6]. The encoded protein, sodium-coupled neutral amino acid transporter 4 (SNAT4), belongs to the amino acid transport system (known as system A). Given that SNAT4 facilitates the cationic amino acid transport independently from Na^+ and pH, it has a crucial role in fetal growth and development. Alterations in the *SLC38A4* gene due to polymorphisms have been implicated in the development of impaired gluconeogenesis in the adult population[7,8].

The activity of this system is important for hepatic gluconeogenesis through the conversion of amino acids and glucose. The neutral amino acid transporter SNAT4 role in the placenta is crucial in fetal growth and development[9,10].

In this context, the objective of this study was to determine whether placental polymorphisms 1304 G > A (rs11183610) and 292 C > T (rs2429467) in the *SLC38A4* gene are associated with glucose > 95 mg/dL in normal weight full-term healthy newborns.

Methods

The study was conducted following the Code of Ethics of the Declaration of Helsinki. The Mexican Social Security Institute Ethics Committee approved the protocol. We obtained the written informed consent from the mothers of all the infants who participated in the study.

We conducted a case–control study with normal weight healthy newborns born from non-diabetic mothers with no history of gestational diabetes, high blood pressure, or malnutrition. Apgar score was > 8 in all newborns. The corresponding placentas were analyzed as reported elsewhere[8]. Newborns with umbilical cord venous blood glucose levels ≥ 95 mg/dL were considered cases and those with cord venous blood glucose levels < 95 mg/dL as controls. We used a cut point between the normal values and hyperglycemia. Some mothers' characteristics, such as age, smoking, alcohol intake, number of gestations, weight, and body mass index (BMI) before pregnancy, were matching criteria.

For identifying polymorphisms of the *SLC38A4* gene (1304 G > A and 292 C > T), DNA was obtained from

formalin-fixed, paraffin-embedded (FFPE) placental fragments. Maternal weight and height before pregnancy were obtained from medical records. BMI was calculated according to the following formula:

BMI = weight (kg)/height^2 (m^2)[11].

Definitions

Hyperinsulinemia was defined by umbilical cord venous insulin levels ≥ 5 μU/mL[12]. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated[13] as follows:

insulin levels (μU/mL) x glucose levels (mmol/dL)/22.5

Assays

We determined serum glucose levels using the glucose oxidase method. Insulin levels were measured by microparticle enzyme immunoassay (Abbott Axsym System, Alameda, CA, USA). Measurements were performed using an automated device (VITROS 250, Ortho-Clinical Diagnostics Inc., Raritan New Jersey, USA).

Umbilical cord venous blood was collected at birth for measuring serum glucose and insulin levels. The FFPE placenta blocks were processed as previously described[8]. DNA integrity was verified through 1% agarose gel electrophoresis, while purity and concentration by spectrophotometry at 260/280 nm using Nanodrop 2000c equipment (Thermo Scientific®).

Using TaqMan technology, we performed real-time PCR (quantitative polymerase chain reaction) system StepOne™ (Applied Biosystems®) for the genotyping. A total of 25 ng of genomic DNA were used under the following reaction conditions: one cycle of initial denaturation at 95°C/10 min, followed by 42 cycles of denaturation (95°C/15 s), annealing (60°C/1 min), and extension (60°C/30 s). The TaqMan probe used to recognize the SNP 1304 G > A (rs11183610) was C_25751555_10 (TaqMan® SNP Genotyping Assays, Thermo Fisher Scientific), whereas the SNP 292 C > T (rs2429467) was identified using the TaqMan probe C_15797142_10 (TaqMan® SNP Genotyping Assays, Thermo Fisher Scientific).

Statistical analysis

The data are presented as mean ± standard deviation for variables with normal distribution or median (25th and 75th percentiles) for skewed data. Differences between numerical variables were established using a Student's t-test (Mann–Whitney *U*-test for skewed data) and Fisher's exact test for categorical variables.

Genotype frequencies were obtained by direct count, and Hardy-Weinberg equilibrium (HWE) was calculated through X^2 goodness-of-fit statistics. Both analyses were carried out using the program SNPstats[14].

The MutationTaster2 software was used as a prediction tool for the functional consequences of the studied polymorphisms[15].

The association between polymorphisms 1304 G > A and 292 C > T of the *SLC38A4* gene (independent variable) and glucose ≥ 95 mg/dL (dependent variable) was determined using multivariate logistic regression analysis.

Statistical significance was established with a 95% confidence interval (95% CI) and $p < 0.05$. The statistical analysis was performed using the SPSS V.15.0 statistical package.

Results

A total of 13 (26%) newborns with glucose levels ≥ 95 mg/dL were compared with 37 (74%) newborns with glucose levels < 95 mg/dL.

Table 1 shows the anthropometric and biochemical variables of newborns and mothers in both case and control groups. In the case group, newborns showed significantly higher HOMA-IR than those in the control group. No significant differences were found neither in gestational age nor in body weight at birth between the groups.

Furthermore, no significant differences were observed regarding mothers' characteristics between the groups (Table 1).

For the SNP 292C > T, the allele C frequency was 58% and 76%, and the allele T frequency was 42% and 24% ($p = 0.08$) for cases and controls, respectively. Regarding the SNP 1304G > A, the allele G frequency was 81% and 82%, and the allele A frequency was 19% and 18% ($p = 1$) for cases and controls, respectively.

Regarding the SNP 292C > T, the genotype TT frequencies were 30.8% and 5.4% ($p = 0.56$); genotype C/C, 46.1% and 57.8% ($p = 0.003$); and genotype C/T, 23.1% and 37.8% ($p = 0.0004$) for cases and controls, respectively.

For the SNP 1304G > A, the genotype AA frequencies were 7.7% and 5.4% ($p = 1$); genotype G/A, 23.1% and 24.3% ($p = 0.04$); and genotype G/G, 69.2% and

Table 1. Demographic characteristics of newborns and mothers

n = 50	Cases	Controls	p-value
	13	37	
Newborns			
Weight at birth (g)	3485.8 ± 729.3	3312.8 ± 731.4	0.47
Gestational age (weeks)	39.4 ± 2.0	39.0 ± 1.5	0.33
Glucose levels at birth (mg/dl)[a]	109.0 (97, 141.1)	75.5 (65.7, 82.2)	<0.0005[b]
Insulin levels at birth (μU/ml)[a]	7.3 (3.3, 12.5)	5.5 (4.1, 7.5)	0.24[b]
HOMA-IR[a]	2.32 (0.85, 4.46)	0.99 (0.81, 1.51)	<0.0005[b]
Mothers			
Age (years)	26.8 ± 3.5	25.3 ± 4.5	0.27
Number of gestations[a]	3 (2, 3)	2 (1, 3)	0.25[b]
Weight before pregnancy (kg)	61.5 ± 10.6	61.9 ± 11.6	0.9
Height (cm)	158 ± 5.9	162.3 ± 4.6	0.01
Weight gain (kg)[a]	12 (11, 20)	14 (11.7, 16)	1[b]
BMI before pregnancy	24.7 ± 4.6	23.5 ± 4.1	0.4

Values are expressed as mean ± SD unless indicated otherwise.
[a]Median (25th, 75th percentile); [b]*p*-value estimated using Mann–Whitney *U*-test.
BMI: body mass index; HOMA-IR: homeostatic model assessment for insulin resistance.

70.3% (p = 0.004) for cases and controls, respectively,

The total population and the case group were in HWE (p = 0.3 and p = 0.086, respectively).

Although the MutationTaster 2 software indicated that polymorphisms 292 C > T and 1304 G > A are probably harmless, the logistic regression analysis showed that the SNP 292 C > T is significantly associated with glucose > 95 mg/dL (odds ratio [OR] 7.78; 95% CI 1.2-49.4, p = 0.02) but not the SNP 1304 G > A (OR 1.46; 95% CI 0.1-17.6, p = 0.77). Results of the SNP 292 C > T fit with a recessive model according to Akaike's criterion value (AIC)[14]. The results regarding inheritance models are shown in tables 2 and 3.

Discussion

Our results suggest that the *SLC38A4* gene SNP 292 C > T but not SNP 1304 G > A is associated with glucose > 95 mg/dL in normal weight full-term healthy newborns. This association is consistent with a recessive inheritance model.

During fetal life, the appropriate maternal amino acid supplementation is essential for the fetus's proper growth and development. The amino acid transport across the human placenta is active and mediated by specific transporters in syncytiotrophoblast plasma membranes[16].

The *SLC38A4* gene belongs to the amino acid transport system A and plays an essential role in hepatic gluconeogenesis and placental amino acid transport through the conversion of amino acids and glucose recycling[9]. Therefore, it has been hypothesized that alterations in the hepatic system A may increase glucose levels through impairing gluconeogenesis[17].

Some polymorphisms in the *SLC38A4* gene are related to glucose alterations[8]. The frequency of mutant alleles of the *SLC38A4* gene varies depending on the population. In Asian and Central American individuals, the frequency of mutant alleles for the SNP 1304 G > A is 8% and 21%, and for the SNP 292 C > T, 24% and 20%, respectively[18,19]. In this study, the frequency of allele A for the SNP 1304 G > A was 18%, and the frequency of the allele T for the SNP 292 C > T was 29%. Both frequencies are similar to those reported by the PAGE study in Mexican subjects (23% and 26%, respectively)[18,19].

According to UniProt Consortium[20], the studied polymorphisms in the *SLC38A4* gene generate amino acid changes that might alter the function of SNAT4. For example, SNP 292 C > T (G29R) is found in an extracellular topological domain, while SNP 1304 G > A (T366M) is found in a cytoplasmic topological domain. In this regard, the SNAT4 protein contains a total of six potential *N*-linked glycosylation sites, and *N*-glycosylation occurs typically at the extracellular side of the membrane proteins[21,22].

The *SLC38A4* gene is subjected to imprinting, and the placental expression of this gene is determined by a paternal allele, suggesting the critical role in promoting fetal growth[23]. Topological domains in the protein may exert different effects depending on gene expression.

Table 2. Inheritance models for SNP 292 in a biallelic locus C > T of the *SLC38A4* gene

Inheritance model	Genotype	Controls	Cases	OR	95% CI	p-value	AIC
		n (%)	n (%)				
Codominant	CC	21 (57.8)	6 (46.1)	1	—	—	
	CT	14 (37.8)	3 (23.1)	0.75	0.2-3.5	0.07	48.4
	TT	2 (5.4)	4 (30.8)	7.0	1.02-48.0	0.05	40.2
Dominant	CC	21 (56.8)	6 (46.1)	1	—	—	
	CT-TT	16 (43.2)	7 (53.9)	1.53	0.4-5.4	0.5	60.9
Recessive	CC-CT	35 (94.6)	9 (69.2)	1	—	—	
	TT	2 (5.4)	4 (30.8)	7.78	1.2-49.4	0.02	56.1
Overdominant	CC-TT	23 (62.2)	10 (79.9)	1	—	—	
	CT	14 (37.8)	3 (23.1)	0.49	0.1-2.1	0.32	60.3
Log-additive	—			2.04	0.8-5.0	0.12	58.8

AIC: Akaike's criterion value; 95% CI: 95% confidence interval; OR: odds ratio; SNP: single-nucleotide polymorphism.

Table 3. Inheritance models for SNP 1304 in a biallelic locus G > A of the *SLC38A4* gene

Inheritance model	Genotype	Controls	Cases	OR	95% CI	p-value	AIC
		n (%)	n (%)				
Codominant	GG	26 (70.3)	9 (69.2)	1	—	—	
	GA	9 (24.3)	3 (23.1)	0.96	0.2-4.4	0.96	57.4
	AA	2 (5.4)	1 (7.7)	1.44	0.1-18.0	1	47.7
Dominant	GG	26 (70.3)	9 (69.2)	1	—	—	
	AG-AA	11 (29.7)	4 (30.8)	1.05	0.3-4.1	0.94	61.3
Recessive	GG-AG	35 (94.6)	12 (92.3)	1	—	—	
	AA	2 (5.4)	1 (7.7)	1.46	0.1-17.6	0.77	61.2
Overdominant	GG-AA	28 (75.7)	10 (76.9)	1	—	—	
	AG	9 (24.3)	3 (23.1)	0.93	0.2-4.1	0.93	61.3
Log-additive	—			1.10	0.4-3.1	0.86	61.3

AIC: Akaike's criterion value; 95% CI: 95% confidence interval; OR: odds ratio; SNP: single-nucleotide polymorphism.

The monoallelic expression of both the gene and mutation 292 C > T, located in the extracellular domain, would be enough to disturb protein function, as no functional alleles would be present. Therefore, we hypothesize that the paternal transmission of only one 292 T mutant allele could be enough to disturb the gene function in the placenta, giving rise to glucose imbalance.

SLC38A4 gene expression is postnatally downregulated in the lungs and kidneys. In contrast, the expression of the *SLC38A4* gene in the liver increases as the rate of organ growth slows down. This finding suggests an essential role for this gene in gluconeogenesis[24]. Such increase could be related to the hepatic biallelic expression of the *SLC38A4* gene. For this reason, it is

possible that the double mutant genotype 292 T/T or the inheritance of heterozygous genotype 1304 G > A could increase the risk of developing hyperglycemia in adult life.

Previous findings support this idea. In a population with diabetes, the linkage disequilibrium between these two SNPs was D' > 0.82, and the presence of the A/T haplotype was associated with hyperglycemia[8].

According to Desforges et al.[6], evidence shows complex changes in placental transporters' expression and activity during pregnancy. The *SLC38A4* gene generates five *SLC38A4* isoforms (201-205), which produce lengthy proteins of 547 aa, 547 aa, 219 aa, 191 aa, and 141 aa, respectively. According to the expression score reported by Bgee (database for gene expression evolution), these proteins are expressed predominantly in the embryonic (94%) and adult liver (94%). However, lower levels can be detected at the placental level (53%) in late embryonic stages[25].

In contrast, our results differ from a previous study that showed an association of the SNP 1304 G > A but not of 292C > T with hyperglycemia in individuals with diabetes type 2[8]. These paradoxical results can relate to gestational regulation expression and monoallelic expression of *SLC38A4* mRNA at the placental level and differences in the targeted populations.

Amino acid transport system A is accurately regulated by various hormones and growth factors *in vivo* and substrate availability evidenced *in vitro*[26]. According to recent studies, the insulin-like growth factor II (*IGF-2*) gene is also imprinted in the placenta. It acts as a controller of nutrient supply by affecting placental development and as a signal of fetal demand by modulating the expression and activity of key placental supply genes such as the system A transporters Slc38a4, Slc38a2, and the glucose transporter Slc2a3 in mouse placenta[26-29]. Therefore, any alterations or interaction between these two genes could develop glucose imbalance in early life[30].

Finally, case group newborns showed a trend for higher insulin levels than those in the control group, which was expected given each group's inclusion criteria. However, no significant statistical differences were observed between the groups. According to Wilcox[31], normal pregnancy is characterized by insulin resistance that reaches its highest levels in the third trimester, which appears to be a physiological response to birth stress or an adaptive response that diverts glucose and lipids to the developing fetus. Furthermore, increased insulin resistance could also be related to increased levels of lactogen, progesterone, and cortisol, which act as counter-regulatory factors for insulin. This response is typically observed in pregnancy, and its imbalance is related to the development of gestational diabetes and hypertension[31].

Moreover, we found that newborns in the case group exhibited higher HOMA-IR than those in the control group; thus, it is possible that the lack of insulin action at peripheral tissues also contributes to the elevated glucose levels in the newborn. Further research is required in this field.

The following recommendations are mandatory to understand the role of *SLC38A4* function: (1) to measure placental *SLC38A4* gene expression for the transcripts and correlate them with the genotype, (2) to correlate IGF-2 serum concentration in pregnant women with *SLC38A4* placental expression, and (3) to evaluate the gene methylation pattern and the parental origin of the alleles.

Some limitations of this study should be mentioned. First, as no data regarding mothers' serum glucose levels was available, we could not exclude their potential role on newborns' glycemia. Second, given the small sample size, the statistical power was low (0.47). As a II error was possible in the statistical analysis, our results should be considered preliminary. Third, we could not determine the parental origin of the alleles and analyze samples from siblings. Undoubtedly, further research in this field is necessary to confirm our findings.

Our results suggest that the presence of 292 C > T of *the SLC38A4* gene is associated with glucose levels ≥ 95 mg/dL in normal weight full-term healthy newborns.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

Funding was provided by FONSEC-Salud CONACYT-Mexico, grant number 2011-162338, Secretaría de Investigación y Posgrado (SIP)-IPN with the projects 20201479 and 20201482.

Acknowledgments

We thank physicians and nursing personnel of the participant hospitals who provided the facilities to carry out the clinical work.

References

1. Şimşek DG, Ecevit A, Hatipoğlu N, Çoban A, Arısoy AE, Baş F, et al. Neonatal hyperglycemia, which threshold value, diagnostic approach and treatment? Turkish Neonatal and Pediatric Endocrinology and Diabetes Societies consensus report. Turk Pediatri Ars. 2018;53:S234-S238.
2. Falcão MC, Ramos JL. Prediction of hyperglycemia in preterm newborn infants. Rev Hosp Clin Fac Med Sao Paulo. 1999;54:3-8.
3. Reece EA. The fetal and maternal consequences of gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2010;23:199-203.
4. Langley-Evans SC. Nutritional programming of disease: unravelling the mechanism. J Anat. 2009;215:36-51.
5. Angiolini E, Fowden A, Coan P, Sandovici I, Smith P, Dean W, et al. Regulation of placental efficiency for nutrient transport by imprinted genes. Placenta. 2006;27 Suppl A:S98-102.
6. Desforges M, Lacey HA, Glazier JD, Greenwood SL, Mynett KJ, Speake PF, et al. SNAT4 isoform of system A amino acid transporter is expressed in human placenta. Am J Physiol Cell Physiol. 2006; 290:C305-12.
7. Mackenzie B, Erickson JD. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. Pflugers Arch. 2004; 447:784-95.
8. González-Renteria SM, Loera-Castañeda V, Chairez-Hernández I, Sosa-Macias M, Paniagua-Castro N, Lares-Aseff I, et al. Association of the polymorphisms 292 C>T and 1304 G>A in the SLC38A4 gene with hyperglycaemia. Diabetes Metab Res Rev. 2013;29:39-43.
9. Varoqui H, Erickson JD. Selective up-regulation of system a transporter mRNA in diabetic liver. Biochem Biophys Res Commun. 2002;290:903-8.
10. Li Z, Lai G, Deng L, Han Y, Zheng D, Song W. Association of SLC38A4 and system A with abnormal fetal birth weight. Exp Ther Med. 2012;3:309-13.
11. Aparicio MR, Estrada LA, Fernández C, Hernández RM, Ruíz M, Ramos D, et al. Manual de antropometría. Departamento de Nutrición Aplicada y Educación Nutricional, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Ciudad de México: Consejo Nacional de Ciencia y Tecnología; 2004. p. 1-17.
12. Godfrey KM, Hales CN, Osmond C, Barker DJ, Taylor KP. Relation of cord plasma concentrations of proinsulin, 32-33 split proinsulin, insulin and C-peptide to placental weight and the baby's size and proportions at birth. Early Hum Dev. 1996;46:129-40.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9.
14. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22:1928-9.
15. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11:361-2.
16. Jansson T. Amino acid transporters in the human placenta. Pediatr Res. 2001;49:141-7.
17. Handlogten ME, Kilberg MS. Induction and decay of amino acid transport in the liver. Turnover of transport activity in isolated hepatocytes after stimulation by diabetes or glucagon. J Biol Chem. 1984;259:3519-25.
18. National Center for Biotechnology Information. Reference SNP (rs) Report rs11183610. Rockville: National Center for Biotechnology Information; 2020.
19. National Center for Biotechnology Information. Reference SNP (rs) Report rs2429467. Rockville: National Center for Biotechnology Information; 2020.
20. UniProt Consortium T. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2018;46:2699.
21. Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, et al. Topology, subcellular localization, and sequence diversity of the Mlo family in plants. J Biol Chem. 1999;274:34993-5004.
22. Shi Q, Padmanabhan R, Villegas CJ, Gu S, Jiang JX. Membrane topological structure of neutral system N/A amino acid transporter 4 (SNAT4) protein. J Biol Chem. 2011;286:38086-94.
23. Bischoff SR, Tsai S, Hardison N, Motsinger-Reif AA, Freking BA, Nonneman D, et al. Characterization of conserved and nonconserved imprinted genes in swine. Biol Reprod. 2009;81:906-20.
24. Lui JC, Finkielstain GP, Barnes KM, Baron J. An imprinted gene network that controls mammalian somatic growth is down-regulated during postnatal growth deceleration in multiple organs. Am J Physiol Regul Integr Comp Physiol. 2008;295:R189-96.
25. Bastian FB, Parmentier G, Roux J, Moretti S, Laudet V, Robinson-Rechavi M. Integrating and comparing heterogeneous transcriptome data among species. In: Data Integration in the Life Sciences. Berlin: Springer-Verlag; 2008.
26. Lager S, Powell TL. Regulation of nutrient transport across the placenta. J Pregnancy. 2012;2012:179827.
27. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. J Physiol. 2009;587:3459-72.
28. Sibley CP, Coan PM, Ferguson-Smith AC, Dean W, Hughes J, Smith P, et al. Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. Proc Natl Acad Sci U S A. 2004;101:8204-8.
29. Constância M, Angiolini E, Sandovici I, Smith P, Smith R, Kelsey G, et al. Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. Proc Natl Acad Sci U S A. 2005;102:19219-24.
30. Smith RJ, Dean W, Konfortova G, Kelsey G. Identification of novel imprinted genes in a genome-wide screen for maternal methylation. Genome Res. 2003;13:558-69.
31. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26:19-39.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

Preferred sources of help for mental health problems among Chilean adolescents: a descriptive study

Cecilia Olivari[1,2], Carlos Mellado[3], Rocío Casañas[4], Nancy Espinosa-Díaz[1], and Jaime Fuster-Villaseca[5]*

[1]*Departamento de Psicología, Universidad Católica del Maule, Talca, Chile;* [2]*Centro de Investigación en Neuropsicología y Neurociencia Cognitiva, Universidad Católica del Maule, Talca, Chile;* [3]*Escuela de Psicología, Universidad Santo Tomás, Talca, Chile;* [4]*Departamento de Investigación, Asociación Centre Higiene Mental Les Corts, Grup CHM Salut Mental, Barcelona, Spain;* [5]*Doctorado en Psicología, Universidad Católica del Maule, Talca, Chile*

Abstract

Background: *A timely search for professional help regarding mental health issues in adolescents is critical in preventing severe disorders. However, adolescents generally tend not to seek help. This investigation aimed to study Chilean adolescents' willingness to seek help in mental health issues by identifying their preferred help-seeking sources.* **Methods:** *We conducted a cross-sectional-correlational study with 493 high school students between 14 and 19 years of age (mean ± standard deviation = 16.28 ± 1.29). The instruments we used were the general help-seeking questionnaire (vignette version), adapted and validated in Chile, and a sociodemographic questionnaire.* **Results:** *Data showed that adolescents are more willing to seek help from informal rather than from formal sources. We identified no sex differences in terms of willingness to seek help from formal sources. However, males were more willing to seek help from informal sources.* **Conclusions:** *Similar to other cultures, Chilean adolescents are more willing to seek help from informal sources regarding mental health problems.*

Key words: *Help seeking. Mental health. Adolescents.*

Fuentes de ayuda preferidas para problemas de salud mental por adolescentes chilenos: estudio descriptivo

Resumen

Introducción: *La búsqueda de ayuda profesional oportuna para temas de salud mental en adolescentes es fundamental para evitar el desarrollo de trastornos más graves. No obstante, en general los adolescentes tienden a no solicitar ayuda. El objetivo de la presente investigación fue identificar las fuentes de ayuda para problemas de salud mental a las que los adolescentes chilenos están más dispuestos a dirigirse.* **Métodos:** *Estudio transversal-correlacional en el que participaron 493 estudiantes de secundaria de entre 14 y 19 años (media ± desviación estándar = 16.28 ± 1.29). Los instrumentos aplicados fueron el Cuestionario General de Búsqueda de Ayuda (versión viñeta), adaptado y validado en Chile, y un cuestionario de datos sociodemográficos.* **Resultados:** *Los resultados obtenidos muestran que los adolescentes prefieren buscar ayuda en fuentes informales. No se identificaron diferencias por sexo en la disposición a buscar ayuda en fuentes formales, pero sí en la disposición a buscar ayuda en fuentes informales, pues los varones mostraron mayor disposición a hacerlo.*

Correspondence:
*Cecilia Olivari
E-mail: colivari@ucm.cl

Date of reception: 22-06-2020
Date of acceptance: 03-09-2020
DOI: 10.24875/BMHIM.20000182

Available online: 08-06-2021
Bol Med Hosp Infant Mex. 2021;78(3):207-215
www.bmhim.com

Conclusiones: *En concordancia con la evidencia en otras culturas, los adolescentes chilenos presentan una mayor disposición a buscar ayuda para problemas de salud mental en fuentes informales.*

Palabras clave: *Conducta de búsqueda de ayuda. Salud mental. Adolescentes.*

Introduction

Adolescence is a phase of life in which there is a greater vulnerability to initiate and develop risky behaviors such as substance abuse and mental health problems[1,2]. In this context, the World Health Organization reports that depression is the leading cause of morbidity and disability during adolescence[3]. Several epidemiological studies in Chile show a prevalence of depression between 6.1% and 8.3% in the adolescent population[4,5]. Regarding suicidal behavior, another investigation with school enrolled adolescents aged 13-18 years reported a lifetime prevalence of attempted suicide of 14.3%[5]. Between 2009 and 2018, the Ministry of Health recorded 1030 deaths associated with self-harm in the population between 8 and 18 years of age, which constituted the second leading cause of death in this age group in Chile[6].

Regarding substance abuse, the latest national study in the general Chilean population estimated that 18.7% of adolescents between 12 and 18 years of age and 53.2% of young people between 19 and 25 consumed alcohol in the last month. Furthermore, a prevalence of risky alcohol consumption of 3.5% was identified for the 12-18 years old age group. In the previous year, the prevalence of marijuana use was 10.1% in adolescents aged 12-18 years and 32.1% in young people aged 19-25 years[7].

Seeking timely professional help for mental health issues is considered a protective factor that can prevent the development of more severe disorders[8,9]. However, evidence indicates that adolescents tend not to seek professional help when faced with a mental health problem[10-14]. Instead, adolescents tend to keep their problems to themselves; if they seek help, it would be preferably from peers or close adults[11,15], most of whom have little or no knowledge of mental health. Consequently, they do not recognize that they are dealing with a specific problem or where to look for appropriate information about it[16]. Furthermore, it has been observed that adolescents with more emotional symptomatology and even suicidal thoughts are the least willing to seek help[17-19]. Among the main reasons that may influence the tendency to not seek help, even from mental health-care professionals, are the adolescents' need for autonomy[20,21], stigma toward mental health issues[22-24], fear of lack of confidentiality by the health-care professional, and lack of mental health literacy[25-27].

Help seeking is a complex process, with different stages, influenced by individual and social factors[13,15]. The process begins with being aware of the symptoms and accepting that one has a problem. Next, adolescents must be able to express and make themselves understandable to others, which will require the availability and accessibility of sources of help to which adolescents can reveal their needs[13]. Adolescents can obtain support from different formality sources[13,28]. Informal sources of help include casual relationships such as friends and family, and formal sources of help refer to people who have a recognized role and appropriate training in providing support and advice on health, such as health-care and mental health professionals[28]. Seeking informal help has been considered a first step that precedes seeking and accessing professional help[29].

In the present study, we sought to identify the sources of support for mental health problems that adolescents were most willing to approach. It is considered relevant since most of the studies on this subject have been conducted in English speaking populations, and only a few experiences are reported in other cultures[11,30]. Thus, inspired by the study of D'Avanzo et al. conducted in Italy, we studied the willingness to seek help in an adolescent Latino population. In contrast to the cited research, where the instrument used was the general help-seeking questionnaire in its original version, we studied the willingness to seek help with the general help-seeking questionnaire, vignette version, adapted in Chile[31]. Therefore, we explored different and specific mental health problems for which adolescents could indicate the degree to which they would select a particular source of help. As mental health issues are a dimension to be considered, the willingness to seek help is crucial in the help-seeking process[28].

The present results may provide a better understanding of the help-seeking process in a previously unstudied sociocultural environment (Chilean adolescents). New evidence regarding the pattern of willingness to seek help may be useful for developing future interventions to promote timely help seeking.

Methods

Participants

We conducted a non-experimental, cross-sectional, correlational study, including a universe of 17,736 high school adolescents in the commune of Talca, Chile. The sampling was non-probabilistic by convenience. The sample needed a minimum of 377 students to ensure 95% of confidence and 5% margin of sampling error (maximum variance of 50%). The sample finally exceeded this minimum and consisted of 493 adolescents with an age range between 14 and 19 years (mean ± standard deviation = 16.28 ± 1.29), of whom 238 were female (48.3%). Moreover, 67.5% corresponded to young individuals of a medium, medium-low, or low socioeconomic level from the commune's urban area. The remaining 32.5% corresponded to young individuals of medium-high, high, or very high socioeconomic status. Of the young people in the sample, 43.2% studied in municipal schools, 46.9% in private schools with state subsidies, and 9.9% in private schools.

Procedure

Twelve educational institutions were invited to participate in the study, of which seven accepted to participate. From November 2016 to April 2017, participants were recruited. After each institution authorized the study, we requested consent and assent forms. The questionnaires were applied collectively in the classroom. The ethics committee of the Universidad Católica del Maule approved this study.

Instruments

Besides a sociodemographic questionnaire that included age, sex, educational level, and educational institution, the general help-seeking questionnaire, vignette version GHSQ-V[14], adapted in Chile[31], was applied. This instrument assesses the likelihood of participants to seek help from specific proposed sources (partner, friends, parents, other family members, psychologist, physician, psychiatrist, and teacher or counselor) for seven different types of mental health problems (stress, anxiety, depression, suicidal ideation, substance abuse, psychosis, and physical illness). Regarding the questionnaire's reliability indexes (in this adapted version), alpha values ranging from 0.87 to 0.75 were obtained[31]. Each question shows a vignette that can be used independently, describing an adolescent with one mental health problem. Then, eight items with the proposed sources of help and a final item indicating that the adolescent would not seek help from anyone. In the present study, we included only stress, depression, suicidal ideation, and substance abuse. The following is an example vignette:

"Over the past two weeks, Juan has found it difficult to relax. He has also been feeling quite overwhelmed, 'nervous,' and intolerant. He has tended to overreact to things that happen."

Following each vignette, participants rate their intention to seek help for each source of support on a 7-point Likert scale, ranging from 1 = very unlikely to 7 = very likely.

Results

Willingness to seek help from formal and informal sources

We analyzed the willingness to seek help by adolescents according to different mental health problems. We performed a descriptive analysis of means and standard deviations of help seeking by type of problem (stress, depression, suicide, and substance abuse) and sources (informal and formal). Means and standard deviations were reported due to a normal-like distribution of variables. The minimum and maximum scores for each variable were 1 and 7, respectively, where a low score means a low inclination to seek help from each source and a high score, an increased tendency to seek help.

Table 1 summarizes the results by type of problem and source of help seeking. It can be observed that, on average, adolescents prefer to approach their parents and friends among informal sources when they have issues with stress, depression, substance abuse, and suicidal ideation. Among formal sources, they prefer to turn to a psychologist for these problems.

Furthermore, extreme groups of adolescents were characterized according to their willingness to seek help from different help-seeking sources by calculating the percentage of participants who answered options 1 or 2 and 6 or 7 on the response scale for each type of problem. These options represented the lowest and highest intentions to seek help, respectively. Therefore, they were labeled as young people who were *very likely* or *very unlikely* to seek help.

The percentage of adolescents from the extreme groups (*very likely* and *very unlikely*) for the different sources of help, classified by stress, depression, suicide, and substance abuse problems is shown in

Table 1. Means and standard deviations of help-seeking intentions for different problems and sources of help

Source of help	Mental health issue											
	Stress			Substance abuse			Depression			Suicide		
	n	M	SD	n	M	SD	n	M	SD	n	M	SD
Informal sources	485	4.39	1.25	492	4.37	1.44	488	4.43	1.38	487	4.23	1.67
Partner	490	4.19	2.05	493	4.27	2.05	493	4.39	2.13	492	4.15	2.25
Friend	492	4.64	1.93	492	4.73	1.97	492	4.58	1.95	492	4.52	2.14
Parent	492	4.95	2.04	493	4.76	2.12	491	5.05	2.06	490	4.64	2.35
Other relatives	488	3.77	2.11	493	3.72	2.18	491	3.68	2.09	491	3.59	2.29
Formal sources	483	2.56	1.55	485	3.10	1.79	489	2.80	1.71	488	3.11	1.91
Psychologist	491	2.94	2.02	490	3.55	2.17	491	3.31	2.20	493	3.78	2.36
Physician	493	2.32	1.69	490	3.06	2.14	491	2.56	1.93	491	2.78	2.13
Psychiatrist	486	2.40	1.89	492	3.16	2.18	492	2.63	2.02	492	3.23	2.33
Counselor	490	2.60	1.84	491	2.65	1.97	493	2.68	1.94	491	2.68	2.01

The total number of adolescents was 493. The missing data for each type of problem and source were calculated by subtracting the corresponding number of each cell from the total number of participants.
n: frequency; M: mean; SD: standard deviation.

Table 2. Help-seeking differences between informal and formal sources according to the type of mental health problem

	Informal sources M (SD)	Formal sources M (SD)	t (DF)	p*
Stress (n = 476)	4.39 (1.25)	2.56 (1.55)	23.75 (475)	< 0.001
Depression (n = 484)	4.43 (1.38)	2.80 (1.71)	20.99 (483)	< 0.001
Suicide (n = 482)	4.23 (1.67)	3.11 (1.91)	13.47 (481)	< 0.001
Substance abuse (n = 484)	4.37 (1.44)	3.10 (1.79)	15.67 (483)	< 0.001

The total number of adolescents was 493. The missing data for each type of problem were calculated by subtracting the corresponding number of each cell from the total number of participants.
DF: degrees of freedom; M: mean; SD: standard deviation.
*p < 0.05 is considered statistically significant.

figures 1-4. A high percentage of the *very likely* group was observed in the "parents" category among the informal sources, meaning that these young people were very willing to seek help and would go to their parents in the first place. Among the formal sources, a high percentage of the *very likely* group was observed in the "psychologist" category. This pattern was similar for the four mental health issues consulted.

By comparison, a low percentage from the *very unlikely* group to seek help was observed in the "parents" category for stress and depression problems. These findings mean that adolescents who were unwilling to seek help showed less rejection toward their parents when they have stress and depression problems. Alternatively, for suicide and substance abuse problems, these adolescents showed less rejection toward their friends. Finally, in the *very unlikely* group, young people were less likely to reject the "psychologist" as a formal source of help for the problems surveyed.

Differences in the willingness to seek help between informal and formal sources

A Student's t-test for paired samples was performed to compare whether the adolescents sought help from formal or informal sources and identify the adolescents' preferred source of help seeking (Table 2). Significant differences were observed between sources for the different types of mental health problems: on average, adolescents prefer to turn to informal sources for help when they have stress, depression, suicide, and substance abuse issues.

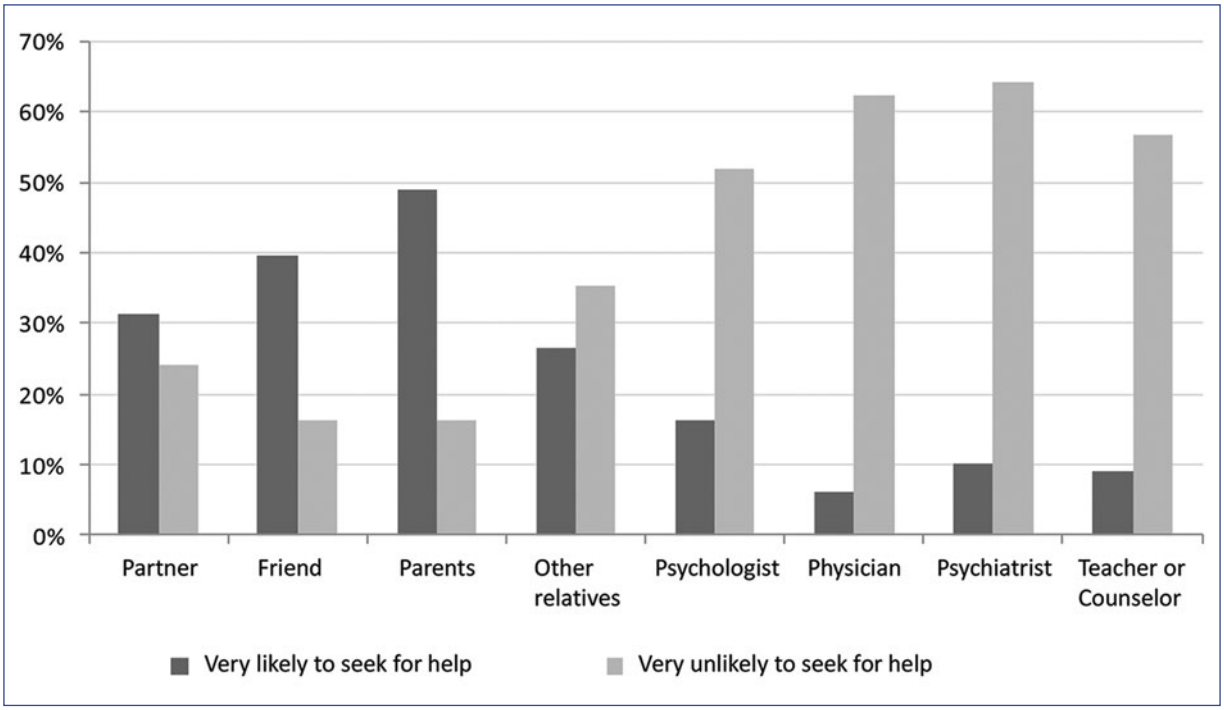

Figure 1. Percentage of young people who considered *very likely* or *very unlikely* to seek help for stress problems by type of source.

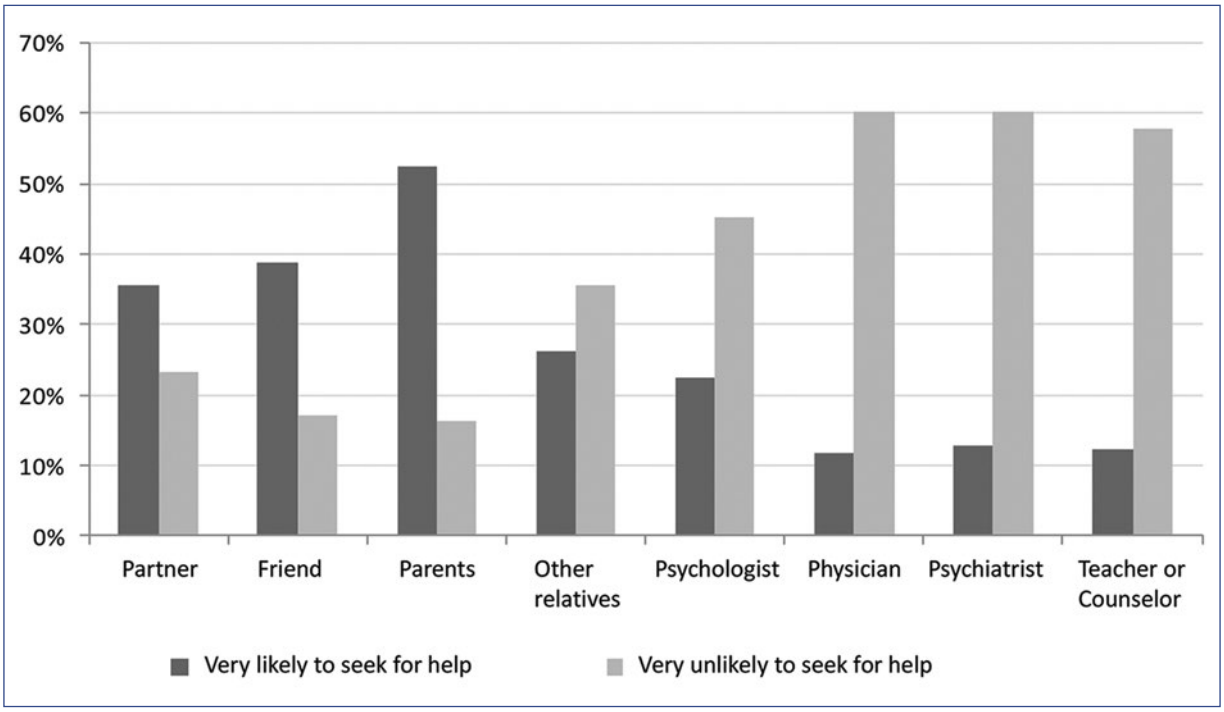

Figure 2. Percentage of young people who considered *very likely* or *very unlikely* to seek help for depression problems by type of source.

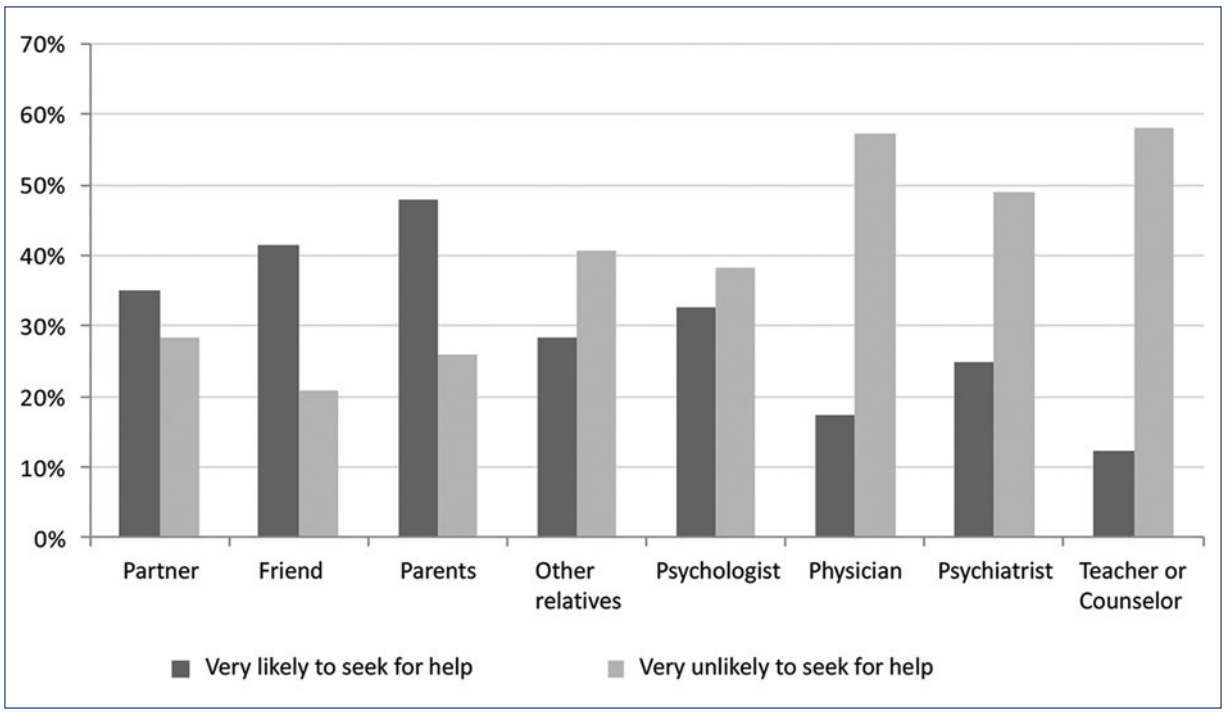

Figure 3. Percentage of young people who considered *very likely* or *very unlikely* to seek help for suicide problems by type of source.

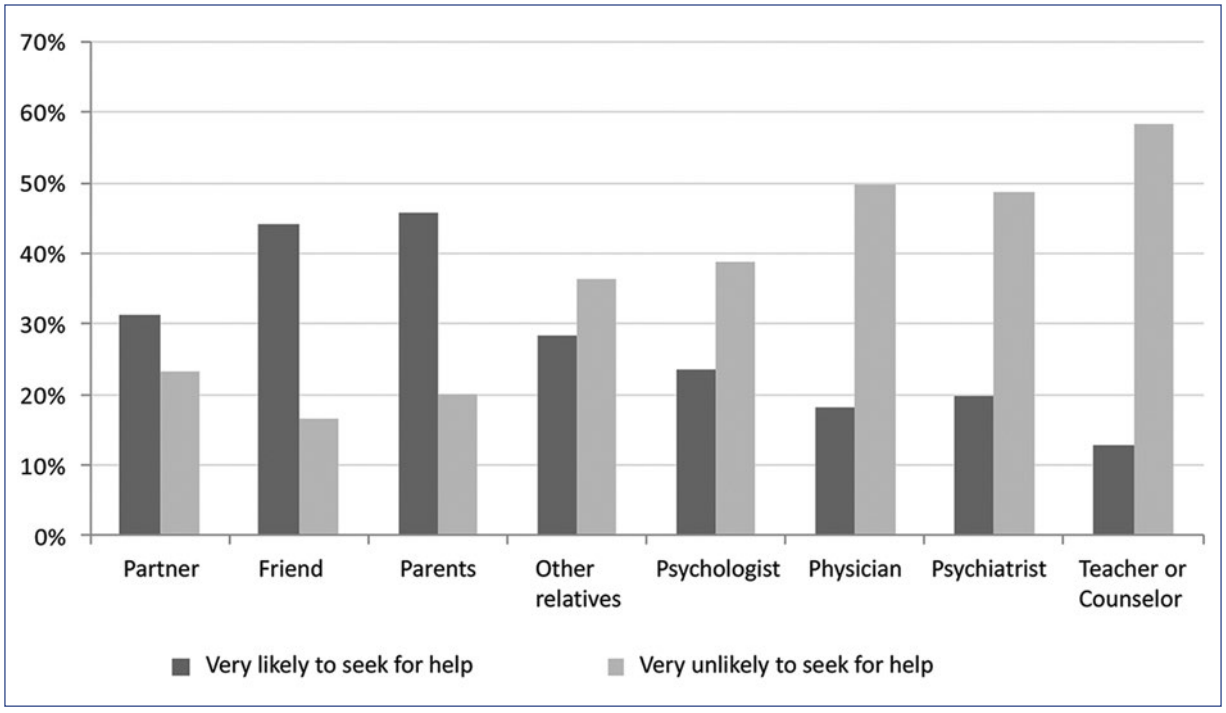

Figure 4. Percentage of young people who considered *very likely* or *very unlikely* to seek help for substance abuse problems by type of source.

Table 3. Differences in help-seeking intentions by sex

Type of problem	Source of help seeking	Male (n = 232) M (SD)	Female (n = 220) M (SD)	t (DF = 450)	p*
Stress	Informal	4.52 (1.21)	4.25 (1.31)	2.26	**0.024**
	Formal	2.50 (1.54)	2.61 (1.58)	-0.73	0.461
Depression	Informal	4.62 (1.30)	4.20 (1.45)	3.24	**0.001**
	Formal	2.78 (1.71)	2.77 (1.72)	0.78	0.938
Suicide	Informal	4.54 (1.54)	3.86 (1.75)	4.40	**< 0.001**
	Formal	3.23 (1.87)	2.99 (1.94)	1.31	0.189
Substance abuse	Informal	4.59 (1.38)	4.14 (1.49)	3.34	**0.001**
	Formal	3.18 (1.82)	3.00 (1.76)	1.03	0.303

The total number of adolescents was 493. The missing data for each type of problem were calculated by subtracting the number of males plus females from the total number of participants.
DF: degrees of freedom; M: mean; SD: standard deviation.
*p < 0.05 is considered statistically significant.

Table 4. Correlation matrix between the willingness to seek help according to the type of source of help and problem

Type of problem	1	2	3	4	5	6	7	8
Informal sources								
Stress	—	481	480	485	477	482	481	479
Depression	0.74**	—	484	489	480	486	485	482
Suicide	0.54**	0.63**	—	488	479	485	484	482
Substance abuse	0.57**	0.61**	0.61**	—	484	490	489	486
Formal sources								
Stress	0.29**	0.35**	0.29**	0.26**	—	482	481	478
Depression	0.27**	0.41**	0.34**	0.30**	0.78**	—	486	484
Suicide	0.25**	0.38**	0.49**	0.35**	0.65**	0.75**	—	482
Substance abuse	0.28**	0.35**	0.40**	0.40**	0.62**	0.71**	0.78**	—

Above the diagonal, the number for each correlation. The total number of adolescents was 493. The missing data for each type of problem were calculated by subtracting the corresponding number of each cell from the total number of participants.
*p < 0.05; **p < 0.01.

Differences in help seeking by sex, age, and socioeconomic level

A Student's t-test for the variable sex and correlation analysis for age and socioeconomic level (SES) variables were performed to identify differences in the willingness to seek help according to these sociodemographic variables.

Regarding differences in help seeking by sex, we observed that males seek help from informal sources more than females for problems related to stress, depression, suicide, and substance abuse (Table 3). No significant differences regarding sex were observed in seeking help from formal sources for none of the studied problems. Furthermore, no significant association was found between age, SES, and help seeking from formal or informal sources for these mental health problems.

Characterization of adolescents with low willingness to seek help

Adolescents who showed a low willingness to seek help were characterized, so we calculated the percentage of participants who answered options 6 or 7 in the item *I would not seek help from anyone*. These options represent the highest likelihood of not seeking help. Adolescents indicated that they are very likely not to seek help for stress (13.8%), depression (13.4%), suicide (15.9%), and substance abuse (11.6%).

To identify differences by sex, age, and SES in low willingness to seek help, we performed a Student's

t-test for the sex variable and correlation analysis in the case of age and SES variables. On average, females reported a greater probability of not seeking help for stress-related problems (t [489] = 2.06; p = 0.040). For problems related to depression, suicide, and substance abuse, no significant differences were observed according to sex. Furthermore, no significant association was found between age or SES and the reported probability of not seeking help for stress, depression, suicide, and substance abuse problems.

Relationship between willingness to seek help from formal and informal sources

To establish whether the willingness to seek help from informal sources is related to seeking help from formal sources, we performed a Pearson correlation analysis considering the different types of problems surveyed. Table 4 shows a significant correlation of all the variables. The correlations between seeking help from informal sources for stress, depression, suicide, and substance abuse problems were all positive and high, as well as the correlations between seeking support from formal sources for each of these problems. Finally, the correlations between seeking help from informal sources and seeking help from formal sources were positive and moderate according to the different issues.

Discussion

The present research aimed to identify the sources of help for mental health problems that Chilean adolescents are most willing to turn to, considering both formal and informal sources.

Adolescents considered help seeking as an option when facing the mental health issues investigated. Consistent with international evidence, adolescents exhibited a greater willingness to seek informal than formal help[11,14,15]. On average, we found that Chilean adolescents first choice were their parents, followed by their friends as their favorite alternatives for support. These findings are consistent with most studies in similar populations, which identify these two options as the primary informal sources adolescents turn to; however, in some of these studies, friends were their first choice followed by their parents as the preferred source of help[11,12]. In contrast, other studies[13] showed that younger adolescents tend to be more influenced by their parents, who are their preferred source of help, especially their mother. Consistent with previous scientific literature, these results should raise the need to promote mental health literacy for parents (and guardians) and adolescents themselves[32,33].

Formal sources of help were less chosen than informal sources, with the psychologist being the preferred source. This observation is consistent with other studies that have identified this same source as the one selected by adolescents (over the psychiatrist or general practitioner)[11]. Furthermore, we identified a higher level of preference for counselors or teachers than general practitioners and similar to the psychiatrist in some topics. This study points to high school teachers as potential sources of help to which Chilean adolescents would be willing to turn when faced with mental health concerns or problems. As similar results have already been observed in other studies[34], we should focus on increasing teachers' knowledge of mental health issues and sensitizing them on their role in help seeking by adolescents with mental health problems in our setting.

Regarding sex, we found mixed results depending on whether adolescents sought formal or informal help. Concerning informal help seeking, males tended to seek more help than females. In contrast to other studies in which females were identified to have a greater willingness to seek help[13,35], our findings showed that males tend to seek more informal help. However, regarding formal help seeking, no significant differences were observed between female and male adolescents. Furthermore, we identified no gender differences in seeking help from formal sources, which coincides with other studies[11,12]. Therefore, it should be considered that our analysis differentiated between formal and informal sources of help, which allows us to know more precisely the people to whom adolescents would prefer to turn to when faced with a given mental health problem.

One of the limitations of this study is the age range of the sample (14-19 years). Although it was a representative non-consulting sample of schoolchildren in the Maule region, Chile, it did not include the entire range that defines the adolescent stage. Therefore, it will be of interest to conduct further studies of the help-seeking pattern by broadening the sample's age range and consulting on specific mental health issues.

Knowing the pattern of help seeking in the population studied is essential to identify the preferred sources that adolescents turn to, which can undoubtedly be relevant for developing interventions for these groups and favor more effective support for their needs.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This research has been funded by the Fondo Nacional de Desarrollo Científico y Tecnológico of the Chilean Government, Proyecto Fondecyt N° 11130473, granted to the first author.

References

1. Jones PB. Adult mental health disorders and their age at onset. Br J Psychiatry Suppl. 2013;54:s5-10.
2. Lubman DI, Berridge BJ, Blee F, Jorm AF, Wilson CJ, Allen NB, et al. A school-based health promotion programme to increase help-seeking for substance use and mental health problems: study protocol for a randomised controlled trial. Trials. 2016;17:393.
3. World Health Organization. Health for the World's Adolescents: A Second Chance in the Second Decade. Geneva: World Health Organization; 2014. Available from: https://www.who.int/maternal_child_adolescent/documents/second-decade/en.
4. De la Barra F, Vicente B, Saldivia S, Melipillán R. Estudio de epidemiología psiquiátrica en niños y adolescentes en Chile. Estado actual. Rev Med Clin Condes. 2012;23:521-9.
5. Silva D, Valdivia M, Vicente B, Arévalo E, Dapelo R, Soto C. Intento de suicidio y factores de riesgo en una muestra de adolescentes escolarizados de Chile. Rev Psicopatol Psicol Clin. 2017;22:33-42.
6. Ministerio de Salud. Recomendaciones Para la Prevención de la Conducta Suicida en Establecimientos Educacionales. Desarrollo de Estrategias Preventivas Para Comunidades Escolares. Santiago: Ministerio de Salud; 2019. Available from: https://www.minsal.cl/wp-content/uploads/2019/03/GUIA-PREVENCION-SUICIDIO-EN-ESTABLECIMIENTOS-EDUCACIONALES-web.pdf.
7. Observatorio Chileno de Drogas. Décimo Tercer Estudio Nacional De Drogas En Población General De Chile, 2018. Santiago: Ministerio de Salud; 2019. Available from: https://www.senda.gob.cl/wp-content/uploads/2020/02/ENPEG-2018.pdf
8. Barker G, Olukoya A, Aggleton P. Young people, social support and help-seeking. Int J Adolesc Med Health. 2005;17:315-35.
9. Del Mauro JM, Jackson Williams D. Children and adolescents' attitudes toward seeking help from professional mental health providers. Int J Adv Couns. 2013;35:120-38.
10. Carlisle J, Shickle D, Cork M, McDonagh A. Concerns over confidentiality may deter adolescents from consulting their doctors. A qualitative exploration. J Med Ethics. 2006;32:133-7.
11. D'Avanzo B, Barbato A, Erzegovesi S, Lampertico L, Rapisarda F, Valsecchi L. Formal and informal help-seeking for mental health problems. A survey of preferences of Italian students. Clin Pract Epidemiol Ment Health. 2012;8:47-51.
12. Leavey G, Rothi D, Paul R. Trust, autonomy and relationships: the help-seeking preferences of young people in secondary level schools in London (UK). J Adolesc. 2011;34:685-93.
13. Rickwood D, Deane FP, Wilson CJ, Ciarrochi J. Young people's help-seeking for mental health problems. Aust E-J Adv Ment Health. 2005;4:218-51.
14. Rughani J, Deane FP, Wilson CJ. Rural adolescents' help-seeking intentions for emotional problems: the influence of perceived benefits and stoicism. Aust J Rural Health. 2011;19:64-9.
15. Gulliver A, Griffiths KM, Christensen H. Perceived barriers and facilitators to mental health help-seeking in young people: a systematic review. BMC Psychiatry. 2010;10:113.
16. Johnco C, Salloum A, McBride NM, Cepeda SL, Guttfreund D, Novoa JC, et al. Mental health literacy, treatment preferences, and barriers in Salvadorian parents. Int J Ment Health. 2019;48:139-64.
17. Wilson CJ. General psychological distress symptoms and help-avoidance in young Australians. Adv Ment Health. 2010;9:63-72.
18. Wilson CJ, Deane FP. Help-negation and suicidal ideation: the role of depression, anxiety and hopelessness. J Youth Adolesc. 2010;39:291-305.
19. Wilson CJ, Deane FP, Marshall KL, Dalley A. Adolescents' suicidal thinking and reluctance to consult general medical practitioners. J Youth Adolesc. 2010;39:343-56.
20. Vogel DL, Wester SR, Larson LM. Avoidance of counseling: psychological factors that inhibit seeking help. J Counsel Dev. 2007;85:410-22.
21. Wilson CJ, Deane FP. Brief report: Need for autonomy and other perceived barriers relating to adolescents' intentions to seek professional mental health care. J Adolesc 2012;35:233-7.
22. Clement S, Schauman O, Graham T, Maggioni F, Evans-Lacko S, Bezborodovs N, et al. What is the impact of mental health-related stigma on help-seeking? A systematic review of quantitative and qualitative studies. Psychol Med. 2015;45:11-27.
23. Saporito JM, Ryan C, Teachman BA. Reducing stigma toward seeking mental health treatment among adolescents. Stigma Res Action. 2011;1:9-21.
24. Schnyder N, Panczak R, Groth N, Schultze-Lutter F. Association between mental health-related stigma and active help-seeking: systematic review and meta-analysis. Br J Psychiatry. 2017;210:261-8.
25. Kutcher S, Bagnell A, Wei Y. Mental health literacy in secondary schools: a Canadian approach. Child Adolesc Psychiatr Clin N Am. 2015;24:233-44.
26. Wei Y, McGrath PJ, Hayden J, Kutcher S. Mental health literacy measures evaluating knowledge, attitudes and help-seeking: a scoping review. BMC Psychiatry. 2015;15:291.
27. Yamasaki S, Ando S, Shimodera S, Endo K, Okazaki Y, Asukai N, et al. The recognition of mental illness, schizophrenia identification, and help-seeking from friends in late adolescence. PLoS One. 2016;11:e0151298.
28. Rickwood D, Thomas K, Bradford S. Help-seeking Measures in Mental Health: a Rapid Review. Glebe NSW: The Sax Institute; 2012. Available from: https://www.saxinstitute.org.au/wp-content/uploads/02_Help-seeking-measures-in-mental-health.pdf.
29. Rickwood DJ, Mazzer KR, Telford NR. Social influences on seeking help from mental health services, in-person and online, during adolescence and young adulthood. BMC Psychiatry. 2015;15:40.
30. Cakar FS, Savi S. An exploratory study of adolescent's help-seeking sources. Proc Soc Behav Sci. 2014;159:610-4.
31. Olivari C, Guzmán-González M. Validación del cuestionario general de búsqueda de ayuda para problemas de salud mental en adolescentes. Rev Chil Pediatr. 2017;88:324-31.
32. Hurley D, Allen MS, Swann C, Okely AD, Vella SA. The development, pilot, and process evaluation of a parent mental health literacy intervention through community sports clubs. J Child Fam Stud. 2018;27:2149-60.
33. Ratnayake P, Hyde C. Mental health literacy, help-seeking behaviour and wellbeing in young people: implications for practice. Educ Dev Psychol. 2019;36:1-6.
34. Venkataraman S, Patil R, Balasundaram S. The need for assessing mental health literacy among teachers: an overview. Int J Res Med Sci. 2019;7:3210-5.
35. Houle J, Chagnon F, Lafortune D, Labelle R, Paquette KB. Correlates of help-seeking behaviour in adolescents experiencing a recent negative life event. Can J Fam Youth. 2013;5:39-63.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

How the COVID-19 contingency affects children

J. Carlos Reséndiz-Aparicio[1,2]*

[1]*Departamento de Neurología Pediátrica, Hospital Psiquiátrico Infantil Dr. Juan N. Navarro;* [2]*Oficina del Programa Prioritario de Epilepsia, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez. Mexico City, Mexico*

Abstract

Background: *Due to the pandemic, children are undergoing many changes in their daily lives.* **Methods:** *We analyzed how parents perceive the effects of the contingency on their children through an online survey shared by digital media for 7 days.* **Results:** *We obtained 4000 responses. The most frequent difficulty of the children was online homeschooling (30.4%), followed by sleeping disorders (20.3%). The use of screens increased 30-80% in over 65% of children. Tantrum was detected in 34% and mood swings in 30% of children. The majority of parents (77.8%) considered that distance education does not guarantee children's education and that the level of learning acquired through online classes is not the same as that of face-to-face education (83.5%). In contrast, 70.6% of parents considered that it is not yet time to reopen schools, 78.8% believed that there is sufficient evidence to keep them closed, and 45% indicated that it is better not to return to campus this year. Regarding activities to improve mental health during the contingency, 51.3% have created home games, and 23.6% perform physical activity. However, 74.4% do not have the peace of mind to restart daily life. Among the positive aspects of the contingency, adaptability (35%) and family union (33.5%) were reported.* **Conclusions:** *Health professionals in contact with children must be prepared for the problems that this contingency is generating and sensitize parents to observe their children and seek professional help on any alarm data on the emotional or behavioral state of the child.*

Key words: *Coronavirus disease 2019. Contingency. Children. Survey.*

Cómo afecta a los niños la contingencia por COVID-19

Resumen

Introducción: *Debido a la pandemia, la población infantil ha experimentado cambios en varios aspectos de su vida cotidiana.* **Métodos:** *Se analizó la percepción de los padres con respecto a los efectos de la contingencia en sus hijos a través de una encuesta en línea compartida por medios digitales durante 7 días.* **Resultados:** *Se obtuvieron 4000 respuestas. La dificultad más frecuente a la que se enfrentan los niños en la pandemia son las clases en línea en casa (30.4%), seguida de problemas para dormir (20.3%). Se identificó un incremento en el uso de pantallas del 30-80% en más del 65% de los niños. Se detectaron berrinches en el 34% y cambios de humor en el 30% de los niños. La mayoría de los padres (77.8%) consideraron que la educación a distancia no garantiza la educación de los niños, y que el nivel de aprendizaje de las clases en línea no es igual que el de las clases presenciales (83.5%). Por otro lado, el 70.6% de los padres opinaron que no es momento para abrir los colegios, el 78.8% indicaron que existe evidencia para mantenerlos cerrados y el 45% consideraron que es mejor no regresar este año al plantel. En cuanto a actividades para mejorar la salud mental en la contin-*

Correspondence:
*J. Carlos Reséndiz Aparicio
E-mail: jc_doc@yahoo.com

Date of reception: 29-05-2020
Date of acceptance: 03-08-2020
DOI: 10.24875/BMHIM.20000140

Available online: 08-06-2021
Bol Med Hosp Infant Mex. 2021;78(3):216-224
www.bmhim.com

gencia, el 51.3% han creado juegos en casa y el 23.6% realizan actividad física. Sin embargo, el 74.4% no tienen tranquilidad para reiniciar su vida cotidiana. Dentro de las cosas positivas de la contingencia, se reportó adaptabilidad (35%) y unión familiar (33.5%). **Conclusiones:** *Los profesionales de la salud en contacto con niños deben estar preparados para los problemas generados por esta contingencia y sensibilizar a los padres para observar a sus hijos y solicitar ayuda si detectan datos de alarma del estado emocional o conductual del niño.*

Palabras clave: *COVID-19. Contingencia. Niños. Encuesta.*

Introduction

Knowing the potential repercussions of a contingency that forces children to stay at home all the time is a transcendental subject for the professionals who dedicate themselves to their care.

The coronavirus family are not a new group of viruses that affect humans[1]. The current epidemic is caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), named by the International Virus Classification Committee. This virus produces a disease that received its name from the World Health Organization[2] on February 11, 2020, the coronavirus disease 2019 (COVID-19).

Severe COVID-19 pulmonary disease in children is uncommon[3]. Neurological symptoms can be divided into three categories: (1) neurological manifestations of the underlying disease symptoms (headache, dizziness, consciousness disorders, ataxia, acute symptomatic seizures, and cerebral vascular disease); (2) symptoms of peripheral nervous system origin (hypogeusia, hyposmia, and neuralgia); and (3) symptoms of skeletal muscle damage, often associated with liver and kidney damage[4], although data on severe neurological involvement are fortunately infrequent in children[5].

Each country has considered its official infection figures to create contingency phases to manage the pandemic. In Mexico, on March 20, 2020, the suspension of classes was officially announced in every school in the country, which meant that children had to stay at home, regardless of the benefits or consequences that this national contingency would bring.

In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association[6], trauma-related disorders and stressors are included in acute stress disorder (ASD) and adjustment disorders, which can occur after a disaster or a radical change in the pattern of activities. Other psychological and psychiatric conditions may also be observed, such as depressive episodes, anxiety disorder, insomnia, and even suicidal behavior[7].

In children, we find the subtle (or sometimes not so subtle) symptoms that may occur due to confinement, in addition to those directly caused by SARS-CoV-2 infection. Due to different life circumstances, family behaviors and routines change to reduce childhood distress, minimize exposure to stimuli that provoke fear or anxiety, and relieve distress; this process has been called family accommodation[8]. One modification of family routines may be staying at home to work or canceling family trips. However, most of the time, these changes are not voluntary, as in contingency, and may play a positive or negative role: just as they may alleviate distress in the short term, they may maintain anxiety and avoidance in the long term in children[8]. Parental care is critical as part of any child's upbringing. However, some parents become overly vigilant and think that everything is a threat to their child. This parental behavior has been considered a behavior that causes a major neurocognitive factor in developing and maintaining pediatric anxiety disorders[9].

Insomnia is a common symptom in children, which may increase in special situations where changes in established patterns occur. Insomnia is associated with a decrease in school performance and an increase in psychopathology[10]. Behavioral profiles associated with objective sleep duration have been studied in young children with insomnia symptoms, and sleep duration and various physiological changes such as increased cortisol have been associated. Some studies show that children with insomnia symptoms have more behavioral problems in general than control groups[11].

In children in whom sleep duration is typical, insomnia has been associated with high scores of externalized behavioral problems, such as restlessness, inattention, impulsivity, irritability, mood changes, and school problems. Conversely, children with insomnia and short sleep duration are associated with a more frequent profile of internalized problems, such as anxiety or low mood[11]. These children show a lower quality of life associated with a higher risk of other medical conditions[10].

The difficulties of not attending school are a problem that should be continuously monitored in children. Several factors can result in a child having a good educational level. Specific measures have been studied, such as the socioeconomic level (a factor that directly impacts the educational opportunities and offerings provided by schools, including the quality of their teachers), maternal education, and paternal education. These three events impact children's educational level. If these factors are modified, they can have a negative influence. Differences in children's temperament must also be considered when dealing with school learning[12,13].

In countries where the death toll has been significant and continues to rise, there will be many children and young people dealing with loss and grief in addition to their already challenging circumstances. There are no easy answers, but parents and caregivers are expected to be vigilant and work with children to provide a high level of interaction and support them through this contingency[13].

Methods

Scientific information on COVID-19 appeared in February 2020, and the number of publications has been vast. The majority has been shared online to accelerate awareness. The transmission of data has been second only to viral and human-to-human transmission.

To analyze the parents' opinion of how confinement affected children, we conducted and shared a survey on Google Forms 2 months after the suspension of classes. The survey consisted of 12 questions and was addressed to parents of children aged 4-15 years, considering that they were the most sensitive population, and was sent by email, WhatsApp, and Facebook. The survey was active online for 7 days. The first response was received on May 20, and the last response was received on May 26. The questionnaire was elaborated to obtain the following information: if children had any problems, from the parents' perspective, with emphasis on sleeping difficulties; how they were living their school life from home; how distance learning was working out; how the use of screens had increased (which we know is a problem at present); how confident and informed parents felt with the current information to return to everyday life, including the return to school, and when the ideal time to return to school would be; if they had to implement any activity to improve the child's quality of time at home; and finally, if they considered that the contingency could leave something positive for their children.

Results

A total of 4000 responses were obtained with the Google Forms count. Some of those who entered to respond did not answer all the questions (marked as "no response").

The first question was related to the problems that parents had observed in their children during confinement. The most-reported problem was related to online homeschool, access to a computer and internet, a physical space, or the difficulty for pre-school children to be sitting in front of a computer for online classes. Difficulties with online homeschool were reported in 30.4% of subjects. We should remember that schools' responses have been very variable, as no one was prepared for the contingency. In general, homework prevailed, but some with TV classes, some with sporadic online tutoring, and some with formal online classes every day. In addition to sleeping problems, these problems represent half of the difficulties encountered by parents in their children (Fig. 1).

Secondly, we explored the kind of sleep-related problems in children during the contingency. We know that frequent expressions of anxiety in children are sleeping problems, including insomnia, nightmares, and night terrors; in general, insomnia symptoms are associated with a wide range of behavioral problems in children[11]. One of UNICEF's first efforts to care for children during the contingency was to provide easy-to-understand material, in addition to not overexposing children to COVID-19 issues[14]. The percentage of sleeping problems reported by parents was 46.7%, mainly related to difficulty falling asleep. This finding may be related to the loss of sleep and wake times during the contingency rather than a purely anxiety-related issue. However, it could be a sum of several factors (Table 1).

The third question was related to the increase in using screens, which has been a growing problem in children worldwide and has influenced their motor and social development[15]. Approximately 11% of parents perceived an increase of screen use > 90% in their children, 31.3% have seen an increase of 60-80%, and 33% observed an increase of 30-50% of screen use (Table 2). A study published a couple of years ago reported that 34% of preschoolers have an electronic device with screens and that the 16-24 age group spends more time playing than sleeping[15]. Thus, a current concern is developing addictive behavior in children and young people, now known as screen dependence disorder[15]. Characteristics of this problem

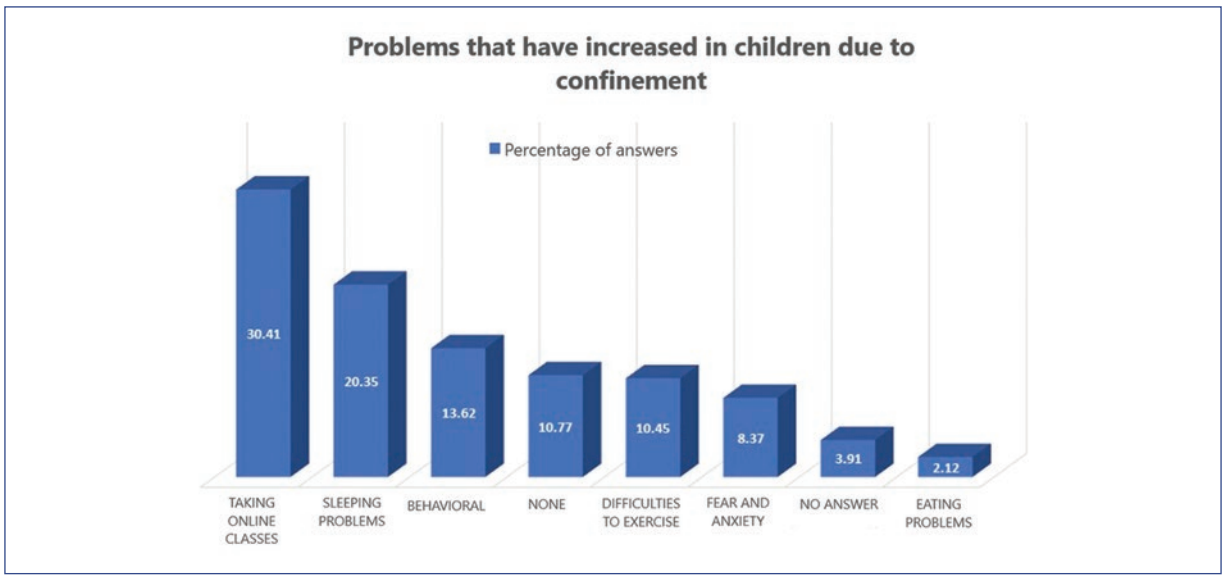

Figure 1. Some of the problems that parents identified in their children due to confinement.

Table 1. Sleeping troubles during the contingency in children according to their parents

Possible responses	Number of answers*	Percentage
Difficulty starting to sleep	1868	46.7
None	1446	36.15
Awakenings at night	286	7.15
No answer	198	4.95
Nightmares	82	2.05
Night terrors	56	1.40
Bed-wetting	40	1
Sleepwalking	24	0.6

*From a total number of answers = 4000.

Table 2. Screen time increase in children during the contingency, according to their parents

Percentage of increase (%)	Number of answers	Percentage
From 30 to 50	1322	33.05
From 60 to 80	1252	31.30
From 10 to 20	563	14.07
More than 90	440	11.0
It has not increased	247	6.17
No answer	176	4.4

*From a total number of answers = 4000.

focus on reducing or stopping screen use, loss of interest in other activities, the continuation of the behavior despite consequences, lying to hide the behavior, and its use as an emotional outlet[15]. The development and maintenance of this disorder are related to a dysfunction of brain structures. Brain imaging studies have demonstrated structural changes in the white and gray matter in the prefrontal cortex and limbic structures and reduced frontostriatal neuronal circuits[15]. Although the use of screens increased during the contingency, parents should make sure that this situation will not be detrimental to their children's future functioning and decrease this behavior as soon as the contingency is over.

We also investigated children's behavior during the contingency. Due to this situation, anyone may show signs of low frustration tolerance. As it is important to learn to postpone pleasure, we should recognize that many vulnerable children cannot handle stress[13]. The responses to this question are split into three possibilities of almost one-third of answers each. Tantrum or low tolerance was detected, followed by frequent changes in mood. However, more than 20% of the parents did not perceive behavioral problems, which was very good for this group of children who could handle the situation with no alterations (Fig. 2).

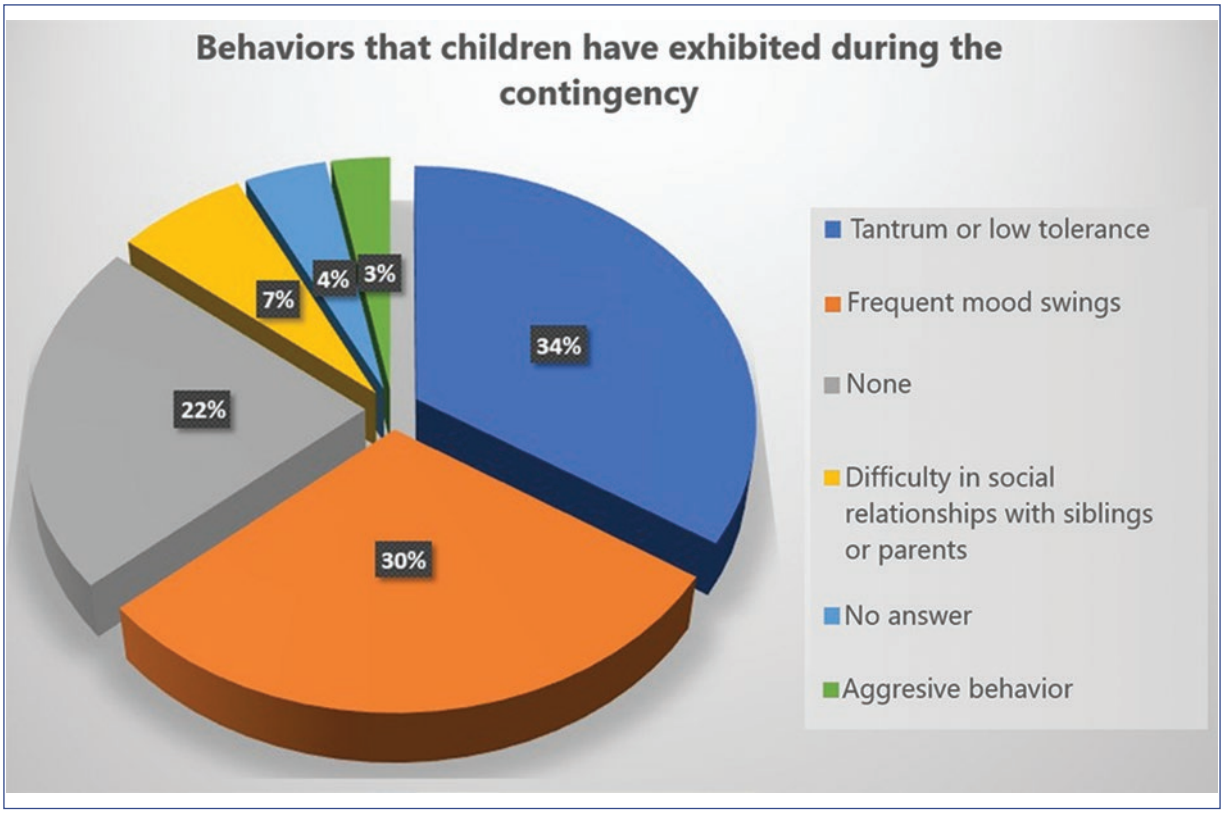

Figure 2. Main behaviors identified in children during the contingency.

Table 3. Distance education and learning in children during the contingency according to the parental perception

"Distance education guarantees the right to education for all children equally"		"Do you consider that the quality of learning of your child has been the same with distance classes?"	
Answer	**Percentage**	**Answer**	**Percentage**
False	77.8	No	83.57
True	17.85	Yes	12.11
No answer	4.35	No answer	4.32

Table 4. Parents' opinions regarding reopening schools and keeping schools closed during the contingency

"In your opinion, is it time to reopen schools?"		"Is there enough evidence to keep schools closed?"	
Answer	**Percentage**	**Answer**	**Percentage**
Yes	9	Yes	78.85
No	70.57	No	16.65
Perhaps	16.25	Perhaps	4.5
No answer	4.17		

*From a total number of answers = 4000.

Regarding education, 77.8% of parents consider that distance education does not guarantee equal education for all children (Table 3). We know the economic differences and access to products and services in many families. Schools have tried to adopt different modalities that cope with the contingency. However, online classes require a computer and internet services, which is complicated for some children who do not have these requirements or even access electricity. In addition to these potential liabilities, families have been impacted socially and economically by the contingency[13].

Concerning distance education, nearly 85% of parents consider that the quality of learning is not the same as face-to-face classes. We are used to in-person learning and supervision accompanied by teachers' personalized guidance. Each child's temperament and learning styles are drivers of learning and educational success even with distance education[13]. Many families have complained, especially at the beginning

Table 5. Parents' opinions on the ideal time to return to school

Answer	Number of answers	Percentage (%)
In June 2020	226	5.65
In July 2020	725	18.12
Not returning this school year	1798	44.95
Repeating the school year	353	8.82
That everyone passes this school year	704	17.61
No answer	194	4.85

of the contingency, about the enormous homework load. In the future, the mix of in-person classes can certainly be complemented by distance support (Table 3).

About returning to schools, 70.6% of the parents considered that it is not time to reopen schools, although 16.25% were not sure, while 9% considered that schools should be reopened (Table 4). These results suggest that parents may not be aware of the risks of opening schools at present, which implies bringing back groups of children and adults in a classroom together.

Furthermore, regarding evidence to keep schools closed, 16.65% of parents considered insufficient evidence to keep schools closed (Table 4). As a society, we need to receive clear information about the rationale for the closing, the risk of family mobility, the risk of children being infected, the risk that they may serve as vectors and transfer the disease to family members, and even the possibility that they may become ill and present severe symptoms (although this situation has been relatively infrequent)[3,5]. It is essential to be particularly careful with young children, as it is more challenging to maintain a proper distance, wearing masks, and maintaining hygiene measures in the classroom[14].

Due to variations in official data and the unknown, the answers related to the ideal time to return to school are complex because no one has the absolute truth. However, 45% of parents believe that children should not return to school this year and almost one-fifth think that July 2020 could be an option for their return. Strikingly, almost 10% consider an option to repeat the school year, which would undoubtedly complicate the country's school system (Table 5).

The majority of parents took measures to improve the family's mental health during the contingency: strategies to entertain children at home throughout the day. We found that 51.3% have implemented games at home, which is a good strategy that also allows parents to observe the behavior of their children during an activity that involves rules and shifts; therefore, games imply working on tolerance to shifts, times, and the possibility of winning or losing. Physical exercise at home has been something that parents have engaged in almost 25% of the cases. We have long argued that engaging in physical activity can improve mood for several reasons, including endorphin production. It has been confirmed that more significant physical activity is consistently associated with a lower probability of developing depression and that the protective effect of physical activity is observed independently of age and sex, which is significant in all geographic regions[16]. Therefore, daily activity seems to be an excellent recommendation. In contrast, only a low percentage of parents have felt the need to seek professional help to improve mental health in their children (Fig. 3).

Regarding the next question, "With the information you have, do you feel comfortable with restarting your daily activities?", we obtained a total of 2975 responses indicating that parents were not comfortable with starting their activities, which represents 74.4% of the responses. On the contrary, 849 participants (21.22%) indicated that they were comfortable. A total of 176 people did not answer this question.

Everybody is uncertain about the pandemic, how to reverse the contingency, how to do it, which places to avoid, and which will be the safest. Although general information has been given and we can establish that hospitals are undoubtedly the riskiest places[2], we will certainly have to avoid crowded and poorly ventilated spaces. We will have to change some habits to return to usual activities, but the parents' responses show that almost 75% are uncomfortable resuming their daily activities.

Finally, we wanted to know if the contingency left positive things to children from their parents' perspective. Adaptability and family unity were the most frequent answers, followed by learning to manage tolerance. A low percentage expressed taking advantage of the time to acquire a new skill (Fig. 4).

Discussion

This survey has shown the difficulties parents have observed in their children's behavior, learning, and

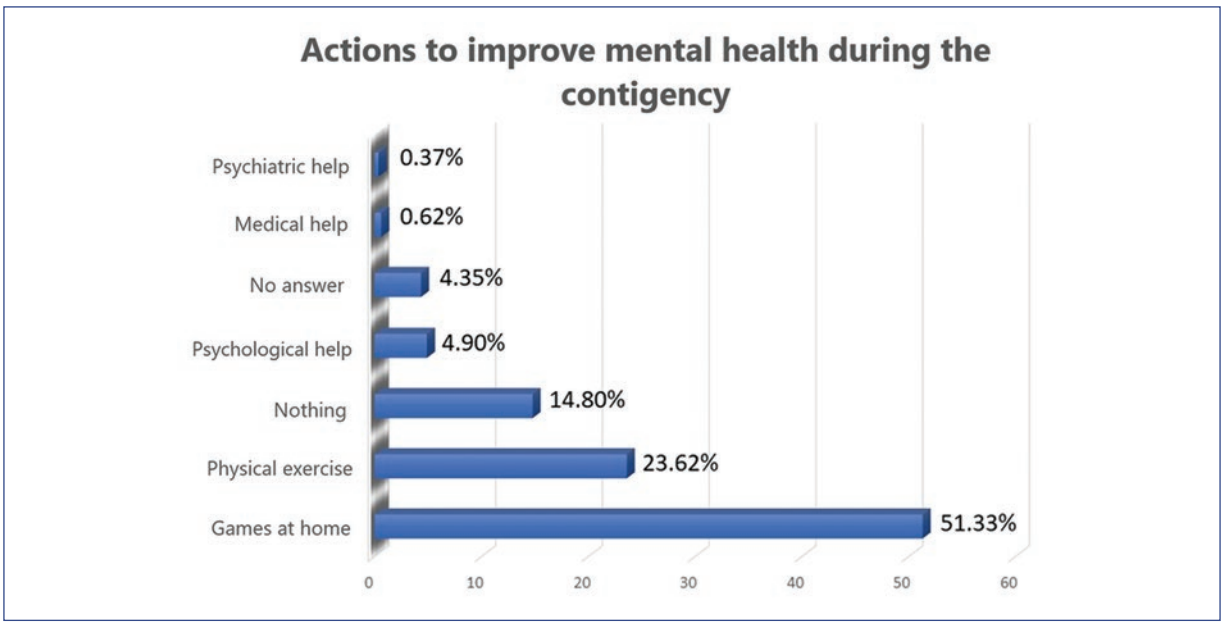

Figure 3. Actions that parents took to improve mental health during the contingency.

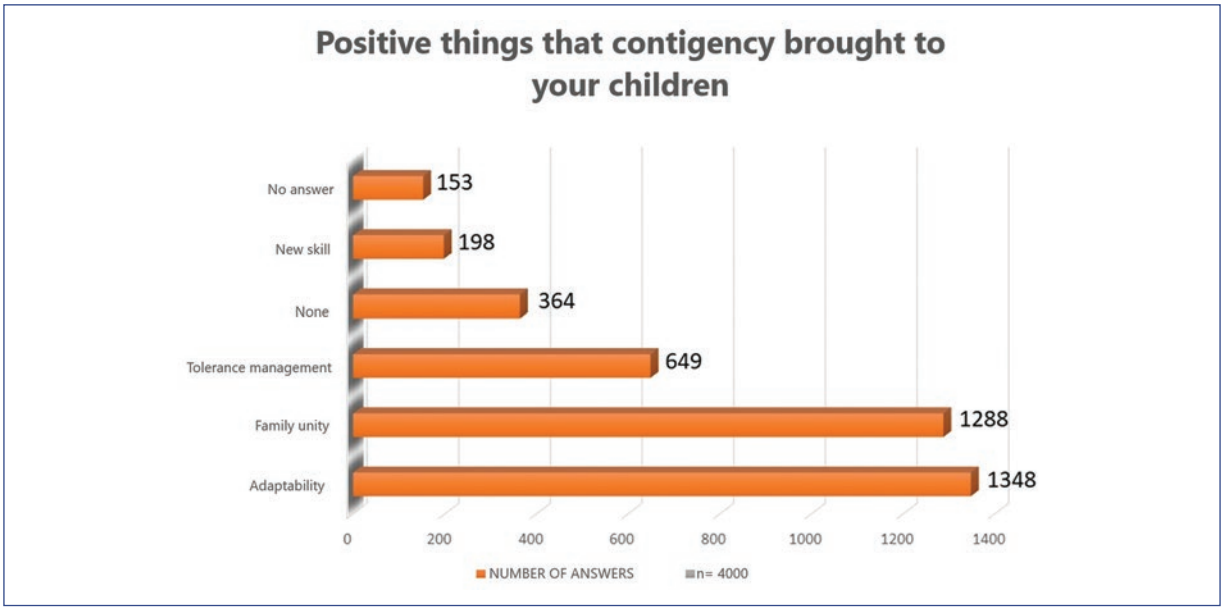

Figure 4. Positive behaviors that parents identified in their children during the contingency.

emotional state at the beginning of the contingency. Regarding COVID-19, neither children nor adults know what is happening and what is coming concerning health, education, and economy. In general, children are afraid of the unknown or what they do not understand.

In a population exposed to potentially traumatic experiences, emotional, cognitive, behavioral, and somatic symptoms can be triggered. Although most of them disappear spontaneously, a proportion could evolve into more serious mental health disorders[17], such as ASD, which should be monitored in children during and after the contingency. Although almost 13-37% of individuals who underwent any stressful situation have been reported with ASD, this disorder depends on several factors. In 2017, Bryant mentioned that the

temporary nature and different causes of this condition have made it difficult to obtain accurate information about the prevalence of ASD[18].

Although anxiety is prevalent in the general population, including children, and can be initiated in stressful situations such as the pandemic, it often goes unnoticed and untreated[19]. If children are timely diagnosed and treated, the risk of anxiety persisting into adulthood can be reduced. Restlessness, irritability, or sleep disorders such as insomnia are data that may be present in generalized anxiety disorders[20].

Anxiety diagnosis is based on clinical data since there is no biological marker, for which the clinician's clinical experience is significant[9]. Among other scales used to assess anxiety at any age, the SCARED scale for children[21] explores some of the following items: "When I am afraid I cannot breathe well," "I cannot swallow food," or "I get dizzy," which are somatization data; "people tell me that I look nervous"; or data related to sleep such as "I worry when I have to sleep alone," "at night I dream that bad things are going to happen to my parents," or "at night I have nightmares that something bad is going to happen to me." Also, other data such as "I sweat a lot when I am afraid," "I worry that something will happen to my parents," among others. For this reason, these questions can be incorporated into the routine pediatric consultation when anxiety is suspected, even though a formal scale is not applied. These data are non-specific. However, if considered together, these data could lead to suspect anxiety in children[9,21].

Stress directly impacts sleep, such as sleep reactivity, vulnerability to insomnia, and circadian disorders[22]. Sleep reactivity is the degree to which stress disrupts sleep, resulting in difficulty falling and staying asleep[22]. The neurobiological basis for sleep reactivity involves disrupted cortical networks, dysregulation in the autonomic nervous system, and the hypothalamic-pituitary-adrenal axis, an axis involved in stress and anxiety[22]. Poor sleep quality and insomnia are associated with decreased school performance, increased psychopathology, increased risk of self-harm, and even suicidal ideation[10]. Treatment of insomnia should be a priority in children upon diagnosis, as chronic insomnia has explicitly been associated with increased health risk and interpersonal, psychological, and daily functioning[10].

While it is true that use of screens and the internet has become indispensable to take classes or be in communication with other people, we should be alert with children since they can fall into excesses using these tools only to play. Internet gaming disorder has been documented, which is a severe disorder that leads to impairment in self-regulation, mood, reward regulation, problems in decision-making, and impact on social skills[23].

In our country, previous studies have detected that over half of the population seeks self-treatment, even when they perceive that they have a severe illness, mainly if they live in poverty or rural communities[24]. Therefore, constant campaigns should be carried out to promote mental healthcare. For the management of children with adaptive disorders due to the changing situation, drugs may be used; however, in many cases, breathing techniques, relaxation exercises, or self-help techniques have been tested and proven to be sufficient[12,25].

Moreover, creative approaches to staying functional during this confinement have emerged: people must find fun ways to express their confinement experiences and isolation at home[13]. Some studies have analyzed parenting during the contingency, what to do with children's stress and the best measures to deal with it[13]. Some suggested measures to address the contingency are online soft skills programs, remote work capability, resilience-supported work guides, and monitoring of vulnerable children[13].

Some limitations of this study were that the survey covered a pediatric population in a wide age range, and children's behavior is not the same at different stages of development; dividing the responses by age group would allow finding specific differences. Furthermore, only one response option was allowed, and some people may feel the need to answer multiple responses. Finally, there may have been confusion about who should answer the survey in families with more than one child since it was established that only one person per device could answer.

Undoubtedly, many things will change after the pandemic, including modifications in our hygiene and cleaning habits at home, workplaces, and school, and encourage online meetings to reduce mobilization of people. However, the difficulties reported by parents in this survey should be monitored in the short, medium, and long term, as children could exhibit behavioral and mood changes or sleep problems, which could increase if not timely addressed. We suggest watching for feeding, sleeping, responses to external stimuli, tolerance to frustration, hand sweating, nail-biting, enuresis or encopresis, repetitive behaviors, easy crying, overly sensitivity, and mood swings. If these symptoms are

observed, it is worthwhile to have them checked by a health-care professional.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

None.

Acknowledgments

The author would like to thank Berenice Reséndiz Rodríguez, who guided me on using Google Forms surveys, and Rosario Busquets Nosti, who kindly uploaded the survey in her social networks, which almost tripled the number of responses.

References

1. Woo P, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. J Virol. 2012;86:3995-4008.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. JAMA. 2020;323:1239-42.
3. Sinha IP, Harwood R, Semple MG, Hawcutt DB, Thursfield R, Narayan O, et al. COVID-19 infection in children. Lancet Resp Med. 2020;8:446-7.
4. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China. JAMA Neurol. 2020;77:683-90.
5. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Pediatr. 2020;109:1088-95.
6. American Psychiatric Association. DSM-5. Manual Diagnóstico y Estadístico de Los Trastornos Mentales. Madrid: Editorial Médica Panamericana; 2018.
7. Ahuja JR, Aparicio JC, Hernández EI, Flores RE. Manual de Intervención en Salud Mental en Situaciones de Desastre 2018. Mexico City: Gobierno de México; 2018.
8. Schleider JL, Lebowitz ER, Silverman WK. Anxiety sensitivity moderates the relation between family accommodation and anxiety symptom severity in clinically anxious children. Child Psychiatry Hum Dev. 2018;49:187-96.
9. Abend R, de Voogd L, Salemink E, Wiers RW, Pérez EK, Fitzgerald A, et al. Association between attention bias to threat and anxiety symptoms in children and adolescents. Depress Anxiety. 2018;35:229-38.
10. Combs D, Goodwin JL, Quan SF, Morgan WJ, Shetty S, Parthasarathy S. Insomnia, health-related quality of life and health outcomes in children: a seven-year longitudinal cohort. Sci Rep. 2016;6:27921.
11. Calhoun SL, Fernandez-Mendoza J, Vgontzas AN, Mayes Sd, Liao D, Bixler EO. Behavioral profiles associated with objective sleep duration in young children with insomnia symptoms. J Abnorm Child Psychol. 2017;45:337-44.
12. Weinberg D, Stevens GW, Finkenauer C, Brunekreef B, Smit HA, Wijga AH. The pathways from parental and neighborhood socioeconomic status to adolescent educational attainment: an examination of the role of cognitive ability, teacher assessment, and educational expectations. PLoS One. 2019;14:e0216803.
13. Lehmann J. Responding to COVID19: what is happening for our vulnerable children and young people? Children Australia. 2020;45:88-90.
14. Patricia Núñez. Hablando Sobre el Coronavirus-19 con los Niños y Niñas Más Pequeños. Guía Para Hablar Sobre el Coronavirus de Manera Sencilla, Clara y Tranquilizadora. Panama: Fondo de las Naciones Unidas para la Infancia (UNICEF); 2020. Available from: https://www.unicef.org/lac/informes/hablando-sobre-el-coronavirus-19-con-los-ninos-y-ninas-mas-pequenos.
15. Sigman A. Screen dependency disorders: a new challenge for child neurology. J Int Child Neurol Assoc. 2017;17:119.
16. Schuch FB, Vancampfort D, Firth J, Rosenbaum S, Ward PB, Silva SE, et al. Physical activity and incident depression: a meta-analysis of prospective cohort studies. Am J Psychiatry. 2018;175:631-48.
17. Álvarez-Icaza D, Medina-Mora ME. Impacto de los sismos de septiembre de 2017 en la salud mental de la población y acciones recomendadas. Salud Publica Mex. 2018;60:52-8.
18. Bryant RA. Acute stress disorder. Curr Opin Psychol. 2017;14:127-131.
19. Vega L, Ollinger E, Zimerman R, Figueroa J, Gutiérrez R. La representación infantil de los sismos. Salud Ment. 1987;10:66-71.
20. Cárdenas EM, Feria M, Palacios L, de la Peña F. In: Berenzon S, del Bosque J, Alfaro J, Medina ME, editors. Guía Clínica para los Trastornos de Ansiedad en Niños y Adolescentes. Mexico: Instituto Nacional de Psiquiatría Ramón de la Fuente; 2010.
21. Birmaher B, Brent DA, Chiappeta L, Bridge J, Monga S, Baugher M. Psychometric properties of the screen for child anxiety-related emotional disorders (SCARED): a replication study. J Am Acad Child Adolesc Psychiatry. 1999;38:1230-6.
22. Kalmbach DA, Anderson JR, Drake CL. The impact of stress on sleep: pathogenic sleep reactivity as a vulnerability to insomnia and circadian disorders. J Sleep Res. 2018;27:e12710.
23. Paulus FW, Ohmann S, Von Gontard A, Popow C. Internet gaming disorder in children and adolescents: a systematic review. Dev Med Child Neurol. 2018;60:645-59.
24. Orozco R, Borges G, Benjet C, Medina-Mora ME, López-Carrillo L. Traumatic life events and posttraumatic stress disorder among Mexican adolescents: results from a survey. Salud Publica Mex. 2008;50:S29-37.
25. Sarmiento-Hernández EI, Ulloa-Flores RE, Ávila-Rodríguez VM, Ramírez-Ahuja J, Velázquez-López VM, Reséndiz-Aparicio JC, et al. Intervención del hospital psiquiátrico infantil Dr. Juan N Navarro tras el sismo del 19 de septiembre de 2017. Salud Publica Mex. 2019;61:551-2.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

CLINICAL CASE

Hematopoietic stem cell transplantation in a patient with osteopetrosis and mutation in CLCN7: long-term follow-up

*José F. Gaytán-Morales[1], Iván Castorena-Villa[1], Fredenet O. Mendoza-Camargo[1], Dolores C. Cortés-Flores[1], Yazmin A. Gómez-Domíguez[1], Pamela D. Montenegro-Chahar[1], Paulina García-Maldonado[1], and Israel Parra-Ortega[2]**

[1]Servicio de Trasplante de Médula Ósea; [2]Departamento de Laboratorio Clínico. Hospital Infantil de México Federico Gómez, Mexico City, Mexico

Abstract

Background: *Osteopetrosis is a rare hereditary bone dysplasia characterized by insufficient osteoclast activity that results in increased bone mineral density. Hematopoietic stem cell transplantation (HSCT) can reverse skeletal abnormalities and restore hematopoiesis.* **Case report:** *We present the case of a 3-year and 2-month-old male patient with the diagnosis of osteopetrosis. The patient underwent allogeneic HSCT (Allo-HSCT) using 100% compatible bone marrow from a related donor and received a myeloablative conditioning regimen and a CD34 cell dose (4.7 × 10^7/kg). In the early post-transplant, frequent complications such as pneumonitis, hypercalcemia, and hyperphosphatemia ocurred. With a suitable granulocytic graft and chimerism of 100%, it was considered a successful transplant. However, the patient showed a delayed platelet graft treated with a platelet-stimulating factor for 6 months. The patient is currently disease-free, outpatient follow-up, with no data on graft-versus-host disease, and no progressive neurological damage.* **Conclusions:** *Osteopetrosis is a childhood disease that requires clinical suspicion and early diagnosis. HSCT is necessary at an early age to prevent disease progression and sensorineural, hematological, and endocrinological functions damage that can lead to death.*

Key words: *Osteopetrosis; Hematopoietic stem cell transplantation; Bone marrow stem cell transplant.*

Trasplante de células progenitoras hematopoyéticas en un paciente con osteopetrosis y mutación en CLCN7: seguimiento a largo plazo

Resumen

Introducción: *La osteopetrosis es una displasia ósea hereditaria poco común, caracterizada por una actividad osteoclástica deficiente que aumenta la densidad mineral ósea. Se considera que el trasplante de células progenitoras hematopoyéticas (TCPH) puede revertir las anormalidades esqueléticas y restaurar la hematopoyesis.* **Caso clínico:** *Se presenta el caso de un paciente de sexo masculino, de 3 años y 2 meses de edad, con diagnóstico tardío de osteopetrosis. Se realizó un TCPH alogénico de donador relacionado 100% compatible con médula ósea. Se utilizaron un régimen de acondicionamiento mieloablativo y una dosis celular de CD34 de 4.7 × 10^7/kg de peso. En el postrasplante temprano, el paciente desarrolló complicaciones como neumonitis, hipercalcemia e hiperfosfatemia. Con un injerto granulocítico adecuado y quimerismo del*

Correspondence:
*Israel Parra-Ortega
E-mail: i_parra29@hotmail.com

Date of reception: 10-04-2020
Date of acceptance: 11-07-2020
DOI: 10.24875/BMHIM.20000105

Available online: 02-03-2021
Bol Med Hosp Infant Mex. 2021;78(3):225-233
www.bmhim.com

100% se consideró un trasplante exitoso. Sin embargo, el paciente presentó retraso en el injerto plaquetario, por lo que se administró factor estimulante de plaquetas por 6 meses. Actualmente el paciente se encuentra libre de enfermedad, en seguimiento ambulatorio, sin datos de enfermedad del injerto contra el hospedero y con pruebas de neurodesarrollo sin deterioro neurológico progresivo. **Conclusiones:** *La osteopetrosis es una enfermedad infantil que requiere una sospecha clínica y un diagnóstico temprano, ya que es necesario un TCPH a corta edad como tratamiento para evitar la progresión de la enfermedad y el deterioro de las funciones neurosensoriales, hematológicas y endocrinológicas que puede derivar en la defunción del paciente.*

Palabras clave: *Osteopetrosis. Trasplante de células progenitoras hematopoyéticas. Trasplante de médula ósea.*

Introduction

Osteopetrosis, also known as marble bone disease, is a rare hereditary bone dysplasia characterized by a decreased resorptive activity of osteoclasts leading to inadequate resorption and abnormal bone turnover, causing an increased bone mineral density and increased risk of fractures[1]. Inadequate bone remodeling produces skeletal deformities, and the lack of bone elasticity coupled with bone fragility increases the risk of fractures[2]. The endochondral bone in the medullary cavity alters hematopoietic function causing life-threatening anemia, thrombocytopenia, and leukopenia.

Alterations in bone restructuring, which produce a narrowing of the bone canals and limits blood supply, can cause neurological disorders[3]. Different gene mutations can cause neurological alterations: the mutation of the T-cell immune regulator 1 (TCIRG1) gene causes neuronal compression, while the mutation of the CLCN7 gene causes significant progressive encephalopathy and retinopathy in some patients[4].

Clinical suspicion is essential to achieve early diagnosis and timely intervention with allogeneic hematopoietic stem cell transplantation (Allo-HSCT), a curative treatment that can reverse skeletal abnormalities and restore hematopoiesis[5].

Epidemiology

Autosomal recessive osteopetrosis incidence is 1 in 250,000 births, with a higher incidence in Costa Rica (3.4 in 100,000 births). In contrast, autosomal dominant osteopetrosis incidence is 1 in 20,000 births[6].

Classification

Osteopetrosis has a broad clinical spectrum: from a minimal disease, even asymptomatic, to the severe form known as infantile or malignant osteopetrosis (autosomal recessive), which is life threatening, especially during the first year of life (Table 1).

For decades, before identifying gene mutations affecting osteoclasts activity, osteopetrosis had been categorized depending on clinical characteristics and inheritance patterns. The *malignant* or *infantile* osteopetrosis with autosomal recessive inheritance pattern is the most severe form and the one with the earliest presentation. *Intermediate* osteopetrosis also has an autosomal recessive inheritance pattern, and *benign* or *adult* osteopetrosis is inherited in an autosomal dominant pattern.

Clinical description

The increase in bone mineral density results in particular physical characteristics, such as macrocephaly, craniofacial deformities, and generalized sclerosis. However, the primary involvement is in the bone marrow and central nervous system. The bone marrow's infiltration produces bone marrow failure; thus, hematopoiesis is compromised, and extramedullary hematopoiesis appears, which manifests itself as hepatosplenomegaly.

Etiology

Osteopetrosis is caused by a failure in osteoclasts' development and function. Furthermore, mutations in at least 10 genes are found in approximately 70% of patients. This disorder has an autosomal dominant or autosomal recessive inheritance pattern, being the last one the most severe form of presentation[7].

Osteoclasts are highly specialized cells derived from mononuclear precursors from the myeloid lineage. Their function is bone resorption, a fundamental process for remodeling and maintaining bone stability and mineral homeostasis.

Some osteopetrosis cases are caused by mutations in genes involved in pH acidification, which depends on the proper function of V-ATPase and CLCN-7. Homozygous mutations in these genes can cause malignant osteopetrosis.

Table 1. Characteristics of the different types of osteopetrosis and genetic classification

Population	Inheritance	Bone marrow failure	Diagnosis	Clinical features	Prognosis	Associated genes (% of presentation)	Life expectancy
Adult	AD	No	Incidental	Asymptomatic. However, in type I, there is skull affection, and in type II, there is the involvement of the cranial base, vertebrae, pelvis, and long bones. In some cases, bone pain or fractures have been reported	Good	CA II RANKL	Normal
Childhood	AR	Severe	One year of age	Blindness, hearing loss, growth retardation, frequent fractures, severe hypocalcemia, hydrocephalus, neurodegeneration	Poor	TCIRG1 (51-53%)* CLCN7 (13-16%)** OSTM1 (2-6%)*	0-10 years 0-3 years 0-2 years
Intermediate	AR	Variable	Childhood/ adolescence	Bone pain, arthritis, blindness, hearing loss, osteomyelitis, anemia	Variable	PLEKHMI CLCN7 LRP5 CA II	Variable
Gene and transcript	*Location*	*Variant*			*Genotype*	*Classification*	
CLCN7	Exon 5/25	ENST00000382745.4:c.376C>T ENSP00000372193.4:p.Arg126Cys Grch37 (hg19) chr16: 1510925 Grch38 (hg38) chr16: 1460924			Homozygous	Probably pathogenic variant Missense variant	

*Percentage of presentation of genes in malignant childhood osteopetrosis.
**Detected in our patient by genetic sequencing.
AR: autosomal recessive; AD: autosomal dominant.

Diagnosis

The diagnosis of childhood osteopetrosis is based on clinical and radiographic evaluations and confirmed by genetic testing[2]. It is essential to consider the diagnosis based on clinical suspicion and corroborate it by radiography and image studies, where cortical atrophy and generalized sclerosis mainly affecting the skull, pelvis, and spine can be appreciated. The characteristic image of *bone within a bone*, particularly in vertebrae and phalanges, can be observed. Age of presentation, inheritance, and associated clinical features such as pancytopenia, neurodegeneration, mental retardation, tubular acidosis, neuronal compression, and visual and auditory impairment support the diagnosis.

In case of suspicion, laboratory studies should be requested, such as serum calcium, parathyroid hormone, phosphorus, creatinine, 25-hydroxyvitamin D, and lactic dehydrogenase levels, which provide mineral homeostasis data. Also, a complete blood count with differential is necessary for bone marrow failure evaluation. Bone biopsy is useful for differentiating rich or deficit osteoclasts forms of osteopetrosis; however, it should not be performed routinely and is not essential for diagnosis.

Creatine kinase BB isoenzyme has been detected in patients with malignant osteopetrosis with CLCN-7 mutation; however, its levels do not correlate with the severity of the disease, and normal levels do not exclude the diagnosis[8].

Finally, the specific mutation must be confirmed by genetic testing to provide follow-up, prognosis, a probable response to treatment, recurrence, and genetic counseling.

Multidisciplinary management of these patients is vital since multiple organs and systems are affected. Endocrinologically, serum calcium levels should be

monitored since bone cannot mobilize calcium due to the osteoclasts' defect, which causes hypocalcemia and, secondary to this, tetany and seizures.

It is necessary to periodically evaluate patients' visual capacity to detect and control the emergence of major visual complications, such as optic nerve atrophy. Patients often have dental abnormalities, such as delayed or failed tooth eruption, malformation, and easy teeth loss. Fractures and their complications with osteomyelitis are frequent. Therefore, an expert orthopedic surgeon should evaluate patients. Neurologically, developmental delay, compression neuropathies, and craniofacial deformities should be intentionally sought.

Regarding hearing, nerve compression can lead to sensorineural deafness. Concerning hematological complications, pancytopenia is the primary life-threatening condition of patients with osteopetrosis, leading to severe infections and bleeding[5].

Differential diagnosis

The differential diagnosis should be made with any disease that causes bone sclerosis, such as berylliosis, myelofibrosis, Paget's disease, and malignant diseases. X-rays may show an increase in bone mineral density; however, compared to osteopetrosis, this alteration improves overtime. When osteopetrosis is diagnosed, it is essential to distinguish the subtype because each has a different evolution, progression, prognosis, and treatment.

Genetic alterations

The primary characteristic mutation of osteopetrosis is found in the TCIRG1 gene, which encodes the $\alpha 3$ subunit of the vacuolar H^+ATPase (V-ATPase), which pumps protons through the membranes, helping to regulate cellular and extracellular pH.

The V-ATPase plays an essential role on the surface of osteoclasts. This protein is embedded in a membrane that contacts the bone's surface and forms a compartment between the osteoclast and the bone surface. Its function is to pump protons into the bone, acidifying it and creating a suitable bone resorption environment[9]. This mutation is responsible for 50% of patients diagnosed with recessive osteopetrosis[10].

The CLCN7 gene encodes a chloride channel (CLC-7) that provides electroneutrality during the acidification process[4]. The mutation of this gene is present in 10-15% of patients with malignant osteopetrosis.

The third gene involved in this disease is OSTM1, which causes a more aggressive disease phenotype, shortening survival.

Treatment

HSCT is a procedure in which stem cells (HSC) from a disease-free donor are infused into patients with the disease. This procedure is performed to restore bone marrow function. There are different types of HSCT, depending on the type of HSC donor: autologous transplant (donor and recipient are the same subject), allogeneic transplant, which is performed from a donor with human leukocyte antigen (HLA) 100% compatible with the recipient and can be a related donor (family member) or an unrelated donor (non-family member), and haploidentical transplant, in which the family donor has an HLA 50% compatible with the recipient.

The initial treatment of osteopetrosis is mainly supportive and focuses on treating the main complications, such as pancytopenia, infections, and bleeding.

Fractures and arthritis require specialized treatment and follow-up since there is usually a delay in consolidation, osteomyelitis, and recurrent fractures.

Developmental delay and seizures associated with normal calcium levels often indicate childhood osteopetrosis. Neurological evaluations, including magnetic resonance imaging (MRI) and electroencephalogram, periodic ophthalmological examinations, including visual evoked potentials, and fundus examination to detect optic nerve atrophy, should be performed as soon as possible.

Hearing function evaluations should also be performed because, initially, neurosensory damage is mild; however, it may contraindicate HSCT as it progresses to severe forms.

Bone marrow failure can be treated initially with platelet and red blood cell concentrates transfusions; however, the definitive treatment of osteopetrosis is Allo-HSCT. In this pathology, HSCT is considered a therapeutic emergency because, as the disease progresses, sensory, visual, and hearing alterations become irreversible if treatment is late.

Transplant-related complications include transplant rejection, delayed graft function, hepatic sinusoidal occlusive syndrome, pulmonary hypertension, and hypercalcemic crises[11].

It is necessary to keep this disease under consideration when evaluating a patient with multiple dysmorphia, retarded growth and development, as well as sensory alterations, accompanied by metabolic and hematological alterations since timely diagnosis will

allow to perform an effective treatment and thus avoid a progression of the disease with more significant neurosensory impairment. Highlighting the importance of this pathology's treatment, we present the case of a patient with osteopetrosis treated with an Allo-HSCT, who had a successful evolution, even with a late disease diagnosis and a mild neurosensorial impairment.

Clinical case

We present the case of a 3-year and 2-month-old male patient from the State of Mexico. In his family medical history, healthy parents with no chronic degenerative diseases or consanguinity, a healthy 8-year-old sister, and no history of cancer, genetic or metabolic diseases, or immunodeficiency were reported.

At 1 year of age, the patient attended the Hospital Infantil de México Federico Gómez (HIMFG) for presenting community-acquired pneumonia (CAP), and later required hospitalizations for the same diagnosis (Fig. 1).

At 3 years of age, he was admitted again for CAP. On physical examination, generalized integument paleness, macrocephaly, hypertelorism, microretrognathia, prominent forehead, telecanthus, trichiasis, prominent eyes, flat nasal bridge, short neck, and narrow chest were observed. The skull X-ray showed the *mask* sign, characterized by generalized bone hyperdensity, predominantly at the base of the skull, which gave it the appearance of a mask in the orbital region. A chest X-ray was performed, observing an increase in bone density at the rib, sternal, and vertebral level. In addition, the *bone within a bone* sign, which consists of lines parallel to the tibia's cortical bone, was observed in the long bone X-ray. In laboratory studies, bicytopenia was reported in the complete blood count (hemoglobin 5.7 mg/dl, hematocrit 17.7%, leukocytes 13,400 cells/μL, total neutrophils 4020 cells/μL, and platelets 22,000 cells/μL). Therefore, as part of the approach, a bone marrow biopsy was requested, which reported hypocellularity, small intertrabecular spaces with fibrous tissue, and endochondral ossification. Due to the presence of dysmorphic syndrome, the patient required evaluation by the genetics service.

The patient was diagnosed with osteopetrosis at 3 years and 2 months of age, and as part of the evaluation by the HSCT service, the molecular study was carried out using second-generation sequencing in the HIMFG Research Unit. A mutation in the CLCN7 gene was reported, and the patient was classified as a homozygous genotype (Table 1). With this mutation, the patient met the criteria for an Allo-HSCT.

During hospitalization, the patient presented bilateral serous otitis media with secondary conductive hearing loss, for which ventilation tubes were placed. Influenza was detected, requiring antiviral treatment; another event of otitis media with otorrhea occurred, for which the patient required myringotomy and placement of ventilation tubes again.

A skull MRI was performed in the pre-transplant evaluations, where frank cortical atrophy with secondary ventricular dilation associated with its underlying pathology was observed. We detected a slight alteration in the bilateral visual pathways' conduction, together with a normal audiometry report, employing visual evoked potentials. Due to these results and the significant risk of developmental delay, the Infant Development Assessment test was performed, in which the patient met the criteria for risk of developmental delay. In the Battelle developmental test, a total development ratio of 63 was reported, corresponding to an equivalent age of 2 years and 3 months of age (deficit of 1 year and 5 months [37%]). Finally, the patient was diagnosed with global neurodevelopmental delay.

According to the HSCT protocol of the HIMFG, comorbidities were evaluated by the following services: ophthalmology, endocrinology, cardiology, infectology, otorhinolaryngology, pulmonology, nephrology and gastroenterology, and nutrition. Neurosensory, severe visual, or hearing damage were ruled out. In the admission blood count, before the HSCT, the following results were reported: total leukocytes 5300 cells/μL, total neutrophils 2070 cells/μL, platelets 35,000 cells/μL, and hemoglobin 9.1 mg/dL. At 3 years and 9 months of age, Allo-HSCT was performed from 100% compatible related donor (sister), using a myeloablative conditioning regimen: on days −7, −6, −5, and −4, the patient received busulfan (1.2 mg/kg/day), and on days −3 and −2, cyclophosphamide (60 mg/kg/day). Subsequently, a dose of 4.7×10^7 cells/kg obtained from bone marrow was infused.

As part of the prophylaxis for graft-versus-host disease (GvHD), immunosuppression was used based on cyclosporine (CsA, 3 mg/kg/day) and methotrexate (15 mg/m^2 day +1 and 10 mg/m^2 day on days +1, + 3, +5, +7, and +11). However, due to the lack of graft data on day +22, the calcineurin inhibitor dose was decreased, continuing with stimulation using granulocyte stimulating factor (Filgrastim). On day +27, granulocytic graft data with a total neutrophil count of 1200/μL were observed. On day +35, the patient showed 100% chimerism. Therefore, the dose of Filgrastim was decreased, and treatment with platelet-stimulating factor (Eltrombopag) was added, which continued for 6 months.

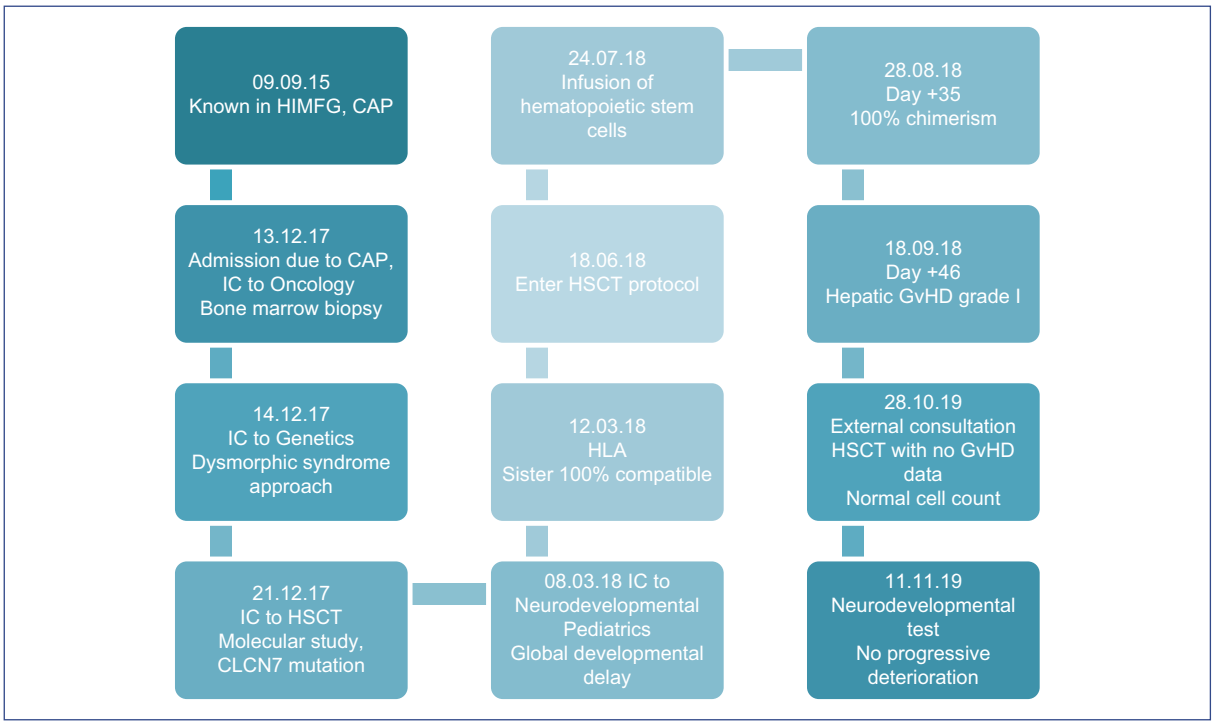

Figure 1. Chronological evolution of a patient with osteopetrosis. CAP: community-acquired pneumonia; GvHD: graft-versus-host disease; HIMFG: Hospital Infantil de México Federico Gómez; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation; IC: interconsultation.

During this period, the patient developed pneumonitis –requiring supplemental oxygen support through nasal prongs and inhaled steroid management–and presented hypercalcemia and hyperphosphatemia–which responded to management with hydration, forced diuresis, and phosphorus chelator, with no other complications. Subsequently, on day +46, Grade II acute GvHD was diagnosed, supported by altered liver function tests with elevated bilirubin, for which treatment with methylprednisolone (1 mg/kg/day), cyclosporine, and ursodeoxycholic acid was administered. The treatment was gradually decreased according to the clinical course and CsA levels. Due to the adequate response to treatment, it was possible to withdraw immunosuppressive drugs (currently, the patient is not immunosuppressed).

According to the follow-up protocol, a periodic quantification of chimerism was carried out (on days +15, +21, +28, +35, +100, and +150) to verify the transplant graft. On day +15, a chimera of 16.6% was found; subsequently, on day +21, the percentage increased to 59.88%, and on days +35, +100, and +150 after HSCT, 100% chimerism was documented (Fig. 2). At present, at 5 years of age, the patient continues in monthly outpatient follow-up and is in the process of entering preschool education. Furthermore, cell numbers are normal, with no platelet-stimulating factor or erythropoietin requirement, no GvHD data, and deferasirox treatment to avoid iron overload. Due to delayed growth, a weight of 14.2 kg (PZ –2), and a height of 90 cm (PZ –3) at 5 years and 3 months of age, the patient is being followed up by the endocrinology service. The patient maintains a visual capacity with a slight deficit but no progression and under ophthalmology service follow-up.

In addition, the patient undergoes an annual audiological evaluation, with no apparent deficit. As part of the follow-up, the EDI test that reported significant developmental delay improved compared to the previous results. An increase in the score of six evaluated domains was observed, finding that the personal-social and adaptive sphere significantly improved. Since the patient showed no added neurological deficit and an improvement was reported in neurodevelopmental tests, a new neurological imaging study was not considered necessary at the time. At present, the patient is being monitored by the neurodevelopment and neurology service.

Discussion

Infantile osteopetrosis is a rare hereditary disease, with a characteristic phenotype of short stature,

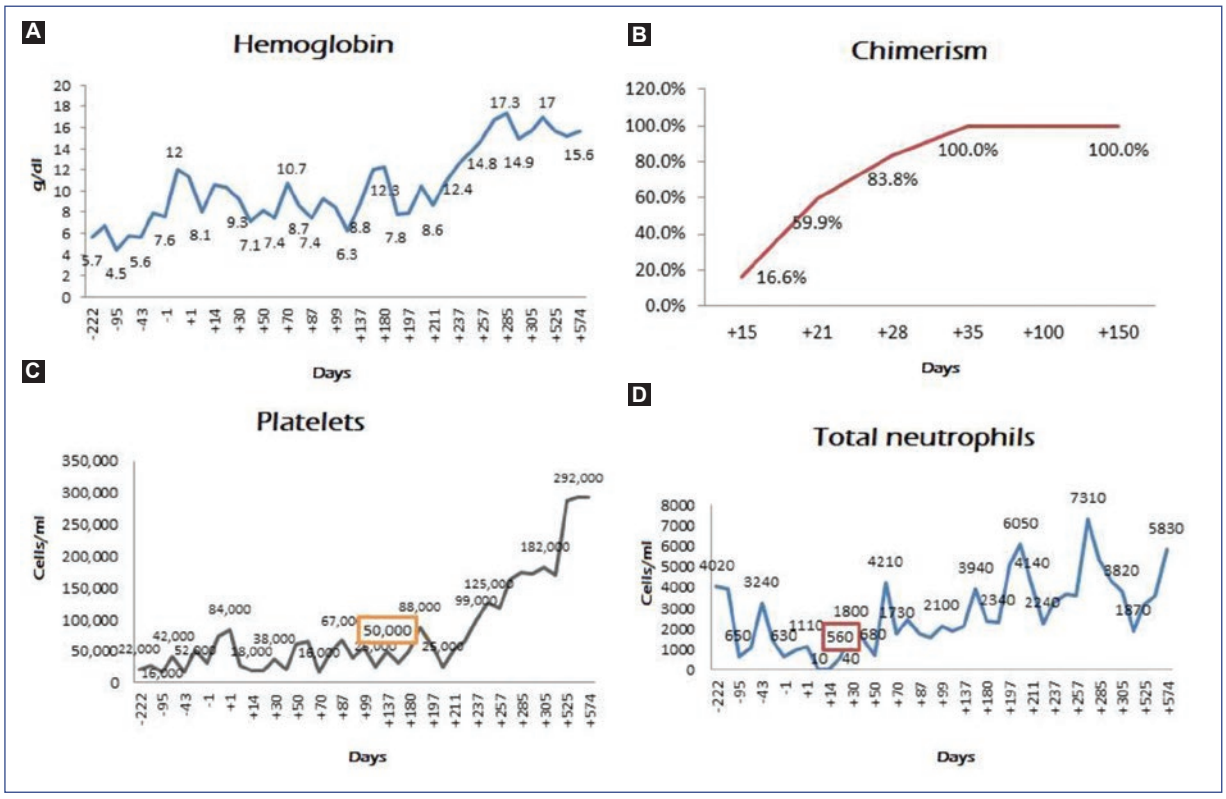

Figure 2. Laboratory studies of the patient with post-transplant osteopetrosis. **A.** Hemoglobin value after transplantation. **B.** Molecular chimerism after transplantation. **C.** Platelet value after transplantation. **D.** Granulocytic value after transplantation.

hypocalcemia, fractures, compression neuropathy, and pancytopenia; it is caused by the failure in the development and function of osteoclasts. We present the case of a 5-year-old male with a CLCN7 gene mutation and a 1-year follow-up after Allo-HSCT. Because osteopetrosis is caused by a defect in the osteoclasts derived from the hematopoietic cell line, bone marrow transplantation is the definitive treatment. Our patient underwent Allo-HSCT at 3 years and 9 months of age and currently has normal cell counts. The skeletal deformities and bone sclerosis shown on the X-rays improved since treatment (Fig. 3). Since the first approach, this patient exceeded the 3-year life expectancy reported for osteopetrosis patients with the CLCN-7 mutation (Table 1). Over 600 days after the transplant, the patient has shown a favorable evolution, decreasing the need for transfusions and hospitalization. Furthermore, skeletal deformities and neurodevelopmental tests have improved, leading to a better quality of life.

Severe osteopetrosis with increased bone mineral density results from the absence of osteoclasts, caused by a defect in the differentiation of osteoclast precursors or by dysfunctional osteoclasts. Therefore, the definitive treatment must be the transplant of hematopoietic stem cells with a bone marrow origin to obtain bone marrow-derived osteoclasts.

Abnormal bone expansion interferes with hematopoiesis, causing life-threatening pancytopenia and extramedullary hematopoiesis in the spleen and liver, and frequent hepatosplenomegaly. This patient presented pancytopenia at diagnosis. Initially, an infiltrative syndrome was suspected but ruled out; later, osteopetrosis was diagnosed.

Patients with some mutation usually have neurosensory, visual, and auditory alterations caused by bone compression[12]. In this patient, cortical atrophy accompanied with secondary ventricular dilation and bilateral visual pathway conduction alteration was documented, consistent with some reports in which MRIs show myelination delay and progressive cortical and subcortical atrophy[13]. The leading causes of death in these patients are infections, bleeding, and severe anemia, and allogeneic hematopoietic progenitor cells transplant is the only curative option[14]. A 5-year survival rate of 73% is reported when 100% compatible donor transplant is performed[15]. Risk of death increases in the first

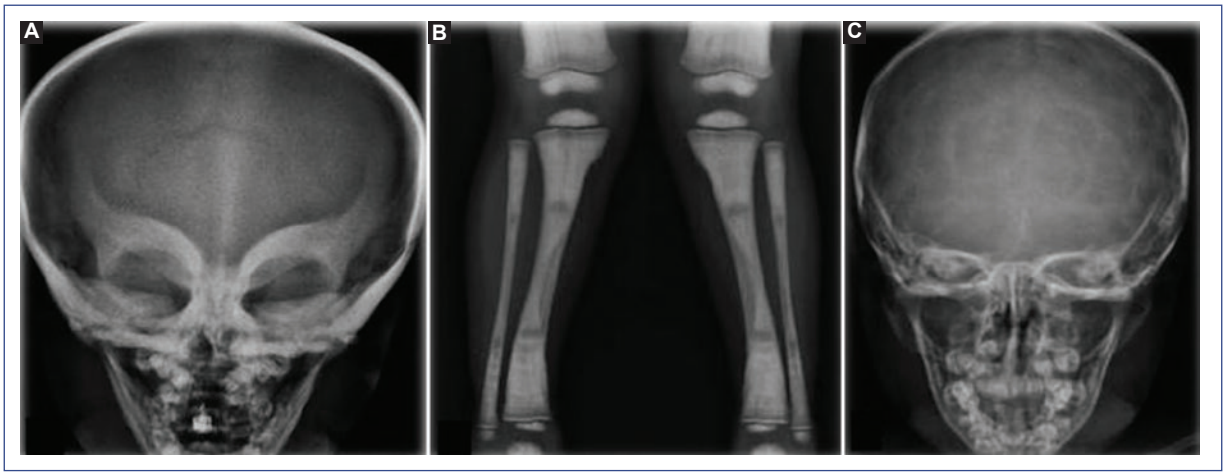

Figure 3. Osteopetrosis patient imaging studies. **A.** X-ray of the skull showing *mask* sign. Predominant generalized bone hyperdensity is observed at the base of the skull (before transplantation). **B.** X-ray of tibia and fibula with the *bone within a bone* sign. **C.** X-ray of the skull after transplantation with decreased bone hyperdensity.

10 years if Allo-HSCT is not performed due to the suppression of hematopoiesis in the bone marrow and its consequences.

The highest survival is reported in patients who received a transplant 100% HLA compatible of a related donor. The 5- and 10-year survival in patients with 100% compatible related donor Allo-HSCT is 62% in both, while for transplant of unrelated donor decreases to 42% and 39%, respectively. Therefore, it is of critical importance to obtain a related donor Allo-HSCT[16]. As for the complications that can occur after transplant in patients with osteopetrosis, primary and late graft failure has been described. In the present case, we observed delayed platelet engraftment with an adequate response to the platelet-stimulating factor. This complication imposes a higher mortality risk in transplanted patients[17].

The leading cause of transplant-related mortality is graft failure, accounting for 50% of deaths in transplants from related donors and 43% from unrelated donors.

Allo-HSCT improves bone lesions within 2 months, and they resolve entirely within a year of transplant[18]. However, survivors reported that only 7% experience improvement regarding the optical damage, 69% show no progression, and 25% show deterioration.

This clinical case generates essential information about HSCT and osteopetrosis despite the age at the time of diagnosis because the evolution has been favorable. Therefore, we emphasize the importance of clinical suspicion for timely diagnosis and treatment. As no documented evidence exists in our country, it is imperative to generate this kind of reports due to the remarkable improvement that a timely treatment means in patients' quality of life and survival.

There is only one previous report in our country, documenting a 9-month-old patient who required a second transplant at 13 months of age, using the umbilical cord as a cellular source on both occasions[19]. It is necessary to continue investigating and reporting these cases to enrich this knowledge.

In this case, we emphasize the importance of early diagnosis. Suspicion of the disease in a patient with pancytopenia, craniofacial and thoracic dysmorphia, hepatosplenomegaly, and radiological evidence of bone sclerosis should prompt a comprehensive approach and early osteopetrosis diagnosis. Therefore, timely interventions and early treatment with Allo-HSCT could be established to improve the patient's clinical conditions. It is essential to perform an Allo-HSCT, preferably before 1 year of age, to avoid progressive and irreversible neurological damage and decrease mortality. It has been shown that patients who have 100% compatible donor for Allo-HSCT have a 5-year survival of 73%. However, with an unrelated donor, survival of 40% has been reported.

Timely Allo-HSCT has an impact on the patient's prognosis and quality of life. It is possible to perform late Allo-HSCT in a patient with mild neurosensory deficits and achieve a cure for osteopetrosis, stop the progression of neurological damage, and achieve an adequate quality of life on the condition where a multidisciplinary treatment is available.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

None.

References

1. Wu CC, Econs MJ, DiMeglio LA, Insogna KL, Levine MA, Orchard PJ, et al. Diagnosis and management of osteopetrosis: consensus guidelines from the Osteopetrosis Working Group. J Clin Endocrinol Metab. 2017;102:3111-23.
2. El-Sobky TA, Elsobky E, Sadek I, Elsayed SM, Khattab MF. A case of infantile osteopetrosis: the radioclinical features with literature update. Bone Rep. 2015;4:11-6.
3. Srinivasan M, Abinun M, Cant AJ, Tan K, Oakhill A, Steward CG. Malignant infantile osteopetrosis presenting with neonatal hypocalcaemia. Arch Dis Child Fetal Neonatal Ed. 2000;83:F21-3.
4. Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, et al. Loss of the chloride channel ClC-7 leads to lysosomal storage disease and neurodegeneration. EMBO J. 2005;24:1079-91.
5. Tolar J, Teitelbaum SL, Orchard PJ. Osteopetrosis. N Engl J Med. 2004;351:2839-49.
6. Stark Z, Savarirayan R. Osteopetrosis. Orphanet J Rare Dis. 2009;4:5.
7. Penna S, Capo V, Palagano E, Sobacchi C, Villa A. One disease, many genes: implications for the treatment of osteopetroses. Front Endocrinol (Lausanne). 2019;10:85.
8. Waguespack SG, Hui SL, White KE, Buckwalter KA, Econs MJ. Measurement of tartrate-resistant acid phosphatase and the brain isoenzyme of creatine kinase accurately diagnoses type II autosomal dominant osteopetrosis but does not identify gene carriers. J Clin Endocrinol Metab. 2002;87:2212-7.
9. Kornak U, Schulz A, Friedrich W, Uhlhaas S, Kremens B, Voit T, et al. Mutations in the a3 subunit of the vacuolar H(+)-ATPase cause infantile malignant osteopetrosis. Hum Mol Genet. 2000;9:2059-63.
10. Sobacchi C, Frattini A, Orchard P, Porras O, Tezcan I, Andolina M, et al. The mutational spectrum of human malignant autosomal recessive osteopetrosis. Hum Mol Genet. 2001;10:1767-73.
11. Steward CG, Pellier I, Mahajan A, Ashworth MT, Stuart AG, Fasth A, et al. Severe pulmonary hypertension: a frequent complication of stem cell transplantation for malignant infantile osteopetrosis. Br J Haematol. 2004;124:63-71.
12. Steward CG. Neurological aspects of osteopetrosis. Neuropathol Appl Neurobiol. 2003;29:87-97.
13. Maranda B, Chabot G, Décarie JC, Pata M, Azeddine B, Moreau A, et al. Clinical and cellular manifestations of OSTM1-related infantile osteopetrosis. J Bone Miner Res. 2008;23:296-300.
14. Stepensky P, Grisariu S, Avni B, Zaidman I, Shadur B, Elpeleg O, et al. Stem cell transplantation for osteopetrosis in patients beyond the age of 5 years. Blood Adv. 2019;3:862-8.
15. Driessen GJ, Gerritsen EJ, Fischer A, Fasth A, Hop WC, Veys P, et al. Long-term outcome of haematopoietic stem cell transplantation in autosomal recessive osteopetrosis: an EBMT report. Bone Marrow Transplant. 2003;32:657-63.
16. Orchard PJ, Fasth AL, Le Rademacher J, He W, Boelens JJ, Horwitz EM, et al. Hematopoietic stem cell transplantation for infantile osteopetrosis. Blood. 2015;126:270-6.
17. Ramírez P, Brunstein CG, Miller B, Defor T, Weisdorf D. Delayed platelet recovery after allogeneic transplantation: a predictor of increased treatment-related mortality and poorer survival. Bone Marrow Transplant. 2011;46:981-6.
18. Cheow HK, Steward CG, Grier DJ. Imaging of malignant infantile osteopetrosis before and after bone marrow transplantation. Pediatr Radiol. 2001;31:869-75.
19. González LL, Jaime PJ, Cantú RO, Mancias GC, Gutiérrez AH, Herrera GJ, et al. Allogeneic hematopoietic stem cell transplantation using a reduced-intensity conditioning regimen in infants: experience at a single institution in Mexico. Pediatr Hematol Oncol. 2008;25:39-47.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

CLINICAL CASE

Clinical, radiological, and molecular diagnosis of progressive fibrodysplasia ossificans

*Vianey Ordóñez-Labastida[1], Alan Cárdenas-Conejo[1], Juan C. Huicochea-Montiel[1], Guadalupe E. Paredez-Rivera[2], Alberto Hidalgo-Bravo[3], Lucero M.J. Monterde-Cruz[4], and María A. Aráujo-Solís[1]**

[1]Departamento de Genética, Unidad Médica de Alta Especialidad Hospital de Pediatría Dr. Silvestre Frenk Freund, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS); [2]Servicio de Genética, Unidad Médica de Alta Especialidad Hospital de Oncología, Centro Médico Nacional Siglo XXI, IMSS; [3]Servicio de Genética y Genómica, Instituto Nacional de Rehabilitación; [4]Fundación Teletón México. Mexico City, Mexico

Abstract

Background: *Progressive fibrodysplasia ossificans is a rare genetic disease with heterozygous mutations (autosomal dominant inheritance) in the ACVR1 gene, which causes progressive heterotopic ossification in muscles, tendons, and ligaments, usually secondary to trauma. The ossification foci generate pain, joint ankyloses, and restricted movement. Congenital shortening and medial deviation first metatarsal of the foot is a distinctive feature. This report aimed to present an educational value case of a patient with clinical, imaging, and molecular diagnosis of progressive fibrodysplasia ossificans, recognized as a rare condition that severely affects the quality of life.* **Case report:** *We present the case of a 6-year-old female patient with lumps in the right scapular and dorsal region, progressive joint rigidity, and short first metatarsal medially deviated since birth. By imaging studies, we established the diagnosis of progressive fibrodysplasia ossificans. Sanger sequencing of ACVR1 reported c.617G>A (p.Arg206His).* **Conclusions:** *Confirmation of the diagnosis allowed genetic counseling, including a comprehensive explanation of the disease's natural history and measures to prevent its rapid progression.*

Key words: *Myositis ossificans. Diagnostic. Genetics. Genetic counseling.*

Diagnóstico clínico, radiológico y molecular de fibrodisplasia osificante progresiva

Resumen

Introducción: *La fibrodisplasia osificante progresiva es una enfermedad genética poco frecuente, causada por variantes patogénicas en estado heterocigoto (herencia autosómica dominante) en el gen ACVR1, que provoca osificación heterotópica progresiva en músculos, tendones y ligamentos, comúnmente secundaria a traumatismos. Los focos de osificación generan dolor, anquilosis articular y restricción del movimiento. Es característico el acortamiento congénito y la desviación medial del primer metatarsiano del pie. El objetivo de este reporte es presentar un caso de alto valor educativo de una paciente con diagnóstico clínico, imagenológico y molecular de fibrodisplasia osificante progresiva, reconocida como una condición infrecuente y que afecta de manera grave la calidad de vida.* **Caso clínico:** *Paciente de sexo femenino con tumoraciones induradas en la región dorsal y escapular, detectadas a los 6 años de vida. Cursaba además con rigidez articular*

Correspondence:
*María A. Aráujo-Solís
E-mail: tonyarasol@yahoo.com.mx

Date of reception: 02-05-2020
Date of acceptance: 21-07-2020
DOI: 10.24875/BMHIM.20000119

Available online: 03-05-2021
Bol Med Hosp Infant Mex. 2021;78(3):234-238
www.bmhim.com

progresiva y primer metatarsiano del pie acortado y con desviación en sentido medial desde el nacimiento. Por estudios de imagen se estableció el diagnóstico de fibrodisplasia osificante progresiva. Por secuenciación Sanger se reportó c.617G>A (p.Arg206His) en ACVR1. **Conclusiones:** *La confirmación del diagnóstico permitió ofrecer un asesoramiento genético integral, incluyendo una amplia explicación de la evolución natural del padecimiento y de las medidas preventivas para disminuir su rápida progresión.*

Palabras clave: *Miositis osificante. Diagnóstico. Genética. Asesoramiento genético.*

Introduction

The prevalence of fibrodysplasia ossificans progresiva (FOP) is 0.6 to 1.36 cases per million people worldwide, with no predisposition by ethnicity or sex[1]. It is caused by pathogenic variants in the heterozygous state (autosomal dominant inheritance) in the *ACVR1* gene (located in 2q24.1) that encodes the activin A receptor type I (ACVR1)[2]. The c.617G>A (p.Arg206His) mutation has been detected in 95% of the cases (classic form)[3].

The soft tissue (muscles, tendons, and ligaments) is continuously regenerated in healthy individuals on a daily basis and following trauma[4]. At the cellular level, this happens through various members of the transforming growth factor-beta family (TGF-β). TGF-β family members are classified as osteogenic such as bone morphogenic proteins (BMPs) and non-osteogenic such as activin A. In turn, activin A intervenes in the differentiation of interstitial myogenic and mesenchymal cells during muscle fiber regeneration[5].

In the classic FOP, a gain of function in the ACVR1 receptor produces the abnormal overactivation of the intracellular signaling pathway by non-osteogenic ligands, causing the transformation of soft tissue precursor cells into chondrocytes and osteoblasts, which are then responsible for heterotopic endochondral bone formation[4,6].

Heterotopic ossification mainly affects the axial, dorsal, and cranial skeleton. This process starts from the central part with progression towards the ventral, appendicular, and caudal regions[7,8]. Trauma, surgeries, and intramuscular injections damage the soft tissue and create inflammatory foci, which subsequently undergo heterotopic ossification, causing stiffness and limited movement of adjacent joints[9].

In patients with the classic form of FOP, shortening and medial deviation or monophalangism of the first metatarsal or both are present from birth[10]. Cervical vertebrae fusion (from C2 to C7), high and narrow vertebral bodies, short and wide femoral necks may also be present, as well as osteochondromas in the medial part of the tibia[11]. These patients frequently present thoracic insufficiency syndrome, which predisposes to frequent pneumonia episodes that are usually an important cause of mortality[8]. Also, they can show conductive hearing loss due to the middle ear's ossification, while sensorineural hearing loss is also reported due to the affection of the auditory nerve or the cochlea[7]. In the presence of clinical and radiographic suspicion of this disease, the diagnosis is confirmed by molecular studies in search of pathogenic variants in *ACVR1*[7,9].

This report aims to describe a patient with clinical, radiological, and molecular FOP diagnosis. Awareness of this pathology will allow timely identification of patients with this condition, thus, avoiding unnecessary interventions that worsen their already precarious quality of life.

Clinical case

We report the case of a 10-year-old female patient product of the second pregnancy of healthy non-consanguineous parents and with two apparently healthy siblings. At birth, the patient was hospitalized for 5 days for low birth weight (1700 g) and height (42 cm). Subsequently, the patient showed normal development during infancy and childhood, with no other relevant antecedents. At 6 years of age, two indurated tumors were identified: one of 3.5 cm in the right scapular region and another of 5.5 cm at the dorsal vertebrae level. The patient underwent an orthopedic evaluation, and the biopsy of these tumors concluded myofibromatosis. After performing the diagnostic procedure, the tumors increased in size. The patient also presented joint stiffness of the upper extremities, limitation for rotation and flexion of the neck, and severe scoliosis. The Rheumatology department ruled out inflammatory pathological conditions. The patient underwent reevaluation by the Medical Genetics department. Physical examination indicated that the patient's weight was found on the 10th percentile and height and head circumference on the 50th percentile. Also, the patient showed reduced neck mobility (rotation and flexion), shoulder, elbow, and wrist joints

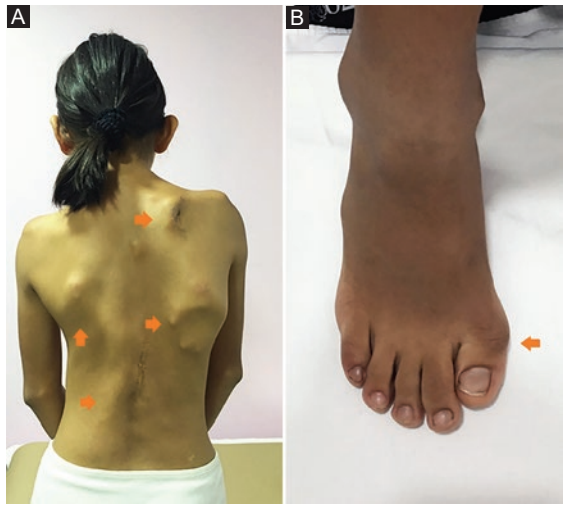

Figure 1. A. Back of the patient with ossification foci on scapulas, dorsal vertebrae (scars from previous surgical procedures), and body asymmetry by scoliosis. **B.** Shortening of the first toe with medial deviation.

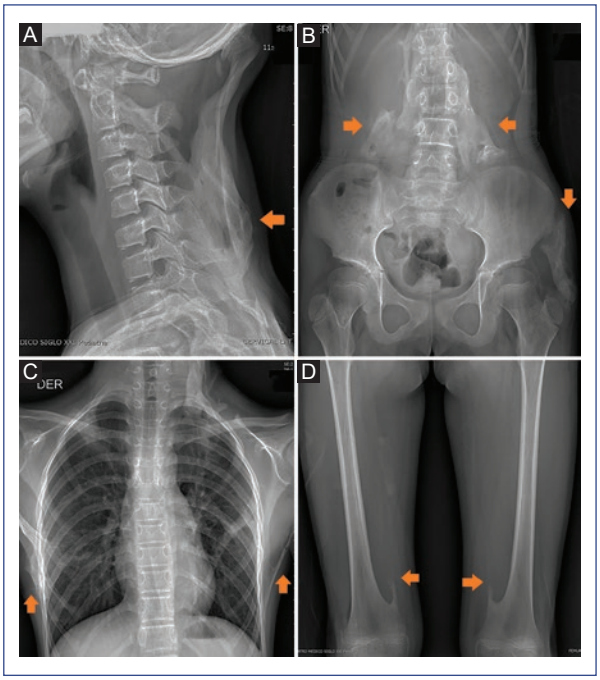

Figure 2. X-rays of the patient. **A.** Heterotopic ossification in the left cervical paravertebral region **B.** Heterotopic ossification in the lumbar paravertebral level bilaterally and the left coxofemoral joint. **C.** Heterotopic ossification in scapular and infraaxillary region **D.** Bone protrusions in femoral metaphyses due to heterotopic ossification of the iliotibial ligament.

stiffness, body asymmetry secondary to scoliosis, indurated tumors in the scapular and infrascapular areas (sites where biopsies had been performed), and a right infra-axillary tumor (Figure 1A). Short first toes with medial deviation were observed and present since birth according to the mother (Figure 1B). The anteroposterior feet X-ray showed bilateral fusion of the fourth and fifth metatarsals' proximal portion and shortening of the phalanges of the first toe with medial deviation. Dorsal, lumbar, and long bone X-rays showed soft tissue calcifications in several regions: the left cervical paravertebral region with an extension towards the left pectoral region (Figures 2A and 2C), the bilateral lumbar paravertebral level (Figure 2B), the left hip joint and femoral metaphyses bony projections (Figures 2B and 2D).

Computed tomography of the spine reported scoliosis, heterogeneous, poorly defined, multiple images with calcium depositions in the spine (bilaterally), trapezius, and back muscles (Figure 3).

The diagnosis of fibrodysplasia ossificans progressiva was established based on the radiological findings and physical examination. Subsequently, molecular studies were carried out at the Instituto Nacional de Rehabilitación (National Institute of Rehabilitation). Sanger sequencing of *ACVR1* reported the pathogenic variant in heterozygous state c.617G>A (p.Arg206His) in exon 6. Upon diagnosis confirmation, preventive measures were indicated, such as the use of protective cushions, administration of intramuscular injections only of vital importance, and not allowing surgical removal of the tumors.

The patient's last evaluation showed limitation of arm elevation above the 180° plane, difficulty in dressing and writing, and pain when remaining in a sitting or standing position for long periods. An audiological evaluation reported probable ossification of the middle ear ossicles. An MRI of the ear was indicated but has not been performed to date. The patient continues to be monitored by the Orthopedic and Rehabilitation services to treat scoliosis and for physical therapy and pain management with non-steroidal anti-inflammatory drugs, which, so far, have been sufficient to reduce the discomfort caused by tumors and joint stiffness.

Discussion

Based on clinical and radiological features and the molecular results of the *ACVR1* sequencing, the classic FOP form with the most frequently reported

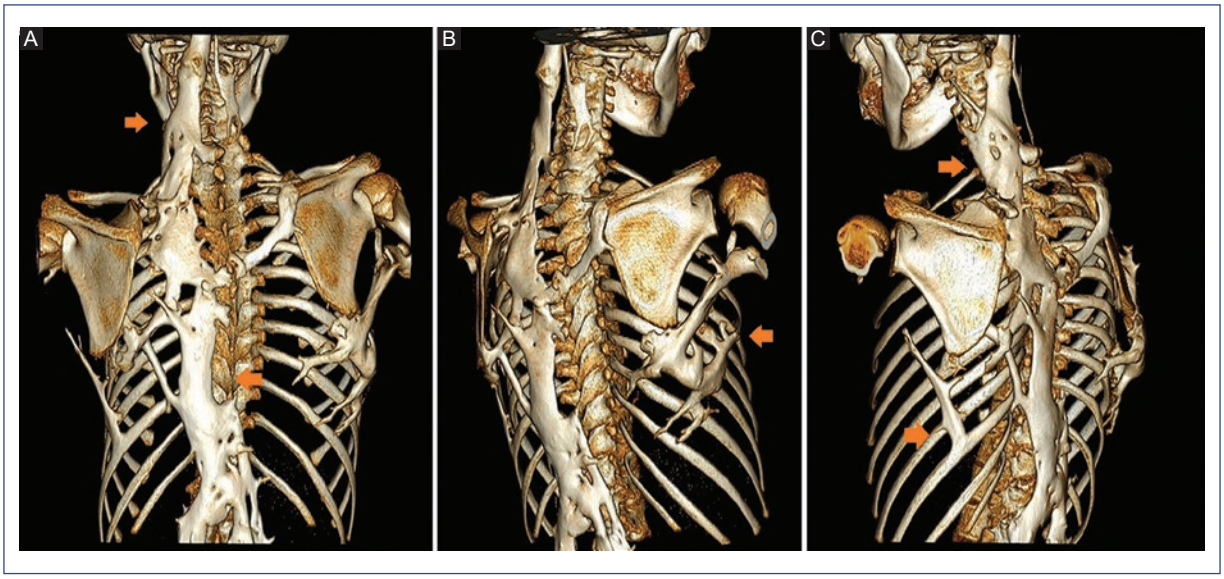

Figure 3. Tomography with a 3D reconstruction of the patient where heterotopic ossification is observed in posterior soft tissues, extending from the skull base to the lumbar region **(A)**, involving paravertebral and infrascapular muscles **(B)**. Fusion is observed along the tenth left posterior costal arch and dorsal kyphoscoliosis **(C)**.

pathogenic variant worldwide was diagnosed. The evolution in the present case is similar to previous reports in patients with the same variant, with heterotopic ossification foci appearing in the back, neck, and scapulas between one and 15 years of age, bilateral shortening with a medial deviation of the first toe since birth (virtually pathognomonic) and progression of the disease with the appearance of more ossification foci, and ankylosis limiting movement[12-14]. This mutation leads to the abnormal function of *ACVR1*, resulting in increased activation of the BMP pathway (osteogenic ligand) when exposed to the inflammatory stimulus secondary to trauma, giving rise to heterotopic ossification foci. FOP timely diagnosis allows avoiding unnecessary procedures that aggravate the clinical presentation. Due to late diagnosis, most patients have already undergone invasive studies that aggravate their condition. As this disease is highly disabling, it must be suspected in the presence of heterotopic ossification foci. Shortening of the first toe identification provides an excellent opportunity for clinical suspicion from the first years of life, allowing the differential diagnosis with entities that also present heterotopic ossifications, such as disorders of *GNAS* inactivation and Klippel Feil syndrome[15,16].

Once the FOP diagnosis has been established, the following preventive measures can improve the quality of life: restriction of physical activities to avoid falls or other traumas (without being strict, since the functional state of the joints must be maintained); physical rehabilitation, which should be focused on exercises that avoid passive movement—that can result in tissue inflammation leading to ossification—and exercises that help patients perform their daily activities. Intramuscular injections should be avoided as much as possible (subcutaneous injections and venipuncture do not represent the same risk). Dental care is also important; however, it is necessary to avoid keeping the mouth open for prolonged periods to prevent joint ankylosis. Removal of the tumors should be avoided, as it will cause a recurrence of heterotopic ossification foci and, thus, disease progression[17].

Interdisciplinary treatment by Pneumology (for evaluating lung function), Physical Medicine and Rehabilitation, Orthopedics, and Audiology services is important[17]. Due to the autosomal dominant inheritance pattern of this disease, the offspring's risk for an affected individual is 50% per gestational event regardless of the sex of the product. Although family cases are very infrequent, comprehensive genetic counseling should be offered, including planning and preventing complications in the pre-pregnancy stage since this kind of pregnancy is classified as a high obstetric risk. The patient should be advised on the offspring's risk of developing the disease and the obstetric risks (thromboembolism due to prolonged immobilization, respiratory distress due to restrictive lung disease, delivery complications due to pelvic

muscles' ossification, prematurity, and fetal distress)[17]. Some studies on diagnosis during pregnancy have reported shortening and medial deviation of the first metatarsal on structural ultrasound, leading to suspicion of this disease[18]. As no curative treatment currently exists, therapy is primarily directed at supportive measures such as those mentioned above. Non-steroidal anti-inflammatory drugs and corticosteroids are used to decrease excessive inflammation and control pain; some drugs such as bisphosphonates and leukotriene inhibitors are also used but with little efficacy[6]. Currently, therapies aimed at slowing the progression and preventing the formation of ossification foci are being developed[6]. In phase 2 clinical study, promising results have been shown: evidence showed a decrease in the size of heterotopic ossification foci and arrest of disease progression using a selective retinoic acid receptor gamma agonist (palovarotene)[19,20].

Manifestations of this condition are incapacitating and seriously affect the quality of life, for which a timely diagnosis is imperative to prevent situations that trigger ossification foci. In this case, comprehensive genetic counseling was provided, including a broad explanation of the condition's natural history. The patient and the family were made aware of the risks of recurrence for the parents of the index case (less than 1% because it is a *de novo* variant) and for the patient's offspring (50%). The patient started an interdisciplinary treatment that has been of great benefit to the patient.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

Conflicts of interest

The authors declare no conflict of interest.

Funding

None.

Acknowledgments

We thank the family of the patient and all those who collaborated in the preparation of this work.

References

1. Baujat G, Choquet R, Bouée S, Jeanbat V, Courouve L, Ruel A, et al. Prevalence of fibrodysplasia ossificans progressiva (FOP) in France: an estimate based on a record linkage of two national databases. Orphanet J Rare Dis. 2017;12:123-32.
2. Kaplan FS, Xu M, Seemann P, Connor JM, Glaser DL, Carroll L, et al. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. Hum Mutat. 2009;30:379-90.
3. Valer JA, Sánchez-de-Diego C, Pimenta-Lopes C, Rosa JL, Ventura F. ACVR1 function in health and disease. Cells. 2019;8:1366-92.
4. Katagiri T, Tsukamoto S, Kuratani M. Heterotopic bone induction via BMP signaling: potential therapeutic targets for fibrodysplasia ossificans progressiva. Bone. 2018;109:241-50.
5. Alessi-Wolken DM, Idone V, Hatsell SJ, Yu PB, Economides AN. The obligatory role of activin A in the formation of heterotopic bone in fibrodysplasia ossificans progressiva. Bone. 2018;109:210-7.
6. Wentworth KL, Masharani U, Hsiao EC. Therapeutic advances for blocking heterotopic ossification in fibrodysplasia ossificans progressiva. Br J Clin Pharmacol. 2019;85:1180-7.
7. Pignolo RJ, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva: clinical and genetic aspects. Orphanet J Rare Dis. 2011;6:1-6.
8. Pignolo RJ, Kaplan FS. Clinical staging of fibrodysplasia ossificans progressiva (FOP). Bone. 2018;109:111-4.
9. Pignolo RJ, Bedford-Gay C, Liljesthrom M, Durbin-Johnson BP, Shore EM, Rocke DM, et al. The natural history of flare-ups in fibrodysplasia ossificans progressiva (FOP): a comprehensive global assessment. J Bone Miner Res. 2016;31:650-6.
10. Barnett CP, Dugar M, Haan EA. Late-onset variant fibrodysplasia ossificans progressiva leading to misdiagnosis of ankylosing spondylitis. Am J Med Genet A. 2011;155A:1492-5.
11. Bauer AH, Bonham J, Gutierrez L, Hsiao EC, Motamedi D. Fibrodysplasia ossificans progressiva: a current review of imaging findings. Skeletal Radiol. 2018;47:1043-50.
12. Hüning I, Gillessen-Kaesbach G. Fibrodysplasia ossificans progressiva: clinical course, genetic mutations and genotype-phenotype correlation. Mol Syndromol. 2014;5:201-11.
13. Zhang JM, Li CF, Ke SY, Piao YR, Han TX, Kuang WY, et al. Analysis of clinical manifestations and treatment in 26 children with fibrodysplasia ossificans progressiva in China. World J Pediatr. 2020;16:82-8.
14. Pignolo RJ, Baujat G, Brown MA, De Cunto C, DiRocco M, Hsiao EC, et al. Natural history of fibrodysplasia ossificans progressiva: a cross-sectional analysis of annotated baseline phenotypes. Orphanet J Rare Dis. 2019;23;14:1-11.
15. Bastepe M. GNAS mutations and heterotopic ossification. Bone. 2018;109:80-5.
16. Frikha R. Klippel-Feil syndrome: a review of the literature. Clin Dysmorphol. 2020;29:35-7.
17. Kaplan FS, Al Mukaddam M, Baujat G, Brown M, Cali A, Cho T-J, et al. The medical management of fibrodysplasia ossificans progressiva: current treatment considerations. Proc Intl Clin Council FOP. 2019;1:1-111.
18. Maftei C, Rypens F, Thiffault I, Dubé J, Laberge AM, Lemyre E. Fibrodysplasia ossificans progressiva: bilateral hallux valgus on ultrasound a clue for the first prenatal diagnosis for this condition—clinical report and review of the literature. Prenat Diagn. 2015;35:305-7
19. Lees-Shepard JB, Nicholas SE, Stoessel SJ, Devarakonda PM, Schneider MJ, Yamamoto M, et al. Palovarotene reduces heterotopic ossification in juvenile FOP mice but exhibits pronounced skeletal toxicity. Elife. 2018;18;7:1-20.
20. ClinicalTrials.gov. Clementia Pharmaceutical. An efficacy and safety study of palovarotene to treat preosseus flare-ups in FOP subjects. USA, 2020. https://clinicaltrials.gov/ct2/show/NCT02190747.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

CASO CLÍNICO

Disfunción del transportador del surfactante pulmonar ABCA3: reporte de un caso peruano

*Héctor Nuñez-Paucar[1], Carlos Valera-Moreno[1], Mariela K. Zamudio-Aquise[1], Roxana Lipa-Chancolla[2], Fabio Pérez-Garfias[1], Ana G. Moncada-Arias[3] y Noé Atamari-Anahui[1,4]**

[1]Instituto Nacional de Salud del Niño-Breña, Lima; [2]Instituto Nacional de Salud del Niño-San Borja, Lima; [3]Escuela Profesional de Medicina Humana, Facultad de Ciencias de la Salud, Universidad Nacional de San Antonio Abad del Cusco, Cusco; [4]Universidad San Ignacio de Loyola, Vicerrectorado de Investigación, Unidad de Investigación para la Generación y Síntesis de Evidencias en Salud, Lima. Perú

Resumen

Introducción: *Los trastornos genéticos que afectan la homeostasis del surfactante pulmonar son una causa importante del síndrome de dificultad respiratoria en el recién nacido a término y de enfermedad pulmonar intersticial difusa en niños. El transportador ABCA3 (ATP binding cassette A3) interviene en la producción normal del surfactante que recubre el interior de las paredes alveolares y funciona como agente tensioactivo.* **Caso clínico:** *Recién nacido a término que presentó dificultad respiratoria a los 3 días de vida y requirió ventilación mecánica. Los estudios para determinar otras causas de enfermedad pulmonar fueron negativos. Se realizó una biopsia de pulmón para realizar estudios de microscopía óptica y microscopía electrónica. Esta última mostró pequeños cuerpos lamelares anómalos, además de condensaciones electrodensas periféricas, características de las mutaciones del transportador ABCA3. Se inició tratamiento con pulsos de metilprednisolona, hidroxicloroquina, azitromicina y corticoides inhalados a dosis altas, y la respuesta clínica y radiológica fue favorable durante el seguimiento.* **Conclusiones:** *La correlación de las características clínicas y de las imágenes (tomografía y microscopía electrónica) puede ser útil para el diagnóstico de la disfunción del surfactante pulmonar, especialmente en los países de bajos y medianos recursos que no disponen de estudios genéticos para determinar las diferentes mutaciones del transportador ABCA3. Este es uno de los primeros casos reportados en Perú con respuesta adecuada al tratamiento y evolución favorable durante el seguimiento.*

Palabras clave: *Surfactante pulmonar. Transportador ABCA3. Enfermedades pulmonares. Niño. Recién nacido.*

Surfactant ABCA3 transporter dysfunction: a case report from Peru

Abstract

Background: *Genetic disorders affecting pulmonary surfactant homeostasis are a major cause of respiratory distress syndrome in full-term newborn and childhood interstitial lung disease. The ABCA3 transporter (ATP binding cassette A3) intervenes in the normal production of surfactant that covers the interior of alveolar walls and plays a fundamental role as a surfactant.* **Case report:** *Male term newborn who presented respiratory distress 3 days after birth and required mechanical ventilation. Studies to determine other causes of lung disease were negative. Lung biopsy was performed for the study with*

Correspondencia:
*Noé Atamari-Anahui
E-mail: noe.atamari@gmail.com

Fecha de recepción: 06-05-2020
Fecha de aceptación: 03-07-2020
DOI: 10.24875/BMHIM.20000123

Disponible en internet: 12-01-2021
Bol Med Hosp Infant Mex. 2021;78(3):239-244
www.bmhim.com

light microscopy and electron microscopy. Electron microscopy showed small abnormal lamellar bodies in addition to peripheral electrodense condensations characteristic of ABCA3 transporter mutation. Treatment was started with pulses of methylprednisolone, hydroxychloroquine, azithromycin, and high-dose inhaled corticosteroids, finding a favorable clinical and radiological response to follow-up. **Conclusions:** *Correlation of clinical characteristics and images (tomography and electron microscopy) can be useful for the diagnosis of lung surfactant dysfunction, especially in low and medium-income countries where genetic studies to determine the different ABCA3 transporter mutations are not available. This is one of the first cases reported in Peru with an adequate response to treatment and favorable evolution to follow-up.*

Key words: *Pulmonary surfactants. ATP binding cassette A3. Lung diseases. Child. Newborn.*

Introducción

El surfactante es un agente tensioactivo secretado por los neumocitos de tipo II presentes en los alvéolos. Está conformado por un complejo lipoproteico, cuya función fundamental es reducir la tensión superficial de las paredes alveolares, evitando el colapso de los alvéolos al final de la espiración[1]. Existen trastornos genéticos que alteran el metabolismo del surfactante; aunque son poco frecuentes, pueden causar una morbimortalidad significativa en la población neonatal y pediátrica[2,3].

El transportador ABCA3 *(ATP binding cassette A3)* es una proteína transmembrana encontrada en los cuerpos lamelares de los neumocitos de tipo II, cuya función es el transporte, desde el citosol hacia el interior de los cuerpos lamelares, de las proteínas y los fosfolípidos que conforman el surfactante[4,5]. Las mutaciones genéticas que afectan la expresión del transportador ABCA3, la síntesis de las proteínas surfactantes B y C, y las mutaciones del gen NKX2-1 originan la enfermedad pulmonar intersticial de gravedad variable en el recién nacido. Debido a la superposición de signos clínicos y radiológicos, es necesario plantearse diagnósticos diferenciales[1,4,6].

Aunque se desconoce la verdadera incidencia, las mutaciones del gen que codifica el transportador ABCA3 son la causa más frecuente de disfunción del surfactante[7,8]. Se ha estimado una frecuencia de presentación de 1/3100 a 1/18,000 en individuos de diferentes grupos étnicos[9].

La prueba diagnóstica definitiva para los errores innatos del metabolismo del surfactante es el estudio genético de las mutaciones específicas de estos genes[4,10]. Sin embargo, las características clínicas y radiológicas de la enfermedad, asociadas con los hallazgos de la biopsia pulmonar en la microscopía óptica, la identificación de las proteínas del surfactante por inmunohistoquímica y el estudio ultraestructural de los cuerpos lamelares de los neumocitos de tipo II mediante microscopía electrónica son de gran ayuda para el diagnóstico de estas disfunciones del surfactante, en especial en países con escasos recursos, donde no se dispone de estudios genéticos para identificar las mutaciones causantes[11].

Debido a la infrecuente presentación de los errores innatos del metabolismo del surfactante y el mal pronóstico que, en general, acompaña a esta patología, el objetivo del presente caso fue reportar una adecuada respuesta terapéutica de un recién nacido a término con cuadro clínico, imágenes y estudio de microscopía óptica y electrónica compatibles con una mutación del transportador ABCA3.

Caso clínico

Paciente de sexo masculino, recién nacido a término por parto eutócico con 39 semanas de edad gestacional (primera gestación), con peso al nacer de 2480 g y Apgar 8[1]-9[5], sin complicaciones durante el parto. A los 3 días de nacido presentó dificultad respiratoria marcada, por lo cual fue intubado y conectado a un equipo de ventilación mecánica; además, se le administró nutrición parenteral durante 2 semanas. Posteriormente dejó la ventilación mecánica y se observó taquipnea persistente, incremento progresivo del esfuerzo respiratorio, hipoxemia persistente y dependencia de oxígeno para mantener una saturación adecuada. Debido a la falta de mejoría clínica, el paciente fue transferido al Instituto Nacional de Salud del Niño de Breña (Lima, Perú) a los 24 días de nacido. No tenía antecedentes familiares de enfermedades pulmonares o congénitas, y tampoco se reportaron intercurrencias durante la gestación.

A su ingreso al servicio de emergencia se le encontró taquipneico, con frecuencia respiratoria de 90 respiraciones por minuto, saturación de oxígeno al 95% y oxígeno suplementario con fracción inspirada de oxígeno al 50%. En la exploración física se le encontró despierto, reactivo a estímulos, con tórax excavado y politirajes. En la auscultación pulmonar se encontraron subcrépitos difusos en ambos hemitórax. El resto de la exploración fue normal.

En los exámenes de laboratorio se encontró en rango normal el título de anticuerpos IgM-IgG para perfil TORCH (toxoplasmosis, rubeola, citomegalovirus, herpes simple), adenovirus y *Mycoplasma pneumoniae*. La serología para el virus de inmunodeficiencia humana fue negativa. Los hemocultivos seriados fueron negativos. La inmunofluorescencia para virus sincitial respiratorio, adenovirus, influenza tipo A y B y parainfluenza tipo 1, 2 y 3 fue negativa. El dosaje de inmunoglobulinas séricas totales fue normal para IgM, IgG, IgA e IgE. La citometría de flujo con recuento y fenotipo de linfocitos T, linfocitos B y linfocitos NK estaba en rango normal para la edad. También se realizaron fondo de ojo y ecocardiografía, con resultados normales.

La radiografía de tórax y la tomografía espiral multicorte de tórax mostraron compromiso intersticial difuso bilateral con patrón de vidrio esmerilado (Fig. 1).

Debido a la hipoxemia y la taquipnea persistente, y ante la sospecha de enfermedad pulmonar intersticial difusa de la infancia, el paciente fue programado para una biopsia de pulmón a cielo abierto. La microscopía óptica de la biopsia de pulmón mostró engrosamiento de los septos alveolares, constituido fundamentalmente por hiperplasia de los neumocitos de tipo II, y la presencia de macrófagos espumosos PAS (ácido peryódico de Schiff) positivos en el interior de la luz alveolar. La microscopía electrónica de la biopsia de pulmón mostró cuerpos lamelares pequeños y anómalos, con presencia de condensaciones electrondensas periféricas, muy características de la deficiencia del transportador ABCA3 (Fig. 2).

A los 3 meses de edad, con resultados de la biopsia de pulmón sugerente de disfunción del surfactante, se inició un tratamiento con pulsos de metilprednisolona (dosis de 30 mg/kg al día por 3 días consecutivos), que se repitió mensualmente durante 6 meses y luego fue espaciado con intervalos de 2 meses. Además de los pulsos de metilprednisolona se indicó tratamiento coadyuvante antiinflamatorio e inmunomodulador con hidroxicloroquina (dosis de 10 mg/kg al día fraccionados en dos tomas) y azitromicina (dosis de 10 mg/kg tres veces por semana). Adicionalmente se indicaron corticoides inhalados a dosis altas.

Como la evolución clínica-radiológica fue favorable, el paciente dejó el oxígeno luego del tercer ciclo de pulsos de metilprednisolona. El paciente no requirió atención en la unidad de cuidados intensivos y no presentó infecciones agregadas ni otro tipo de complicaciones, por lo que fue dado de alta. Toleró adecuadamente los pulsos de metilprednisolona, recibiendo seis ciclos mensuales seguidos de seis ciclos bimensuales de manera ambulatoria. Asimismo, toleró la hidroxicloroquina por 18 meses sin complicaciones. Se le realizó un fondo de ojo basal y luego controles periódicos, los cuales fueron normales. Actualmente el paciente se encuentra estable en tratamiento con azitromicina tres veces por semana y corticoides inhalados a dosis altas.

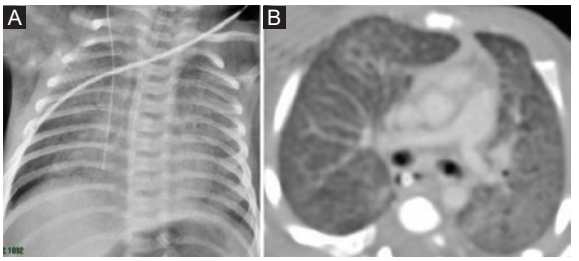

Figura 1. A: Radiografía de tórax en la que se observa un patrón intersticial moderado en ambos campos pulmonares. **B:** Tomografía de tórax en la que se observa compromiso intersticial difuso bilateral moderado con patrón en vidrio esmerilado y pequeñas imágenes quísticas intraparenquimales (1 mes de edad).

Durante el seguimiento, el paciente ingresó brevemente al hospital por una infección viral intercurrente sin complicaciones, a la edad de 1 año. Actualmente, a los 3 años de edad, se sigue periódicamente en el consultorio ambulatorio de neumología y su evolución clínica y radiológica es estacionaria (Fig. 3).

Discusión

Se presenta el caso de un recién nacido a término, con signos de dificultad respiratoria, cuyas manifestaciones clínicas y radiológicas fueron compatibles con enfermedad pulmonar intersticial difusa.

En los niños menores de 2 años, la causa más frecuente de enfermedad pulmonar intersticial difusa es la disfunción del surfactante o error innato del metabolismo del surfactante. La disfunción del surfactante se origina por mutaciones genéticas que afectan la síntesis de las proteínas precursoras del surfactante o su transporte[12,13]. La familia de transportadores ABC consta de más de 40 proteínas; la subfamilia ABCA está involucrada en el transporte de fosfolípidos y colesterol, dentro y entre las células. El ABCA3 es un transportador de fosfatidilcolina, el ABCA1 transporta colesterol y fosfolípidos, el ABCA4 transporta fosfatidiletanolamina, el ABCA7 transporta fosfatidilcolina

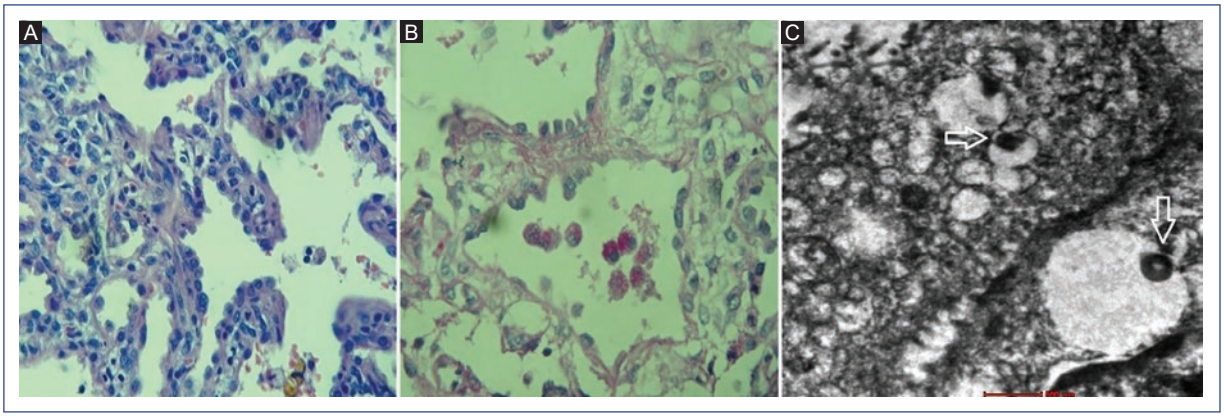

Figura 2. Microscopía óptica. **A:** Septos alveolares engrosados constituidos fundamentalmente por hiperplasia de neumocitos de tipo II y leve infiltrado inflamatorio linfohistiocitario. Ausencia de fibrosis intersticial (tinción de hematoxilina-eosina, × 200). **B:** Macrófagos espumosos PAS positivos en el interior de la luz alveolar (× 400). Microscopía electrónica. **C:** Cuerpos lamelares pequeños y anómalos con presencia de condensaciones electrondensas periféricas concéntricas (flechas) (tamaño de barra: 500 nm).

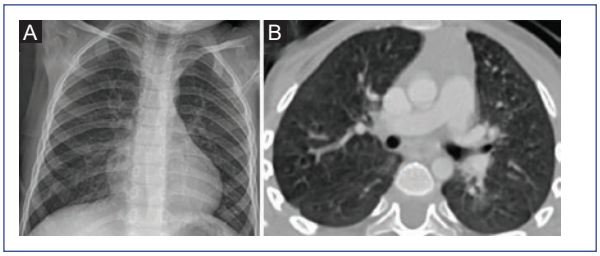

Figura 3. A: Radiografía de tórax en la que se observa un tenue patrón intersticial en ambos campos pulmonares. **B:** Tomografía de tórax en la que se observa un tenue patrón intersticial en vidrio esmerilado (3 años de edad).

y esfingomielina, pero no colesterol, y el ABCA12 participa en el flujo de lípidos. Por tanto, la familia de transportadores ABCA posee especificidad por determinados sustratos lipídicos. Al tener esta especificidad por sustratos lipídicos, los pacientes con mutaciones en el transportador ABCA3 presentarían una disminuida proporción de fosfatidilcolina en el surfactante pulmonar, además de ciertas características que se pueden identificar por microscopía electrónica, como los cuerpos lamelares anormales, lo que respalda el diagnóstico en el paciente[7,11].

El transportador ABCA3 desempeña un papel fundamental en la homeostasis del surfactante pulmonar, pues es el encargado de transportar las proteínas y los fosfolípidos precursores del surfactante desde el citosol del neumocito de tipo II hacia el interior de los cuerpos lamelares y a través de la membrana celular a la superficie alveolar. Las mutaciones del gen ABCA3 son la causa más frecuente de enfermedad pulmonar intersticial difusa secundaria a disfunción del surfactante. La gravedad y el pronóstico de la enfermedad dependen del tipo y la zigocidad de la mutación, de la expresión fenotípica y de factores asociados, por lo que la patología puede manifestarse por primera vez en el niño mayor o el adulto[4,5].

Se deben sospechar deficiencias genéticas de las proteínas surfactantes en todo recién nacido a término y en aquellos prematuros que no siguen un curso clínico normal, que presentan dificultad respiratoria progresiva inexplicable, hipoxemia y alteraciones radiológicas difusas, siempre que se hayan excluido causas comunes y de acuerdo con los protocolos internacionales sobre el manejo de la enfermedad pulmonar intersticial en niños. En el paciente de este caso se realizaron diferentes estudios que incluyeron exámenes de laboratorio, radiografía y tomografía de tórax, ecocardiografía y biopsia pulmonar. La biopsia pulmonar proporciona un diagnóstico histopatológico cuando el diagnóstico genético no es concluyente y la progresión de la enfermedad no da tiempo para estudios genéticos, o cuando no se dispone de estos[13].

En la tomografía se evidenció compromiso pulmonar intersticial con patrón en vidrio esmerilado y algunos quistes pequeños intraparenquimales, similar a lo reportado por Doan, et al.[14] en nueve niños con deficiencia en el transportador ABCA3. Debido a que estos hallazgos no son exclusivos de la deficiencia del transportador ABCA3, fue necesario realizar una biopsia

pulmonar para su análisis por microscopía óptica y electrónica; esta última para realizar el estudio ultraestructural de los cuerpos lamelares en el interior de los neumocitos de tipo II[15].

Cuando no se dispone de estudios genéticos, las características de las anormalidades de los cuerpos lamelares identificadas mediante microscopía electrónica permiten realizar una aproximación diagnóstica bastante cercana de la mutación del transportador ABCA3 como causa de la disfunción del surfactante[11]. En los casos de mutación del transportador ABCA3 son característicos los cuerpos lamelares pequeños con membranas de fosfolípidos densamente empaquetados y formaciones electrondensas periféricas (excéntricas), lo que la diferencia de otras disfunciones del surfactante[3,10].

Por otro lado, no fue posible realizar el estudio de fosfolípidos y proteínas del surfactante en el líquido del lavado broncoalveolar. Se sabe que los pacientes con mutaciones de ABCA3 presentan una proporción significativamente disminuida de fosfatidilcolina en el lavado broncoalveolar. Si se hubiera realizado este estudio, podría haber orientado el diagnóstico[7].

Se conoce que la producción de surfactante aumenta notablemente al final de la gestación y se acelera con los glucocorticoides. Los estudios en modelos animales han demostrado que los glucocorticoides regulan la expresión de la proteína ABCA3 presente en la membrana limitante de los cuerpos lamelares. Este mecanismo se debe a la unión del receptor del glucocorticoide a un sitio específico en la región promotora del gen ABCA3, denominada elemento de respuesta de glucocorticoide (GRE), que induce la activación transcripcional y la subsecuente expresión de la proteína ABCA3[15]. También se han descrito otros factores de transcripción que regulan la expresión del gen ABCA3 en el pulmón, como la proteína de unión a elementos reguladores de esteroles (SREBP) y el factor de transcripción tiroideo-1 (Nkx2.1)[16].

La eficacia terapéutica en pacientes con disfunción del transportador ABCA3 es variable. Ocasionalmente se observa una respuesta transitoria a la administración de surfactante exógeno[17]. No existen ensayos clínicos aleatorizados sobre el tratamiento de la enfermedad pulmonar intersticial difusa en niños; sin embargo, algunas instituciones con experiencia en el manejo de patologías pulmonares en niños recomiendan pulsos de metilprednisolona, prednisona y terapias complementarias con hidroxicloroquina y azitromicina[13]. Estos medicamentos han sido utilizados en diferentes reportes de casos con una respuesta clínica variable[14,17-20].

El paciente del presente caso recibió terapia combinada triple con metilprednisolona, hidroxicloroquina y azitromicina por un periodo prolongado, logrando una mejoría clínica significativa. En la actualidad, el paciente no requiere oxígeno suplementario y se mantiene estable. Sin embargo, el pronóstico a largo plazo es incierto y varía de acuerdo con el tipo de mutación[21]. En algunos centros europeos se realiza el trasplante combinado de corazón y pulmón en pacientes que no responden al tratamiento[2]. En Perú no se ha realizado trasplante pulmonar en niños, por lo que debe optarse por el tratamiento médico prolongado.

Los pacientes con esta afección presentan episodios hipóxicos intermitentes, nutrición subóptima y disminución de la estimulación del desarrollo psicomotor, y deben recibir apoyo ventilatorio cuando lo requieran, terapia respiratoria, soporte nutricional y terapia física[22].

La mutación del gen que codifica al transportador ABCA3 es una causa infrecuente de enfermedad pulmonar intersticial secundaria a disfunción del surfactante en recién nacidos y niños. La confirmación diagnóstica se consigue mediante estudios genéticos para determinar la presencia de mutaciones en el gen ABCA3. Sin embargo, a falta de estos estudios, la correlación de las características clínicas, radiológicas y tomográficas con los hallazgos en la microscopía óptica y electrónica de la biopsia de pulmón permiten realizar el diagnóstico presuntivo cercano de la mutación del transportador ABCA3.

Responsabilidades éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad de los datos. Los autores declaran que han seguido los protocolos de su centro de trabajo sobre la publicación de datos de pacientes.

Derecho a la privacidad y consentimiento informado. Los autores han obtenido el consentimiento informado de los pacientes o sujetos referidos en el artículo. Este documento obra en poder del autor de correspondencia.

Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

Financiamiento

Ninguno.

Bibliografía

1. Gower WA, Nogee LM. Surfactant dysfunction. Paediatr Respir Rev. 2011;12:223-9.
2. Dinwiddie R, Sharief N, Crawford O. Idiopathic interstitial pneumonitis in children: a national survey in the United Kingdom and Ireland. Pediatr Pulmonol. 2002;34:23-9.
3. Griese M. Chronic interstitial lung disease in children. Eur Respir Rev. 2018;27:170100.
4. Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med. 2004;350:1296-303.
5. Beers MF, Mulugeta S. The biology of the ABCA3 lipid transporter in lung health and disease. Cell Tissue Res. 2017;367:481-93.
6. Schindlbeck U, Wittmann T, Höppner S, Kinting S, Liebisch G, Hegermann J, et al. ABCA3 missense mutations causing surfactant dysfunction disorders have distinct cellular phenotypes. Hum Mutat. 2018;39:841-50.
7. Garmany TH, Moxley MA, White FV, Dean M, Hull WM, Whitsett JA, et al. Surfactant composition and function in patients with ABCA3 mutations. Pediatr Res. 2006;59:801-5.
8. Garmany TH, Wambach JA, Heins HB, Watkins-Torry JM, Wegner DJ, Bennet K, et al. Population and disease-based prevalence of the common mutations associated with surfactant deficiency. Pediatr Res. 2008;63:645-9.
9. Wambach JA, Wegner DJ, DePass K, Heins H, Druley TE, Mitra RD, et al. Single ABCA3 mutations increase risk for neonatal respiratory distress syndrome. Pediatrics. 2012;130:e1575-82.
10. Wert SE, Whitsett JA, Nogee LM. Genetic disorders of surfactant dysfunction. Pediatr Dev Pathol. 2009;12:253-74.
11. Comité Nacional de Neumonología, Sociedad Argentina de Pediatría. [Childhood interstitial lung disease in infancy. Classification, diagnosis and management]. Arch Argent Pediatr. 2019;117:S120-34.
12. Hamvas A. Evaluation and management of inherited disorders of surfactant metabolism. Chin Med J (Engl). 2010;123:2943-7.
13. Bush A, Cunningham S, De Blic J, Barbato A, Clement A, Epaud R, et al. European protocols for the diagnosis and initial treatment of interstitial lung disease in children. Thorax. 2015;70:1078-84.
14. Doan ML, Guillerman RP, Dishop MK, Nogee LM, Langston C, Mallory GB, et al. Clinical, radiological and pathological features of ABCA3 mutations in children. Thorax. 2008;63:366-73.
15. Yoshida I, Ban N, Inagaki N. Expression of ABCA3, a causative gene for fatal surfactant deficiency, is up-regulated by glucocorticoids in lung alveolar type II cells. Biochem Biophys Res Commun. 2004;323:547-55.
16. Besnard V, Xu Y, Whitsett JA. Sterol response element binding protein and thyroid transcription factor-1 (Nkx2.1) regulate Abca3 gene expression. Am J Physiol Lung Cell Mol Physiol. 2007;293:L1395-405.
17. Kröner C, Wittmann T, Reu S, Teusch V, Klemme M, Rauch D, et al. Lung disease caused by ABCA3 mutations. Thorax. 2017;72:213-20.
18. Akil N, Fischer AJ. Surfactant deficiency syndrome in an infant with a C-terminal frame shift in ABCA3: a case report. Pediatr Pulmonol. 2018;53:E12-4.
19. Mitsiakos G, Tsakalidis C, Karagianni P, Gialamprinou D, Chatziioannidis I, Papoulidis I, et al. A new ABCA3 gene mutation c.3445G>A (p.Asp1149Asn) as a causative agent of newborn lethal of newborn lethal respiratory distress syndrome. Medicina (Kaunas). 2019;55:389.
20. Williamson M, Wallis C. Ten-year follow up of hydroxychloroquine treatment for ABCA3 deficiency. Pediatr Pulmonol. 2014;49:299-301.
21. Peca D, Cutrera R, Masotti A, Boldrini R, Danhaive O. ABCA3, a key player in neonatal respiratory transition and genetic disorders of the surfactant system. Biochem Soc Trans. 2015;43:913-9.
22. Gupta A, Zheng SL. Genetic disorders of surfactant protein dysfunction: when to consider and how to investigate. Arch Dis Child. 2017;102:84-90.

Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

CASO CLÍNICO

Síndrome de Bannayan-Riley-Rubalcaba en pediatría

Beth Sainz de la Peña-Hernández[1] y Bertha E. Guillén-Palacios[2]*

[1]Departamento de Pediatría; [2]Departamento de Dermatología Pediátrica. Hospital de Especialidades Pediátricas, Tuxtla Gutiérrez, Chiapas, México

Resumen

Introducción: *El síndrome de Bannayan-Riley-Ruvalcaba (SBRR) forma parte de la enfermedad de PTEN tumor-hamartoma, que comprende los síndromes de Cowden, Proteus y similar a Proteus, los cuales presentan un espectro de lesiones cutáneas, mucosas, de mama, tiroides y tracto gastrointestinal, así como polipomatosis hereditaria autosómica dominante. El SBRR se caracteriza por macrocefalia, lipomatosis, hemangiomatosis, pólipos intestinales, lentiginosis genital y discapacidad intelectual. El diagnóstico clínico y de variantes patogénicas en el gen PTEN, detectables en el 60% de los afectados, brinda la oportunidad de un manejo adecuado y de asesoramiento genético.* **Caso clínico:** *Se reporta el caso de un paciente en edad escolar que fue enviado a valoración inicial a dermatología por presentar antecedente de macrocefalia al nacimiento, lentiginosis genital, retraso en el desarrollo psicomotor y posteriormente rectorragia secundaria a polipomatosis intestinal. Se le realizó el diagnóstico clínico y molecular de SBRR.* **Conclusiones:** *El SBRR es poco frecuente, lo que puede retrasar el diagnóstico para los pacientes y los familiares en riesgo, por lo que es importante conocer sus características clínicas en el paciente pediátrico para lograr un diagnóstico y un manejo oportunos.*

Palabras clave: *Síndrome Bannayan-Riley-Ruvalcaba. PTEN. Polipomatosis. Lentiginosis genital. México.*

Bannayan-Riley-Rubalcaba syndrome in pediatrics

Abstract

Background: *Bannayan-Riley-Ruvalcaba syndrome (BRRS) is part of the PTEN tumor-hamartoma disease, which includes the Cowden, Proteus and Proteus-like syndromes, which present a spectrum of skin, mucosal, breast, thyroid, and gastrointestinal tract lesions, as well as autosomal dominant hereditary polypomatosis. BRRS is characterized by macrocephaly, lipomatosis, hemangiomatosis, intestinal polyps, genital lentiginosis, and intellectual disability. Clinical diagnosis and diagnosis of pathogenic variants in the PTEN gene, detectable in 60% of those affected, provides the opportunity for appropriate management and genetic counseling.* **Case report:** *We report the case of a school-age patient who was sent to an initial dermatological evaluation for presenting a history of macrocephaly at birth, genital lentiginosis, delayed psychomotor development and later rectal bleeding secondary to intestinal polypomatosis. A clinical and molecular diagnosis of BRRS was carried out.* **Conclusions:** *BRRS is rare, which can delay the diagnosis for patients and relatives at risk, so it is important to know its clinical characteristics in pediatric patients to achieve a timely diagnosis and management.*

Key words: *Bannayan-Riley-Ruvalcaba syndrome. PTEN. Hamartomatous polyps. Genital lentiginosis. Mexico.*

Correspondencia:
*Bertha E. Guillén-Palacios
E-mail: guillenpa@hotmail.com

Fecha de recepción: 20-06-2020
Fecha de aceptación: 06-11-2020
DOI: 10.24875/BMHIM.20000172

Disponible en internet: 27-04-2021
Bol Med Hosp Infant Mex. 2021;78(3):245-250
www.bmhim.com

Introducción

En 1960, Riley y Smith[1] agruparon la tríada de macrocefalia, pseudopapiledema y hemangiomas. En 1971, Bannayan[2] describió el conjunto de macrocefalia, lipomatosis y hemangiomas, mientras que Ruvalcaba, en 1980, asoció macrocefalia, pólipos hamartomatosos intestinales, lentiginosis genital y discapacidad intelectual. Finalmente, en 1990, Cohen[4] sugirió que todas estas características podrían entrar en una única enfermedad denominada síndrome de Bannayan-Riley-Ruvalcaba (SBRR; OMIM158350). El SBRR se incluye entre los síndromes de PTEN y tumores hamartomatosos, en el cual se agrupan otros como los síndromes de Cowden y de Proteus[1-4].

En este reporte se presenta un caso de SBRR diagnosticado en un hospital de tercer nivel de atención de México, al mismo tiempo que se realiza una breve revisión de la literatura para familiarizar a los médicos generales y a los pediatras con esta enfermedad de baja prevalencia con predisposición a diversas neoplasias.

Caso clínico

Paciente de sexo masculino, producto de primera y única gesta, con ambos progenitores aparentemente sanos, no consanguíneos; se desconocen más datos del padre biológico. Control prenatal referido como adecuado; la madre cursó con infección de vía urinarias en el primer trimestre, y el resto del embarazo se reportó sin complicaciones. A falta de progresión del trabajo de parto, se realizó cesárea secundaria sin complicaciones al nacimiento. La somatometría neonatal indicó un peso de 3800 g. Al nacimiento se detectó macrocefalia, pero la madre no recuerda el perímetro cefálico. El binomio egresó sin eventualidades. Los hitos del desarrollo se refirieron con la presencia de bisílabos a los 9 meses, bipedestación a los 15 meses y control de esfínteres a los 4 años.

El paciente acudió por primera vez al Hospital de Especialidades Pediátricas a los 4 años de edad por presentar hipertrofia amigdalina de grado IV con amigdalitis crónica, por lo que fue programado por el servicio de otorrinolaringología para realización de adenoamigdalectomía en julio de 2012. A los 6 años, se detectó la presencia de máculas pigmentadas de color café en los genitales, por lo fue atendido por un médico en el Centro de Salud de Bochil, quien lo refirió al servicio de dermatología del Hospital de Especialidades Pediátricas, en Tuxtla.

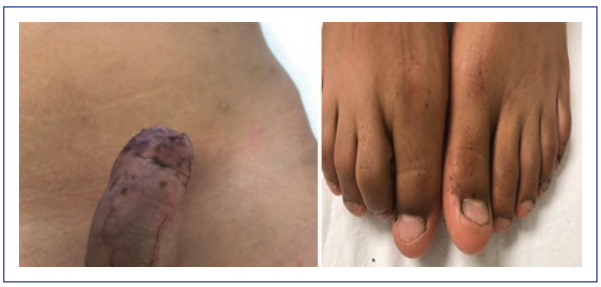

Figura 1. A: lentiginosis en zona genital. **B:** lentiginosis en ortejos.

En la exploración física se detectó macrocefalia con un perímetro cefálico de 57 cm (> P75), peso de 16.6 kg, talla de 100 cm, pequeñas máculas de color café diseminadas en la cara, el tronco y las extremidades, que afectaban los labios, el pene (Figura 1 A) y los ortejos (Figura 1 B), la mayoría entre 2 y 4 mm, y presencia de extremidades con braquidactilia. En ese momento se consideraron diagnósticos diferenciales de síndrome de Peutz-Jeghers y SBRR. Se exploró de forma dirigida a la madre, sin encontrar estigmas cutáneos. Durante el seguimiento, el paciente presentó rectorragia recurrente, por lo que se le practicó una colonoscopia. Se resecaron dos pólipos rectales, con reporte de anatomía patológica como pólipos rectales juveniles, el mayor de 0.7 × 0.5 cm y el menor de 0.5 × 0.4 cm, sin evidencia de neoplasia. Continuando con el abordaje diagnóstico, se realizó de forma externa un análisis molecular en sangre periférica mediante exoma dirigido para síndromes de predisposición a cáncer hereditario de 94 genes, basado en la tecnología de captura con sondas de oligonucleótidos (Nextera Rapid Capture Illumina Inc.) y secuenciación de siguiente generación en el equipo MiSeq. Se encontró la variante NM_000314.6:c.955delA del gen *PTEN (phosphatase and tensin homolog deleted on chromosome 10),* que origina el cambio en la proteína NP_000305.3:P. Thr319LefusTer2. Para el análisis bioinformático se emplearon los programas Trimmomatic, BWA (algoritmo mem) y GATK, y para la anotación de las variantes se empleó SnpEff, Mutation Taster e información de bases de datos y de la literatura internacional. La variante se clasificó como patogénica, pese a que no ha sido reportada en dbSNP, ClinVar del NCBI (National Center for Biotechnology Information) ni HGMD (Human Gene Mutation Database) (último acceso en agosto del 2020). Sin embargo, otras variantes que afectan al nucleótido 955 por deleción o duplicación han sido reportadas y clasificadas como patogénicas. No se

Tabla 1. Cronología de los hallazgos y valoraciones del caso

Aparatos y sistemas	Edad de presentación	Hallazgos
Neurológico	1. Al nacimiento 2. Detectado en febrero de 2017 por psicología 3. Detectado en febrero de 2017 por psicología	1. Macrocefalia 2. Déficit de atención de acuerdo con el *Kiddie Schedule for Affective Disorders and Schizophrenia* 3. Capacidad intelectual limítrofe con coeficiente intelectual de 74 según la Escala de Wechsler de inteligencia para niños
Cardiovascular	Sin problemas detectados	Sin problemas detectados
Respiratorio	4 años	Infecciones de vías respiratorias altas de repetición, se detecta hipertrofia amigdalina de grado IV con amigdalitis crónica (amigdalectomía)
Gastrointestinal	10 años	Rectorragia Colonoscopia: poliposis múltiple + polipectomía + cauterización de pólipos sésiles. Se observa en recto y sigmoides múltiples pólipos sésiles y un par pediculados de 5 mm
Endocrinología	Sin problemas detectados	Sin problemas detectados
Tegumentario	6 años	Máculas pigmentadas de color café en cara, tronco y extremidades que afectan labios, pene, mucosa genital y ortejos
Renal	Sin problemas detectados	Sin problemas detectados

realizó ningún análisis *in silico,* ya que no se cuenta con el recurso. A la madre del paciente se le realizó el estudio molecular en búsqueda de la variante encontrada; sin embargo, se reportó como normal y se brindó asesoramiento genético.

De acuerdo con los hallazgos clínicos y moleculares se realizó el diagnóstico de SBRR *de novo*.

En la Tabla 1 se resumen las valoraciones y los abordajes por aparatos y sistemas, con su cronología.

Discusión

El SBRR es una enfermedad poco frecuente; en todo el mundo se han reportado cerca de 100 casos, con una incidencia de 1 en 200,000. Sin embargo, la cifra podría ser mayor debido a la dificultad para realizar el diagnóstico ante síntomas inespecíficos[5-7]. Se ha observado un predominio de casos en el sexo masculino que podría relacionarse con las máculas hiperpigmentadas en el pene como hallazgo específico[8].

El SBRR se origina por variantes patogénicas en línea germinal del gen *PTEN,* que en su mayoría corresponden a un patrón de herencia autosómico dominante; sin embargo, el 10-45% de los casos son *de novo*[9]. El 65% de las variantes patogénicas se encuentran en regiones codificantes y el 11% se deben a duplicaciones o deleciones que, en su mayoría, recaen en el exón 5, en el centro catalítico de la fosfatasa. Recientemente se han reportado en el exón 9, ya que no se habían documentado variantes[10]. En diversos tumores (tiroides, mama, endometrio, próstata y riñón) se han encontrado mutaciones somáticas en el gen *PTEN*[10].

El gen *PTEN,* locus 10q23, consta de nueve exones y se expresa de forma ubicua en diversos órganos, en particular en el tejido graso y el bazo. *PTEN* codifica una fosfatasa con sustratos lipídicos y proteicos con actividad constitutiva de supresor tumoral[7], facilitando la apoptosis o el arresto del ciclo celular en la fase G1 mediante la actividad fosfatasa, y regulando de forma negativa las concentraciones intracelulares de fosfatidilinositol trifosfato, así como la vía de señalización de AKT/PKB[11]. Las mutaciones de este gen resultan en síndromes de sobrecrecimiento y malignidad, por lo cual se ha sugerido mantener el tamizaje ante el riesgo aumentado de desarrollar cáncer de mama, tiroides, endometrio, carcinomas renales y melanoma cutáneo. Se han observado mutaciones de *PTEN* en numerosas neoplasias esporádicas (hematológicas y sólidas)[11,12]. Clínicamente, se presentan con múltiples lipomas, hemangiomas, polipomatosis intestinal hamartomatosa, malformaciones arteriovenosas, retraso en el neurodesarrollo, autismo, macrocefalia y lentiginosis genital. Otras características que se han asociado son hueso frontal prominente, hipertelorismo, alteraciones tiroideas, alteraciones retinianas, escoliosis, *pectus excavatum,* hiperlaxitud articular y miopatía proximal con hipotonía[13,14].

Aunque no existen criterios diagnósticos definidos, Parisi, et al.[14] propusieron al menos dos de los

Tabla 2. Criterios diagnósticos para síndromes de PTEN y tumores hamartomatosos[16]

Criterios diagnósticos para PHTS
Criterios mayores Cáncer de mama Cáncer endometrial (epitelial) Cáncer de tiroides (folicular) Hamartomas gastrointestinales (incluye ganglioneuromas, pero se excluyen pólipos hiperplásicos; ≥ 3) Macrocefalia (> P75) Pigmentación macular del glande del pene Múltiples lesiones mucocutáneas (cualquiera de las siguientes): – Triquilemoma múltiple (> 3, al menos uno comprobada por biopsia) – Queratosis acral (≥ 3 fosas queratósicas palmoplantares o pápulas hiperqueratósicas acrales) – Neuromas mucocutáneos (≥ 3) – Papilomas orales (en particular en lengua y encías) múltiples (≥ 3) o comprobado por biopsia o diagnosticado por el dermatólogo
Criterios menores Trastornos del espectro autista Cáncer de colon Lipomas (≥ 3) Discapacidad intelectual Carcinoma de células renales Lipomatosis testicular Cáncer tiroideo (papilar o variante folicular del papilar) Lesiones estructurales tiroideas (adenoma)
Aplicación de criterios El diagnóstico operacional debe incluir uno de los siguientes: – Tres o más criterios mayores, pero uno debe incluir macrocefalia o hamartomas gastrointestinales o – Dos criterios mayores y tres criterios menores El diagnóstico operacional en una familia en la que un individuo cumpla los criterios clínicos para PHTS o tenga una variante patogénica del PTEN debe incluir lo siguiente: 1. Dos criterios mayores, o 2. Un criterio mayor y dos criterios menores, o 3. Tres criterios menores

PHTS: síndrome de tumor hamartoma PTEN.

siguientes: macrocefalia, hamartomas (incluyendo al menos un lipoma, hemangioma o pólipo intestinal) y máculas en el pene para pacientes de sexo masculino. El diagnóstico se confirma mediante la identificación de mutaciones en el gen *PTEN*[15]. Pilarski, et al.[16] han propuesto algunos criterios diagnósticos para síndromes de *PTEN* y tumores hamartomatosos (Tabla 2).

Se han demostrado mutaciones idénticas en *PTEN* en pacientes con síndrome de Cowden. Existen familias con superposición de síndrome de Cowden y SBRR, con miembros que manifiestan ambos fenotipos. Debido a tal superposición clínica y genética, el SBBR y el síndrome de Cowden ahora se aceptan como expresiones fenotípicas diferentes del mismo síndrome alélico y se denominan colectivamente síndrome de hamartoma-tumor PTEN[16]. Se han reportado todos los tipos de variantes patogénicas en las mutaciones de *PTEN* (deleciones completas del exón, pérdida de función, sentido equivocado y promotor), aunque sin una clara correlación genotipo-fenotipo, ya que incluso pacientes con la misma variante patogénica presentan fenotipos distintos del SBRR[16]. Ante la falta de una correlación firme genotipo-fenotipo, es recomendable que los pacientes con una variante de *PTEN* patógena o probablemente patógena presente en la infancia sigan las pautas de vigilancia para la detección oportuna de cáncer a medida que envejecen[16-18].

En este reporte se expone el caso de un paciente que acudió por primera vez a la consulta de dermatología por lentiginosis genital, asintomático, en quien se sospechó el diagnóstico de SBRR por las máculas en el pene y la macrocefalia. Posteriormente, en el abordaje integral se detectaron problemas conductuales, retraso del desarrollo y discapacidad intelectual. Además, en un periodo corto, el paciente presentó rectorragia secundaria y polipomatosis intestinal. Se detectó una variación de la mutación del gen *PTEN* que incluye un número importante de las alteraciones descritas para este síndrome.

En México se tienen reportados muy pocos casos de SBRR. En una revisión de reportes de caso se identificaron al menos cinco, de los cuales cuatro se

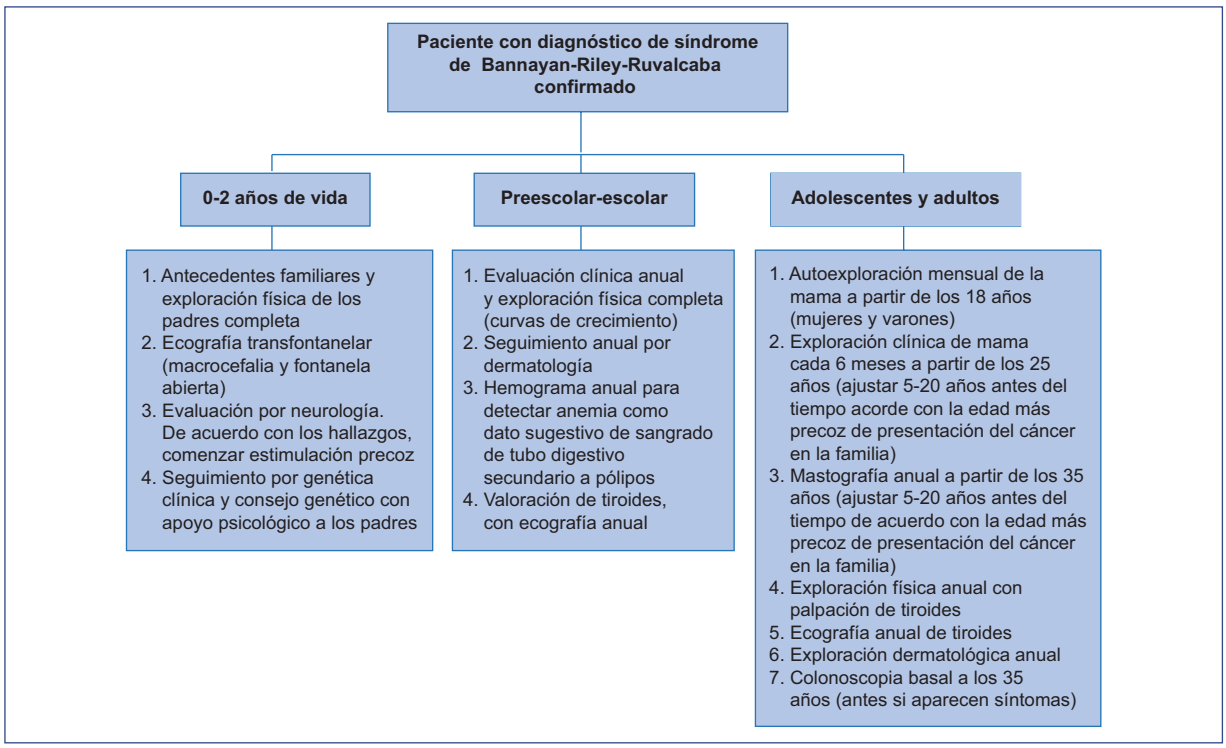

Figura 2. Diagrama de flujo para el seguimiento del paciente con síndrome de Bannayan-Riley-Rubalcaba.

reportaron en un estudio de polipomatosis gastrointestinal en pediatría. El otro caso correspondió a un estudio de manchas de color café con leche en una consulta externa de dermatología[19,20].

Familiarizarse con este tipo de enfermedades poco frecuentes permitirá buscar de manera intencionada el diagnóstico del paciente: los hallazgos clínicos descritos (macrocefalia, lentiginosis genital, retardo del neurodesarrollo y discapacidad intelectual) por sí solos llevarán a la sospecha de este síndrome, el cual debe ser confirmado con estudios moleculares. En la Figura 2 se propone un flujograma para el seguimiento de los pacientes con SBRR de acuerdo con la edad.

La importancia del diagnóstico de SBRR recae en su asociación con el desarrollo de neoplasias en la edad adulta y con otras complicaciones que requieren cribado para su detección y manejo oportuno.

Responsabilidades éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad de los datos. Los autores declaran que han seguido los protocolos de su centro de trabajo sobre la publicación de datos de pacientes.

Derecho a la privacidad y consentimiento informado. Los autores han obtenido el consentimiento informado de los pacientes o sujetos referidos en el artículo. Este documento obra en poder del autor de correspondencia.

Financiamiento

Ninguno.

Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

Bibliografía

1. Riley HD, Smith WR. Macrocephaly, pseudopapilledema and multiple hemangiomata. Pediatrics. 1960;26:293-300.
2. Bannayan GA. Lipomatosis, angiomatosis, and macrencephalia. A previously undescribed congenital syndrome. Arch Pathol. 1971;92:1-5.
3. Zigman AF, Lavine JE, Jones MC, Boland CR, Carethers JM. Localization of the Bannayan-Riley-Ruvalcaba syndrome gene to chromosome 10q23. Gastroenterology. 1997;113:1433-7.
4. Cohen MM. Bannayan-Riley-Ruvalcaba syndrome: renaming three formerly recognized syndromes as one etiologic entity. Am J Med Genet. 1990;35:291.
5. Hendriks YMC, Verhallen JTCM, van der Smagt JJ, Kant SG, Hilhorst Y, Hoefsloot L, et al. Bannayan-Riley-Ruvalcaba syndrome: further delineation of the phenotype and management of PTEN mutation-positive cases. Fam Cancer. 2003;2:79-85.

6. Lachlan KL, Lucassen AM, Bunyan D, Temple IK. Cowden syndrome and Bannayan Riley Ruvalcaba syndrome represent one condition with variable expression and age-related penetrance: results of a clinical study of PTEN mutation carriers. J Med Genet. 2007;44:579-85.
7. Schreibman IR, Baker M, Amos C, McGarrity TJ. The hamartomatous polyposis syndromes: a clinical and molecular review. Am J Gastroenterol. 2005;100:476-90.
8. Blumenthal GM, Dennis PA. PTEN hamartoma tumor syndromes. Eur J Hum Genet. 2008;16:1289-300.
9. Nosé V. Genodermatosis affecting the skin and mucosa of the head and neck: clinicopathologic, genetic, and molecular aspect PTEN-Hamartoma Tumor Syndrome/Cowden Syndrome. Head Neck Pathol. 2016; 10:131-8.
10. Pezzolesi MG, Li Y, Zhou XP, Pilarski R, Shen L, Eng C. Mutation-positive and mutation-negative patients with Cowden and Bannayan-Riley-Ruvalcaba syndromes associated with distinct 10q haplotypes. Am J Hum Genet. 2006;79:923-34.
11. Zhou XP, Waite KA, Pilarski R, Hampel H, Fernandez MJ, Bos C, et al. Germline PTEN promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the phosphoinositol-3-kinase/Akt pathway. Am J Hum Genet. 2003;73:404-11.
12. Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res. 2012;18:400-7.
13. Hobert JA, Eng C. PTEN hamartoma tumor syndrome: an overview. Genet Med. 2009;11:687-94.
14. Parisi MA, Dinulos MB, Leppig KA, Sybert VP, Eng C, Hudgins L. The spectrum and evolution of phenotypic findings in PTEN mutation positive cases of Bannayan-Riley-Ruvalcaba syndrome. J Med Genet. 2001;38:52-8.
15. Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, et al. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet. 1999;8:1461-72.
16. Pilarski R, Burt R, Kohlman W, Pho L, Shannon KM, Swisher E. Cowden syndrome and the PTEN hamartoma tumor syndrome: systematic review and revised diagnostic criteria. J Natl Cancer Inst. 2013;105:1607-16.
17. Wanner M, Celebi JT, Peacocke M. Identification of a PTEN mutation in a family with Cowden syndrome and Bannayan-Zonana syndrome. J Am Acad Dermatol. 2001;44:183-7.
18. Macken WL, Tischkowitz M, Lachlan KL. PTEN hamartoma tumor syndrome in childhood: a review of the clinical literature. Am J Med Genet. 2019;181C:591-610.
19. Cázeres-Méndez JM, Zamudio-Vázquez VP, Gómez-Morales E, Ortiz-Aguirre SG, Cadena-León JF, Toro-Monjaraz EM. Pólipos gastrointestinales en pediatría. Acta Pediatr Mex. 2015;36:158-63.
20. Pérez-Hernández J, Frías-Ancona G, Vergara-López A. Prevalencia de las manchas café con leche en pacientes de consulta externa del servicio de dermatología del Centro Médico Nacional 20 de Noviembre. Dermatol Rev Mex. 2010;54:315-20.