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REVIEW ARTICLE

Normal development of the heart: a review of new findings

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Abstract

Development and formation of the heart, the central organ of the circulatory system in vertebrates, starts early during embryonic development (second week), reaching maturity during the first few postnatal months. Cardiogenesis is a highly complex process that requires the active and orderly participation of different cardiac and non-cardiac cell populations. Thus, this process is sensitive to errors that may trigger a variety of heart-development defects, called congenital heart defects, which have a worldwide incidence of 8-10/1000 live births. A good understanding of normal cardiogenesis is required for better diagnosis and treatment of congenital heart diseases. This article reviews normal cardiogenesis by comparing information from classic studies with more recent findings. Information from descriptive anatomical studies of histological sections and selective in vivo marking of chicken embryos were emphasized. In addition, the discovery of heart fields has fueled the investigation of cardiogenic events that were believed to be understood and has contributed to proposals for new models of heart development.

Keywords: *Cardiogenesis. First and second heart fields. Heart.*

Desarrollo normal del corazón: revisión de nuevos hallazgos

Resumen

El corazón, órgano central del aparato circulatorio de los vertebrados, comienza a formarse muy temprano en el desarrollo embrionario (segunda semana de gestación) y alcanza su forma madura durante los primeros meses posteriores al nacimiento. La cardiogénesis se caracteriza por ser un proceso altamente complejo, dependiente de la participación activa y ordenada de diferentes poblaciones celulares cardiacas y no cardiacas. Lo anterior hace que este proceso sea sensible a errores que pueden desencadenar una variedad de defectos del desarrollo cardiaco, llamados cardiopatías congénitas, con una incidencia mundial de 8 a 10/1000 nacidos vivos. Para mejorar el diagnóstico y el tratamiento de las cardiopatías congénitas es necesario comprender adecuadamente los eventos implicados en la cardiogénesis normal. En este artículo se revisa el desarrollo cardiaco normal, contrastando la información de los estudios clásicos con la de hallazgos recientes. Se hace hincapié en la información obtenida de los estudios de anatomía descriptiva de cortes histológicos y marcaje selectivo in vivo en embriones de pollo. Adicionalmente, el descubrimiento de los campos cardiogénicos ha estimulado la investigación de eventos cardiogénicos que se creían comprendidos, contribuyendo con propuestas de nuevos modelos del desarrollo del corazón.

Palabras clave: *Cardiogénesis. Primer y segundo campos cardiogénicos. Corazón.*

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Introduction

The heart is the central organ of the circulatory system of vertebrates; it functions as a pump that aspirates and propels blood throughout the body. Of all the organs in the body, it is the first to acquire functionality during embryonic development, supplying nutrients and oxygen to the embryo, as well as eliminating waste substances[1-3]. In humans, the heart develops from the second to the eighth week of gestation; however, various physiological and structural modifications occur during and after birth that completes its formation[3]. Cardiogenesis is a complex process that involves the gradual integration of different cardiac cell populations, called heart fields[4-6], and other non-cardiac cell populations, such as neural crest[7] and proepicardial organ cells[8,9].

The classical view of cardiac development was established mainly by descriptive studies of human embryos resulting from spontaneous abortions[10-17]. Although these studies provided valuable information, they did not reflect the true dynamism of the process of cardiogenesis as they were based on histological sections; moreover, the embryos likely presented errors associated with defects in cardiac development. These limitations were initially resolved using *in vivo* selective labeling experiments with plastic labels carried out in chick embryos[18-28]. This technique allowed longitudinal studies to be carried out since it is possible to temporally trace the structural changes of an embryonic region previously marked with a label. Furthermore, molecular labeling studies in mouse[5,6,29] and chick[4] embryos established the concept of the "heart field" as an embryonic region in which cells destined to become myocardium are located. With this new approach, *in vivo*, and *in vitro* selective labeling experiments in chick embryos[30-32] have been developed, which collectively have succeeded in proposing new models of heart development.

It is important to emphasize that errors in the normal development of the heart cause congenital heart disease, a condition with a worldwide incidence of 8-10/1,000 live births, of which approximately 50% die during the first year if they are not clinically and surgically treated[3,33]. The frequency and lethality of congenital heart defects make it imperative to study normal cardiogenesis to improve diagnosis and treatment. In this review, we contrast some of the classical concepts of cardiac development with current anatomical descriptions and the general events of gene expression and their cellular repercussions. We hope this work will provide an overview for understanding the anatomical, cellular, and genetic processes of normal cardiogenesis.

Cardiogenic areas

The first signs of cardiogenesis appear at the blastula stage, when the embryonic disc consists of only two cell layers: the epiblast and the hypoblast (Fig. 1). Two compact groups of cells positioned in the epiblast on both sides of the primitive streak are known as pre-cardiogenic areas (Fig. 1A)[34,35]. The cells of the pre-cardiogenic areas remain undifferentiated through positive Wnt/β-catenin signaling[36-38]. Once gastrulation has begun, the cells of the cardiogenic precardiac areas migrate through the primitive streak until they are incorporated into the splanchnic mesoderm, where they form two cardiogenic areas located contralateral to the primitive streak at the level of Hensen's node (Fig. 1B)[34,35,39]. In the splanchnic mesoderm, cells from cardiogenic areas are already determined to differentiate into cardiac cells[34] by molecular signals secreted by the underlying endoderm[40], including bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and Wnt signaling inhibitors, which together promote cardiac phenotype genes, such as NKX2-5, GATA4, and TBX5, and the chromatin remodeling protein SMARCD3 (BAF60c)[36-38,41,42]. Even ectopic activation of 3SMARCD3, GATA4, and TBX5 is sufficient to drive cardiomyogenesis in non-cardiogenic regions of the embryo[43].

Cardiogenic crescent

During late gastrulation, cells from the cardiogenic areas migrate in a cephalomedial direction and fuse to form the cardiogenic crescent,[44] named for its lunar crescent-like appearance (Fig. 1C and 2A). Figure 2 depicts normal cardiac development based on observations in chick embryos. The cell population that makes up the cardiogenic crescent is recognized as the "first heart field" (FHF), which expresses genes characteristic of the cardiac phenotype and is the precursor of the left ventricle and part of the atria in the amniotic heart (reptiles, birds, and mammals)[5,6]. The rest of the heart in amniotes derives from another cell population located dorsal to the cardiogenic crescent, known as the "second heart field" (SHF)[4-6,29,45]. The cells of the SHF are undifferentiated and can be observed by molecular labeling of Isl1 or Tbx1, considered characteristic transcription factors of the SHF[46]. In fact, knockout mice for Isl1 develop hearts lacking the

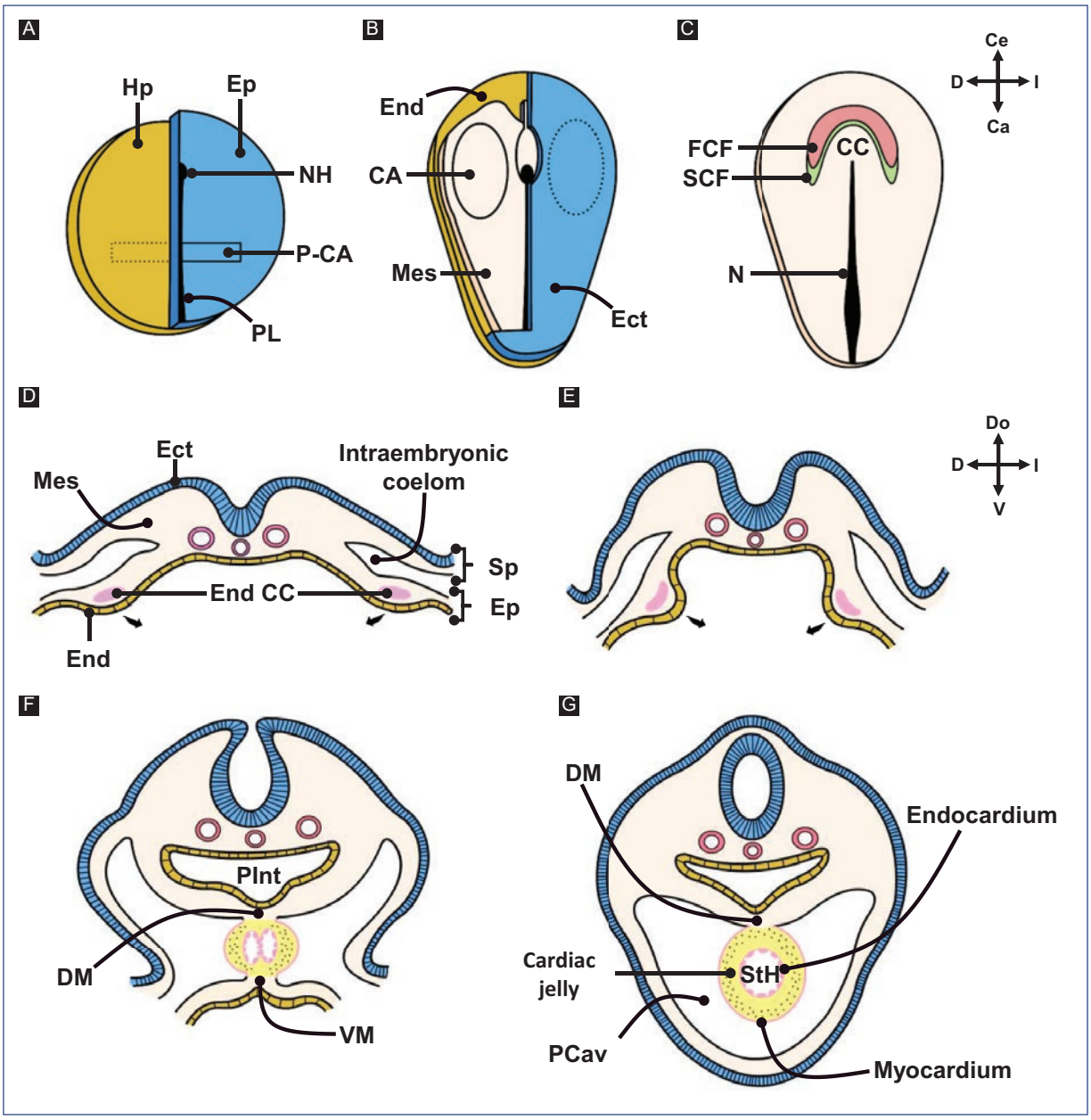

Figure 1. Early cardiogenesis. **A:** blastula. Two pre-cardiogenic areas are present in the epiblast. **B:** early gastrula. The pre-cardiogenic areas migrate through the primitive streak to incorporate into the splanchnic mesoderm, forming the cardiogenic areas. **C:** late gastrula. The cardiogenic areas migrate in an cephalomedial direction and fuse to form the cardiogenic crescent. **D:** the cardiogenic crescent is mobilized in the splanchnopleura, showing its ends as two endocardial tubes. **E-G:** the ends of the cardiogenic crescent move in a ventro-medial direction until they fuse and form a single myo-endocardial tube, called a straight-tube heart. Note that the dorsal wall of the heart is attached to the ventral wall of the primitive gut tube (A and B modified from García-Peláez[82], D-G modified from Arteaga et al[3].).
CA: cardiogenic areas, DM: dorsal mesocardium, Ect: ectoderm, End CC: cardiogenic crescent endings, End: endoderm, Ep: epiblast, FHF: first heart field; Hp: hypoblast, Mes: mesoderm, N: notochord, NH: node of Hensen, P-CA: pre-cardiogenic areas, PCav: pericardial cavity, PGT: primitive gut tube; PS: primitive streak, SHF: second heart field, Sp: somatopleure, splanchnopleure, TH: straight-tube heart, VM: ventral mesocardium.

embryonic outflow tract (classically called conotruncus), right ventricle, and a large part of the atria[47]; also, mice with a deletion in Tbx1 present defects in the embryonic outflow tract[48].

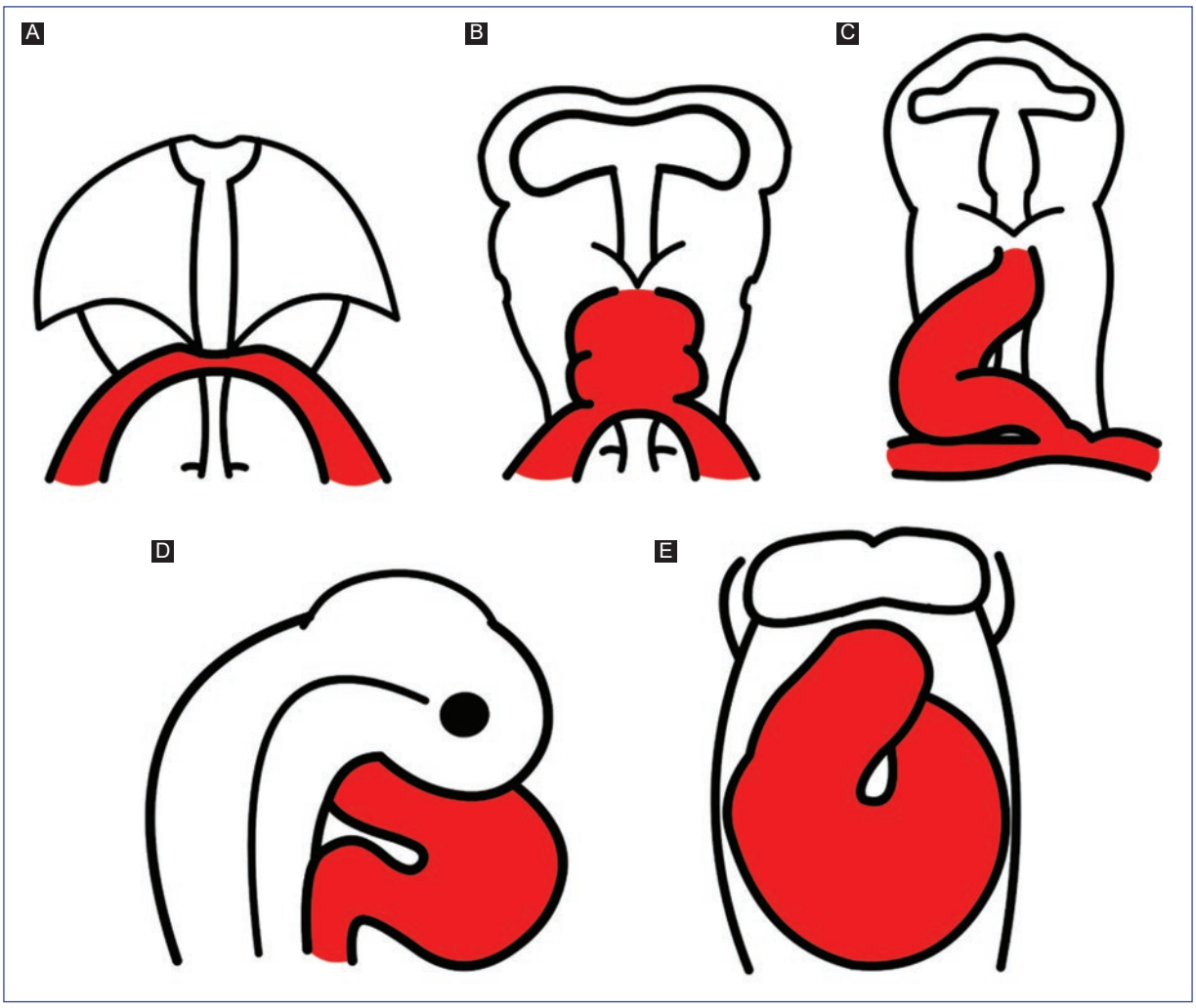

Figure 2. Schematic representation of normal cardiac development, based on observations of chick embryos. **A:** cardiogenic crescent. **B:** straight tube heart. **C:** C-loop. **D:** S-loop. **E:** advanced loop.

Straight-tube heart

Once gastrulation is completed, embryonic tubulation begins, a process by which the trilaminar embryo adopts a tubular and elongated morphology (Fig. 1D-G), manifesting the segmentation of the mesoderm into three layers (paraxial, intermediate, and lateral) and the development of the neural tube, the primitive gut tube and the walls of the body[3]. The lateral mesoderm is delaminated in two layers; one is associated with the ectoderm (somatopleure) and the other with the endoderm (splanchnopleure), causing the formation of the intraembryonic coelom (Fig. 1D)[3]. The cardiogenic crescent is located in the splanchnopleure[11,39] and is organized to form two endocardial tubes (Fig. 1D-F), right and left[26], which are the ends of the cardiogenic crescent. As a result of the embryonic tubulation process, the ends of the cardiogenic crescent move in a ventromedial direction until they fuse and form a single tube called a straight-tube heart (Fig. 1F-G and 2B)[39].

The straight-tube heart is composed of a lumen delimited by a layer of endocardial cells and another layer of myocardial cells; in between these layers exists an extracellular matrix rich in mucopolysaccharides, glycoproteins, and collagen, called cardiac jelly (Fig. 1G)[11,13]. Several authors accept that cardiac jelly only has a purely septal function in the embryonic heart[13,16,20], while others claim that it also has a provisional valvular activity[49,50].

During this stage, the heart is incorporated within the cephalic portion of the intraembryonic coelom (primitive pericardial cavity) and is positioned ventrally to the

primitive gut tube. In fact, the straight-tube heart initially has a canal shape because its dorsal wall corresponds to the ventral wall of the primitive gut tube; however, after the myocardium invades and closes the canal to form a tube, the heart remains attached to the primitive gut tube by a band of mesoderm, called the dorsal mesocardium (Fig. 1G)[26,39,51,52]. It is likely that this temporary junction, heart with primitive gut tube, allows the cardiac loop to twist to the right during embryonic flexion.

In the 1920s, the preformation model of cardiogenesis was proposed based on descriptive studies of human embryos. This model considered that all the components of the mature heart were already present in the straight-tube heart, which only grew during development (Fig. 3A). Decades later, through *in vivo* selective labeling studies in chick embryos[20,25-27,53], it was described that the heart is formed by the gradual integration of several cardiac components (Fig. 3B-F). It was accepted that the straight-tube heart is composed of the trabeculated region of the right ventricle and the trabeculated region of the left ventricle, both regions flanked by interventricular grooves (Fig. 3B)[26,27]. However, Villavicencio et al.[32] have recently proposed a segmental model of the heart (Fig. 4A-D) where they suggest that the straight-tube heart (Fig. 4A) is composed of the primordia of the interventricular septum, the left ventricle, and the atrioventricular canal (A-V canal). Figure 4 shows selective labeling experiments in chick embryos.

Molecular expression studies have described that the straight-tube heart contains the primordia of the left ventricle and part of the atrial segment[5,6]. Concomitantly, SHF cells in the pharyngeal mesoderm are in a state of continuous proliferation and delayed differentiation due to positive FGF signaling, which stimulates proliferation by inhibiting the pro-differentiation signal of BMP[47,54].

It is currently accepted that the straight-tube heart undergoes a series of morphological changes, mainly the addition of new segments from its venous and arterial ends, and consequently undergoes a process of torsion that establishes the definitive spatial position of the cardiac cavities. Cardiac torsion is divided into three stages: C-loop (Fig. 2C), S-loop (Fig. 2D), and advanced loop (Fig. 2E). The letters correspond to the similarity of the pathway followed by blood flow within the heart.

C-SHAPED LOOP HEART

The straight-tube heart increases in size due to the differential aggregation of cells from the SHF[29,45] from its venous and arterial poles[26,27]. Different studies have shown that Isl1[47] and Tbx1[55,56] play a fundamental role in the admixture by promoting cell migration from the SHF to the developing heart. Cell differentiation toward the myocardium is mediated by positive BMP signaling, which by inhibiting FGF signaling and silencing Isl1 and Tbx1, promotes the expression of cardiac phenotype genes, such as NKX2-5 and GATA4[47,54]. The increase in size causes the middle portion of the heart to begin to twist to the right, thus obtaining the characteristic shape of the letter "C" (Fig. 2C)[51,53]. The recruitment of cell populations that are added to the poles of the heart at this stage results in the emergence of new anatomical components. Initially, it was proposed that three new structures were incorporated into the heart: the A-V canal[25-27], the primitive atria at the caudal end[18,27,39] and the cone at the cephalic end (Fig. 3C)[20,26,27]. In contrast, it is currently proposed that during the C-loop stage, the conus, recognized as the right ventricular primordium including its outflow tract (Fig. 4E and F)[31], is recruited cephalad and the primitive atria are recruited caudally (Fig. 4B)[32].

S-SHAPED LOOP HEART

The continuous differential growth of the heart and the separation of the dorsal mesocardium from the cardiac midline cause the torsion of the heart to become increasingly accentuated until it acquires the shape of the letter "S" (Fig. 2D)[51,53]. De la Cruz et al.[25-27] point out that the atria acquire a dorsal position due to their upward displacement (Fig. 3D). Similarly, Villavicencio et al.[32] mention that during this stage, the primordia of the ventricles and the interventricular septum begin to descend, causing the primitive atria to ascend (Fig. 4C). Conversely, the results of Lazzarini et al.[31] suggest that the incorporation of the myocardial conus into the ventricular segment is responsible for the ascent of the atria in the dorsal cephalic direction.

ADVANCED LOOP HEART

During the advanced loop stage (Fig. 2E), the atria and ventricles acquire their definitive position and spatial relationship[51,53]. De la Cruz et al.[28] describe that the conus finishes incorporating into the heart, which contrasts with the recent findings of Lazzarini et al.[31], who describe how the myocardium of the conus transforms into a large part of the myocardium of the right

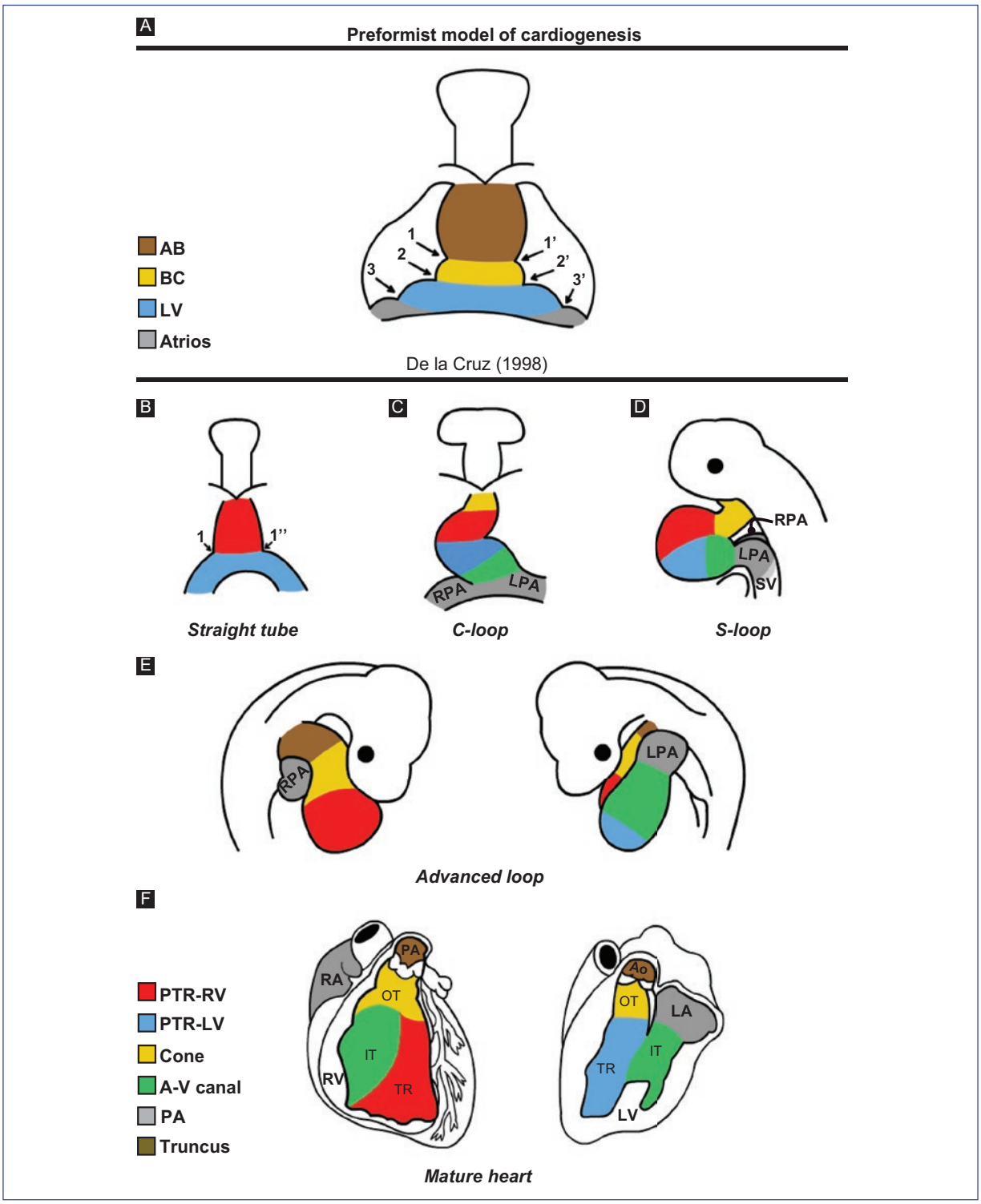

Figure 3. Theories of cardiac development **A:** according to descriptive embryology studies in humans and **B-F:** selective labelling studies in chick.
1 and 1': right and left interbulbar sulci, 1 and 1'': right and left interventricular sulcus, 2 and 2': right and left bulboventricular sulcus, 3 and 3': right and left atrioventricular sulcus, A-V canal: atrioventricular canal, AB: aortic bulb, Ao: aorta, BC: *bulbus cordis*, IFT: inflow tract, LA: left atrium, LF: left ventricle, LPA: left primitive atrium, OFT: outflow tract, pA: primitive atria, PA: pulmonary artery, pTRRV: primordium of the trabeculated region of the LV, pTRRV: primordium of the trabeculated region of the RV, RA: right atrium, RPA: right primitive atrium, RV: right ventricle, SV: sinus venosus, TR: trabeculated region.
(Figure A was modified from De la Cruz et al.[26] and figures B-F from De la Cruz[53].).

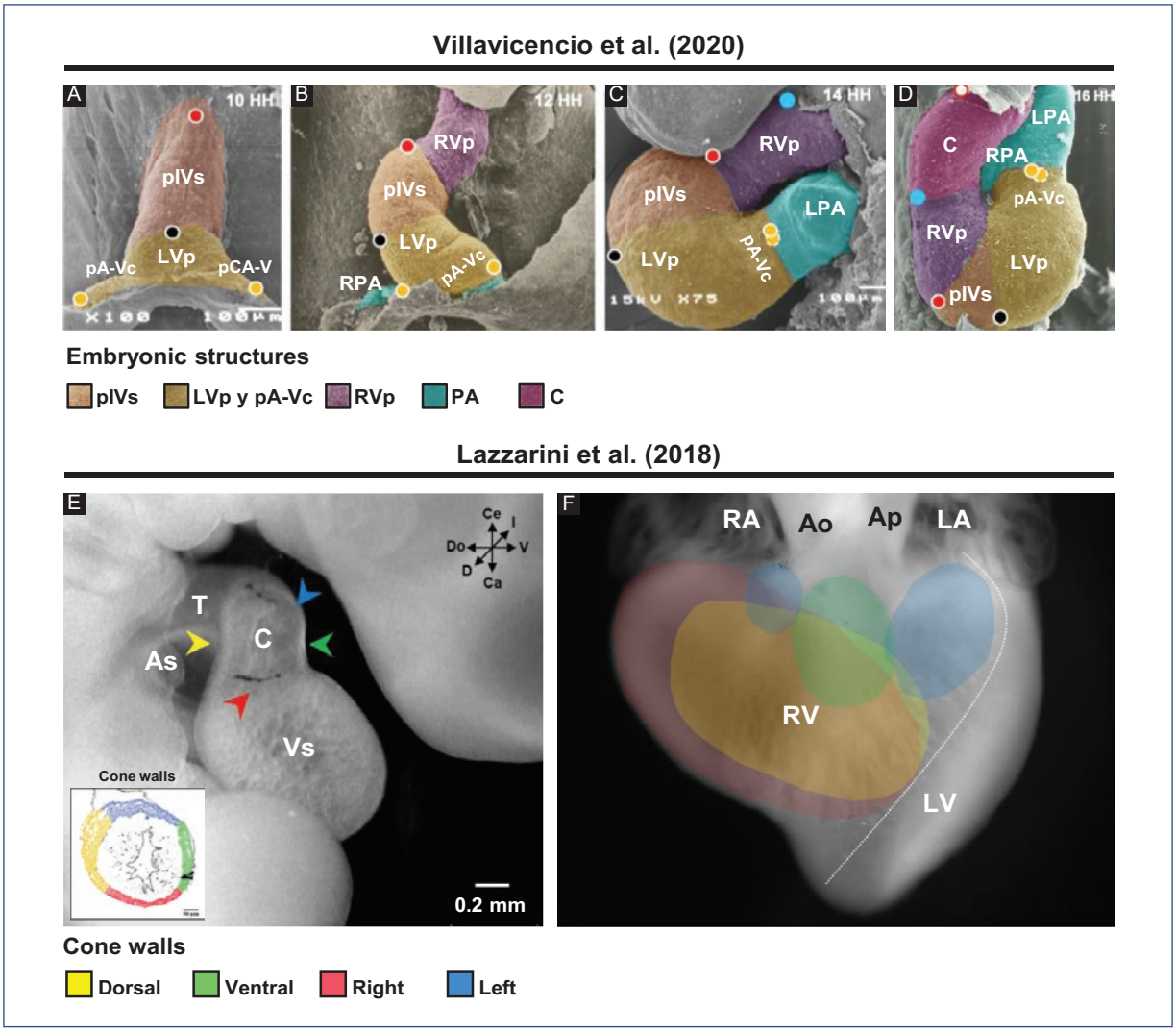

Figure 4. Selective labeling experiments in chick embryos. Segmental model of the heart development proposed by Villavicencio et al.[32]. **A:** straight tube heart. **B:** C-loop. **C:** S-loop. **D:** U-loop (advanced loop). The circles represent the selective markings performed, in A (red, black and yellow) and in C (blue), while in B-D the tracing of these markings is shown. Destination of myocardial cone walls by Lazzarini et al.[31]. **E:** location in space of the different cone walls: dorsal (yellow), ventral (green), left (blue), and right (red). **F:** map of the destination of all conal walls in the right ventricle, ventral view of the heart.
Ao: aorta, As: atrial segment, C: conus, LA: left atrium, LPA: left primitive atrium, LV: left ventricle, LVp: LV primordium, pA-Vc: primordium of the atrioventricular canal, pA: primitive atria, PA: pulmonary artery, pIVs: primordium of the interventricular septum, RA: right atrium, RPA: right primitive atrium, RV: right ventricle, RVp: RV primordium, T: truncus, Vs: ventricular segment.
Figures A-D were modified from Villavicencio et al.[32] and E-F from Lazzarini et al.[31]. Since these are different experiments, the interpretations are not the same, so different terminologies are used.

ventricle. Moreover, the distal segment of the conus begins to appear, being recognized as a truncal segment that joins the heart with the aortic sac (Fig. 3E and 4D)[20]. This segment, like the conus, originates from the incorporation of cells from the SHF[29,45]. In most of the literature, the conus and the truncus are considered to be the same structure, called the conotruncus or embryonic outflow tract. However, despite having anatomical homology, both structures undergo different developmental processes, for example, apoptosis[31].

During the advanced loop heart stage, the proepicardial organ appears, which is recognized as a bulge of mesothelial cells positioned on the surface of the

venous sinus[8]. The cells of the proepicardial organ invade the myocardium so that they cover it and subsequently transform into the epicardium[8,9,57]. In addition, some of these cells contribute to the formation of coronary arteries and veins[58].

Cardiac septation

Cardiac septation is a process by which the heart with a single blood flow is physically divided into four chambers, thus creating a dual pathway flow: systemic circulation and pulmonary circulation, characteristic of birds and mammals. Thus, three septums are recognized in the heart: the interatrial septum (IAS), the interventricular septum (IVS), and the atrioventricular septum (AVS). In addition, aorticopulmonary septation and conus remodeling are important events for cardiac septation and the establishment of definitive cardiac circulation.

According to De la Cruz et al.[23,28], the first indication of the cardiac septum is the primitive cardiac septum (pCS), which they describe as being shaped like an eye mask (Fig. 5A). This septum is constituted by the septum primum (SP), the ventro-superior (VSc) and dorso-inferior (DIc) cushions of the A-V canal and the primordium of the muscular IVS (pmIVS)[23,28]. However, the pCS is likely an erroneously described structure since it does not fulfill a proper septal function due to the temporary valvular function of the cardiac jelly and its derivatives: the cushions of the A-V canal and the conal and truncal crests[49,50]. Thus, we propose that all the structures that constitute the pCS have an indispensable role in cardiac septation but do not form a single septum.

Figure 5A shows the primitive cardiac septum proposed by De la Cruz et al.[28]; figures 5B-F the atrial septum, and 5G-I the ventricular septum.

Epithelial-mesenchymal transition

The proliferation, delamination, and invasion of endocardial cells into the cardiac jelly of the A-V canal cushions and conotruncal crests are considered fundamental events for septation and are also part of a process known as epithelial-mesenchymal transition (EMT)[49,59]. In the embryonic heart, the surrounding myocardium stimulates endocardial EMT when it secretes adherens that induce the loss of cell adhesion molecules, such as E-cadherin, causing the cells to delaminate and acquire an invasive mesenchymal phenotype characterized by the expression of N-cadherin, vimentin, and fibronectin[60-62]. The transforming growth factor beta (TGF-β) signaling pathway is considered the most important in EMT; it has even been shown that in cell cultures, TGF-β treatment is sufficient to induce EMT in epithelial cells[63]. However, numerous studies propose that this process is regulated by a complex network consisting of TGF-β/Smad, BMP, Wnt/β-catenin, Notch, and Smad-independent TGF-β signaling pathways, which together induce the expression of transcription factors, such as Snail, Slug and Twist, and promote or inhibit EMT[64-68].

Atrial septation

Atrial septation is a cardiac event that, despite its complexity, has been almost completely described since its pioneering studies[10,69-71]. However, one of the drawbacks to understanding this phenomenon is the developmental variations presented in the animal models, which result from evolutionary modifications[72,73]. For this reason, we will focus only on IAS formation in birds and placental mammals (Fig. 5B-F), as these are the animal models most commonly used for research.

Atrial septation begins with the appearance of the SP, a muscular structure in the dorsal cephalic wall of the common atrium (Fig. 5B)[69,73,74]. The SP has a crescent shape, with one end directed toward the VSc and the other toward the DIc. In addition, the SP is covered at its leading edge by a mesenchymal cap originating from the dorsal mesenchymal protrusion (DMP)[73-75], a mesenchymal bulge expressing Isl1 derived from the SHF (Fig. 5B and B')[76]. The orifice bounded by the SP's mesenchymal cap and the A-V canal's two cushions is known as the foramen primum (FP)[23,28,73]. Some authors include the DMP as part of the perimeter of this foramen[73,74,77,78] and even mention that these structures form the atrioventricular mesenchymal complex (A-VMC)[74]. The growth of the SP and the subsequent fusion of the A-VMC components results in the FP closure (Fig. 5C and D)[23,69,70,74,78,79].

According to Anselmi and De la Cruz[80], experiments by De la Cruz et al.[22,23] demonstrated that only the DIc of the A-V canal cushions participates in the closure of the FP. Before the closure of the FP, several perforations appear in the cephalic region of the SP (Fig. 5C), which allow unidirectional blood flow between the atria[69,73]. In the chicken, the interatrial septation process remains in this state until the time of eclosion, which is when these perforations are eventually closed by the growth of the myocardial and endothelial tissues

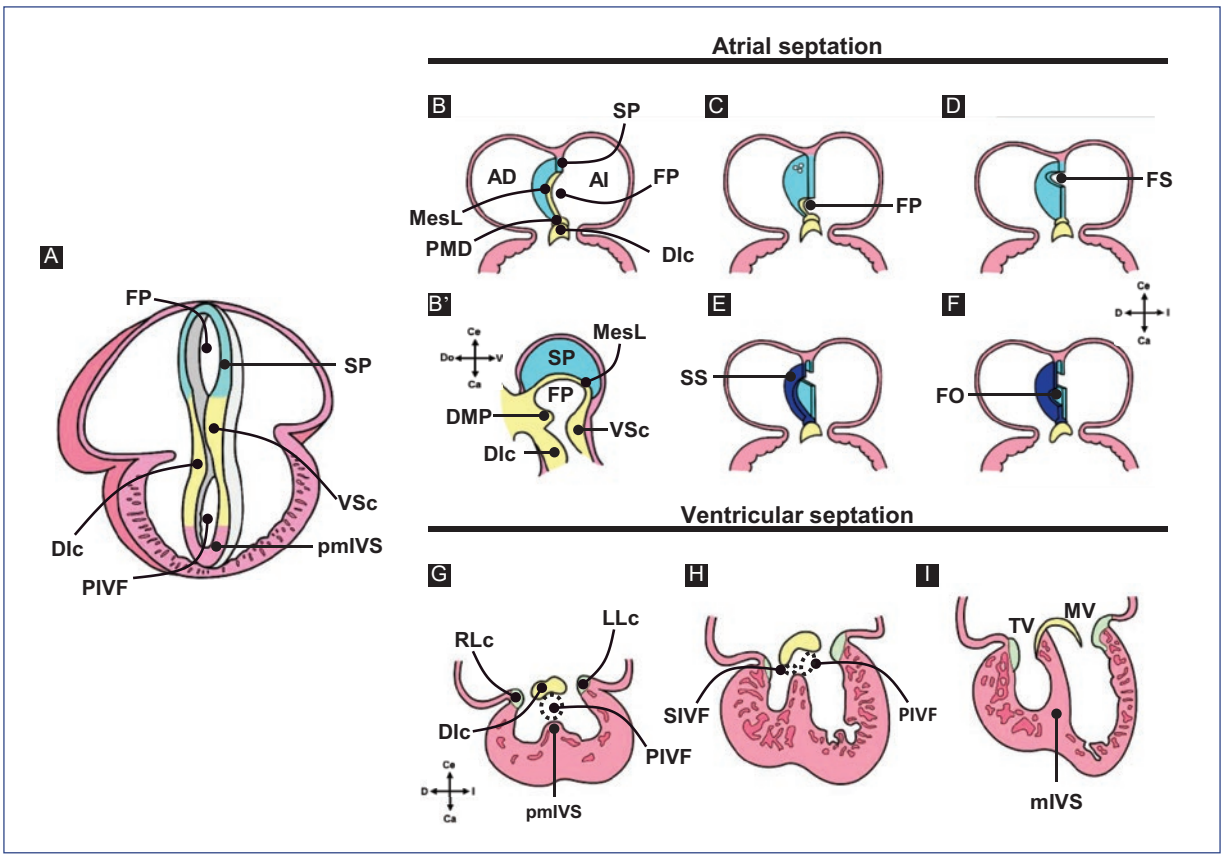

Figure 5. Cardiac septation. **A:** primitive cardiac septum proposed by De la Cruz et al. **B-F:** atrial septation. **G-I:** ventricular septation.
DIc: dorso-inferior cushion, DMP: dorsal mesenchymal protrusion, FO: fossa ovalis, FP: foramen primum, LLc: left lateral cushion, MesC: mesenchymal cap, mIVS: muscular interventricular septum, MV: mitral valve, PIVF: primary interventricular foramen, pmIVS: primordium of the muscular interventricular septum, RLc: right lateral cushion, SIVF: secondary interventricular foramen, SP, septum primum, SS: septum secundum, TV: tricuspid valve, VSc: ventro-superior cushion. Figure A was modified from De la Cruz et al.[28] and B-I from Arteaga et al.[3].

at their margins[69,72,81]. In contrast, in placental mammals, the perforations of the SP coalesce to originate the foramen secundum (FS) (Fig. 5D)[10,73]. Subsequently, another muscular structure, the septum secundum (SS), appears to the right of the SP (Fig. 5E) due to the folding of the right atrial roof and myocardial differentiation of the mesenchyme that closed the FP[10,75,77,79]. The ends of the SS grow until they meet and fuse, resulting in the formation of an orifice just below the FS, known as the fossa ovalis (FO) (Fig. 5F)[3,71]. The location of the FS and FO determines that the SP functions as a valve, which maintains right-to-left blood flow[3,73]. Finally, during birth, physiological closure of the interatrial communication occurs due to increased pressure in the left atrium, which ends up compressing both septa[3,73]. In humans, anatomical closure of the interatrial communication occurs in the first 6 months after birth[3].

Ventricular and atrioventricular septation

The IVS is an anatomically and embryologically complex structure because it originates from different embryonic structures in a "mosaic" fashion. In general, the IVS comprises a muscular portion, the muscular interventricular septum (mIVS), and a fibrous portion, known as the membranous septum (MS), with the mIVS being the most prominent component of the IVS[1]. The muscular and fibrous nature of the IVS is responsible for the lack of consensus on its embryonic origin; however, it has been reported that the pmIVS, the DIc and VSc cushions of the A-V canal, and the left conal crest (LCc) participate in its formation[12,14,15,21-23,28,77].

Regarding the formation of the MS, it is considered that the cushions of the A-V canal and the conal crests participate[21,22]. Therefore, the fusion of the main

cushions of the A-V canal (Fig. 6) is the most relevant event in the formation of the MS[15,17]. Initially, due to the appearance of cardiac jelly and its subsequent transition to mesenchyme, four endocardial cushions are formed in the A-V canal: one dorso-inferior, oneventro-superior, and two lateral[3,82]. Afterward, the DIc fuses with the VSc, which occurs in a cephalocaudal direction, leaving no demarcation line to distinguish one from the other (Fig. 6A-C)[3,24,82]. The fusion of the cushions divides the A-V canal into the right and left atrioventricular orifices, (A-VV) will form, tricuspid valve (VT) and mitral valve (MV), respectively (Fig. 6D and E)[3,82]. In addition, the DIc participates in the formation of the septal leaflet of the TV and the septal portion of the anterior leaflet of the MV, whereas the VSc contributes to the origin of the free portion of the latter leaflet[23]. For their part, the lateral cushions contribute to the development of the valvular rings and the lateral leaflets of the A-VV[3,82]. The A-VV does not coincide in the horizontal plane within the fibrous skeleton, as the septal leaflet of the TV inserts closer to the cardiac apex than the anterior leaflet of the MV[1,3,82,83]. This misalignment of A-VV insertion is attributed to the development of lateral protrusions in the DIc and VSc cushions, termed the right and left tubercles, during the fusion process. Specifically, the DIc is curved at its caudal edge, so the right tubercle is also positioned closer to the cardiac apex than the left tubercle (Fig. 5H)[3,82]. It is relevant to mention that this difference in the level of the A-VV leaflets delimits the AVS region, which separates the right atrium from the left ventricle[1,3,82,83]. Despite being recognized as independent septa, the MS and AVS share the same embryonic origin, which is reflected in the lack of an anatomical boundary beyond the gap between the atrioventricular leaflets.

Descriptive cardiac embryology work concluded that the AVS is formed by the contribution of the DIc and VSc cushions of the A-V canal[15,17]. In contrast, an *in vivo* labeling experiment of the A-V canal cushions described that the DIc forms the entire AVS and the adjacent portions of the IAS and IVS[22,23]. Despite both perspectives, Webb et al.[84] concluded that simple fusion of the A-V canal cushions is not the only event involved in atrioventricular septation. This process requires remodeling different cardiac structures, including the development of the venous sinus, the formation of the atrial and ventricular septa, the expansion of the right atrioventricular junction, and the junction of the LCc with the VSc of the A-V canal[84].

It is commonly described that the first sign of mIVS is a myocardial ridge located between the trabecular pouches of the ventricles; in fact, the formation of these trabecular pouches (a process called diverticulization) is responsible for its appearance[3,82]. Classical studies of human embryos[12,14,15] attributed the emergence of the mIVS to the coalescence of the trabecular pouches. Likewise, the authors suggested that this septum grows passively toward the ventricular cavity as the trabecular pouches develop. However, this coalescence origin was not fully supported by the *in vivo* selective labeling results in chick embryos[19,21,22,28]. De la Cruz et al.[28,85] described that pmIVS appears in the straight-tube heart, precisely at the midline of fusion of the cardiac primordia and at the level of the interventricular grooves (Figure 3B). They also observed that the first morphological manifestation of this septum appears in the apical region of the interventricular groove and that its growth occurs in a caudal-cephalic direction due to the continuous incorporation of cells from the ventricular free walls and cell multiplication. Despite this, Villavicencio et al.[32] recently proposed that the IVS primordium occupies the entire cephalic segment of the straight-tube heart. In contrast, the results of Contreras-Ramos et al.[30] showed neither the coalescence of the trabecular pouches suggested by classical studies[12,14,15] nor the continuous incorporation of cells from the ventricular free walls proposed by De la Cruz et al.[28,85]. Conversely, these authors proposed that the formation is due to the association of the trabeculae to the pmIVS suggesting that the septum grows in a cephalic-caudal direction, not caudal-cephalic as described by De la Cruz et al.[28,85].

Regardless of its development, anatomically, it is recognized that the mIVS has two ends: a dorsal one that continues with the DIc and a ventral one that joins with the VSc and LCc[3,28,82]. Thus, the mIVS and these mesenchymal structures delimit the perimeter of the primary interventricular foramen (PIVF) (Fig. 5G)[3,28,82].

Subsequently, remodeling and fusion of the A-V canal cushions determine the inclination of the PIVF and the formation of the secondary interventricular foramen (SIVF) (Fig. 5H)[3,61]. The SIVF will eventually close by fusion of the DIc with the dorsal end of the mIVS, while the PIVF will form the left ventricular outflow tract (Fig. 5I)[3,82]. In addition to the above, Lazzarini et al.[31] suggest that the supraventricular crest fulfills a septal function at the level of the ventricular outflow tracts (Fig. 6E), thus discarding the idea that the supraventricular crest separates the inflow outflow tracts of the right ventricle, as described by De la Cruz et al.[20].

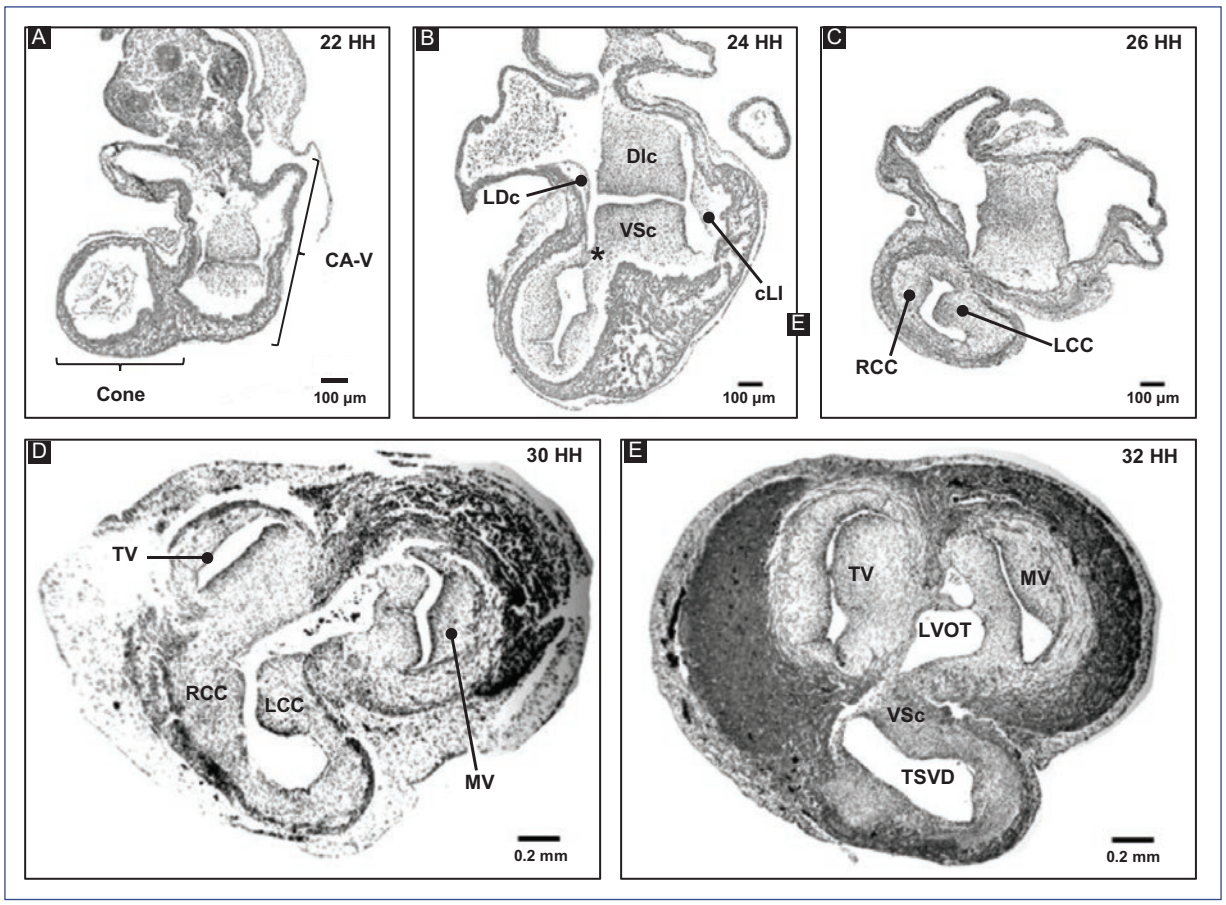

Figure 6. A-E: remodeling of the conus and A-V canal in chick embryo.
DIc: dorso-inferior cushion, LCc: left conal crest, LVOFT: left ventricular outflow tract, MV: mitral valve, RCc: right conal crest, RVOFT: right ventricular outflow tract, SVr: supraventricular ridge, TV: tricuspid valve, VSc: ventro-superior cushion (modified from Lazzarini et al.[31]).

Aortopulmonary septation

Aorticopulmonary septation is a process that has been controversial for different reasons: the variety of techniques used, spatiotemporal misinterpretation of embryonic events, morphological differences between biological models, and lack of consensus on the terms used[86]. In addition, for years, a disagreement prevailed regarding the number of truncal crest. Some authors have described two crests in the chick[20,87,88], while others suggest the existence of three crests[89-92], and it has even been reported that two of the three truncal crests are fused in their cephalic position to form a common crest[90,91]. In contrast, two truncal crests have been described in mammals[13,17,93], although it has even been mentioned that both crests should be considered conotruncal because they continue longitudinally to the conus[16]. Both biological models mention the existence of intercalary crests, which, together with the truncal crests, participate in the formation of the leaflets of the arterial valves[13,92]. In addition, there are different proposals regarding the pattern of fusion of the truncal and intercalary crests, as well as the participation of the aorticopulmonary septum (APS) and conal crests. It is currently accepted that aorticopulmonary septation involves the participation of both the truncal crests and the APS, the latter being a contribution of non-cardiac cells.

Kirby et al.[7] discovered that APS formation requires a cell population that originates from the neural crest between the otic placode and the third somite through ablation experiments. The neural crest cells delaminate from the neural tube and migrate to the third, fourth, and sixth pharyngeal arches (Fig. 7A)[91,94], where they support the endothelium of the aortic arch arteries[95]. A neural crest cell subpopulation in the pharyngeal

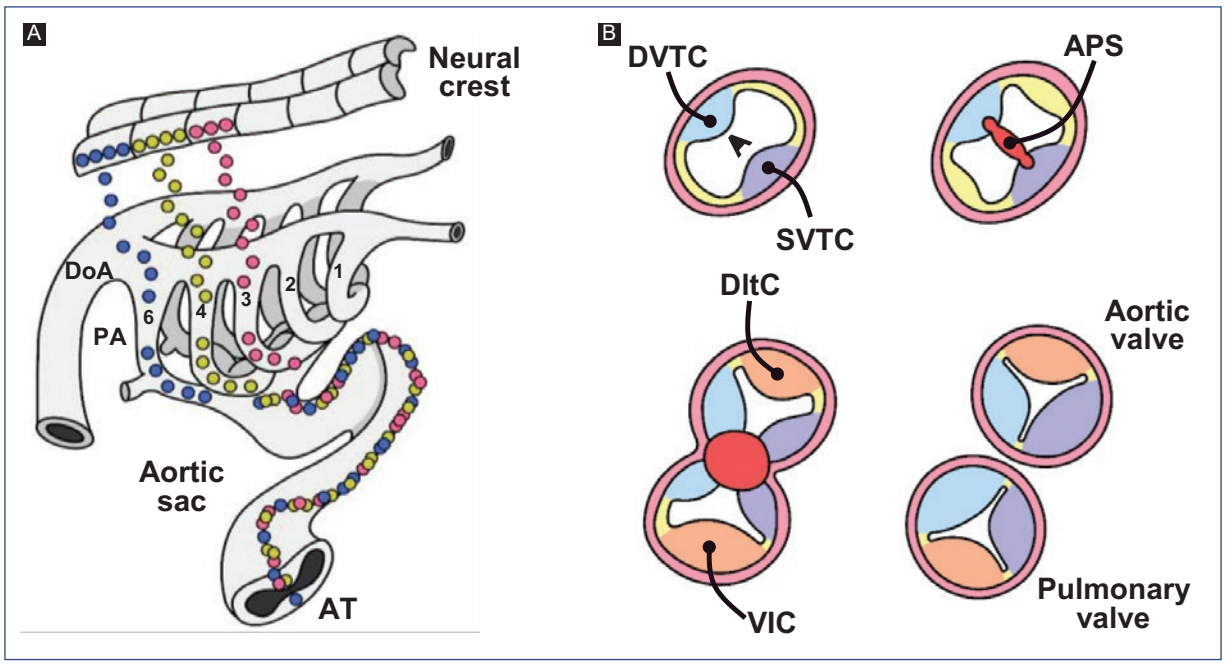

Figure 7. Aortic-pulmonary septation. **A:** migration of neural crest cells into the truncus (modified from Kirby and Waldo[96]). **B:** formation of the trunk of the great arteries, at the level of the arterial valves.
APS: aortic-pulmonary septum, T: Truncus, DInc: dorsal intercalated crest, DoA: dorsal aorta, DDoTc: dextro-dorsal truncus crest, PA: pharyngeal arches, SVTc: sinistro-ventral truncus crest, VInc: ventral intercalated crest.

arches continues to migrate through the aortic sac until settling in the truncus, where they invade the vascular border of the truncal crests and give rise to APS (Fig. 7)[78,96]. The truncal crests and APS initially function as a septum, dividing the aortic and pulmonary components; however, they will eventually lose this function and separate the truncus into the trunk of the great arteries (Fig. 7B)[92].

Neural crest cells express specific genes, including *FoxD3*, *Snai1*, and *AP-2*[97-100], induced by several signaling pathways such as BMP, FGF, Notch, and Wnt[101,102]. It is considered a pre-EMT stage, where the *Slug* promoter is activated, a SOX9-dependent event[103]. During delamination and migration, the action of the extracellular secreted signaling molecule WNT1 is considered essential[104,105]. *Wnt1* is expressed in early migrating cells, but expression declines rapidly as they reach their final destinations[106].

Conus remodeling

Over the years, the understanding of embryonic conus development has been misinterpreted by classical studies in human embryos and selective labeling experiments in chick embryos. It is widely accepted that the fusion of the right and left conal crests gives rise to two independent conduits, anterior and posterior; thus, the "anterior conus" would become the right ventricular outflow tract (RVOFT) and the "posterior cone" the left ventricular outflow tract (LVOFT)[13,16,20,107]. It has also been claimed that the conus is shortened longitudinally[11,16,17,20,108], and it has even been proposed that it disappears completely[108-110]. Similarly, it has been suggested that the conotruncus's shortening and rotation result from cardiomyocyte apoptosis[111,112]. However, Lazzarini et al.[31] describe that the spatiotemporal apoptotic pattern affects mostly the truncus, even suggesting that the myocardium of the conus of tubular structure loses continuity in its dorsal-left wall by an independent process of apoptosis and is transformed into a lamellar structure that corresponds to a large part of the anterior free wall of the right ventricle (Fig. 4E and F). Internally the cone crests fuse in their dorsal portions and participate in the formation of the RVOFT[31] (Fig. 6), an event that contrasts with classical selective labeling experiments[20]. It is currently suggested that the ventricular outflow tracts have distinct embryonic origins, the RVOFT originating in the conus and the LVOFT in the cushions of the A-V canal[31], descriptions that are consistent with the concepts of the FHF and SHF[4-6,29].

Final considerations

Cardiac development is a highly complex process that depends on the active and orderly contribution of different cardiac and non-cardiac cell populations. This complexity makes cardiogenesis sensitive to developmental defects, which tend to give rise to various congenital heart diseases. For this reason, it is essential to elucidate the events involved in cardiogenesis to improve the diagnosis and treatment of these conditions. In this review, we discussed the contrast between information from classic studies and recent findings that propose new models of heart development. Interestingly, research is now being conducted that studies cardiogenic events, which have been assumed to be understood since the last century.

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.

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The authors dedicate this work to the memory of Dr. Concepción Sánchez Gómez.

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ARTÍCULO DE REVISIÓN

El binomio causalidad-azar: ¿principio explicativo del conocimiento científico en medicina?

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Resumen

Este ensayo cuestiona las verdades matemáticas como principio explicativo del conocimiento científico médico. Se analiza, en primer término, el concepto de normalidad actual basado en una distribución de valores probabilísticos, y se destacan sus limitaciones y equívocos para captar la complejidad de la condición humana. Los sistemas cerrados (juegos de azar), origen de la teoría de las probabilidades y del binomio causalidad-azar, se comparan con los sistemas abiertos propios del proceso vital y se argumentan sus diferencias extremas. Se destaca el despropósito de depositar en el binomio causalidad-azar el significado de asociaciones entre sucesos propios de la complejidad de la vida humana en salud y enfermedad. Se confrontan las características de la causalidad mecanicista (puntual, lineal, unidireccional, homogénea y fija), que equipara al organismo con una máquina y es la única explicación científica aceptada del acontecer de la vida humana, con las de la causalidad contextual (difusa, heterogénea, jerárquica, multidireccional y cambiante), que especifica diversos órdenes causales interactuantes que dan forma a la condición humana: el histórico, el social, el político, el económico, el cultural o el biológico, que representa una mirada escrutadora y penetrante de la complejidad de los seres humanos. Se concluye la superioridad de la causalidad contextual sobre la mecanicista, que abre posibilidades explicativas de sucesos vitales que suelen arrumbarse como «efectos del azar». Esta aproximación integradora a la complejidad humana puede enriquecer y fortalecer el método clínico, hoy degradado y en riesgo de extinción.

Palabras clave: *Normalidad estadística. Sistemas cerrados. Binomio causalidad-azar. Sistemas abiertos. Causalidad mecanicista. Causalidad contextual.*

The causality-chance binomial: explanatory principle of scientific knowledge in medicine?

Abstract

This essay questions mathematical truths as an explanatory principle of the medical scientific knowledge. It analyzes, in the first place, the current concept of normality based on a distribution of probabilistic values and its limitations and mistakes to capture the complexity of the human condition are highlighted. The closed systems (gambling) origin of the theory of probabilities and the binomial causality-chance are compared with open systems typical of the complexity of the vital process, and their extreme differences are argued. The nonsense of depositing in the causality-chance binomial the meaning of associations between events typical of the complexity of human life in health and disease is highlighted. The characteristics

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of mechanistic causality (punctual, homogeneous, linear, unidirectional and fixed), which equates the organism with a machine and is the only accepted scientific explanation of events of human life, are confronted with those of contextual causality (diffuse, heterogeneous, hierarchical, multidirectional and changing), which specifies various interacting causal orders that shape of human condition: the historical, the social, the political, the economical, the cultural or the biological that represents a scrutinizing and penetrating look at the complexity of human beings. It concludes the superiority of contextual causality over mechanistic causality that opens up explanatory possibilities of the vital events that are usually put away as "effects of chance". This integrative approach to the human complexity can enrich and strengthen the clinical method that is now degraded and at risk of extinction.

Keywords: *Statistical normality. Closed systems. Binomial causality-chance. Open systems. Mechanistic causality. Contextual causality.*

«Recurrir al azar es "delegar" la explicación en lo inexplicable.»
El autor

Introducción

En las revistas científicas médicas se describen los métodos y los procedimientos de observación y de experimentación, y el análisis y la interpretación de los datos, utilizando las matemáticas para describir, analizar, sistematizar y validar los hallazgos, donde entran en juego diversas herramientas: concepto de normalidad, escalas de medición, teoría de las probabilidades o pruebas estadísticas, entre las más destacables. Todo esto nos revela que los números poseen un poder de persuasión incontrastable y sui géneris: se asumen como realidades o verdades contundentes, incuestionables; en el fondo, se trata de la creencia ancestral de ubicar en las matemáticas —«las ciencias exactas»— el auténtico soporte de toda verdad científica. Este trabajo pretende sembrar dudas acerca del valor incuestionable de las «verdades matemáticas» como fundamento explicativo del conocimiento científico en medicina, en especial la teoría de las probabilidades con sus conceptos de *causalidad* y de *azar,* alusivos a dos formas de relación entre sucesos.

El concepto de normalidad

Una vía de acceso a las entrañas del quehacer científico en medicina es el vocablo «normal», que tiene un uso extraordinariamente extendido, desde el lenguaje más vernáculo («es normal que así suceda») hasta sus acepciones técnicas más precisas («este resultado es anormal porque se encuentra dos desviaciones estándar por debajo de la media de la población estudiada»). Esta polisemia de lo «normal» plantea la necesidad de rastrear su significado a fin de especificar su utilización y así aproximarse a la *teoría de las probabilidades,* a la *causalidad* y al *azar*.

En la investigación médica, el concepto de normalidad representa el núcleo que da apoyo y fundamento a numerosas ideas, enfoques y métodos. Designa, de manera genérica, la dispersión de los valores de conjuntos de mediciones de moléculas, metabolitos, especímenes, pacientes, grupos o poblaciones, dentro de un cierto patrón de distribución o norma. Conviene precisar, de inicio, que el concepto *normal* con sus implicaciones modernas proviene de la noción de norma (jurídica), que se originó en parajes muy apartados del ámbito científico. En efecto, desde un punto de vista estricto, fue con el advenimiento de la Revolución Francesa y la aparición en el escenario histórico de un «nuevo sujeto jurídico» que surgieron las leyes y las normas en su sentido moderno y no como privilegios, directos o indirectos, de la divinidad[1]. La legislación tiene el fin de regular la convivencia entre personas en un marco de igualdad y libertad; son esas nociones de ley y de norma las que se diseminaron a diferentes campos de conocimiento, incluyendo las matemáticas y la biología.

Dada la relación entre el concepto de normalidad actual y la teoría de las probabilidades, en primer término se analizarán las condiciones de surgimiento de esta en el ámbito de las matemáticas, y a continuación algunas de sus aplicaciones en el campo de la medicina a través de los conceptos de causalidad y de azar. El propósito es incitar la reflexión y el debate sin pretender agotar el tema ni resolver todas las interrogantes. Los destinatarios de estas reflexiones son los que no están «tan seguros de sus certezas metodológicas», habida cuenta de que, en cuestiones conceptuales, la evidencia es más teórica que empírica.

Los prolegómenos de la teoría de las probabilidades pueden ubicarse en el siglo xvii, cuando la curiosidad de los matemáticos por los llamados «juegos de azar» inicia un largo devenir que llega hasta nuestros días.

Los nombres de Pascal, Fermat, Buffon, Huyghens, Bernoulli, Bayes, Laplace, Gauss, Poincaré, Frécher o Bourbaki[2] dejaron su impronta en ese derrotero, donde ocurrieron sistematizaciones, correcciones y reacomodos conceptuales, sin los cuales sería inconcebible la moderna teoría de las probabilidades. En lo tocante a «lo normal», hace su aparición en el continente de las matemáticas —sin olvidar que es una «exportación» jurídica— como resultado de la búsqueda incesante que, entre otros logros, desarrolla la teoría del azar y aporta criterios para la *predicción* de diferentes tipos de acontecimientos físicos, con base en la teoría de las probabilidades[3].

En contra de lo que se piensa, la curva normal gaussiana designaba, en un principio, la distribución que exhibían las mediciones repetidas de un mismo objeto, adoptándose el término «normal» para calificar ese patrón específico de distribución; es decir, los «errores» de medición se distribuían de un modo característico y simétrico[4]. Conforme se extendió la aplicación de ese patrón de distribución a las mediciones en otras ramas del saber, lo «normal» se empezó a utilizar para calificar la distribución de mediciones únicas de conjuntos de objetos; en otras palabras, el concepto originario que aludía a la forma de distribución de los valores de las mediciones de un mismo objeto fue sustituido por el de la distribución de los valores de las mediciones de múltiples objetos, y más aún, la distribución normal se consideró atributo de la medición de conjuntos de objetos de una misma clase. Así las cosas, esta «normalidad» se tomó como base de sustentación para entender los fenómenos biológicos, complementada con la estadística y la teoría de las probabilidades. Tal situación fue un poderoso impulso para que la mirada epidemiológica alcanzara predominio metodológico en detrimento de lo clínico o cualitativo, que ahonda en la individualidad.

En el campo de la medicina, lo «normal» era de uso corriente antes de su aparición en las matemáticas; en efecto, puede afirmarse que la noción de normalidad, con sus variantes, era equiparable a sano, y anormalidad a enfermo. Tales acepciones han persistido, si bien han sufrido diversas modificaciones para llegar a nuestros días; los cambios ocurridos se deben, en buena medida, a la introducción creciente de los conceptos matemáticos antes referidos en la investigación médica.

El concepto de normal está ahora asentado sobre bases estadísticas y probabilísticas; de esta manera, la anormalidad biológica es equiparable con una medición que se ubique fuera del rango de distribución que incluye el 95% de una muestra representativa de «sanos». En otros términos, el criterio de normalidad se hizo cada vez más dependiente del resultado de una medición; se concretó así el reduccionismo matemático como concepto clave de la práctica médica que, por lo mismo, ha perdido su carácter propiamente biológico. Este reduccionismo facilita la tarea de condensar, describir y precisar en números las observaciones (estadística descriptiva), pero funciona como cortina de humo si pretendemos una inteligibilidad profunda de las cualidades distintivas del proceso vital (ver más adelante).

Si en un principio se tenía una idea clara de que los métodos de cuantificación entrañaban variaciones que se distribuían normalmente, muy pronto tal evidencia dejó de representar un criterio relevante al interpretar los resultados de las mediciones. Así, la dispersión de los resultados de la medición de algún rasgo o molécula en un grupo de individuos se interpreta como variabilidad atribuible solo a los individuos; se pierde de vista que el cálculo de la media, la desviación estándar y la varianza del grupo estudiado incluyen no solamente las diferencias interindividuales, sino también las variaciones del método de medición. ¿Cómo arribar a conclusiones, sin la menor sombra de duda, a partir de ciertos resultados, sin haber considerado las variaciones del método utilizado? La literatura biomédica está saturada de datos, disquisiciones, discusiones, proposiciones o extrapolaciones que desestiman lo relativo a la precisión y la exactitud del método empleado* .

La investigación en el campo de la salud recurre, casi con exclusividad, al método epidemiológico, dejando cada vez más de lado el estudio a profundidad de los casos (método clínico). El uso generalizado de baterías de exámenes de laboratorio como principal recurso de detección de problemas potencialmente curables o prevenibles enfrenta la situación paradójica de que «la normalidad» de base probabilística y estadística fija un rango que engloba el 95% de los integrantes de cierto universo poblacional, y según este porcentaje, un sujeto normal (verdadero) tendrá solo una oportunidad de tres de tener resultados uniformemente normales en una rutina de 20 pruebas diferentes[5], debido a que el rango normal se determina independientemente para cada prueba (0.95^n). Así, suponiendo la aplicación a sanos de una sola prueba de laboratorio, el 95% será considerado normal; con

* Si bien esta variabilidad ha disminuido considerablemente con las actuales tecnologías de cuantificación, se trata de un fenómeno inherente a los métodos de medición.

cinco pruebas, el 77% (0.95^5); con diez determinaciones diferentes, el 60% (0.95^{10}), y con 20 solo el 35% (0.95^{20}) estará incluido dentro de la normalidad en todas las mediciones[6]. A tales equívocos cabe agregar la observación de la escasa utilidad del uso *rutinario* (independiente de lo sugerido por el estudio clínico) de los exámenes de laboratorio para detectar problemas nuevos no sospechados clínicamente[7].

Lo anterior revela lo inconveniente de la realización rutinaria de baterías de exámenes de laboratorio para «chequeos del estado de salud», que suelen arrojar datos «anormales» sin ninguna base clínica y que estrictamente corresponden a «ficciones de laboratorio». El desconocimiento de tales eventualidades obliga a repetir estudios o solicitar otros nuevos —igualmente innecesarios— para finalmente concluir, en el mejor de los casos, que no hay alteración alguna, con el consecuente desperdicio de tiempo y de recursos, y el malestar físico y psicológico de la persona que se somete a estos rituales estresantes para «cuidar de su salud».

Otro aspecto que se ha desestimado es la existencia de variaciones intraindividuales reales en la concentración de ciertas sustancias que no son atribuibles al método de medición[8-10]. El conocimiento de que los ritmos biológicos (ultradianos, circadianos, infradianos), la edad, el sexo o la etnia se asocian con diferentes patrones de fluctuación de diversas variables fisiológicas planteaba, desde mucho tiempo atrás, la necesidad de ponderar y reinterpretar algunos de nuestros supuestos que consideramos incontrovertibles; además, tal conocimiento constituye un motivo de peso para reorientar algunos de nuestros esfuerzos hacia el entendimiento de la individualidad biológica, psicológica y cultural que representa toda persona, sana o enferma. El asunto se vuelve más intrincado si consideramos que la medición de ciertas variables no tiene una distribución normal[11,12], lo que representa otra objeción al constructo de normalidad que ha sido soslayada en detrimento de los pacientes.

Lo argumentado pone en entredicho ese criterio rector del proceder médico. Si el interés es profundizar en el conocimiento de cada individualidad, es preciso reconocer que el criterio de normalidad que traza una línea de demarcación entre la salud y la enfermedad es inapropiado; en ausencia de una comprensión penetrante de la vida individual, difícilmente avanzaremos hacia mejores interpretaciones del significado de las mediciones en un sujeto o una población, y tomar decisiones pertinentes.

Causalidad y azar

El componente de las matemáticas que tiene una influencia decisiva en el devenir de las ciencias naturales y de la medicina es la teoría de las probabilidades, con los conceptos de *causalidad* y de *azar* que la acompañan. La teoría de las probabilidades constituye una poderosa herramienta de utilización universal; es difícil imaginar un campo del conocimiento en que no se utilicen las probabilidades, sin dejar de reconocer que los matemáticos, desde tiempo atrás, convencidos de la naturaleza impredecible de muchos fenómenos naturales, han desarrollado las «matemáticas del caos» (ver más adelante)[13].

Para entrar en materia, consideremos esta definición de *azar:* «fuerza de naturaleza desconocida u oscura a la que, por exclusión, se atribuye la aparición de ciertos sucesos». Recíproco de esta se encuentra la de *causalidad,* que alude a «un tipo de vínculo entre sucesos, con ocurrencia y secuencia temporal consistentes, que permite atribuir a uno la aparición del otro». Advirtamos cómo, indefectiblemente, la aceptación del azar lleva aparejada la de causalidad, y viceversa: en todo fenómeno en el que no se «comprueban» relaciones causales se invoca al azar, y este se disipa en cuanto la causalidad se verifica.

Todas las modalidades de investigación en medicina que utilizan métodos cuantitativos y estadística inferencial sustentan el significado de sus observaciones en los conceptos antes mencionados; es decir, en esta herramienta matemática se deposita el poder explicativo de los sucesos de la vida humana en salud y enfermedad. Sin embargo, un concepto no es un hecho, y *causalidad* y *azar* son ideas acerca del porqué y el cómo de fenómenos, al igual que existen otras al respecto, o sea, la dualidad causa-azar no es la única concepción posible ni la más penetrante (ver más adelante); lo que es obvio es que constituye la «explicación» que goza de exclusividad en este campo de indagación.

La primera objeción a tal exclusividad es que las leyes del *azar* se desarrollaron a partir de los juegos de azar, creados con la intención deliberada de suprimir la causalidad en su dinámica interna. Estos juegos pueden equipararse con lo que termodinámicamente se designa como *sistemas cerrados* —a cualquier intercambio de energía con el exterior—, que en los juegos de azar alude al cierre a cualquier influencia externa al sistema que pueda modificar su forma de operar. Son características de los juegos de azar:

- La supresión de la causalidad: «el azar en su máxima expresión».
- Su funcionamiento y posibilidades se establecen de antemano. Se anula así toda posibilidad de que surjan contingencias espontáneas no previstas que modifiquen su dinámica.
- Son heterónomos. Es decir, su movimiento no es espontáneo, depende de la intervención del exterior, y de ahí la tentación y la posibilidad de hacer «trampas» por parte del operador.
- Todo cambio intrínseco está excluido, y si llega a ocurrir es consecuencia de una acción humana exterior.
- Obviamente, carecen de lo que denominamos «medio ambiente». Son sistemas virtuales que existen cuando un operador externo los pone en funcionamiento.
- Son una creación humana, lo cual significa, entre otras cosas, que tienen una finalidad.

Estos sistemas, con características opuestas a los seres vivos, son los que dieron fundamento a la teoría de las probabilidades y a la moderna herramienta matemática de hoy día. Dicha teoría, en su complejidad y refinamiento actuales, conservó sus bases originarias: *el comportamiento del azar propio de los sistemas cerrados*. En cuanto a las pruebas estadísticas modernas aplicadas a la investigación médica, si bien no se derivaron de los juegos de azar, basan el criterio de *significación causal* en esa relación recíproca entre causalidad y azar†. El valor *p* expresa la probabilidad de que los resultados obtenidos al aplicar una prueba estadística sean atribuibles al efecto del azar. Convencionalmente este valor se ha fijado en 0.05, o sea, cinco probabilidades entre cien de que esos valores obtenidos se expliquen por el azar; tal proporción se considera con suficiente solidez para sostener que la relación causal es real. Así las cosas, se piensa que las leyes del azar propias de los sistemas cerrados tienen la misma aplicabilidad y análogo «poder explicativo» de sucesos del orden biológico, psicológico o social. Lo endeble de tal razonamiento se hará ostensible al caracterizar el proceso vital que equiparamos, para efectos comparativos, con los «sistemas abiertos», a sabiendas de que el concepto de sistema es de estirpe física.

† Debe diferenciarse la causalidad que incluye todo aquello que no pueda ser explicado por las leyes del azar de la que constituye un criterio metodológico clasificatorio. Por ejemplo, en los diseños de investigación clínico-epidemiológica, el experimento, la cohorte y los casos y controles, en este orden, se consideran de mayor a menor fuerza para sustentar relaciones causales.

Son características de los procesos vitales como «sistemas abiertos»:

- Una complejidad apenas imaginable, resultado de una dilatada filogenia donde lo aleatorio debió ser forzosamente neutralizado en gran medida para posibilitar el surgimiento de estructuras moleculares inéditas por su estabilidad antientrópica y poder replicante, que hicieron posible su persistencia, diversificación y evolución.
- Su cualidad autopoiética: se producen a sí mismos y se transforman por sí mismos[14].
- Sus posibilidades son mayores que todas las suposiciones teóricas sobre ellos: «hay en nosotros, en cada instante, muchas más posibilidades fisiológicas que las que dice la fisiología, pero se necesita la enfermedad para que se nos revelen»[15].
- Poseen su propio entorno: espacio de intercambio selectivo con los objetos significativos del medio ambiente que es, al propio tiempo, condición de su existencia y cualidad vital por excelencia[16]; es decir, están en perpetua interacción con el exterior, y de ahí su carácter «abierto».
- Permanentemente sufren cambios y oscilaciones cíclicas en su dinámica interna y en su organización, que son efecto de las características de sus interacciones (entorno), de los ciclos ambientales y de sus distintas fases etarias.
- Toda pretensión de controlar desde el exterior su funcionamiento es, las más de las veces, un esfuerzo insuficiente y en buena medida ilusorio.
- Su finalidad, el *para qué,* son suposiciones subjetivas propias de ideologías.

Esta caracterización destaca las diferencias de raíz entre los sistemas cerrados y los sistemas abiertos; no obstante, las ciencias médicas reconocen a la teoría de las probabilidades y a sus conceptos de causalidad y azar igual aplicabilidad, validez y poder explicativo en ambos sistemas. ¡Es como equiparar la dinámica de la ruleta o la lotería con los aconteceres vitales! Asumir que las leyes del azar propias de los sistemas cerrados son las mismas en los sistemas abiertos es un ejemplo típico de *reduccionismo* (matemático) que, lejos de aproximarnos al entendimiento de la complejidad, propicia la simplificación y oscurece su comprensión.

Hacia otra causalidad

La relación recíproca entre causalidad y azar, que son los cimientos de la teoría de las probabilidades, prefigura una forma de *causalidad mecanicista* propia

de los sistemas cerrados (máquinas), donde destacan varios atributos:

– Es *puntual,* con efectos muy circunscritos en el espacio y en el tiempo; evidente en investigaciones que buscan identificar la mayor cantidad posible de «factores» causales. Esta concepción fragmentaria, atomizada e inconexa del proceso vital propicia que los investigadores consideren que pueden aproximarse a la complejidad asociando uno a uno los diversos y minúsculos fragmentos identificados.
– Es *homogénea*. No existen jerarquías entre las diferentes causas posibles: los factores biológicos, los psicológicos o los sociales tienen, en principio, el mismo peso explicativo (causal). Aun los llamados análisis multivariados, que pueden asignar valores numéricos diferentes a las distintas variables estudiadas, hacen recaer en el número el criterio para diferenciar entre ellas. Si se obtienen valores similares, se considera que los diversos factores tienen la misma influencia, y se puede llegar al absurdo, por ejemplo, de atribuir a un factor psicológico cuatro veces más influencia que a uno social porque el riesgo calculado fue de 6.0 vs. 1.5.
– Es *lineal.* Las matemáticas que se utilizan se basan en relaciones lineales: los coeficientes de correlación, las regresiones lineales o las logísticas, son pruebas estadísticas sustentadas en las matemáticas lineales.
– Es *unidireccional*. Invariablemente, el sentido de la influencia se considera en una sola dirección: de la causa al efecto. Ni por asomo se plantea que los términos de esa relación se puedan invertir. El que en ocasiones se cambie de dirección al indagar del efecto a la causa (estudios de casos y controles, o *ex postfacto*), en nada cambia lo anterior: la causa y el efecto seguirán considerándose tales.
– Es *fija*. Una vez establecida la evidencia de una relación causal, suele permanecer y tomarse como supuesto para futuras indagaciones.

El concepto de *azar* también se inscribe en ese tipo de pensamiento de corte mecanicista, típico del empirismo reduccionista. Para este empirismo solo existe la explicación causal o por azar de los hechos observados; desestima las grandes teorías explicativas porque no son susceptibles de reducirse a esa causalidad de tipo puntual, lineal y unidireccional, y a las leyes del azar de los sistemas cerrados. Se infiere de lo dicho que para este empirismo tampoco tiene lugar la investigación teórica en la ciencia (como el presente ensayo), la cual consiste, de manera sucinta, en indagar en el universo de las ideas, confrontar planteamientos teóricos, sopesar sus alcances y limitaciones a fin de optar por el punto de vista más penetrante y esclarecedor, o proponer alternativas promisorias.

Para ejemplificar lo que la investigación teórica puede aportar y lo restrictivo del mecanicismo expresado en la teoría de las probabilidades, analicemos las llamadas «enfermedades de la pobreza», como la desnutrición, las enfermedades infecciosas y parasitarias gastrointestinales o la tuberculosis, que son estigmas de la miseria y la marginación que, a su vez, son efecto de la desigualdad social imperante que se genera porque los intereses de las minorías, en cada etapa histórica, han predominado sobre los de las mayorías. Este predominio persiste, a través de las épocas, porque los valores, las tradiciones, los hábitos y las costumbres que han tenido mayor presencia en los sucesivos escenarios sociales resultan permisivos para la preponderancia de esos intereses dominantes, manteniendo la desigualdad. En los tiempos modernos, ese predominio no ocurre por coerción física, como antaño, sino por medio de las diferentes instituciones estatales —las leyes, los tribunales, las dependencias del poder ejecutivo, las instancias del poder legislativo, las instituciones educativas, los medios de (des)información masiva— cuyos efectos ideológicos hacen aparecer el interés de pocos como el interés general de la sociedad. Ante tal perspectiva de causalidad —condensada por razones de espacio—, la explicación causal mecanicista aportaría más confusión que esclarecimiento.

Antes de abordar la *causalidad contextual,* una breve digresión. La ciencia moderna ha degradado la *causalidad,* soslayando el pensamiento fundacional aristotélico que reconocía su diversidad (causas eficiente, material, formal y final) al simplificarla y pulverizarla instituyendo la *causalidad mecanicista* como la única aceptable; de ahí lo inconveniente de recurrir a la *causalidad* para referirse a procesos diversos en tiempo y cualidad, intermitentes, que interactúan y originan *contextos*. Sin embargo, se conserva para facilitar su contrastación con la *causalidad mecanicista*.

La *causalidad contextual* reconoce las diferencias cualitativas que existen entre los diversos órdenes o esferas de la existencia humana: lo histórico, lo social, lo político, lo económico, lo cultural o lo técnico; estos, en interacción perpetua, configuran *contextos* en incesante cambio. Son características de la *causalidad contextual:*

– Es *difusa* y *diversa* en el espacio-tiempo. Sus influencias se deben a cierto tipo de atmósferas, de ambientes o de procesos, no a factores circunscritos. Las

influencias de lo histórico, lo social o lo cultural de ninguna manera son puntuales; sus efectos configuran formas de división del trabajo, ambientes de convivencia, tradiciones y costumbres, creencias, convicciones, sentimientos o ideas inveteradas.

- Es *heterogénea.* Al coexistir órdenes causales cualitativamente diferentes (social, psicológico o biológico), los efectos son también cualitativamente distintos. Los efectos biológicos no son equiparables a los psicológicos, y mucho menos a los sociales o a los históricos. Esto significa que las expresiones numéricas no pueden dar cuenta de la especificidad diferencial de los efectos de cada orden causal.
- Es *jerarquizada*. Como complemento de la heterogeneidad, se reconoce que algunos órdenes causales tienen efectos más penetrantes o de mayor duración, lo cual les confiere poder de subordinación sobre otros. Por ejemplo, el orden social subordina al psicológico o al biológico matizando sus expresiones. En la situación del médico que se esfuerza para que su paciente modifique ciertos hábitos y costumbres indeseables para su salud, el orden causal cultural (tradiciones arraigadas, que se resisten tenazmente al cambio) tiene mayor efecto que el técnico (recomendaciones para que cambie sus hábitos cual si se tratara de decisiones sobre las que el paciente tiene pleno control), para explicar los precarios resultados que suelen acompañar a este proceder del médico.
- Es *no lineal*. Cada orden causal es envolvente, serpenteante, y tiene, además de características singulares que lo especifican y lo hacen cualitativamente diferente de otros, su propia temporalidad. Las implicaciones del tiempo para un cambio histórico son distintas de las modificaciones en el orden biológico o técnico. Se entiende de lo anterior que las interacciones de diferentes órdenes causales no tienen relaciones lineales entre sí, ni en el espacio ni en el tiempo; de ahí la incongruencia de relacionar con matemáticas lineales lo social con lo biológico o lo psicológico.
- Es *multidireccional*. La influencia que va de la causa al efecto se invierte del efecto a la causa. Por ejemplo, los efectos del orden causal psicológico, modificados por poderosas tendencias del orden causal social, se revierten e influyen a su vez, matizando los efectos de lo social; de igual manera, un orden causal subordinado a otros de mayor jerarquía (el biológico vs. el social) modifica o matiza, al propio tiempo, los efectos del orden causal superior en la escala jerárquica. Por ejemplo, la resistencia o la menor susceptibilidad genética a ciertas enfermedades crónicas propias de la modernidad provocan efectos diferenciales (sujetos no afectados) en presencia de una atmósfera social que condiciona una alta prevalencia de aquellas.
- Es *cambiante*. Los efectos pueden volverse causas, y viceversa. Por ejemplo, la internalización temprana de sólidos principios morales (efecto) se convierte en causa en la adultez al influir en cierto contexto por una forma consistente de proceder ético. Además, constantemente surgen, en todo contexto, nuevas tendencias que influyen y modifican la constelación de causalidades interactuantes que conforman *contextos*. Por ejemplo, el surgimiento de una organización no gubernamental influye y es influida por el orden causal social, político y económico, contribuyendo a cambiar los efectos de la *causalidad contextual*.

Cuando una perspectiva de conocimiento incorpora diversos órdenes causales, constituye una aproximación transdisciplinaria a la inteligibilidad de una situación problema. En el ejemplo citado de las enfermedades de la pobreza se integran lo histórico, lo social, lo político, lo cultural y lo biológico en el entendimiento de una problemática de salud: la desnutrición y el cortejo que la acompaña. ¿Qué ocurriría si, en vez de una aproximación transdisciplinaria, nos ciñéramos a la *causalidad mecanicista* afincada en la teoría de las probabilidades? Encontraríamos que los efectos históricos, sociales, políticos o culturales serían prácticamente imposibles de encajonar en los estrechos moldes de esta causalidad, y por ende no podrían considerarse como causas «científicamente comprobables o comprobadas». En el mismo sentido, imagine el lector cómo operacionalizar variables como «sociedad pasiva», «ideología dominante», «división social del trabajo», «población redundante», «masificación», «burocratismo médico», «colonialismo», etc., para probar sus efectos y valorar su grado de asociación; aun en la remota posibilidad de construir variables complejas de cierta relevancia teórica, cómo pretender valorar, sin la menor sombra de duda, las influencias entre ellas con base en una idea de causalidad puntual, homogénea, unidireccional, lineal y fija para, posteriormente, ¡dar significado estadístico a los hallazgos por medio del binomio causalidad-azar! El absurdo de subordinar un enfoque epistemológico *transdisciplinario* al *mecanicista* y *reduccionista* salta a la vista.

Para abundar en lo anterior, analicemos un ejemplo: la búsqueda de relaciones causales lineales entre baja escolaridad y desempleo. Tal pretensión pierde de vista que la escolaridad es efecto, principalmente, de dos órdenes causales distintos: el cultural, que determina, entre otras cosas, el valor que se asigna al conocimiento, y el político, que explica la desigualdad y el nivel de ingresos. El desempleo, por otra parte, es fundamentalmente efecto de un orden causal económico globalizado, que determina la forma en que se organizan y desarrollan la producción y la comercialización de bienes y servicios, altamente tecnificadas —para competir por los mercados—, que requieren del trabajo intensivo de pocos y convierten en redundantes a crecientes contingentes de la población económicamente activa. Aun soslayando las consideraciones anteriores, es muy probable que en un estudio de esa índole se encuentre una asociación significativa entre baja escolaridad (causa) y desempleo (efecto), de lo que podrían derivarse recomendaciones y acciones para elevar el nivel de escolaridad en la población y disminuir el desempleo. Tal situación ha ocurrido y se trataría de acciones que, por sí mismas, tendrían escasa incidencia en el nivel de empleo (esto no supone desconocer la prioridad universal de elevar los niveles de escolaridad).

Es preciso insistir en que la lógica intrínseca de órdenes causales como el histórico (tradiciones), el social, el político y hasta el biológico es ajena al *mecanicismo* erigido como orden causal único. Incluso los matemáticos convencidos de la imposibilidad de predecir incontables sucesos de la naturaleza, no por limitaciones del conocimiento actual, sino por una cualidad intrínseca de dichos sucesos, han desarrollado alternativas como la teoría de las relaciones no lineales, las matemáticas del caos o de las catástrofes, y la geometría fractal[17-20]. No obstante, llevar estos desarrollos para entender la vida conllevaría el vicio de origen: matematizar un mundo cuya lógica intrínseca está en el otro polo del mecanicismo. El monopolio de la *causalidad mecanicista* deriva de su papel clave en la esfera de actividad humana instrumental y técnica de acciones deliberadas y controladas con fines predeterminados; fuera de tal ámbito resulta en una metáfora simplificadora que oscurece la inteligibilidad de la vida.

El evanescente concepto del azar

Ahora toca el turno del *azar:* «fuerza desconocida u oscura que provoca ciertos sucesos», que asociada a la de *causa* dio forma al binomio causalidad-azar. Si se confirman los criterios causales, se descarta el azar; en cambio, si no se cumplen, se invoca el azar como responsable. Este razonamiento que atribuye al azar un poder explicativo, que por supuesto no tiene, debe cuestionarse así se trate de la visión científica actual de los hechos «evidentes por sí mismos». Para tal efecto, primero especificaremos los contrastes entre los sistemas cerrados y los sistemas abiertos:

- En los sistemas cerrados, las leyes del azar (mecanicistas) no pueden ser subvertidas por influencias exteriores (salvo por un operador tramposo que manipula la causalidad a su favor en un proceso en el que está excluida).
- En los sistemas abiertos, ámbitos de la *causalidad contextual,* coexisten e interactúan diversos *órdenes* y *subórdenes causales.* La dinámica de algún orden es trastocada incesantemente por las influencias «exteriores» de otros órdenes; es decir, las perturbaciones («inexplicables») son inherentes al contexto de cada quien.

Deriva de lo previo que, al investigar una enfermedad bajo la óptica de la *causalidad mecanicista,* ante la presencia de sucesos «inexplicables» surgirá la necesidad de invocar el azar. Por el contrario, si al investigar se proyecta la *causalidad contextual,* los mismos sucesos pueden interpretarse como efectos de las interacciones de órdenes causales. Así, si nuestra perspectiva de aproximación e interpretación de sucesos es transdisciplinaria, lo atribuido al azar son «perturbaciones» provocadas por las interacciones incesantes de los órdenes causales involucrados. En otros términos, el azar propio de la *causalidad mecanicista* corresponde, en la visión transdisciplinaria de la *causalidad contextual,* a efectos provocados por esas interacciones de *órdenes causales* diversos. Unos ejemplos al respecto:

- Los médicos que se enfrentan a cierta enfermedad y disponen de estimados pronósticos sobre la esperanza de vida promedio pueden encontrar grupos de pacientes, semejantes en edad, factores de riesgo, apego al tratamiento y a las medidas higiénico-dietéticas, en los que unos fallecen prematuramente por la enfermedad y otros rebasan ampliamente la media de sobrevida, para lo que solo encuentra la «explicación» del azar según la *causalidad mecanicista;* sin embargo, si considera el *contexto* de esos pacientes, en particular el entorno psicosocial, puede aproximarse al esclarecimiento. En los de curso clínico desfavorable puede

«descubrir» un ambiente psicosocial adverso, con estresores intensos y persistentes, y soportes o apoyos precarios; en el caso opuesto, puede hallar estresores semejantes, pero soportes de bienestar robustos, diversos y consistentes. Lo anterior revelaría, según la perspectiva de la *causalidad contextual,* que un orden causal de mayor jerarquía, el *orden cultural* en su expresión psicosocial, influyó sobre el *orden causal biológico* en dos sentidos: 1) como ambiente deletéreo desencadenante o situación agravante de la enfermedad, o 2) como circunstancia protectora y atenuante de los estresores, que explicaría la evolución diferencial de la enfermedad y los contrastes observados entre los grupos.

– La observación de resultados contrastantes de intervenciones farmacológicas en pacientes con paralelismos en cuanto a edad, tiempo de evolución, gravedad y ambiente psicosocial. De un lado, el médico que suele propiciar atmósferas de empatía y respeto, al suscitar en ellos gran confianza y esperanza de mejoría, incita el *efecto placebo,* cuyos beneficios se suman a los farmacológicos (orden causal fisicoquímico) o contrarrestan los secundarios. Del otro, el médico reservado y escueto, que elude el plano afectivo de intercambio y se limita a los aspectos técnicos de la consulta, al «silenciar» el efecto placebo tendrá resultados menos efectivos, con mayores efectos secundarios y hasta contraproducentes[21]. Lo anterior ilustra cómo pacientes en análogas circunstancias (salvo el médico tratante) y que reciben el mismo tratamiento pueden tener efectos contrastantes en virtud de la ausencia o presencia del *efecto placebo* (orden causal psicobiológico).
– El último ejemplo alude al error en medicina, específicamente el que se atribuye a una negligencia. Podemos encontrar situaciones disímiles: unas donde el error sea particularmente frecuente y otras donde su incidencia sea sensiblemente menor aun en circunstancias análogas. La primera situación se favorece cuando en una institución de salud la actividad técnico-médica está subordinada al control impersonal, cuantitativo y burocrático impuesto por la administración[22]; en tales condiciones, cada acción debe justificarse con «un papel» aunque sea simulada. Así, cuando lo prioritario es cumplir con «el papeleo», los intereses de los pacientes pasan a un plano secundario, y de esta manera se propicia el descuido y se favorece la mala práctica. En tal situación, el orden causal institucional configura un orden técnico-médico proclive al error, lo que suele coincidir con un orden causal *social pasivo* ante la calidad de los servicios que recibe la población, lo que da lugar a una atmósfera social de impunidad ante las consecuencias, en este caso del error médico, que mantiene el burocratismo y un orden técnico-médico desvirtuado. Por contraste, en condiciones laborales y sociales análogas, algunos médicos con sólidos principios morales (interiorizados en etapas tempranas) pueden resistir el burocratismo y priorizar el cuidado del paciente, disminuyendo considerablemente la incidencia del error médico; aquí, el orden causal moral emerge para conferir reciedumbre al médico, lo que explica la «sobrevivencia» de comportamientos éticos a favor de la vida y de los intereses de los pacientes, en circunstancias adversas.

Estos ejemplos relativos al proceder de los médicos (tenues esbozos de la *complejidad* que entraña toda situación concreta) revelan, por contraste, la *simplicidad* y la pobreza explicativa de la *causalidad mecanicista,* de la teoría de las probabilidades y del binomio *causalidad-azar;* empero, permanecen como el único orden causal que considera el saber científico en medicina, inmerso en el *empirismo reduccionista* que propicia certidumbres engañosas y visiones cada vez más fragmentarias del organismo; de ahí el desinterés por captar la complejidad y por incursionar en el universo de las ideas explicativas y comprehensivas‡, todo lo cual implica «renunciar» al esclarecimiento de la condición humana, referente obligado de toda pretensión de penetrar las enfermedades crónicas que, salvo excepciones, no son desviaciones de «lo normal», sino acompañantes indefectibles de tal condición[23].

‡ Resulta paradójico que, por un lado, las ciencias médicas afirmen que su mayor aspiración es el entendimiento cabal del organismo y, por el otro, se afanen en «desmenuzar» *ad infinitum* lo que existe integrado, profundicen en el *micro* y el *nano* cosmos generando miríadas de hechos científicos comprobados, donde depositan «la verdad sobre la vida humana», soslayando la *complejidad* que es condición de tal entendimiento y marco interpretativo necesario para dar significado biológico a sus hallazgos. Cabe precisar que la *complejidad* no es un *problema empírico* que se resuelva asociando infinidad de variables simples; es un *problema teórico* cuya concienciación puede significar, para científicos sensibilizados, un reto ineludible que los lleve a la búsqueda de ideas explicativas y comprehensivas con poder esclarecedor de la condición humana, que es el objeto de conocimiento prioritario que se ha extraviado en el reduccionismo.

Epílogo

Lo precedente nos permite dar respuesta a la interrogante que encabeza el ensayo: es inconveniente que el binomio *causalidad-azar* sea el *principio explicativo* exclusivo al validar y dar significado a los «hechos científicos» sobre las causas de las enfermedades, porque ha relegado o vedado la búsqueda de la complejidad integradora, vía de acceso para desentrañar *quiénes somos*.

Contrapuesta a la *causalidad mecanicista,* que equipara la *simplicidad* de las máquinas con la *complejidad* de los organismos y detenta el monopolio explicativo de los hechos científicos, se propuso la teoría de la *causalidad contextual,* que reconoce la gran diversidad de *órdenes y subórdenes causales* (perspectiva transdisciplinaria), en perpetua interacción, que configuran los contextos de existencia propios de cada persona en «salud y enfermedad», que se expresan como procesos entrelazados con influencias difusas, heterogéneas, jerarquizadas, multidireccionales y cambiantes que configuran cada individualidad.

Antes de seguir, una precisión: la *causalidad mecanicista* y su binomio *causalidad-azar,* como marco analítico e interpretativo que valida el tipo de relación existente entre conjuntos de sucesos, donde la individualidad está diluida o es irrelevante, opera *a posteriori*. En cambio, la *causalidad contextual,* como mirada escrutadora y penetrante de individualidades, opera *a priori,* y al juzgar el significado biológico[24] de las asociaciones validadas con el binomio *causalidad-azar* opera *a posteriori*.

El concepto de *causalidad contextual* nos permite apreciar que la ocurrencia de sucesos de origen oscuro, que desde el *mecanicismo* son «efectos del azar», puede entenderse como manifestación de las *interacciones incesantes y cambiantes de órdenes causales cualitativamente distintos*. Al respecto, cada persona es un enclave singular de interrelaciones, donde se condensan e interactúan los más diversos *órdenes causales:* físico, químico, biológico, psicológico, político, económico, ecológico, histórico, moral y jurídico, configurando el *contexto* privativo de cada quien. Esta complejidad es aún mayor si consideramos que dentro de cada orden causal coexisten e interactúan subórdenes que amplifican, casi al infinito, la multiplicidad de influencias de la *causalidad contextual* que configura una inmensa variabilidad interindividual, a lo que se agregan las variaciones que surgen constantemente en el contexto de cada individuo en su devenir.

La construcción —así sea esquemática y simplificada— de una perspectiva transdisciplinaria de la *causalidad* permite captar que el azar opera como obstáculo epistemológico[25] al propiciar la convicción-ilusión de que ya probamos que se trata de «eventos debidos al azar», soslayando la búsqueda de auténticas explicaciones; el azar no explica, sino que encubre lo que deberíamos indagar y esclarecer. La *causalidad contextual,* con sus características, abre posibilidades explicativas de los aconteceres vitales de cada individualidad (las enfermedades crónicas, por ejemplo), en especial los inusuales que suelen atribuirse al azar. Esta mirada integradora podría enriquecer y fortalecer el método clínico, hoy degradado y en riesgo de extinción, al ampliar considerablemente su perspectiva de aproximación al doliente que lo lleve a diversificar sus ámbitos de indagación con fines de esclarecimiento, aproximando al clínico a captar la integridad compleja que tiene ante sí, a fin de incitar pertinentemente el cambio de ciertos hábitos deletéreos, jerarquizar e individualizar sus prescripciones, con mayor beneficio potencial.

Estos planteamientos están lejos de la añeja pretensión de un mundo determinista donde se encuentren las causas de todos los sucesos relevantes y sean predecibles; todo lo contrario, penetrar la inefable complejidad de la vida abre posibilidades de aproximación novedosas a todo aquel comprometido con su esclarecimiento. El carácter irreversible e impredecible de la vida, en todas sus formas y manifestaciones, no es óbice para una inteligibilidad profunda que dé bases más sólidas a una práctica médica empeñada en su superación y, por ende, en mayores beneficios a los pacientes, y dotarse de un marco interpretativo donde encuentren otros significados los hallazgos de sus investigaciones que lleven, en su caso, a replantear algunas prioridades de investigación impuestas por el orden dominante.

De regreso a la teoría de las probabilidades y su binomio causalidad-azar, que se estima como la quintaesencia de la explicación científica, podemos entrever que las matemáticas no son, necesariamente, el núcleo explicativo de todo campo de conocimiento. En la investigación médica, tales dogmas nos han llevado a ignorar la especificidad de efectos de lo histórico, lo social, lo económico, lo político, lo psicosocial y hasta lo biológico, obstaculizando el desarrollo de miradas transdisciplinarias[§] que representan aproximaciones superiores al proceso vital y brindan marcos

interpretativos apropiados para juzgar la relevancia de nuestras indagaciones que buscan penetrar tal proceso.

Ahora, respecto al propósito de este ensayo, «sembrar dudas acerca del valor incuestionable de las verdades matemáticas», el lector juzgará en qué medida, después de leer este texto, sus ideas al respecto han sufrido alguna modificación. Por nuestra parte, afirmamos que las teorías explicativas y comprehensivas que desde diferentes enfoques tienen como referente a la vida no pueden ni deben ser suplantadas por las teorías matemáticas, so pena de empobrecer el pensamiento y alejarnos en nuestro propósito de profundizar en el entendimiento de la complejidad de la vida como condición de todo verdadero progreso del conocimiento en nuestro campo de reflexión e indagación.

Para concluir, unos aforismos alusivos al conocimiento:

«No existen hechos, solo interpretaciones.»

F. Nietzsche

«Solo podemos conocer de los objetos lo que hemos depositado en ellos.»

I. Kant

«En el saber sobre la vida, no existen comprobaciones, solo aproximaciones.»

El autor

Responsabilidades éticas

Protección de personas y animales. El autor declara que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

§ Perspectivas teóricas de aproximación a la condición humana, que integran y jerarquizan aspectos teóricos de diversas disciplinas que disuelven su identidad, difuminan sus límites y dan lugar a nuevas síntesis que buscan penetrar y esclarecer el objeto de conocimiento (la *causalidad contextual* es una síntesis transdisciplinaria). No confundirlas con la *multidisciplina*, que es resultado de la conjunción de disciplinas donde cada una se suma para incrementar la comprensión del asunto en cuestión, ni con la *interdisciplina*, que consiste en puntos de contacto entre varias disciplinas que conservan su identidad, donde cada una aporta problemáticas o métodos para la resolución del problema identificado.

Confidencialidad de los datos. El autor declara que ha seguido los protocolos de su centro de trabajo sobre la publicación de datos de pacientes.

Derecho a la privacidad y consentimiento informado. El autor declara que en este artículo no aparecen datos de pacientes.

Conflicto de intereses

El autor declara no tener ningún conflicto de intereses.

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RESEARCH ARTICLE

Increased risk of hospitalization and death in Mexican children and adolescents with COVID-19 and comorbidities

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Abstract

Background: *Although COVID-19 (coronavirus disease 2019) in children is usually mild, they need hospitalization and intensive care in exceptional cases. Adverse outcomes have been observed mainly among children with comorbidities, justifying their vaccination. This study aimed to assess the risk of hospitalization and death in Mexican children and adolescents with COVID-19 and comorbidities.* **Methods:** *A cross-sectional study was performed on 366,542 confirmed COVID-19 cases under 18 years, reported by the Mexican Ministry of Health up to July 9, 2022. Logistic regression models were performed.* **Results:** *The mean age was 10.98 years, 50.6% were male, and 7.3% reported at least one comorbidity. The percentage of hospitalization and death in COVID-19 patients with and without comorbidities was 3.52%, and 0.20%, respectively; children with comorbidities presented a higher percentage of hospitalization (14.0%) and death (1.9%). The probability of hospitalization was 5.6 times greater in pediatric patients with COVID-19 and comorbidities, and the comorbidities that showed the greatest risk were immunosuppression (odds ratio (OR) 22.06), chronic kidney disease (CKD) (11.36), and cardiovascular diseases (5.66). The probability of death in patients with comorbidities was 11.01 times higher than in those without diseases, and the highest risk was observed in those with CKD (OR 12.57), cardiovascular diseases (6.87), and diabetes (5.83).* **Conclusions:** *Pediatric patients with comorbidities presented a higher risk of severe COVID-19. It is suggested that vaccination should be promoted with greater emphasis on pediatric patients with comorbidities.*

Keywords: *COVID-19. SARS-CoV-2. Comorbidities. Pediatrics. Hospitalization. Death.*

Riesgo incrementado de hospitalización y muerte en niños y adolescentes mexicanos con COVID-19 y comorbilidad

Resumen

Introducción: *Aunque COVID-19 (enfermedad por coronavirus 2019) en niños es usualmente leve, en casos excepcionales requieren hospitalización y cuidados intensivos. Los resultados adversos han sido observados principalmente en los niños*

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con comorbilidades, justificando su vacunación. El objetivo de este estudio fue evaluar el riesgo de hospitalización y muerte en niños y adolescentes mexicanos con COVID-19 y comorbilidades. **Métodos:** *Estudio transversal en 366,542 casos de COVID-19 confirmados, menores de 18 años y reportados por la Secretaría de Salud de México, hasta el 9 de julio del 2022. Se ejecutaron modelos multivariados de regresión logística.* **Resultados:** *El promedio de edad fue de 10.98 años, 50.6% de sexo masculino, y 7.3% reportaron al menos una comorbilidad. El porcentaje de hospitalización y muerte en pacientes con COVID-19 sin comorbilidades fue 3.52% y 0.20%, respectivamente; mientras que los pacientes con comorbilidades presentaron más elevados porcentajes de hospitalización (14.0%) y muerte (1.9%). La probabilidad de hospitalización fue 5.6 veces más en los pacientes con COVID-19 y comorbilidades, comparando con aquellos sin comorbilidades. Las comorbilidades que mostraron más riesgo fueron inmunosupresión (razón de momios (RM) 22.06), enfermedad renal crónica (ERC) (RM 11.36) y enfermedades cardiovasculares (RM 5.66). La probabilidad de muerte en los pacientes con comorbilidades fue 11.01 veces más que en aquellos sin enfermedades, y fue más elevado en aquellos con ERC (RM 12.57), enfermedades cardiovasculares (RM 6.87) y diabetes (RM 5.83).* **Conclusiones:** *Los pacientes pediátricos con comorbilidades presentaron mayor riesgo de COVID-19 severo, por lo que se sugiere promover con mayor énfasis la vacunación en ellos.*

Palabras clave: *COVID-19. SARS-CoV-2. Comorbilidad. Pediátricos. Hospitalización. Muerte.*

Introduction

Although children and adolescents have a lower risk of infection and severity of coronavirus disease 2019 (COVID-19) compared to adults, in the presence of comorbidities, the risk of developing more severe forms of this disease is higher[1]. Studies have shown that some underlying medical conditions such as asthma, immunosuppression, Type 1 diabetes, obesity, cardiovascular disease, congenital circulatory anomalies, neurodevelopmental disorders, anxiety or fear-related disorders, and depressive disorders have been related to an increased rate of fatal health outcomes[2,3].

Some studies have reported a low incidence of COVID-19 in children and adolescents around the world: from the total cases of COVID-19, the World Health Organization estimated that 8.5% were children aged under 18 years[4], UNICEF reported that 21% of cases occurred in individuals under 20 years[5], and a Mexican nationwide study reported that 2% were cases under 15 years[6].

In Europe, half of the children and adolescents with antibodies against SARS-CoV-2 have experienced symptoms; other studies in Latin America reported that most cases of young people were asymptomatic[7]. Hospitalization due to severe COVID-19 disease in children is rare, and the respiratory symptoms of these inpatients are more apparent than in infected children in the community. Fatality cases in hospitalized children are relatively low at 1%, compared to 27% across all other age groups[8]. Another study reported that < 5% of affected individuals are known to be children and <1% of those required ventilatory support[9].

Scientific evidence has shown that most children with SARS-CoV-2 infection have been asymptomatic or had mild COVID-19 symptoms, and few children are at risk of severe COVID-19; however, some children with comorbidities have had a severe illness and have shown a higher risk of death[10]. Although COVID-19 in children is usually mild, in exceptional cases, they can become seriously ill and need hospitalization and intensive care. One adverse outcome has been termed multisystem inflammatory syndrome in children (MIS-C), characterized by fever, severe inflammation, and multisystem disorders, causing approximately 1–2% of overall mortality[11]. These adverse outcomes have been observed mainly among children with comorbidities. This study aimed to assess the risk of hospitalization and death in children and adolescents with COVID-19 and comorbidities living in Mexico.

Methods

Databases and data extraction

This is a cross-sectional study carried out from the laboratory-confirmed COVID-19 cases reported by the Federal Ministry of Health in Mexico (MoH) through the anonymized and open-access COVID-19 database published by the Epidemiological Surveillance System for Viral Respiratory Diseases[12].

As of July 9, 2022, the MoH database registered 370,947 laboratory-confirmed cases of COVID-19 in people under 18 years of age, 4405 records with missing or unknown comorbidity or condition were excluded from the study. The following variables were extracted and assessed: sociodemographics, modifiable risk

factors, and other health conditions such as smoking and obesity, and diagnosis of non-communicable diseases (NCD): asthma, cardiovascular disease, chronic kidney disease (CKD), diabetes Type 1 and 2, hypertension, and immunosuppression. The main outcomes considered were the type of health care received (outpatient care vs. hospitalization) and death. The descriptors in the database did not define the classification method for comorbidities. The information was obtained through a dichotomous questionnaire that the pollster filled out with the information provided by the patient. Finally, the death variable was analyzed using the date of death.

This study did not require ethical review since it is based on open, anonymized data from the Mexican MoH. The database can be consulted at the following link: https://datos.gob.mx/busca/dataset/informacion-referente-a-casos-covid-19-en-mexico12.

Statistical analysis

Continuous variables were described using measures of central tendency. Categorical variables were described as percentages. The prevalence of COVID-19 cases without and with comorbidities was estimated from a specific population of interest: COVID-19 cases without comorbidities (n = 339,780), then multiplied by 100 and divided by the overall population (n = 366,542). Then, χ^2 test was performed to compare the percentages of patients with and without NCDs or modifiable risk factors between health outcomes of interest: hospitalization or death from COVID-19.

The likelihood of being hospitalized or death was assessed according to NCDs and to modifiable risk factors, estimating odds ratios (ORs) with 95% confidence intervals and their corresponding *p* values, using a multivariate logistic regression model adjusted by age, sex, and for each one of the comorbidities and risk factors analyzed. All statistical analysis was performed using Stata SE version 15.0 software (Stata Corporation, College Station, TX, USA).

Results

From the total 6,303,932 COVID-19 cases accumulated until July 9, 2022, the percentage of pediatric cases under 18 years was 6% (n = 366,542 analyzed in this study) and the average age was 10.98 years. Table 1 shows the general characteristics of Mexican children with COVID-19. From the total number of cases analyzed, the average age was 10.98 years, and 50.66% of the cases were males and most cases (54.57%) were reported by the MoH providers caring for the uninsured, followed by providers of the Mexican Institute of Social Security (IMSS, for its Spanish acronym) caring for social security beneficiaries (36.33%) (Table 1).

Table 2 shows that of the total studied population (n = 366,542), most cases had COVID-19 diagnosis only (92.70%), and 7.30% reported at least one comorbidity or risk factor. Regarding cases without comorbidities (n = 339,780), most were treated as ambulatory patients (96.50%), only 3.52% were hospitalized, and 0.20% died; in contrast, from the total cases with at least one comorbidity (n = 26,762), 86.0% were ambulatory patients, 14.03% were hospitalized, and 1.90% died. Regardless of the presence of comorbidities, the crude percentage of hospitalization was 4.29% (15,729 hospitalized/366,542 infected), and the percentage of death was 0.32% (1,191 deaths/366,542 infected).

Table 2 also shows the percentage of pediatric COVID-19 cases with comorbidities and risk factors across health-care services and the percentage of comorbidities by age groups. From the total cases with at least one comorbidity, the most frequent NCD was asthma (34.0%), followed by immunosuppression (6.48%), diabetes (5.15%), cardiovascular disease (4.83%), hypertension (4.01%), CKD (2.45%), and the most frequent modifiable risk factor was obesity (32.84%). Regarding specific comorbidity and health-care services used, COVID-19 cases with asthma, diabetes, cardiovascular diseases, hypertension, and obesity were significantly more treated as ambulatory cases than hospitalized ($p \leq 0.013$, in all cases); a significantly higher percentage of cases with immunosuppression were hospitalized (57.29%) compared with those treated as ambulatory patients (42.71%) ($p = 0.000$). Furthermore, CKD was the comorbidity with a higher percentage of death (7.61%), followed by cardiovascular disease (6.80%) and immunosuppression (6.63%). When comparing the percentage of death versus hospitalization by comorbidity, all comorbidities showed significantly higher percentages of hospitalization than death ($p = 0.000$).

Concerning the age groups, from the total study population, the group between 12 and 17 years old showed the highest percentage of COVID-19 (54.23%), and cases <5 years presented a higher proportion of hospitalization (14.57%) and death (1.19%) than other age groups (Table 2).

Table 3 shows the likelihood of hospitalization or dying among pediatric patients with comorbidities or

Table 1. General characteristics of children and adolescents with COVID-19 in Mexico (July 9, 2022)

Variables	Frequency	%
Total study population	366,542	100
Gender		
Female	180,868	49.34
Male	185,674	50.66
Health services providers		
Ministry of Health	200,031	54.57
Mexican Institute of Social Security, IMSS (for its Spanish acronym)	133,174	36.33
Private healthcare services	19,378	5.29
Institute for Social Security and Services for State Workers, ISSSTE (for its Spanish acronym)	5,087	1.39
Healthcare services for state employees	4,064	1.11
IMSS-Bienestar	1,852	0.51
Mexican Petroleum Company, PEMEX (for its Spanish acronym)	1,276	0.35
Ministry of the Navy, SEMAR (for its Spanish acronym)	482	0.13
Ministry of Defense, SEDENA (for its Spanish acronym)	462	0.13
Municipal government services	429	0.12
National System for the Integral Development of the Family, DIF (for its Spanish acronym)	138	0.04
University healthcare services	105	0.03
Red Cross	44	0.01
Not specified	20	0.01

risk factors compared to those without comorbidities. Pediatric patients with COVID-19 and NCD comorbidities had 5.65 times greater risk of hospitalization than those without any NCD diagnosis or no risk factors. Immunosuppression is the NCD that poses the greatest hospitalization risk (OR 22.06), followed by CKD (OR 11.36), cardiovascular disease (OR 5.66), and diabetes (OR 4.51). Patients with obesity also had a greater risk of hospitalization of 2.15. Regarding mortality risk, pediatric patients with NCDs and modifiable risk factors presented 11.01 times higher likelihood of death than those without comorbidities. The highest risk was observed in the case of CKD (OR 12.57), followed by cardiovascular disease (OR 6.87) and diabetes (OR 5.83), while asthma was found to be a significant protective factor against death (OR 0.50). Further, considering the odds of hospitalization and death by age groups: COVID-19 cases lower than 5 years old showed significantly higher probabilities of hospitalization (OR 5.45) or death (OR 7.63) than those between 5 and 11 years old. However, the age group between 12 and 17 years old showed a significantly lower probability of hospitalization (OR 0.66) and slightly and non-significant higher odds of death than cases between 5 and 11 years (1.10; p = 0.271).

Table 4 shows the odds of hospitalization in COVID-19 patients with comorbidities or modifiable risk factors by age group. In the group of patients under 5 years old, those with underlying comorbidity had an overall 4.59 times likelihood of hospitalization greater than those without comorbidities, and considering the odds by each comorbidity, the highest risk for hospitalization was observed for immunosuppression (OR 13.41) and cardiovascular disease (OR 6.52); patients between 5 and 11 years had an overall risk of hospitalization of 6.52 higher than those without comorbidities, and the highest risk of hospitalization was observed in patients with immunosuppression (OR 27.03) and CKD (OR 6.12); in the age group between 12 and 17 years, the overall likelihood of hospitalization was 5.70 times greater than in patients without comorbidities, and the highest risk was in immunosuppression (OR 28.27), CKD (OR 16.90), and diabetes (OR 6.71) (Table 4).

Table 5 shows the odds of death in patients with COVID-19 and comorbidities or risk factors by age group. Patients under 5 years had 7.79 times higher overall likelihood of death than those without comorbidities, and this likelihood was higher for cardiovascular diseases (OR 7.09) and CKD (OR 6.75); the group aged between 5 and 11 years had an overall higher risk of death of 14.50-fold risk of death than children without comorbidities, as well children with immunosuppression and CKD had 6.69 and 6.00 times, respectively, higher risk of death; finally, in the group of children between 12 and 17 years their overall risk of death was 11.36 times greater than in those without comorbidities, and the highest risk was observed in CKD (OR 19.57) and immunosuppression (OR 11.42) (Table 5).

Table 2. Percentage of comorbidities and risk factors by healthcare services used among Mexican pediatric patients under 18 years old with COVID-19

	Studied population ambulatory				Hospitalized			Death		
	n = 366,542	Percentage	n = 350,813	Percentage	n = 15,729	Percentage	p-value*	n = 1,191	Percentage	p-value**
COVID-19, diagnosis only	339,780	92.70†	327,805	96.50§	11,975	3.52§	0.000	681	0.20§	0.000
COVID-19 with at least one comorbidity or risk factor	26,762	7.30†	23,008	86.00‡	3,754	14.03‡	0.000	510	1.90‡	0.000
Comorbidities										
Asthma	9,097	34.00‡	8,659	95.18⁎	438	4.82⁎	0.013	17	1.23⁎	0.019
Immunosuppression	1,735	6.48‡	741	42.71⁎	994	57.29⁎	0.000	115	6.63⁎	0.000
Diabetes	1,378	5.15‡	1,107	80.33⁎	271	19.67⁎	0.000	49	3.55⁎	0.000
Cardiovascular disease	1,293	4.83‡	876	67.75⁎	417	32.25⁎	0.000	88	6.80⁎	0.000
Hypertension	1,074	4.01‡	885	82.40⁎	189	17.60⁎	0.000	45	4.19⁎	0.000
CKD	657	2.45‡	420	63.93⁎	237	36.07⁎	0.000	50	7.61⁎	0.000
Risk factors										
Obesity	8,789	32.84‡	8,190	93.18⁎	599	6.81⁎	0.000	84	0.96⁎	0.000
Age groups										
< 5 years	50,082	13.66†	42,786	85.43ϕ	7,296	14.57ϕ	0.000	594	1.19ϕ	0.000
5-11 years	117,689	32.11†	113,850	96.74ϕ	3,839	3.26ϕ	0.000	196	0.17ϕ	0.000
12-17 years	198,771	54.23†	194,177	97.69ϕ	4,594	2.31ϕ	0.000	401	0.20ϕ	0.000

p-value was estimated by means of Chi-squared test, comparing the percentage of COVID-19 patients (with and without comorbidities) regarding healthcare services use: ambulatory versus hospitalization manage, and hospitalization versus death**.
†Percentages were estimated from all COVID-19 cases, without and with comorbidities (n = 366,542).
§Percentages were estimated from COVID-19 cases without comorbidities (n = 339,780).
‡Percentages were estimated from COVID-19 cases with comorbidities (n = 26,762).
⁎Percentages were estimated from study population with a specific comorbidity: asthma (n = 9,097), immunosuppression (n = 1,735), diabetes (n = 1,378), cardiovascular disease (n = 1,293), hypertension (n = 1,074), CKD (n = 657), or obesity (n = 8,789), ϕPercentages were estimated from COVID-19 cases of each age group: <5 years (n = 50,082), 5-11 years (n = 117,689), or 12-17 years (n = 198,771), Some COVID-19 cases used more than one service, for this reason the frequencies in the first column (left) do not correspond exactly to the addition of the second column (ambulatory), third column (hospitalized), and fourth column (dead), CKD: chronic kidney disease.

Table 3. Adjusted odds ratios of hospitalization and death in pediatric patients with COVID-19

Comorbidity/health condition	Risk of hospitalization			Death		
	Adjusted odds ratio	95% CI	p-value	Adjusted odds ratio	95% CI	p-value
At least one comorbidity/risk factor*	5.65	5.42-5.89	0.000	11.01	980-12.37	0.000
Comorbidities*						
Immunosuppression	22.06	19.692-24.72	0.000	5.78	4.54-7.36	0.000
CKD	11.36	9.29-13.88	0.000	12.57	8.69-18.18	0.000
Cardiovascular disease	5.66	4.90-6.55	0.000	6.87	5.23-9.03	0.000
Diabetes	4.51	3.82-5.33	0.000	5.83	4.05-8.40	0.000
Asthma	1.30	1.17-1.45	0.000	0.50	0.29-0.85	0.010
Hypertension	1.06	0.85-1.32	0.583	1.80	1.19-2.74	0.006
Risk factors*						
Obesity	2.15	1.95-2.36	0.000	3.17	2.46-4.07	0.000
Age groups						
< 5 years**	5.45	5.23-5.69	0.000	7.63	6.49-8.98	0.000
5-11 years (Reference group)	1	-	-	1	-	-
12-17 years**	0.66	0.63-0.69	0.000	1.10	0.93-1.31	0.271

*Odds ratio in COVID-19 patients with at least one comorbidity or by each comorbidity/risk factor were compared to COVID-19 patients without comorbidities, and adjusted by sex and age.
**Odds ratio in COVID-19 patients of age group < 5 years and age group between 12 and 17 years were compared to patients of age group between 5 and 11 years old. Odds ratio were adjusted by sex and comorbidities. CKD: chronic kidney disease, CI: confidence interval.

Discussion

This study highlights that the percentage of total accumulated COVID-19 cases under 18 years old was 6%. From the total of this population, more than seven of each hundred cases also reported at least one comorbidity and presented a risk of hospitalization and death close to six-fold and over 11-fold, respectively, compared with cases without comorbidities. Immunosuppression, CKD, cardiovascular diseases, and diabetes were comorbidities that significantly increased the risk of hospitalization and death, and obesity was a modifiable risk factor that also increased the risk of severity of COVID-19. Moreover, considering the odds of hospitalization and death by age groups (Tables 4 and 5, respectively), the COVID-19 cases between 5 and 11 years old that reported at least one comorbidity showed the greatest risk of both outcomes of interest.

The Mexican pediatric population studied here showed a lower percentage of COVID-19 than other pediatric populations worldwide. The WHO data suggests that children under 18 years old represent 8.5% of the total COVID-19 cases in the world[4], UNICEF by July 2022 showed that from a total of confirmed cases of COVID-19 reported by 102 countries, 21% happened in patients under 20 years old[5]. In contrast, our pediatric population showed higher percentages of COVID-19 than other countries: in China, the percentage of laboratory-confirmed cases among children under 19 years of age was of 2%[13]; in England, between January to May 2020, pediatric cases under 16 years old were 4%[14]; in Italy, by March 2020, only 1% were children under 18 years of age[15]. Another Mexican nationwide study, updated in October 2020, also studied the pediatric population under 15 years old independently of comorbidities. This study showed a lower prevalence of COVID-19 (2%) but a higher prevalence of hospitalization and death (13.5% and 1.4%, respectively)[6].

Other countries have reported higher percentages of COVID-19 than our pediatric population: in Canada, by July 2022, among the population of children between 0 and 11 years old, the percentage of them with COVID-19 was 10.5%, and among those between 12 and 19 years was of 8.4%[16]; in the United States, between March 2020 and June 2022, among the population under 18 years old, the percentage with COVID-19 was of 15.5%[17]; further, from March to December 2020, from a total of laboratory-confirmed cases of COVID-19 among population between 0 and 24 years old: 57.4% of cases occurred in young adults aged from 18 to 24 years old, 7.4% in preschoolers (0-4 years), 10.9% in elementary school (5-10 years), 7.9% in middle school (11-13 years), and 16.4% in high school (14-17 years)[18].

Regarding severity and fatality health outcomes related to COVID-19 among children and adolescents,

Table 4. Risk of hospitalization in patients with COVID-19 and preexistence of comorbidity by age groups

Age group	COVID-19 only (n = 174,042)	At least one comorbidity	Immunosuppression	CKD	Cardiovascular disease	Diabetes	Asthma	Hypertension	Obesity
	OR	OR	OR	OR	OR	OR	OR	OR	OR
		(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)
< 5 years	1.00	4.59 (4.23-4.99; 0.000)	13.41 (10.39-17.30; 0.000)	3.22 (1.79-5.78; 0.000)	6.52 (5.18-8.21; 0.000)	1.21 (0.85-1.71; 0.285)	1.73 (1.34-2.22; 0.000)	1.03 (0.75-1.41; 0.850)	0.66 (0.49-0.89; 0.007)
5-11 years	1.00	6.52 (6.04-7.03; 0.000)	27.03 (22.57-32.38; 0.000)	6.12 (4.00-9.35; 0.000)	4.68 (3.41-6.43; 0.000)	5.94 (4.26-8.27; 0.000)	1.85 (1.57-2.18; 0.000)	0.84 (0.46-1.55; 0.580)	1.94 (1.59-2.36; 0.000)
12-17 years	1.00	5.70 (5.35-6.08; 0.000)	28.27 (23.86-33.49; 0.000)	16.90 (13.33-21.43; 0.000)	4.21 (3.18-5.57; 0.000)	6.71 (5.44-8.26; 0.000)	1.09 (0.92-1.29; 0.330)	1.20 (0.87-1.67; 0.271)	2.55 (2.27-2.87; 0.000)

Odds ratio were compared to the COVID-19 patients without NCDs comorbidities, adjusted by sex and age, COVID-19 diagnosis only was the category of comparison. CKD: chronic kidney disease, OR: odds ratio.

Table 5. Risk of death in patients with COVID-19 and preexistence of comorbidity by age groups

Age group	COVID-19 only (n = 174,042)	At least one comorbidity	Immunosuppression	CKD	Cardiovascular disease	Diabetes	Asthma	Hypertension	Obesity
	OR	OR	OR	OR	OR	OR	OR	OR	OR
		(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)	(95% CI; p-value)
< 5 years	1.00	7.79 (6.53-9.28; 0.000)	2.82 (1.82-4.37; 0.000)	6.75 (2.77-16.44; 0.000)	7.09 (5.12-9.81; 0.000)	2.79 (1.57-4.95; 0.000)	0.59 (0.19-1.90; 0.384)	2.80 (1.67-4.69; 0.000)	0.81 (0.41-1.62; 0.556)
5-11 years	1.00	14.50 (10.93-19.23; 0.000)	6.69 (4.11-10.89; 0.000)	6.00 (2.37-15.21; 0.000)	2.56 (0.93-7.02; 0.069)	4.39 (1.51-12.76; 0.007)	0.62 (0.22-1.71; 0.354)	2.63 (0.79-8.80; 0.117)	3.28 (1.82-5.91; 0.000)
12-17 years	1.00	11.36 (9.33-13.83; 0.000)	11.42 (7.92-16.46; 0.000)	19.57 (12.56-30.49; 0.000)	5.70 (3.21-10.11; 0.000)	8.35 (5.13-13.59; 0.000)	0.57 (0.29-1.15; 0.118)	0.54 (0.25-1.21; 0.139)	3.82 (2.82-5.18; 0.000)

Odds ratio were compared to the COVID-19 patients without NCDs comorbidities, adjusted by sex and age, COVID-19 diagnosis only was the category of comparison. CKD: chronic kidney disease, NCD: non-communicable disease, OR: odds ratio.

our findings are similar to other worldwide reports. By July 2022, UNICEF reported that, of the total deaths in 90 countries, 0.40% were in children and adolescents under 20 years[5]. In the United States, by December 2020, from a total of pediatric COVID-19 cases, 11.7% were hospitalized, and 3.6% presented severe illness[1]. In the international network cohort using European primary care records (France, Germany, and Spain), South Korean and US claims, and hospital databases between January and June 2020 among children and adolescents under 18 years old: from the total diagnosed cases, 4% were hospitalized[19]. In a US cohort of pediatric patients under 19 years old, 11.7% were hospitalized for COVID-19, and 31.1% experienced severe COVID-19, showing that patients with one or more chronic conditions presented 3.27 times higher risk of severe COVID-19 than those with none[1]. In a Mexican report from February 2020 to March 2021, the proportion of case fatality was below 0.3% in the population between 1 and 20 years old and 2.2% in infants under 1-year old[10]. Moreover, our results are inconsistent with what is reported in the scientific literature regarding the fact that the age group from 5 to 11 years old showed the highest probabilities of hospitalization and death than those cases under 5 years or those between 12 and 17 years old.

Regarding the comorbidities that most raised the risk of severity by COVID-19 among our Mexican pediatric population: asthma was the most prevalent comorbidity (34%); although immunosuppression was only prevalent in 6.48% of COVID-19 cases, this disease increased the risk of hospitalization by 22.06 times, and death by 5.78 times, compared to COVID-19 pediatric cases without comorbidities; the second most prevalent disease was obesity (32.84%), which increased the risk of hospitalization by 2.15 times, and death by 3.17 times; moreover, our findings also highlight that after immunosuppression: CKD, cardiovascular disease, and diabetes were the diseases that most increased the risk of hospitalization and death. These results are partially consistent with a cross-sectional study done in more than 900 US hospitals that included patients with COVID-19 aged 18 years or younger: 28.7% had underlying medical conditions, asthma being the most common (10.2%), neurodevelopmental disorders (3.9%), anxiety or fear-related disorders (3.2%), depressive disorders (2.8%), and obesity (2.5%). The main risk factors associated with hospitalization were type 1 diabetes (adjusted risk ratio (aRR) of 4.60) and obesity (aRR of 3.07); further, the risk factors for severe COVID-19 were type 1 diabetes (aRR, 2.38) and cardiac and circulatory congenital anomalies (aRR, 1.72)[2]. In a Mexican study with 1443 pediatric patients under 19 years old, 3.3% were admitted to the intensive care unit, 1.8% required assisted mechanical ventilation, and mortality was of 1.9%, where the main risk factors for mortality were pneumonia (OR of 6.45), intubation (OR of 8.75), immunosuppression (OR of 3.66), and cardiovascular disease (OR of 3.1)[3].

In this study, asthma was the most frequent comorbidity, significantly increasing the risk of hospitalization (OR 1.30; 95% confidence interval [CI] 1.17-1.45; p = 0.000), but in contrast, this comorbidity was related with a significant reduction in the risk of death (OR 0.50; 95%CI 0.29-0.85; p = 0.010). There is concern that asthma is a risk factor for developing severe COVID-19 and increases the risk of death in the pediatric population; however, some systematic reviews and meta-analyses have shown that asthma is not an independent factor that significantly increases the risk of hospitalization, care unit admission, or death in children and adult population[20,21]. In contrast, other studies have shown that asthma could be a non-statistically significant protective factor in preventing severe COVID-19. This may be explained by the fact that people with asthma receive treatments that favor low production of IFN-α, thus having a protective role of eosinophils in the airway as well as the immunomodulatory properties of inhaled steroids[22] and montelukast[23].

Compared with adults, the proportion of COVID-19 cases in the pediatric population has been lower, which could be explained by significant differences in the immune system. Children have a robust innate immune response, being the first-line defense against SARS-CoV-2, with more natural killer cells (NKC). In addition, children have 'trained immunity,' which involves epigenetic reprogramming of innate immune cells, including NKCs, following exposure to certain stimuli, including infections and vaccines, leading to 'memory'[24,25]. Children also have a higher proportion of lymphocytes and absolute numbers of T and B cells[26]. Another proposed immunological explanation is that children are less capable of mounting the pro-inflammatory cytokine storm, which plays an important role in the pathogenesis of severe COVID-19 and is responsible for multiorgan failure in critically ill patients[26-29]. In contrast to this theory, other studies have highlighted that hospitalized children with COVID-19 have higher serum levels of IL-17A and IFN-γ but not TNF-α or IL-6; therefore, children are not less prone than adults to develop a cytokine storm and ARDS[28].

The transmission of SARS-CoV-2 among children is a major concern. However, early studies suggest that

children, due to their milder symptoms, do not contribute much to the spread of SARS-CoV-2 since the risk of transmission from an asymptomatic individual with SARS-CoV-2 infection is less than the risk from a symptomatic individual[30]. In Norway, a prospective study showed a minimal transmission of SARS-CoV-2 among children and adults, finding a percentage of transmission of SARS-CoV-2 child-to-child of 0.9% and child-to-adult of 1.7%, supporting that people under 14 years of age are not the main carriers of SARS-CoV-2 transmission[31]. In this regard, a different expression of angiotensin-converting enzyme 2 (ACE2) receptor in children and adults has been proposed as a factor implicated in the reduced transmission and morbidity of SARS-CoV-2 observed at young ages. Furthermore, children have fewer ACE2 receptors in the respiratory tract than adults[32], and these are only in the upper respiratory tract; this could explain why young children are less susceptible to SARS-CoV-2 infection[33] and why they present less severe disease than adults[34].

Although the proportion of COVID-19 cases in pediatrics is lower than in adults, some infected children can also develop serious complications, such as MIS-C, which cause inflammation of the heart, lungs, kidneys, brain, skin, eyes, or gastrointestinal organs[35]. Thus, the vaccination of children is also justified as a strategy to reach herd immunity[36] and to avoid severe COVID-19. However, although Mexico's MoH has announced the vaccination against SARS-CoV-2 in children and adolescents, the Mexican National Health and Nutrition Survey for COVID-19 showed that the refusal and hesitancy to vaccinate against COVID-19 had been related to age, being more elevated among people of 60 and older (34.4% and 11.7%, respectively). However, these percentages had also been elevated among adolescents between 10 and 19 years (28.2% and 6.7%, respectively)[37]. The threshold of 65–70% of the population with flock immunity is the prerequisite to ending this pandemic, either through vaccinations or natural infection; however, widely circulating virus variants and vaccination indecision make this threshold challenging to reach. In addition, novel variants with increased transmissibility and enhanced immune evasion changed the herd-immunity calculation. From an epidemiological perspective, unvaccinated children could become the virus shelter when adults achieve immune protection, given that most COVID-19 cases in children are mild and asymptomatic[36,38].

The most important limitation of this study is that comorbidities collected in the Mexican COVID-19 surveillance system were also frequent diseases of the adult population[12], and specific health conditions in pediatric COVID-19 cases were probably underreported. In addition, the prevalence of confirmed COVID-19 cases found in this study could be underestimated since the pediatric population tends to be asymptomatic or shows mild symptoms of the disease[34]. On the other hand, Mexico has performed few tests to detect SARS-CoV-2: in May 2020, the Organization for Economic Cooperation and Development reported that Mexico only executed 0.6 tests per thousand inhabitants, while other countries performed a greater number of tests, such as Iceland (146.6), Luxembourg (75.8), Lithuania (52.0), Israel (45.4), and Portugal (41.9)[39].

In conclusion, although Mexican children and adolescents have presented much lower percentages of SARS-CoV-2 infection and fatality rates than adults, the pediatric population with comorbidities has presented higher percentages of fatality outcomes. Therefore, children and adolescents with comorbidities should be vaccinated to avoid risks and to prevent transmission of SARS-CoV-2 in schools or communities.

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 conflicts of interest.

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RESEARCH ARTICLE

How the training of ultrasonographers influences the certainty of prenatal detection of congenital malformations of interest to the pediatric surgeon

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Abstract

Background: *The training needed for doing obstetric ultrasounds is rarely reported. The aim of this study was to determine whether the training of the ultrasonographer influences the prenatal diagnostic certainty of some congenital malformations.* **Methods:** *We conducted a retrospective evaluation of antepartum sonographic findings of newborn infants found ultimately to have a congenital anomaly in a tertiary level pediatric reference center. Data were collected on admission for consecutive patients at a tertiary-level pediatric reference center. The mother´s pregnancy and birth demographic variables and those of the prenatal ultrasound (PUS) were analyzed and correlated with the final diagnosis.* **Results:** *Sixty-seven neonates were included. All cases underwent PUS with a mean of 4.6. Prenatal diagnosis was established in 24 cases (35.8%). Thirteen surgical anomalies were detected, particularly anorectal malformation and gastroschisis. The accuracy of PUS was associated with the training of the physician performing the PUS, whereby PUS with the greatest accuracy were performed by gynecologists and maternal-fetal specialists against radiologists and general practitioners (p = 0.005). Patients without an accurate prenatal diagnosis had a greater risk of presenting comorbidities (relative risk [RR]: 1.65, p = < 0.001, 95% confidence interval [CI]: 1.299-2.106).* **Conclusions:** *In our setting, prenatal diagnosis of these malformations is directly determined by the training of the person performing the ultrasound.*

Keywords: *Academic training. Congenital abnormalities. Observer variation. Prenatal diagnosis. Ultrasound.*

Cómo el entrenamiento del ultrasonografista influye en la certeza de la detección prenatal de malformaciones congénitas de interés para el cirujano pediatra

Resumen

Introducción: *Con poca frecuencia se ha reportado el entrenamiento necesario para realizar ultrasonido (US) obstétrico. El objetivo de este estudio fue determinar si el entrenamiento del ultrasonografista influye en la certeza del diagnóstico prenatal de algunas malformaciones congénitas.* **Métodos:** *Se llevó a cabo una evaluación retrospectiva de los hallazgos ultrasonográficos prenatales de neonatos que tuvieron malformaciones congénitas en un hospital de referencia pediátrico de tercer nivel. Se realizó al ingreso de neonatos consecutivos en un hospital de referencia de tercer nivel. Se recolectaron y analizaron datos del embarazo y alumbramiento, así como los de los ultrasonidos prenatales (USP) correlacionando con el diagnóstico final.* **Resultados:** *Se incluyeron 67 neonatos. Todos tuvieron USP con media de 4.6. Se realizó diagnóstico prenatal en 24*

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casos (35.8%). Se detectaron 13 malformaciones congénitas, predominando malformación anorectal gastrosquisis. La certeza del USP se asoció con el entrenamiento del individuo que realizó el US y la mayor certeza se encontró cuando lo realizaron ginecólogos y especialistas materno-fetales contra radiólogos y médicos generales (p = 0.005). Los pacientes sin diagnóstico prenatal certero tuvieron mayor riesgo de presentar comorbilidades (riesgo relativo [RR]: 1.65, p = < 0.001, 95% intervalo de confianza [CI]: 1.299-2.106). **Conclusiones:** *En nuestro medio, el diagnóstico prenatal de estas malformaciones está determinado directamente por el entrenamiento de la persona que realiza el ultrasonido.*

Palabras clave: *Entrenamiento académico. Malformaciones congénitas. Variación del observador. Diagnóstico prenatal. Ultrasonido.*

Introduction

Prenatal ultrasound (PUS) has significantly evolved in the last decades. In the late 80s, a multicenter randomized clinical trial conducted in the USA reported no apparent benefit of ultrasound (US) in women with low-risk pregnancies. A European trial conducted in the early 90s revealed that the routine US detected 73% of all major fetal malformations. The difference between these two studies hinged on the fact that the European study used trained sonographers, unlike the American trial[1]. Subsequent reports showed how PUS modified the age at birth in congenital malformations (CM)[2], and improved advice to parents, thus avoiding postnatal transfers[3]. PUS currently can detect many CM between the 11th and 14th week of pregnancy[4].

The PUS detection rate of the main CM requiring neonatal surgery varies between 31-100%[5-7] but does not specify the characteristics of the ultrasonographer. In our country, official regulations establish that diagnostic ultrasound (DUS) may be performed by physicians specialized in ultrasound or diagnostic imaging; other specialists must provide a certificate of specialization in DUS in the context of their specialty. A general physician trained to perform the diagnostic US, must have a certificate from an accredited institution and have coursed a minimum of one thousand hours of training in DUS[8]. Our hospital is a tertiary care referral pediatric center with no maternity services and the majority of CM that arrive at our hospital requiring neonatal surgery do not have an accurate prenatal diagnosis. We hypothesized that the low certainty of prenatal diagnosis is related to the training of the doctor performing the ultrasound, so this study aimed to determine whether the training of the ultrasonographer influences the diagnostic certainty of some congenital malformations.

Methods

This study was considered without risk for the patients, so it got a formal review and approval by the institutional research committee.

Setting: Tertiary-level pediatric reference center. We retrospectively evaluated antepartum sonographic findings of newborn infants found ultimately to have a congenital anomaly. Data collection was made on admission for consecutive patients between June 1, 2018, and May 31, 2019. The mother´s pregnancy and birth demographic variables as well as those of the prenatal ultrasound (PUS) were collected, analyzed, and correlated with the final diagnosis of the malformation established at our center.

Inclusion criteria: Neonates whose diagnosis upon admission included: anorectal malformation, esophageal, duodenal, or intestinal atresia, intestinal duplication, abdominal wall defects, diaphragmatic hernia, vascular malformations, and tumors.

Once a neonate fulfilled the selection criteria, the patient´s mother completed a questionnaire on her history of prenatal ultrasounds. When the mother was unavailable, the questionnaire was applied to the relative accompanying the baby during the transfer. The questionnaire included demographic information, the performance and findings of prenatal ultrasounds, and the level of care in the labour process. Upon the neonate´s admission, we documented: age at admission, a final diagnosis of the anomaly according to clinical, surgical, imaging, and pathology findings at our hospital, the correlation of the final diagnosis with the prenatal diagnosis, whether the prenatal diagnosis benefitted the patient by modifying the delivery plan, or whether the lack of a prenatal diagnosis led to an untimely transfer (after 2 days of age) or neonatal morbidities.

Statistical analysis

A descriptive analysis was made, and between-group comparative analysis was conducted with the Student's t or Mann-Whitney U tests in the case of quantitative variables, and categorical variables were analyzed by chi-square or Fisher´s exact test with a p-value < 0.05 considered significant. The effect size was quantified with Cramer's V, and relative risks (RR) were calculated if needed.

Results

Sixty-seven consecutive patients were included. The source of information was the mother in 86.6%. The

education level of informants was grade school (10.4%), middle school (49.2%), high school (26.8%), and college (13.4%).

The median maternal age was 23 years (IR: 8), 49.3% were primigravidae, and 97% had prenatal control, 71.8% of whom were at a primary care level. Most cases of prenatal care were managed by a general physician. All pregnant women underwent at least one prenatal ultrasound, with a mean of 4.6 ± 2.6 PUS during gestation, and 53.7% obtained at least one ultrasound per trimester (Table 1).

The US was performed by a radiologist (RX) in 47.8% of cases, an Ob-Gyn in 35.8%, a general practitioner (GP) in 13.4%, and 3% by a maternal-fetal medicine specialist (MFS).

Childbirth occurred in a secondary care public hospital in 61.2% of cases, in a tertiary care public hospital in 22.4%, and in a private hospital in 16.4% of cases.

Of the 67 neonates, 60% were male, the average weight was 2.7 ± 0.62 kg, the average height was 47.6 ± 3.2 cm, and the average gestational age at birth was 37.6 ± 1.9 weeks gestation (WG). Upon admission to our hospital, the median age was 1 (IR: 3) day, and the postnatal diagnosis of malformation was established at a median of 2 (IR: 1) days of life.

According to the final diagnosis, 13 surgical pathologies were detected; the most frequent was anorectal malformation (ARM), followed by gastroschisis (Table 2). In eight patients (11.9%), two associated surgical pathologies were documented: three patients had ARM + type III esophageal atresia, three patients had gastroschisis + intestinal atresia, one patient had omphalocele + intestinal atresia, and one presented duodenal atresia + type III esophageal atresia. These eight patients were classified by the first-mentioned pathology, which was also with the greatest probability of detection by PUS.

Table 1. Epidemiological data

Variable	Sub variable	n (%)
Gestation	Primigravidae	33 (49.3)
	Multigravidae	34 (50.7)
Prenatal control	Yes	65 (97)
	No	2 (3)
Level of care where the pregnancy was followed	Primary care level	48 (71.8)
	Secondary care level	14 (20.9)
	Tertiary care level	3 (4.5)
Training of whom controlled pregnancy	No control	2 (3)
	General practitioner	40 (59.7)
	Ob-Gyn	23 (34.3)
	Maternal-fetal specialist	2 (3)
Pregnant females with ultrasound in each trimester	First trimester	43 (64.2)
	Second trimester	60 (89.6)
	Third trimester	60 (89.6)

Ob-Gyn: obstetrician-gynecologist.

Table 2. Frequencies of diseases by final diagnosis

Postnatal diagnosis	n (%)
Anorectal malformation	15 (22.3)
Gastroschisis	12 (17.9)
Esophageal atresia	12 (17.9)
Bochdalek hernia	8 (11.9)
Omphalocele	6 (8.9)
Duodenal atresia	3 (4.4)
Intestinal atresia	3 (4.4)
Neck vascular malformation	2 (2.9)
Abdominal wall vascular malformation	2 (2.9)
Hepatic hemangioendothelioma	1 (1.4)
Cloacal exstrophy	1 (1.4)
Sacrococcygeal teratoma	1 (1.4)
Intestinal duplication	1 (1.4)
Total	67 (100)

Detection of congenital malformations

A malformation or abnormal finding by PUS was reported in 24 patients (35.8%), and the greatest percentage of structural anomalies was detected in the second (41.6%) and third trimesters (45.8%), with a median of 22.5 (IR: 17) WG. One ultrasound finding was reported in 19 cases and two findings were detected in five cases; the PUS diagnosis was correct in 17/19 patients with one finding. In the other two cases, US findings guided the diagnosis, so they were also classified as a correct diagnosis. In these five patients with two findings, the diagnosis was correct for both findings. Among patients with a prenatal diagnosis, 83.3% benefitted from the diagnosis since it modified the childbirth date or site.

The lack of a prenatal diagnosis occurred in 43 patients (64.2%) and led to complications in 17 (39.5%); the most frequent complications were intestinal necrosis in three patients, pneumonia in three, and sepsis in three, many due to delay in the suspected diagnosis or transfer to our center. A delay in transfer occurred in 27 patients (40.3%), 10 of whom had a prenatal diagnosis while 17 did not, without statistical significance.

Aside from the malformations of interest to be corrected by the neonatal surgeon, there were another 46 associated anomalies detected postnatally: heart disease (28) and renal (five) among others. Only two (4.3%) were correctly diagnosed prenatally. Five (7.4%) patients died, one patient with ARM suffered a delayed transfer, and intestinal perforation, dying because of sepsis; the remaining four deaths were unrelated to the CM.

Accuracy of prenatal ultrasound

For comparative analysis, we divided the patients into those with/without an accurate prenatal diagnosis. The variables associated with an accurate diagnosis were the training of the individual performing the ultrasounds, the training of the individual following the pregnancy, and the level of childbirth care (Table 3). The rest of the variables didn't show statistical association with the accurate diagnosis.

Some malformations such as duodenal atresia, arterio-venous malformations, cloacal exstrophy, and hepatic hemangioendothelioma were associated with an accurate prenatal diagnosis while intestinal atresia, the sacrococcygeal tumor, and the intestinal duplication, were not diagnosed prenatally ($p = 0.005$, Cramer's $V = 0.648$) (Table 4).

We did establish, however, that if there is no accurate prenatal diagnosis, there is a greater risk of developing complications ($p = < 0.001$, RR: 1.654, 95% confidence interval (CI): 1.299-2.106).

Discussion

Prenatal diagnoses and therapies have transformed the practice of neonatal surgery, allowing for the modification of the childbirth plan and, occasionally, fetal intervention leading to a better neonatal outcome[9]. Surprisingly, all our pregnant cases obtained at least one PUS, with a mean of 4.6, reflecting the available windows of opportunity to establish an accurate US diagnosis; however, the percentage of CM detection in our sample was low compared with other CM detection series[10-11].

Comparing our detection rates with the literature, we found some certainties better than the averages reported, like in duodenal atresia (100% vs. 50%)[12] and AVM (100% vs. 88.1%)[13], but there were few patients with each entity. Conversely, the detection rates were lower than those reported in ARM (6.7% vs. 87%)[14], esophageal atresia (16.7% vs. 31%)[5], jejunoileal atresia (0% vs. 50.1%)[15], abdominal wall defects (44.4% vs. 90%)[6,7], and diaphragmatic hernia (50% vs. 70%)[16]. Although there was statistical significance and a good effect size between the diagnostic accuracy of various pathologies or lack thereof (Table 4), these results may need to be revised, given the small number of cases, and a possible referral bias as our hospital is a tertiary-level national referral center.

In our country, government regulations on pregnancy care promote the prenatal detection of anomalies via a minimum of five consultations and at least one obstetric US per trimester[17]; in our study, 86.9% of women obtained a PUS during the second and third trimesters, and still, two-thirds of CM were undetected.

Although all of our patients had a CM, we found three statistically significant variables associated with an accurate prenatal diagnosis: when the pregnancies were followed by a gynecologist/obstetrician or FMS rather than a GP and also when the birth took place in tertiary care hospitals; more than a cause-effect we think that when a CM was detected, the patient was referred to tertiary care as previously reported[18,19]. The training level of the individual performing the PUS was another variable associated with diagnostic accuracy. The number of correct diagnoses increased if it was performed by an Ob-Gyn or MFS rather than an RX or GP; surprisingly, no GP with a US diplomate reached a single correct diagnosis, and radiologists were accurate in only 28.1% of cases. The size effect of this association was moderate (Cramer's $V = 0.440$).

Regarding the qualifications required by an individual that performs PUS to detect CM, Katerndahl stated 40 years ago that a "probable sonographer" must begin by clearly understanding longitudinal and transverse anatomy and possess the ability to modify the study *in situ*. At the time, the American College of Radiology proposed a short-term course for radiologists emphasizing the role of experience and the performance of many studies[20].

In 1999, Grandjean et al. reported a PUS sensitivity of 56.2% in the detection of CM, referring that the PUS had been performed by "qualified personnel, trained in

Table 3. Variables associated with prenatal accurate diagnosis

Variable	Sub variable	With prenatal diagnosis n (%)	Without prenatal diagnosis n (%)	p	Cramer's V
Training of who controlled the pregnancy	GP	11 (27.5)	29 (72.5)	0.047	0.307
	Ob-Gyn	11 (47.8)	12 (52.2)		
	MFS	2 (100)	0 (0)		
Training of who performed the PUS	GP with diplomate	0 (0)	9 (100)	0. 005	0.440
	Radiologist	9 (28.1)	23 (71.9)		
	Ob-Gyn	13 (54.2)	11 (45.8)		
	MFS	2 (100)	0 (0)		
Childbirth hospital level of care	Private hospital	2 (18.2)	9 (81.8)	< 0.001	0.644
	Secondary care public hospital	8 (19.5)	33 (80.5)		
	Third care public hospital	14 (93.3)	1 (6.7)		

GP: general practitioner, MFS: maternal-fetal medicine specialist, Ob-Gyn: obstetrician-gynecologist, PUS: prenatal ultrasound.

Table 4. Prenatal diagnosis related to each malformation

Variable	Sub variable	With prenatal diagnosis n (%)	Without prenatal diagnosis n (%)	p	Cramer's V
Congenital malformation	Duodenal atresia	3 (100)	0 (0)	0.005	0.648
	Hepatic hemangioendothelioma	1 (100)	0 (0)		
	Neck VM	2 (100)	0 (0)		
	Cloacal exstrophy	1 (100)	0 (0)		
	Abdominal wall VM	2 (100)	0 (0)		
	Intestinal atresia	0 (0)	3 (100)		
	SCT	0 (0)	1 (100)		
	Intestinal duplication	0 (0)	1 (100)		
	ARM	1 (6.7)	14 (93.3)		
	Esophageal atresia	2 (16.7)	10 (83.3)		
	Bochdalek hernia	4 (50)	4 (50)		
	Gastroschisis	6 (50)	6 (50)		
	Omphalocele	2 (33.3)	4 (66.7)		

ARM: anorectal malformation, SCT: sacrococcygeal teratoma, VM: vascular malformation.

high-quality equipment" without specifying what exactly they referred to[21].

In the first years of this century, reports on CM detection still failed to specify the sonographers´ training[22-26]. In 2010, the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) emphasized the need for specialized training in the practice of DUS in pregnancy and to establish the following qualifications: training in DUS and its related safety issues, regularly performing fetal ultrasounds and participating in continuous medical education activities[27-28].

In 2016, the American College of Obstetricians and Gynecologists and the American Institute of Ultrasound in Medicine established that the sonographers must be licensed in the practice of Medicine, understand the study´s indications, the complete obstetric US, and be familiar with the study's limitations[29].

In 2021, the Society of Radiographers in conjunction with the British Medical Ultrasound Society defined the "sonographer" as a healthcare professional possessing recognized qualifications in the medical US, capable in a competent manner, to perform the US within their field. The term "sonographer" instead of "ultrasound practitioner" refers to the lengthy training required for the professional certification to be protected under the law[30].

Our results show that, in our milieu, diplomate courses in the US for general physicians are useless in detecting CM in pregnancy. Government regulations are not always followed, and something must change for the diplomate courses to train adequately GPs in detecting CM or leave these specialized studies in the hands of more thoroughly trained personnel. It is pertinent to summon the directors of the medical schools/faculties so that they may implement the subject of ultrasound in their undergraduate training programs, as well as the councils and government agencies to review the current criteria to issue an ultrasound diplomate and certify sonographers.

One of our study´s strengths is its prospective data collection and its conduction in a national referral center. It is limited by the sample size and the fact that all patients harbor a CM, thus precluding the predictive values of PUS; the relation between the physician who followed the pregnancy and the individual who performed the PUS was not further investigated. A possible referral bias could explain the diagnostic accuracy in some pathologies. The study furthers the available information on individuals performing PUS training requirements.

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. This study involved a retrospective review of medical records, for which approval was obtained from a formally constituted review board (Institutional Review Board or Institutional Ethics Committee).

Conflicts of interest

The authors declare no conflicts of interest.

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RESEARCH ARTICLE

Clinical and paraclinical characteristics in pediatric patients with acute recurrent and chronic pancreatitis: a cohort in Mexico

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Abstract

Background: *Acute recurrent pancreatitis (ARP) and chronic pancreatitis (CP) are infrequent clinical entities in pediatric patients, as less than 8% of the literature mentions this population. This study aimed to describe the clinical and paraclinical profile, and the etiology related to patients with ARP and CP attended at a tertiary-level healthcare institute in Mexico.* **Methods:** *We conducted a retrospective study from medical records of patients with ARP and CP attended between 2010 and 2020, analyzing the clinical characteristics, imaging studies, and the etiology associated with each patient.* **Results:** *We analyzed 25 patients: 17 were diagnosed with ARP, and eight with CP. The main etiology identified was an anatomical alteration of the pancreatic duct (32%); pancreas divisum was the most prevalent condition. In 48% of the population, the etiology was not identified. The group with CP was higher in frequency for calcifications and dilation of the pancreatic duct ($p < 0.005$) compared to the ARP group.* **Conclusions:** *The main etiology for ARP and CP was an anatomical alteration of the pancreatic duct; however, in almost half of the cases, no established cause was identified. Although comparing our results with those offered by large cohorts such as the INSPPIRE group can be complex, we found relevant similarities. Currently, the data obtained from this first descriptive study are the foundation for future research in the field of Mexican pediatric pancreatology.*

Keywords: *Acute recurrent pancreatitis. Chronic pancreatitis. Pediatric patients.*

Características clínicas y paraclínicas en pacientes pediátricos con pancreatitis aguda recurrente y crónica: una cohorte en México

Resumen

Introducción: *La pancreatitis aguda recurrente (PAR) y crónica (PC) son entidades poco frecuentes en la edad pediátrica; sin embargo, menos del 8% de la literatura hace referencia a esta población. El objetivo de este estudio fue describir el perfil clínico, paraclínico y etiologías vinculadas en los pacientes con PAR y PC atendidos en una institución de tercer nivel de atención en México.* **Métodos:** *Se realizó un estudio retrospectivo de los expedientes de los pacientes con PAR y PC atendidos entre 2010 a 2020, analizando las características clínicas, estudios de imagen y etiologías asociadas en cada uno de los pacientes.*

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Resultados: *Se analizaron 25 pacientes, 17 con diagnóstico de PAR y ocho con PC. La principal etiología identificada correspondió a las alteraciones anatómicas del conducto pancreático (32%); el páncreas divisum fue la entidad más prevalente. En el 48% de la población no se pudo identificar una etiología. El grupo con PC presentó mayor frecuencia de calcificaciones y dilatación ductal pancreática (p < 0.005) en comparación al grupo de PAR.* **Conclusiones:** *La principal etiología de PAR y PC identificada en nuestro estudio corresponde a las alteraciones anatómicas del conducto pancreático; sin embargo, en casi la mitad de los casos, no se tiene una causa establecida. Aunque es complicado comparar nuestros resultados con los ofrecidos por las grandes cohortes del grupo INSPPIRE, sí encontramos similitudes relevantes. Los datos obtenidos en este primer estudio descriptivo son la base para futuras investigaciones en el ámbito de la pancreatología pediátrica mexicana.*

Palabras clave: *Pancreatitis aguda recurrente. Pancreatitis crónica. Paciente pediátrica.*

Introduction

Currently, most experts studying the pancreas in the pediatric age group are relatively conservative in searching for a cause when dealing with a single episode of acute pancreatitis (AP)[1]; however, a detailed investigation is advised in cases of acute recurrent pancreatitis (ARP) or chronic pancreatitis (CP)[2]. Because PAR and CP are relatively rare in the pediatric population, few studies provide data regarding these conditions[3].

Patients with ARP are considered to be at risk of developing CP over time. Although the diagnostic criteria are different, it is recognized that they share common etiologies[4]. The pathophysiology of recurrent episodes probably resembles that present in patients who experience a single episode; however, these patients may have additional genetic factors that increase the likelihood of developing ARP[5-7]. The following criteria must be met to establish the diagnosis of ARP[2]:

At least two different episodes of AP:

– Presenting complete resolution of pain between both events, with a minimum of 1-month duration between events or
– Complete normalization of serum pancreatic enzyme levels before the next AP event and complete resolution of pain symptoms, regardless of the time interval between the two episodes.

Furthermore, CP consists of an inflammatory process characterized by irreversible morphological changes, with fibrotic replacement of the pancreatic parenchyma resulting from repetitive or long-lasting inflammatory processes. Current theory suggests that CP begins with an AP condition that progresses to fibrosis, resulting from a continuous destructive process in susceptible individuals influenced by environmental and modifiers factors[2,8]. Many of these patients have a history of ARP prior to irreversible changes in pancreatic anatomy and function; however, some may present with diagnostic features of CP without having had a prior diagnosis of ARP. Since making a histopathologic diagnosis of CP at the pediatric age is uncommon, clinical criteria are considered more pragmatic in defining this condition. CP requires for diagnosis at least one of the following criteria[2]:

– Abdominal pain that suggests pancreatic origin associated with imaging findings suggestive of chronic pancreatic damage (irreversible structural changes such as focal or diffuse parenchymal destruction, sclerosis, and ductal abnormalities).
– Evidence of exocrine pancreatic insufficiency plus findings suggestive of chronic pancreatic damage.
– Evidence of endocrine pancreatic insufficiency plus findings suggestive of chronic damage.

As reported by the INSPPIRE consortium, children with ARP have more findings compatible with AP, i.e., inflammatory changes, edema, necrosis, and peri-pancreatic inflammation. In contrast, patients with CP present with persistent pancreatic lesions such as atrophy, irregular contour, heterogeneous texture, calcifications, irregularities, and defects of intraductal filling, calculi, strictures, or dilatations[9].

Although the disorders associated with these forms of pancreatitis are divided into several categories for study, the prevalence of these causes varies significantly among different studies. This appears to result from the inherent limitations of retrospective studies, the bias or experience of physicians caring for children with pancreatitis, incomplete investigations about causes, the greater number of patients recognized as having pancreatitis, and the recognition of new etiologies in childhood[6]. In a cross-sectional study conducted by the INSPPIRE group that included 155 patients diagnosed with ARP and 146 with CP, associated risk factors were sought. The risk factors were divided into four categories: genetic, obstructive, toxic/metabolic, and autoimmune. At least one risk factor was identified in 72% of patients with ARP and 86% of patients with CP.

The authors reported that the most common risk factors for the development of ARP or CP were genetic and obstructive[9].

As in patients who experience a single episode of pancreatitis, many with ARP have no identifiable cause for their disease. Because of this, they are recognized as having an idiopathic cause[6]. In addition, children with ARP or CP are often considered to have multiple risk factors. For example, in a study conducted by the INSPPIRE group, multiple risk factors of different categories were identified in 30% of patients with ARP and 27% of patients with PC, demonstrating the multifactorial nature of these conditions[9].

Pediatric patients with recurrent pancreatitis should be evaluated at least annually to identify early the development of pancreatic insufficiency. In the case of patients with CP, the recommendation is that they should be evaluated annually for both exocrine and endocrine pancreatic insufficiency[4]. The time course for transition to exocrine or endocrine failure is not clearly established. The timing of exocrine failure on the CP timeline spectrum is not fully known. The exocrine function may decline even before imaging findings are evident and thus may be a marker of disease changes[10].

Examining and analyzing a population of Mexican pediatric patients with rare conditions such as ARP or CP allows us to generate greater knowledge and provide relevant information. For this reason, this study aimed to describe the clinical and paraclinical profiles, and related etiologies of patients with ARP and CP treated at a tertiary care institution in Mexico.

Methods

We conducted an analytical and retrospective cross-sectional study of the records of pediatric patients seen at the Hospital Infantil de México Federico Gómez with a diagnosis of ARP and CP from 2010 to 2020. Following the cross-sectional observational study protocol, the STROBE checklist was used. Patients without a complete clinical record, imaging studies, or a history of pancreatic-biliary surgery prior to diagnosis were excluded from the study.

A targeted search was performed in the clinical archive of the institute under the search code "K86.1", corresponding to the diagnoses "recurrent pancreatitis", "repetitive pancreatitis," and "chronic pancreatitis". In addition, an electronic tool (PRC-2021) was created for data collection that covered the most relevant aspects of each individual: demographic, clinical, and radiological. All imaging studies used in the diagnosis and follow-up of patients were intentionally sought, mainly for findings suggestive of chronic pancreatitis. Furthermore, a search was made of the associated etiologies and diagnostic tests used in each case, which were classified as genetic, anatomical, metabolic, or autoimmune. Once the database was obtained, patients were grouped by diagnosis (ARP vs. CP) and by the age of onset (early vs. late), with a cut-off point at six years. Finally, complications such as exocrine or endocrine pancreatic insufficiency were analyzed in each patient.

Statistical analysis

A descriptive analysis of participant characteristics was performed using frequencies for dichotomous variables and medians, minimums, and maximums for quantitative variables. Tests of medians for quantitative variables and Fisher's exact test for nominal variables were performed to identify differences according to the type of diagnosis (ARP vs. CP) and age of presentation (early vs. late). A statistical significance level of $p < 0.05$ was considered. The SPSS V27 program was used for the analysis.

Ethical considerations

The present study was considered a risk-free research as it was a retrospective documentary research in which no intervention or intentional modification of the variables of the study's participants would be performed. Therefore, no letter of informed consent was requested. The confidentiality of the data and the anonymity of the participants were maintained.

Results

A population of 25 patients, who met the INSPPIRE diagnostic criteria were studied. The age range was between 5 and 24 years, according to the date of the last hospital visit. The distribution was similar between genders. The number of patients with ARP was 17 (68%) compared to CP, with eight patients reported (32%) (Table 1). All patients presented abdominal pain; in more than half of the cases, vomiting was reported as an associated symptom, followed by nausea, anorexia, and abdominal distension. In 64% of the patients, placement of a pancreatic prosthesis was required as a temporary measure to manage the inflammatory process and to evaluate the evolution of the

Table 1. General characteristics of the participants (n = 25)

	Median	(min-max)
Age (years)	14	(5-24)
Time of evolution (years)	6	
	n	**(%)**
Sex		
Male	12	(48)
Female	13	(52)
Diagnosis		
Acute recurrent pancreatitis	17	(68)
Chronic pancreatitis	8	(32)
Early onset (< 6 years)	9	(36)
Symptoms reported in acute events		
Abdominal pain	25	(100)
Nausea	7	(28)
Vomiting	16	(64)
Anorexia	2	(8)
Abdominal distention	4	(4)
Other	6	(24)
Etiology		
Genetic	1	(4)
Pancreatic duct alterations	8	(32)
Biliary tract anatomical alterations	3	(12)
Metabolic/Toxic	3	(12)
Cause not identified	12	(48)
Treatment		
Pancreatic prosthesis	7	(28)
Surgical	9	(36)
Complications		
Exocrine pancreatic insufficiency	2	(8)
Secondary diabetes mellitus	1	(4)

clinical picture. This measure was carried out to observe if the patient presented a complete resolution of symptoms or if surgical treatment needed to be considered after 24 months of follow-up.

Twelve percent of the study universe developed exocrine or endocrine pancreatic insufficiency. The main etiology identified corresponded to anatomical alterations of the pancreatic duct (32%); however, in 48%, the etiology could not be identified. *Pancreas divisum* was the most prevalent condition (Figure 1). Concerning genetic causes, only two patients were screened for associated mutations. Only one case of ARP was identified with the *CFRT* gene mutation. In three patients, serum IgG4 levels were requested to indicate autoimmune disease, reporting levels within normal ranges. In one adolescent patient, a fine needle pancreatic biopsy guided by endoscopic ultrasound was performed, reporting the histopathological study as an inconclusive

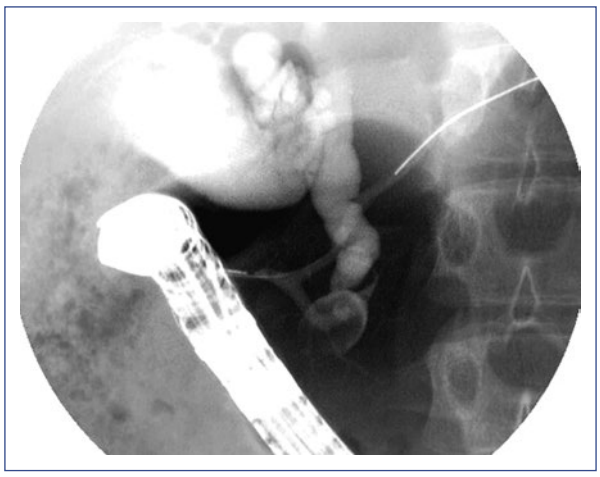

Figure 1. Endoscopic retrograde cholangiopancreatography (ERCP). The image shows a *pancreas divisum,* the course of the duct of Santorini continues with the dorsal duct. In the ventral portion of the pancreas a small duct of Wirsung is observed.

sample; no case of autoimmune pancreatitis could be documented. Metabolic/toxic causes were reported in 3/25 patients, two of them with the presence of hyperlipidemia, and one case associated with L-asparaginase.

The most frequent finding in the imaging studies of the group with CP was the presence of calcifications and pancreatic ductal dilatation (Figures 2 and 3). In patients with ARP, ductal dilatation and pancreas with a heterogeneous pattern were frequently reported.

No differences were identified in the clinical picture, treatment received, or complications (Table 2). In contrast, when comparing groups by the age of onset, there was a higher frequency of pancreatic prosthesis placement in the late-onset pancreatitis group compared to the early form. No other differences were identified between the groups (Table 3).

Discussion

ARP and CP are relatively rare in children; however, these conditions have been recognized more frequently in recent years. Reporting our institution's experience with this nosologic condition is useful to evaluate the current disease landscape in our country. Our results show similarities to those reported by the INSPPIRE group. An earlier presentation of the disease was observed in the ARP group compared to children with CP, similar to that reported by Kumar et al.[9], suggesting the relationship and continuity of the disease between both conditions.

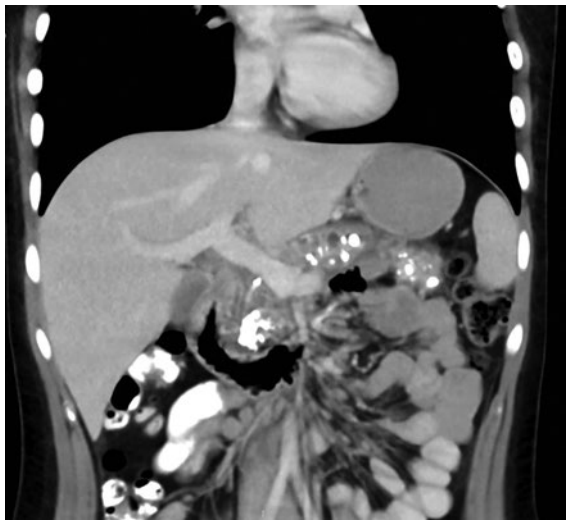

Figure 2. Chronic pancreatitis with calcifications. The CT image shows an enlarged pancreas, heterogeneous parenchyma, with multiple calcifications, poorly demarcated borders, and loss of peri-pancreatic fat.

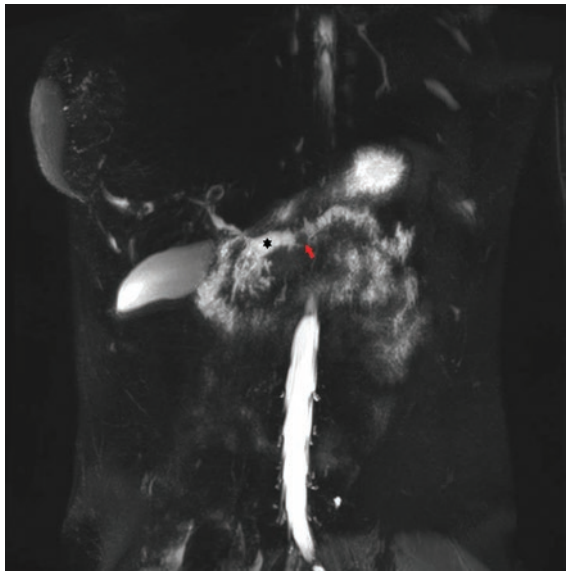

Figure 3. Ductal dilatation in chronic pancreatitis. In the image obtained by magnetic resonance cholangiopancreatography a dilated pancreatic duct of up to 10 mm is observed (asterisk), in addition there are areas of absence of signal in relation to a calculus (arrow).

Table 2. Differences between the participants according to diagnosis

	Acute recurrent (n = 17)	Chronic (n = 8)	p*
Age (years)[a]	13	16	0.031
Time of evolution (years)[a]	5	8.5	0.194
Episodes of pancreatitis[a]	4	4	0.549
Male sex[b]	9	3	0.673
Early onset (< 6 years)[b]	8	1	0.182
Image findings[b]			
Pancreatic atrophy	0	2	0.093
Calcifications	0	6	< 0.001
Heterogeneous pattern	3	5	0.061
Ductal obstruction or stenosis	0	1	0.320
Pancreatic ductal dilation	5	8	0.002
Etiology[b]			
Genetics	1	0	
Pancreatic duct alterations	3	5	0.061
Biliary tract anatomical alterations	3	0	0.527
Metabolic/Toxic	2	1	1.000
Cause not identified	8	3	0.673
Treatment[b]			
Pancreatic prosthesis	5	2	1.000
Surgical	4	5	0.087
Complications[b]			
Exocrine pancreatic insufficiency	0	2	0.093
Secondary diabetes mellitus	0	1	0.320

[a]Difference of medians.
[b]Fisher's exact test.

The INSPPIRE group consensus published by Gariepy et al[3]. noted that a large proportion of patients with these disorders had one or more underlying causes, suggesting the multifactorial nature of these diseases. This could not be observed in our study due to the limitations of the etiological approach, identifying only three cases with more than one associated causal factor.

In our series, a complete etiological approach was not performed in all patients; for example, it is noticeable the lack of identification of genetic alterations. However, we performed a complete anatomical study on all participants, including highly specialized studies such as magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography. In our study, anatomical alterations of the pancreatic duct were the etiological group most frequently found, with the *pancreas divisum* standing out among them. These data are similar to those reported in the large cohorts of the INSPPIRE group, such as those reported by Kumar et al.[9] and Wejnarska et al.[11], with up to 30% of their population presenting these anatomical alterations.

Table 3. Differences of participants according to age of onset

	Late-onset (n = 16)	Early-onset (n = 9)	p*
Age (years)[a]	15	9	0.500
Time of evolution (years)[a]	6	5	0.250
Episodes of pancreatitis[a]	4.5	4	0.250
Male sex[b]	7	5	0.688
Diagnosis[b]			
Acute recurrent pancreatitis	9	8	0.182
Chronic pancreatitis	7	1	
Etiology[b]			
Genetic	1	0	0.661
Pancreatic duct alteration	6	2	0.530
Biliary tract alteration	1	2	1.000
Metabolic/Toxic	2	1	1.000
Cause not identified	7	4	
Treatment[b]			
Pancreatic prosthesis	7	0	0.027
Surgical	5	4	0.671
Complications[b]			
Exocrine pancreatic insufficiency	1	1	1.000
Secondary diabetes mellitus	1	0	1.000

[a]Difference of medians.
[b]Fisher's exact test.

At present, the impact of genetic mutations as a risk factor for the development of ARP and CP is quite underestimated, as reported by Randall et al.[12] In our population, a genetic study was performed in only two patients, identifying the *CFRT* gene mutation in only one. This condition is of great relevance and corresponds to an area of opportunity for improvement in the management of these patients since a severe course of pancreatitis has been found to be mostly related to patients with these mutations[13]. Even an earlier progression to the chronicity of these conditions has been associated with genetic mutations, as Abu-El-Haija et al. reported in their cohort of patients with chronic pancreatitis[10,14]. With imaging studies, we identified a higher frequency of calcifications and ductal obstruction in patients with CP than in those with ARP. Other authors have reported these changes with reports of persistent pancreatic lesion changes in CP (atrophy, calcifications, and ductal irregularities)[9].

Autoimmune pancreatitis is currently recognized as a rare cause of recurrent pancreatitis in the pediatric age group. Large cohorts of patients have reported its presence in 3.9-15% of the population studied[13,15]. In our study, autoimmune pancreatitis was not reported, although we cannot rule it out reliably, given the complex workup required for establishing this etiology[16,17].

The main limitation we face when we want to have a complete study of these patients in whom autoimmunity is suspected is the absence of pancreatic tissue to perform immunohistochemistry. Therefore, obtaining a histological sample with a fine needle guided by endoscopic ultrasound would be the most appropriate method as it is considered the least invasive procedure.

Despite the limitations in the diagnostic approach in our population, we did not identify differences in etiology between the early-onset and late-onset groups. This also agrees with that reported by other authors, who found no significant differences in the distribution of etiological factors according to the age of onset of the disease[12].

In conclusion, the main etiology of ARP and CP identified in our study corresponds to anatomical alterations of the pancreatic duct; however, in almost half of the cases, there was no established cause. Patients with CP have more findings in imaging studies related to calcifications and pancreatic ductal dilatation; however, the risk factors associated with progression to ARP or CP, early or late presentations of the disease are not clear.

Although comparing our results with those offered by the large cohorts of the INSPPIRE group is complicated, we found relevant similarities. The data in this descriptive study provide the precedent and the basis for future research in the field of pediatric pancreatology in the Mexican population. This study allows to propose a comprehensive assessment of these patients by geneticists, pediatric surgeons, nutritionists, endocrinologists, and gastroenterologists to direct efforts on a better etiological diagnosis, follow-up, and timely medical or surgical treatment to avoid sequelae in the endocrine or exocrine pancreatic function.

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 approval from the Ethics

Committee for analysis and publication of routinely acquired clinical data and informed consent was not required for this retrospective observational study.

Conflicts of interest

The authors declare no conflicts of interest.

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RESEARCH ARTICLE

Factors associated with tuberculosis disease in children from a hospital in Western Mexico

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Abstract

Background: *This study aimed to describe the clinical and demographic characteristics of children with confirmed tuberculosis disease and identify associated factors.* **Methods:** *We conducted a retrospective and observational study at the Hospital Civil de Guadalajara Dr. Juan I. Menchaca. Inpatient and outpatient children under 18 years of age who were reported to the National Epidemiological Surveillance System (SINAVE, for its Spanish acronym) for suspected tuberculosis and who had molecular or microbiological tests for mycobacteria were included in the study. Multivariate analysis with logistic regression was used to analyze associated factors.* **Results:** *One hundred and nine patients under 18 years of age with suspected tuberculosis were included in the study. About 50.5% (55/109) were male, and the median age was 11 years. Tuberculosis was confirmed in 55% (n = 60): 15% (9/60) had a pulmonary infection, and the rest (51/60) had an extrapulmonary infection. The diagnostic tests used were histopathological study (n = 26), expectoration or gastric aspirate stains (n = 17), polymerase chain reaction (n = 12), and cultures (n = 5). Positive purified protein derivative (PPD) or interferon-gamma release assay (IGRA) tests were found in 33.9%. Malnutrition (odds ratio [OR] 15.9, 95% confidence interval [CI]: 2.3-109), and consumption of unpasteurized products (OR 7.45, 95% CI: 1.02-54.3) were associated with tuberculosis disease in children.* **Conclusions:** *Malnutrition and consumption of unpasteurized dairy products are associated with tuberculosis.*

Keywords: *Tuberculosis. Latent tuberculosis. Miliary tuberculosis.*

Factores asociados con enfermedad tuberculosa en niños de un hospital del occidente de México

Resumen

Introducción: *El objetivo de este estudio fue describir las características clínicas y demográficas de niños con enfermedad tuberculosa confirmada e identificar los factores asociados.* **Métodos:** *Se realizó un estudio observacional retrolectivo en el Hospital Civil de Guadalajara Dr. Juan I. Menchaca. Se incluyeron menores de 18 años hospitalizados y ambulatorios que se notificaron al Sistema Nacional de Vigilancia Epidemiológica (SINAVE) por sospecha de tuberculosis y que contaron con pruebas moleculares o microbiológicas para micobacterias. El estudio de los factores asociados se realizó mediante análisis multivariado con regresión logística.* **Resultados:** *Se incluyeron en el estudio 109 menores de 18 años con sospecha de*

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tuberculosis. El 50.5% (55/109) fueron de sexo masculino y la mediana de edad fue de 11 años. Se confirmó enfermedad tuberculosa en el 55% (n = 60) de los casos: el 15% (9/60) presentaron infección pulmonar y el resto extrapulmonar. Las pruebas diagnósticas utilizadas fueron el estudio histopatológico (n = 26), tinciones de expectoración o aspirado gástrico (n = 17), reacción en cadena de la polimerasa (n = 12) y cultivos (n= 5). 33.9% de los pacientes presentaron prueba de derivado proteico purificado (PPD) o ensayo de liberación de interferón gamma (IGRA) positiva. Se observó que la desnutrición (razón de momios (RM) 15.9, intervalo de confianza (IC) 95% 2.3 – 109) y el consumo de productos no pasteurizados (RM 7.45, IC 95% 1.02 – 54.3) se asociaron con enfermedad tuberculosa en niños. **Conclusiones:** *La desnutrición y el consumo de lácteos no pasteurizados se asocian con la enfermedad tuberculosa.*

Palabras clave: *Tuberculosis. Tuberculosis latente. Tuberculosis miliar.*

Introduction

In recent decades, global mortality due to tuberculosis has decreased by 31.4%[1]; however, in 2019, the disease caused 1.4 million deaths, and one-third were due to rifampicin-resistant bacteria[2]. In Mexico, the rate of tuberculosis varies from 8.5 to 13.8 cases/100 thousand inhabitants, and 24% of cases are children under 18 years of age[3]; in this age group, tuberculosis has a higher morbidity and mortality rate[4].

The demographic and social characteristics of the pediatric population in Mexico have changed in recent years, which could lead to epidemiological changes in infections such as tuberculosis. Between 1950 and 2020, residency in urban areas in Mexico increased from 43% to 79%[5]; in addition, chronic conditions such as obesity affect 35.6% of preschoolers and 40% of adolescents[6].

It has been reported that in children, the absence of bacillus Calmette-Guerin (BCG) vaccination, contact with bacilliferous patients, and a state of primary or secondary immunosuppression increase the risk of tuberculosis. However, it is pertinent to investigate other factors that are associated with a higher probability of infection.

This study aimed to describe the clinical and demographic characteristics of children with confirmed tuberculosis and compare those with children with discarded infections.

Methods

A retrospective and observational study was conducted at the Hospital Civil de Guadalajara Dr. Juan I. Menchaca (HCGJIM), Mexico. The institution is a referral hospital that serves the general population, mainly with limited economic resources.

In patients who meet the operational definition of a probable case of tuberculosis[7], the Department of Epidemiology is notified to report the case to the National Epidemiological Surveillance System (SINAVE, for its Spanish acronym). Simultaneously samples are collected for molecular, histopathological, and microbiological tests to identify bacteria of the *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum, M. microti, M. canettii, and M. caprae y M. pinnipedii*).

This study included inpatients and outpatients under 18 years of age who were reported to SINAVE from January 2015 to January 2020 and had diagnostic tests to identify mycobacteriae.

In samples of cerebrospinal fluid, sputum, abscess secretion or gastric aspirate, Ziehl Neelsen (ZN) staining, culture in Löwenstein-Jensen medium and, since 2018, automated polymerase chain reaction (PCR) (GeneXpert MTB/RIF®) were performed. Information about clinical and demographic variables was obtained from the clinical records.

For this study, the following definitions were considered:

Confirmed tuberculosis: The presence of signs and symptoms related to tuberculosis infection corroborated by identification of *M. tuberculosis* in stains, culture, or PCR. For extrapulmonary, lymph node, peritoneal, and intestinal infections, histopathological findings characteristic of tuberculosis (caseous granulomas or positive stains for acid-fast bacilli) was also considered confirmatory[7,8].

Probable tuberculosis: Clinical data suggestive of tuberculosis with negative stains, culture, or PCR; however, with exposure to a confirmed contact with tuberculosis or who showed radiographic studies suggestive of infection such as miliary infiltrate or pulmonary caverns[7,8].

Unproven tuberculosis: Patient admitted to the study who presented negative microbiological and/or histopathological tests for the detection of mycobacteria and who was not classified as a probable case[7,8].

Tuberculosis infection: Reactive tuberculin skin test (> 10 mm in immunocompetent patients or > 5 mm in immunocompromised patients) or positive interferon-gamma release assay (IGRA) test[7,9].

Malnutrition: In children of 5 years of age or older, malnutrition was diagnosed if the body mass index (BMI) was less than the 3rd percentile, and for children under 5 years of age, the presence of weight for height < 90%.

The definitions of other symptoms studied were as follows: chronic cough (daily cough lasting more than 4 weeks), adenomegaly (increase in lymph node size > 1.5 in a previously normal region), and fever (temperature > 38°C), with the rectal recording being the most appropriate; for this study, axillary measurement was also considered adequate. Gastrointestinal symptoms included abdominal pain, vomiting, diarrhea, abdominal distension, and constipation[10-12].

Statistical analysis

The frequency of tuberculosis in children reported to SINAVE was estimated. Frequencies and percentages were estimated for qualitative variables, and median and interquartile ranges (IQR) for quantitative variables. For the comparison of proportions to contrast hypotheses, we used a χ^2 test or Fisher's exact test, and for the comparison of medians, we used the Mann–Whitney U test. For the study of factors associated with tuberculosis (dependent variable), patients with a confirmed diagnosis were compared with those with unproven disease (patients with negative microbiological or molecular tests and who did not present suggestive radiographic data or contact with a bacilliferous patient). In a second phase, those with confirmed infection were compared with the rest of the patients, including probable cases. In both phases, bi-variate analysis was performed, and variables with $p < 0.2$ were subjected to multivariate analysis with logistic regression. IBM SPSS® Statistics version 25 was used. The project was approved by the Ethics and Research Committees of the HCGJIM.

Results

During the study period, 732 patients with suspected tuberculosis were registered in SINAVE, of whom 14.9% (109/732) were under 18 years of age. Among those included in the study, 50.5% were male, and 49.5% female; the median age was 11 years (minimum 0.16, maximum 17.0, IQR 11.5). 25.7% were younger than 5 years, and only nine were < 1 year.

About 55% of the patients were classified as having tuberculosis (n = 60), 19.3% as probable (n = 21), and 25.7% with unproven tuberculosis (n = 28). Among patients with the confirmed disease, 15% (9/60) had a pulmonary infection; the rest were extrapulmonary infections (27 lymph node, 12 intestinal, eight miliary, two meningeal, one event of articular, and one cutaneous tuberculosis). The tests that allowed confirming the diagnosis were histopathological study 43.3% (n = 26), sputum or gastric aspirate stains 28.3% (n = 17), PCR 20% (n = 12), and cultures 8.3% (n = 5).

About 33.9% (37/109) of pediatric patients had positive PPD or IGRA skin test results. This result was significantly more frequent in patients with tuberculosis than patients with unproven tuberculosis (43.3% vs. 3.6%, p = 0.006).

The most frequent clinical manifestations in those with confirmed tuberculosis were adenomegaly, fever, and weight loss; however, those symptoms were only observed in slightly more than half of the patients. Less frequent manifestations were asthenia and gastrointestinal symptoms (Table 1).

For the study of the factors associated with tuberculosis in the first phase, patients with confirmed tuberculosis were compared to those with unproven disease. The variables analyzed were sex, age, contact with a person with tuberculosis, BCG vaccination, consumption of unpasteurized dairy products, nutritional status, and human immunodeficiency virus (HIV) infection. In the bivariate analysis, the conditions associated with confirmed tuberculosis were malnutrition, consumption of unpasteurized dairy products, and BCG vaccination. However, in the multivariate analysis, only malnutrition and unpasteurized dairy consumption showed independent association (malnutrition: odds ratio [OR] 15.9, 95% confidence interval [CI] (2.3-109) p = 0.005; unpasteurized dairy consumption: OR = 7.45, 95% CI: 1.02-54.3 p = 0.04). The multivariate analysis compared patients with tuberculosis (dependent variable) and those with unproven disease.

The same factors were analyzed in the second phase, but children with confirmed tuberculosis and those with probable or unproven infection were compared. In the bivariate analysis, an association was observed with age < 5 years, positive PPD or IGRA, and malnutrition; however, in the multivariate analysis, only the last two were independent factors (Table 2).

The results of the PPD skin test or IGRA were compared with any of the methods considered confirmatory for tuberculosis (BAAR staining, culture, PCR, or histopathological study). It was observed that PPD and IGRA showed sensitivity, specificity, and positive and negative predictive values of 43.3%, 96.3%, 96% and 44.2%, respectively. Thus, negative results do not rule out infection, but the probability of tuberculosis infection was > 90% when the results were positive.

Table 1. Comparison of clinical and demographic characteristics of children with tuberculosis, probable tuberculosis, and unproven disease

Clinical and demographic characteristics	Tuberculosis (n = 60)	Probable tuberculosis (n = 21)	Unproven disease 28	p*
Clinical manifestations (%)				
Age < 5 years	18.3	33.3	35.7	0.6
Chronic cough	28.3	28.6	39.3	0.31
Asthenia or hypoactivity	28.3	23.8	53.6	0.01
Adenomegaly	55.0	14.3	42.9	0.29
Weight loss	50.0	42.9	46.4	0.75
Hemoptysis	1.7	0.0	3.6	0.58
Dyspnea/breathing difficulty	11.1 (6/54)	5.0 (1/20)	25.0 (5/20)	0.12
Fever	51.7	71.4	67.9	0.15
Diaphoresis	21.7	19.0	35.7	0.16
Intestinal symptoms	25.5 (15/59)	28.6	48.1 (13/27)	0.04
Positive PPD/IGRA	43.3	47.6	3.6	0.001
Diagnostic tests (%)				
AFB stains	28.3 (17/60)	0	0	-
Löwenstein-Jensen culture	8.3 (5/60)	0	0	-
PCR (GeneXpert MTB/RIF®)	20.0 (12/60)	0	0	-
Histopathological study	43.3 (26/60)	0	0	-
Characteristics included (%)				
Male	43.3	61.9	57.1	0.23
Age in years (median)	11.0	11.0	9.5	0.61
Exposure to a contact with TB	16.1 (9/56)	20.0 (4/20)	6.7 (1/15)	0.32
BCG vaccine	89.6 (52/58)	95.2 (20/21)	71.4 (20/28)	0.03
NP dairy consumption	51.2 (21/41)	46.7 (7/15)	20.0 (2/10)	0.07
Overweight or obesity	4.2 (2/48)	6.7 (1/15)	14.2 (4/28)	0.26
Malnutrition	72.9 (35/48)	73.3 (11/15)	32.1 (9/28)	< 0.001
HIV infection	5.0	9.5	3.6	0.76
Previous TB	3.3	0.0	10.7	0.7

*The P value corresponds to the comparison between patients with confirmed tuberculosis disease and those with unproven disease. In qualitative variables, the hypothesis contrast test was χ^2 or Fisher's exact and in quantitative variables Mann–Whitney U test.
AFB: acid-fast bacillus, BCG: bacillus Calmette-Guerin, HIV: human immunodeficiency virus, IGRA: interferon-gamma release assay, NP: non-pasteurized, PCR: polymerase chain reaction, PPD: purified protein derivative, TB: tuberculosis.

Discussion

In this study, we observed that 14.9% of the total number of patients registered in the SINAVE were under 18 years of age, and among these, 55% presented confirmed tuberculosis. Although culture is considered the reference test, it has limitations since it does not provide immediate results. Therefore, it is recommended to perform staining or PCR as well to establish timely diagnoses.

In adults, a higher risk of tuberculosis has been described in those with chronic degenerative diseases[13]; however, the risk is lower in those with obesity without diabetes[14]. Aibana et al.[15] reported that, in children exposed to adults with tuberculosis, 26.1% presented subsequent infection and noted that the risk was lower if the contact was an adult with obesity.

In HCGJIM patients, no association between overweight or obesity and tuberculosis was observed; however, similar to what has been described in different studies[16-18], a relationship between tuberculosis and malnutrition was identified. It has been described that poor nutritional status is the most frequent comorbidity in infected children (24.3%)[19]. Although being underweight may precede tuberculosis[16,19], weight loss may also be a consequence, given the chronic and progressive evolution of the infection.

At the HCGJIM, we observed that malnutrition was the only independent factor related to tuberculosis in the two phases of the study when comparing children with confirmed tuberculosis and those with probable or ruled-out infections. We also noted that in the initial analysis, the BCG vaccine had a lower frequency of application in uninfected children, but in the multivariate analysis, no association was found. We considered this a confounding effect of the variable and possibly attributable to an overestimation of the infection risk when clinicians were aware of the unvaccinated status or to the socioeconomic characteristics of our population studied.

Similar to the findings of this study, Cohn et al.[20] described a prevalence of tuberculosis of 20.8% in

Table 2. Comparison of clinical and demographic characteristics of children with confirmed tuberculosis and those with probable or unproven infection

Clinical and demographic characteristics	Tuberculosis (n = 60)	Probable tuberculosis or unproven infection (n = 49)	p*	OR (95% CI) Multivariate analysis
Clinical manifestations (%)				
Chronic cough	28.3	34.7	0.48	-
Asthenia or hypoactivity	28.3	40.8	0.19	-
Adenomegaly	55.0	30.6	0.01	-
Weight loss	50.0	44.9	0.6	-
Hemoptysis	1.7	2.0	0.88	-
Dyspnea/breathing difficulty	10.7 (6/56)	15.0 (6/40)	0.14	-
Fever	51.7	69.4	0.06	-
Diaphoresis	21.7	28.6	0.41	-
Intestinal symptoms	25.5 (15/59)	39.6 (19/48)	0.3	-
Characteristics included (%)				
Age < 5 years	18.3	34.7	0.05	0.43 (0.14-1.25)
Male	43.3	59.2	0.1	2.24 (0.85-5.91)
Exposure to a contact with TB	16.1 (9/56)	14.3 (5/35)	0.81	-
BCG vaccination	89.6 (52/58)	81.6 (40/49)	0.21	-
Positive PPD or IGRA	43.3	22.4	0.02	2.9 (1.04-8.39)**
NP dairy consumption	51.2 (21/41)	36.0 (9/25)	0.22	-
Overweight or obesity	4.2 (2/48)	11.6 (5/43)	0.18	0.59 (0.09-4.0)
Malnutrition	72.9 (35/48)	46.5 (20/43)	0.01	3.04 (1.11-8.34) **
HIV infection	5.0	6.1	0.8	-
Previous TB	3.3	6.1	0.49	-

*The p value corresponds to the comparison between patients with confirmed tuberculosis disease and those with unproven disease. In qualitative variables, the hypothesis contrast test was χ^2 or Fisher's exact and in quantitative variables Mann–Whitney U test.
**Statistically significant association.
BCG: bacillus Calmette-Guerin, CI: confidence interval, HIV: human immunodeficiency virus, IGRA: interferon-gamma release assay, NP: non-pasteurized, OR: odds ratio, PPD: purified protein derivative, TB: tuberculosis.

migrant children, the frequency of malnutrition was 29%, and consumption of unpasteurized milk (or dairy pruducts) was found to be associated with tuberculosis (OR: 3.2; 95% CI: 1.4-7.4)[20].

In Mexico, consuming unpasteurized dairy products or their derivatives is frequent. The results of this study highlight the importance of surveillance and control of zoonotic infections related to the consumption of these foods. In the country, up to 7.6% of tuberculosis cases are attributable to *M. bovis*; in states such as Jalisco, it can be up to 28%[21]. A study conducted by Escárcega et al. described that the prevalence of bovine tuberculosis was 15.1%[22].

Consistent with our results, Bapat et al.[23] observed that consumption of unpasteurized dairy products significantly increased the risk of tuberculosis[12]. Gompo et al. also noted an increased disease risk in those exposed to cattle[24].

O'Connor et al.[25] described human infections caused by *M. bovis* originating from species outside cattle. Other authors have emphasized that animal-related occupations increase the risk of tuberculosis[26,27].

The proportion of extrapulmonary tuberculosis varies from 9% to 78% and represents a challenge for the clinician because it requires invasive diagnostic methods. Diriba et al.[28] described that factors associated with extrapulmonary infections include age < 14 years, male sex, and consumption of unpasteurized milk. In HCGJIM patients, 85% of children with tuberculosis showed extrapulmonary involvement and predominately lymph node localization. This high proportion is likely related to the factors described or associated with the institution being a referral center for severe cases of the disease.

According to different guidelines for the diagnosis and treatment of tuberculosis in children[29], the skin test with PPD and IGRA should not be considered the reference tests for the diagnosis of latent infection since different conditions favor false negative or positive results; the recommendation is to consider clinical criteria, risk factors, and the history of BCG vaccination or exposure. The reference test for the diagnosis of tuberculosis is the culture of sputum, gastric aspirate, pleural fluid, cerebrospinal fluid, urine, or tissue biopsies. Nucleic acid identification tests are not a substitute for cultures but allow a rapid diagnosis.

Tuberculosis in children is at greater risk of progressing to severe forms such as miliary or meningeal tuberculosis.

Therefore, universal application of the BCG vaccine, detection and treatment of bacilliferous adults, surveillance and control of zoonoses associated with dairy products, and implementation of prophylactic treatment in children with latent tuberculosis are pertinent.

The limitations of this study are the small number of patients and the low sensitivity of microbiological and molecular tests to identify mycobacteria.

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. This study involved a retrospective review of medical records, for which approval was obtained from a formally constituted review board (Institutional Review Board or Institutional Ethics Committee).

Conflicts of interest

The authors declare no conflicts of interest.

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CLINICAL CASE

Myosin 1g as a high-risk biomarker in a pediatric patient with lineage switch from acute lymphoblastic leukemia to myeloid phenotype

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Abstract

Background: *Myosin 1g (Myo1g) has recently been identified as a potential diagnostic biomarker in childhood acute lymphocytic leukemia (ALL).* **Case report:** *We describe the case of a 1-year-old Mexican female patient. Although initially studied for hepatomegaly, an infectious or genetic etiology was excluded. Liver biopsy showed infiltration by neoplastic B-cell precursors (BCPs), and bone marrow (BM) aspirate showed 14.5% of BCPs. In a joint session of the oncology, hematology, and pathology departments, low-risk (LR) BCP-ALL of hepatic origin with aberrant myeloid markers was diagnosed. Although treatment was initiated, the patient presented early with BM relapse. Modest overexpression of Myo1g was observed from the onset. However, at the end of the steroid window, expression increased significantly and remained elevated during this first relapse to BM. The parents refused hematopoietic stem cell transplantation, but she continued chemotherapy. After a second BM relapse at 5 years of age, the phenotype switched to myeloid. Her parents then opted for palliative care, and the patient died two months later at home.* **Conclusions:** *This case shows the potential use of Myo1g in clinical practice as a high-risk indicator. Myo1g monitoring may reveal a high risk and relapse trend, even when typical parameter values are not altered: Myo1g could be used to classify patients from low to high risk from diagnosis, allowing patients to promptly receive the best treatment and potentially modifying prognosis and survival.*

Keywords: *Acute lymphoblastic leukemia. Lineage switch; Myosin 1g. Biomarker. Relapse.*

Miosina 1g como un marcador de alto riesgo en una paciente pediátrica con cambio de linaje de leucemia linfoblástica aguda a fenotipo mieloide

Resumen

Introducción: *Recientemente se ha identificado a miosina 1g (Myo1g) como un potencial biomarcador de diagnóstico en la leucemia linfoblástica aguda (LLA) infantil.* **Caso clínico:** *Se describe el caso de una paciente mexicana de 1 año de edad. Aunque inicialmente se estudió por hepatomegalia, se descartó una etiología infecciosa o genética. La biopsia hepática mostró infiltración por precursores de células B neoplásicas (PCB) y un aspirado de médula ósea (MO) mostró 14.5%*

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de PCB. En una sesión conjunta de los departamentos de oncología, hematología y patología, se diagnosticó PCB-LLA de bajo riesgo de origen hepático con marcadores mieloides aberrantes. Aunque se inició tratamiento, la paciente presentó tempranamente recaída de MO. Se observó una modesta sobreexpresión de Myo1g. Sin embargo, al final de la ventana de esteroides, la expresión aumentó considerablemente y permaneció elevada durante esta primera recaída a MO. El trasplante de células madre hematopoyéticas fue rechazado por los padres, pero se continuó con la quimioterapia. Tras una segunda recaída de MO a los 5 años, el fenotipo cambió a mieloide. Sus padres optaron entonces por cuidados paliativos y la paciente falleció dos meses después en su domicilio. **Conclusiones:** *Este caso muestra el potencial uso de Myo1g como indicador de alto riesgo en la práctica clínica. El seguimiento de Myo1g puede revelar una tendencia de alto riesgo y recaídas, incluso cuando los valores de los parámetros rutinarios son aparentemente normales; Myo1g podría utilizarse para clasificar a los pacientes de bajo a alto riesgo desde el diagnóstico, lo que permitiría que los pacientes reciban el mejor tratamiento de manera oportuna, modificando potencialmente el pronóstico y la supervivencia.*

Palabras clave: *Leucemia linfoblástica aguda. Cambio de linaje. Miosina 1g. Biomarcador. Recaída.*

Introduction

The class I myosins are a family of actin-dependent molecular motors involved in different functions[1]. Humans possess eight genes that code for these proteins (Myo1a-Myo1h) and are subdivided into short-tailed (Myo1a,b,c,d,g,h) and long-tailed (Myo1e,f) myosins[2]. Class I myosins have attracted interest because some function as tumor suppressors, and others are overexpressed in different types of cancer. We recently identified Myo1g as a potential diagnostic biomarker for childhood acute lymphoblastic leukemia (ALL). Myo1g is expressed exclusively in hematopoietic cells, with a high expression in B and T lymphocytes[3-6]. Myo1g generates the membrane tension required for T and B cell migration, endocytosis, exocytosis, and phagosome closure. We found that Myo1g is overexpressed at different stages of the disease, mainly at diagnosis, and proposed that Myo1g could serve as a marker of high risk and early relapse to contribute to improved patient survival[7].

ALL is the most common pediatric malignancy and accounts for at least 25% of childhood cancer[8]. Although relapse of ALL is frequent, immunophenotype change (lineage switch) rarely occurs at relapse[9,10]. Lineage switch accounts for 6-9% of relapse cases and is more frequent in pediatric patients[11]. Most reports of lineage switch arise from ALL to acute myeloid leukemia (AML) conversions[12-14]. However, the underlying mechanisms are poorly understood. Moreover, the prognosis for these patients is variable, and there is no standard treatment[15].

ALL immunotherapy with blinatumomab has been beneficial in treating patients with refractory or relapsed forms of the disease. Up to 20% of post-blinatumomab relapses are characterized by loss of CD19 from leukemic cells. Some CD19-negative relapses are accompanied by loss of all B-lineage antigens and acquisition of a distinct myeloid immunophenotype. The KMT2A/AFF1 rearrangement in B-ALL is an independent poor prognostic factor associated with a higher rate of treatment failure and an increased risk of linage switch under therapy[16,17].

Diagnosis can be difficult and delayed in the risk staging for atypical or early-stage cases according to the institutional protocol. Since we recently reported that Myo1g is a risk biomarker[7], we decided to follow this case to determine if Myo1g could aid in the diagnosis or follow the etiology of the lineage switch.

Here, we present the first case documented by immunofluorescence (IF) and qRT-PCR of the role of Myo1g in the clinical follow-up of ALL with lineage switch to myeloid phenotype.

Clinical case

We describe the case of a 1-year-old Mexican female patient admitted for hepatomegaly. Her parents were not consanguineous, and she was the youngest of three siblings from a humble community. She was delivered by cesarian section at nine months due to cephalopelvic disproportion (CPD) without complications. She weighed 3,700 kg and was hospitalized for five days due to hyperbilirubinemia. The complete neonatal screening was normal, but the vaccination schedule was incomplete. Physical examination at hospital admission showed left preaxial polydactyly, developmental delay (sitting up at 11 months), and hepatomegaly. No other relevant signs were observed.

Hepatic infiltration of B-cell precursors

In November 2013, different laboratory tests were performed, including blood biometry and serological

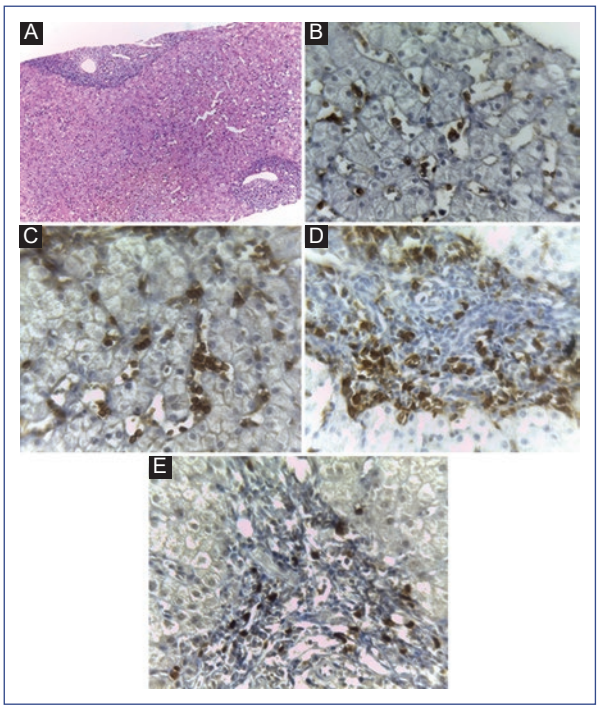

Figure 1. B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in liver biopsy. **A:** hematoxylin and eosin staining (40x). Immunohistochemical staining showed that the neoplastic cells were positive for **B:** CD10 (200×), **C:** CD19 (200×), **D:** CD20 (200×), and **E:** TdT (200x).

tests for TORCH, viral hepatitis, hepatovirus, and Epstein-Barr virus, showing no alterations. The results for Gaucher and Niemann-Pick diseases were negative.

Two days later, she developed a fever and was diagnosed with a fever of unknown origin. Thick blood drop study, BM culture, and complement were also normal. Imaging studies (computerized axial tomography) showed cervical and inguinal lymphadenopathy and hepatosplenomegaly. A liver biopsy showed infiltration by B-cell precursors, CD10 +, CD19 +, CD20 +, and TdT + neoplastic cells (Figure 1). Blood biometry was performed again. The white blood cell count (WBC) was 20,400/μL with 29% neutrophils, 50% lymphocytes, 4% monocytes, 0% eosinophils, and 6% blasts. Hemoglobin was 10.7 g/dL, and platelet count was 77,000/μL (Table 1).

Risk staging

According to the institutional protocol and the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, the patient's conditions indicated a

Table 1. Laboratory, immunophenotypic, and genetic findings

CBCC	ALL diagnosis	First relapse	Second relapse
WBC	20,400/μL	31,800/μL	43,000/μL
Blasts	6%	15%	18%
Segmented	29%	21%	32%
Eosinophils	0%	0%	3%
Basophils	0%	0%	5%
Monocytes	4%	9%	35%
Lymphocytes	50%	70%	25%
RBC	3.80 x10^6/μL	3.65 x10^6/μL	2 x10^6/μL
Hemoglobin	10.70 g/L	10.40 g/L	6.5 g/L
PLT	77,000/μL	95,000/μL	19,000/μL
Bone marrow			
Blasts	14.5%	37%	30%
CD19	+	+	-
CD20	-	-	-
CD22	+	+	-
CD79a	+	+	-
sKAPPA	+	-	-
sLAMBDA	+	-	-
CD13	-	-	+
CD14	-	-	-
CD15	-	+	-
CD33	-	+	+
CD2	-	-	-
CD3	-	-	-
CD5	-	-	-
CD7	-	-	-
CD10	+	-	-
CD34	-	+	+
CD41	-	-	-
CD45	+	+	+
CD117	-	-	+
HLA-DR	-	-	+
Glycophorin	-	-	-
TdT	-	-	-
MPO	+	-	+
TP	-	-	WND

+: positive; –: negative.
ALL: acute lymphocytic leukemia; CBCC: complete blood cell count; MPO: myeloperoxidase; PLT: platelets; RBC: red blood cells; TP: translocation panel; WBC: white blood cells; WND: was not done.

low-risk situation with a good prognosis due to age, absence of hyperleukocytosis and not otherwise specified (NOS) genetic abnormalities. The session with the hematology, oncology, and pathology departments provided the diagnosis of BCP-ALL of hepatic origin. The patient started chemotherapy with a modified National Protocol for Acute Lymphoblastic Leukemias, adapted from the St Jude Children's Research Hospital TOTXV protocol. However, after the steroid window with

prednisone (60 mg/m^2), the patient presented hyperleukocytosis, hyperuricemia, elevated lactate dehydrogenase, and alkaline phosphatase. These results were considered poor steroid responses, so the patient was reclassified as high-risk.

First relapse

On November 30 (2013), remission induction was initiated. On November 30 and December 7, the patient received daunorubicin (30 mg/m^2); on December 1, 3, 5, 8, 10, 12, 15, 17, and 19, L-asparaginase (10,000 IU/m^2), and dexamethasone (6 mg/m^2) for 28 days; on December 7, 14, and 24, vincristine (0.05 mg/kg). Two weeks later, an episode of fever was recorded, along with neutropenia and mucositis. The patient was treated with cefepime and amikacin. Bone marrow aspirate (BMA) on day 7 showed 1.5% blasts, and cerebrospinal fluid (CSF) was negative. Blood count on day 8 showed no blasts. BMA on day 21 showed 0.5% blasts and negative CSF. BMA on day 28 showed 0.5% blasts and negative CSF. On January 7 (2014), hematological remission was documented, and the first intensification with etoposide (100 mg/m^2 4-hour infusion every 24 hours for 5 days) and cytarabine (1000 mg/m^2 1-hour infusion every 12 hours for 3 days) was initiated. Hepatomegaly decreased by 30%.

In February, the first consolidation with methotrexate (2.5 mg/m^2) started. During the third week of maintenance, the patient presented a very early relapse to the BM with 37% blasts (Table 1). Immunophenotyping by flow cytometry suggested that the blasts were positive for CD19, CD22, CD79a, CD15, CD33, CD34, and CD45 (Figure 1B). Similar to the diagnosis phase, a normal cytogenetic study (translocations panel) was reported in this relapse. A hematopoietic stem cell transplantation (HPSCT) was proposed to the parents, but they did not accept it. She continued with second-line chemotherapy according to the institutional protocol with five drugs with cytarabine for myeloid markers. BMA on day 14 showed adequate remission. In August (2014), studies for EBV were conducted again, resulting in a past infection. Eight months later, the patient presented with fever and neutropenia; she received a piperacillin/tazobactam regimen for 19 days. Five months later, a CSF was performed, with negative results for infiltration. Treatment continued until week 98 of maintenance without complications.

Second relapse (lineage switch)

On August 14, 2017, the patient presented to the hospital with petechiae. Laboratory data revealed WBC 43,000/μL, 18% blast cells, 6.5 g/dL hemoglobin, and a platelet count of 19,000/μL (Table 1). The BM was hypercellular and had 30% blasts. Immunophenotyping showed positivity for CD45, CD34, CD117, HLA DR, MPO (myeloperoxidase), CD13, and CD33 (Figure 1C). The patient was diagnosed with acute myeloid leukemia at the age of 5 years. At this point, therapeutic options were explained to the parents considering that chemotherapy would no longer have curative purposes since the prognosis changed due to the lineage switch and not having received HPSCT after the first very early relapse. The parents opted for palliative care; unfortunately, the patient died two months later at home. The family did not authorize an autopsy.

Flow cytometry

Flow cytometry was performed on BMA samples. Samples were processed according to the standard protocol. Mononuclear cells were stained with fluorochromes and antigens from Becton Dickinson Biosciences®, San Diego, CA. Samples were acquired on a FACSCalibur cytometer (Becton Dickinson Biosciences) and subsequently analyzed with CellQuest software (Becton Dickinson Biosciences). Positive antigens were defined as those with fluorochrome expression ≥ 30% of the cell population expressed the fluorescence marker above the cut-off point, using the corresponding isotype control. BM had 14.5% blasts. Flow cytometry showed a population of precursor B cells with aberrant myeloid markers expressing CD45+, CD19+, CD22+, CD79a, sKAPPA+, sLAMBDA+, CD10+, and MPO+ (Figures 2A-2C). Conventional cytogenetic analysis revealed a 46 XX karyotype; the translocation panel was negative for t(9;22) (q34;q11), t(5;14) (q31;q32), t(12;21) (p13;q22), t(1;19) (q23;p13.3), and 11q23 (MLL). No variations were detected in *NRAS*, *KRAS*, *PHF6*, *JAK-STAS*, *RAS*, *NOTCH-1*, or *PAX5* genes. Leukemia chimeric gene screening was negative for *BCR-ABL*. The IKAROS transform study was not performed. Fluorescence in-situ hybridization (FISH) was negative for MLL rearrangements.

Myo1g test

We measured the expression of Myo1g by IF and qRT-PCR at diagnosis, during the poor response to the steroid, and at first relapse. For the second relapse, it was not possible to obtain the sample. As a control, we used a cohort of hematologically healthy children, as recently reported[7]. Briefly, peripheral blood mononuclear

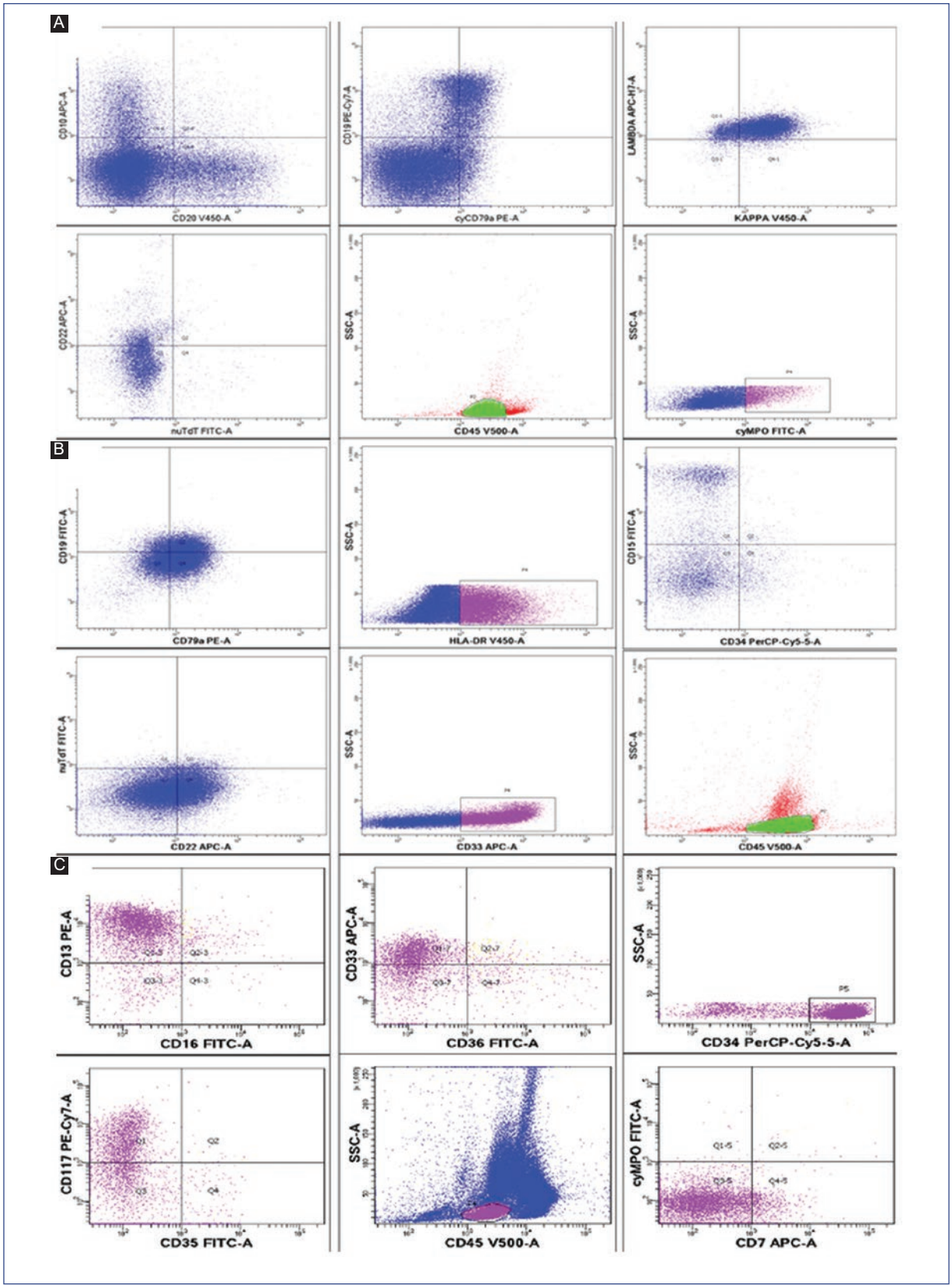

Figure 2. Flow cytometry at different stages of treatment. **A:** diagnosis (CD45+, CD19+, CD22+, CD79a+, sKAPPA+, sLAMBDA+, CD10+, and MPO+). **B:** first relapse (CD19+, CD22+, CD79a+, CD15+, CD33+, CD34+ and CD45+). **C:** second relapse (CD45+, CD34+, CD117+, HLA DR+, MPO+, CD13+ and CD33+). MPO: myeloperoxidase.

cells (PBMC) were isolated by density gradient with Lymphoprep (Axis 250 Shield). Samples were divided into two parts: one was stored in Trizol for mRNA isolation, and the other was used for IF. Samples were placed on slides, fixed with 4% paraformaldehyde for IF, and processed as indicated[7]. We quantified the fluorescence intensity of at least 30 cells at diagnosis and different phases of treatment. Images were analyzed using Fiji, Image J software (NIH)[18].

qRT-PCR

RNA was extracted from PBMCs using the RNeasy mini kit (QIAGEN) and reverse transcribed into cDNA using the Quantitect Reverse Transcription kit (QIAGEN).

Myo1g expression was measured by quantitative PCR using the Agilent Mx3005 P thermocycler, Universal Probe Library (Roche), with a set of Myo1g-specific primers. The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was used as an internal control. Light Cycler 480 master mix (Roche) was used for amplification. Amplification was carried out at 95°C/10s, 56°C-59°C/30s, 72°C/11s for 45 cycles. Fold change values of gene expression were calculated with the $2^{-\Delta\Delta Ct}$ method using the mean of triplicate measurements[7]. Myo1g expression at diagnosis was moderately high in bone marrow (BM) compared to controls (Figure 3A, 3B). However, at the end of the steroid window (Figure 3C) and during the first BM relapse (Figure 3D), significant overexpression was observed by qRT-PCR and immunofluorescence (Figures 3E and 3F), respectively. The sample from the second relapse could no longer be collected because the patient entered palliative care, and the parents no longer allowed further studies.

Discussion

The incidence of ALL is higher in Latin America than in other parts of the world, with rates of up to 120 patients per million per year[19,20]. Therefore, it is likely that patients with ALL in this region present biological variations compared to other areas. As a result of epidemiological studies[21], it is known that 1-9-year-old female patients have better survival than those outside this age range, such as our patient. At the same time, the prognosis is unfavorable for patients with a central nervous system (CNS) infiltration and leukocytosis (> 50,000/mm^3) at diagnosis[22]. On this basis, in addition to the response to chemotherapy and the leukemia phenotype, the patient's risk of relapse could be established[23].

In general, leukemia patients with BM relapse show decreased survival. Moreover, with further complications such as a second BM relapse, myeloid lineage switch, and liver as the primary origin of leukemia, the possibility of cure becomes < 10%. Therefore, third-line chemotherapy could be administered in these cases, but not for curative purposes. Also, the possibility of a third relapse is latent, and secondary toxicity due to chemotherapy could cause organic, infectious, and severe bleeding that would increase mortality. Another possibility is a refractory state of leukemia that would require transplantation. However, this procedure does not have a good prognosis after a second BM relapse. At the time of the second relapse, our patient was not in remission, had tumor activity, and a 100% compatible donor was unavailable. Therefore, the survival prognosis was very poor. Moreover, neither a new chemotherapy cycle for curative purposes nor the HPSCT procedure could be guaranteed, so there were no curative options from the oncological point of view. Therefore, palliative care was the best option to maintain the patient's comfort, seeking the best quality of life.

The steroid window is part of the initial treatment in ALL and is used to assess drug response as a prognostic factor[24]. In this case, the patient was staged as LR; however, Myo1g overexpression at the end of the steroid window and the increase in leukocytes showed in BC served as biomarkers of HR and potentially as a biomarker of relapse. This report suggests using Myo1g as a biomarker to aid in the classification of patients who meet the characteristics of LR to HR from the onset of the disease; consequently, this will help to start treatment in a targeted and timely manner, thus potentially improving patient survival. Leukemic transformation and clonal expansion of ALL can occur at different stages of the lymphoid maturation and differentiation[25,26], making the course of the disease even more complex, as in this case. Not all cases of ALL express antigens of a single lineage[27]; there are cases of ALL expressing associated myeloid antigens (My + ALL), such as our patient, and cases of acute myeloid leukemias expressing associated lymphoid antigens (Ly + AML)[28]. In contrast, acute leukemias of mixed lineage (ambiguous lineage) represent a heterogeneous group of rare and poorly differentiated leukemias with features of both lymphoid and myeloid lineages.

The diagnosis of BCP-ALL represents a challenge considering the new classifications, such as the WHO

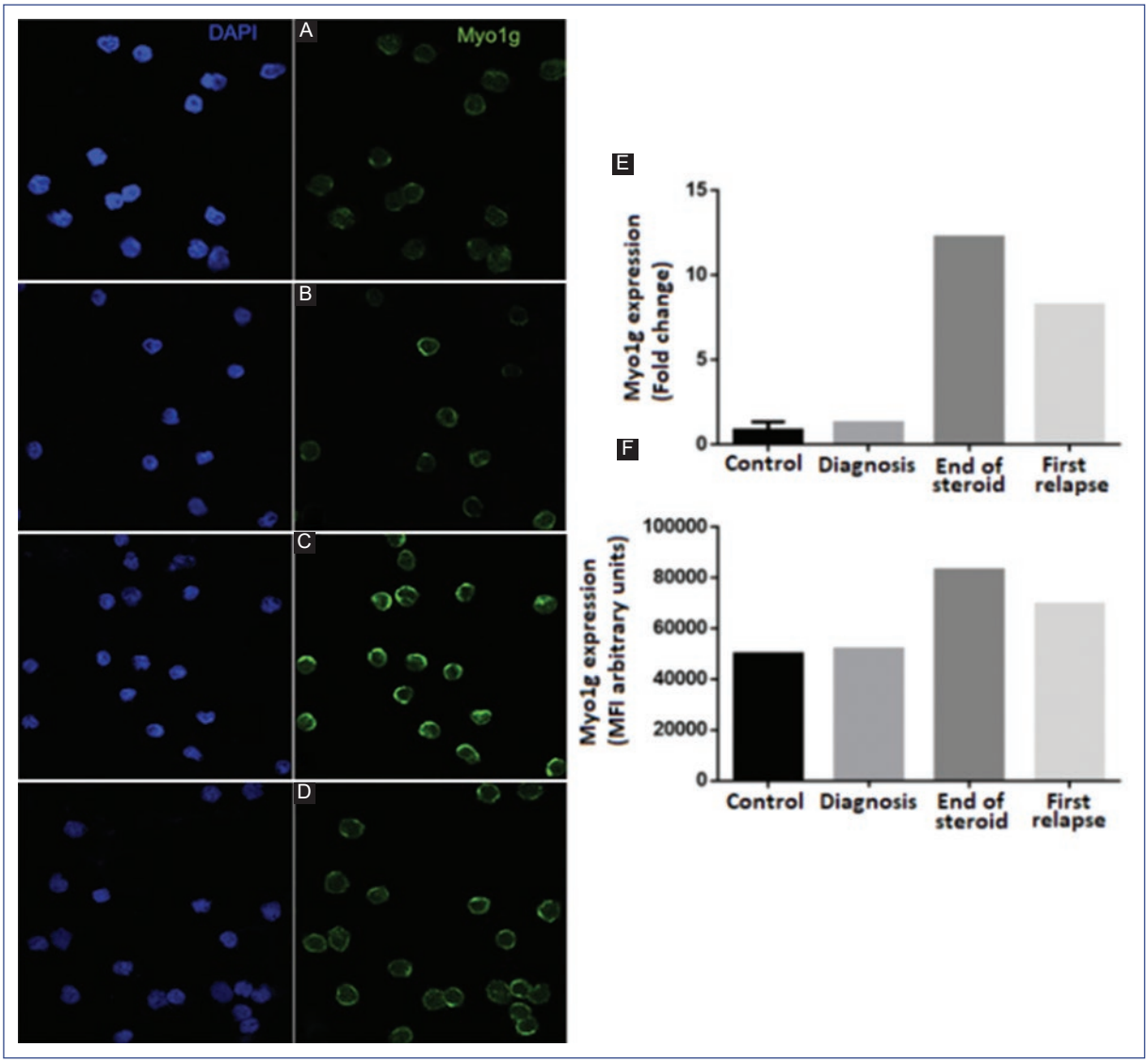

Figure 3. Expression of myo1g at different stages of the disease. Representative confocal images of the expression of Myo1g in **A:** control; **B:** diagnosis; **C:** end of steroid window; **D:** first relapse; **E:** expression of Myo1g by qRT-PCR (real-time quantitative reverse transcription polymerase chain reaction) expressed as fold change; **F:** quantification of Myo1g expression of at least 50 cells, measured by fluorescence intensity (arbitrary units).

2017 classification. In this case, the patient initially presented BCP-ALL expressing a myeloid marker (MPO) and negative CD14, so mixed phenotype acute leukemia (MPAL) was considered a differential diagnosis as MPO was the only myeloid marker weakly expressed[29].

New subtypes of BCP-ALL with lineage-switching have been described. Novakova et al. found an early switch to monocytic lineage and loss of the B-cell immunophenotype, including CD19 expression[30]; therefore, more complexity is added to diagnosing this type of neoplasm. The presence of monocytosis (4%) supports the diagnosis. Moreover, a decrease in lymphoid blasts but not in myeloid blasts was observed with chemotherapy, which from the first relapse already showed CD15 + and CD33 +. The expression of myeloid markers was unclear compared to lymphoid blasts, so the diagnosis was agreed as lineage switch and not MPAL. Since this topic may be debated and discussed, it should be supported with cytogenetic studies for better staging and treatment.

Some conditions could explain the lineage switch at relapse; one is the possibility of a second neoplasm after two or three years of treatment with high doses of etoposide. However, this is more frequent when the

initial neoplasm is of myeloid phenotype. Another hypothesis to explain the changes in the immunophenotype during relapse is clonal selection[15]. A previous study showed that MLL rearrangements were prevalent in almost 80% of pediatric patients with B-ALL who underwent lineage conversion to AML after chemotherapy with or without hematopoietic stem cell transplantation[11]. These rearrangements were negative in the present case.

Currently, there is no standard recommended therapy for lineage-switching leukemia. A case report of an infant with lineage-switching leukemia showed that allogeneic hematopoietic stem cell transplantation (alloHSCT) as consolidation therapy after remission resulted in a well-controlled disease without relapse over 2 years[31]. In the present case, alloHSCT was not feasible due to the patient's advanced condition and lack of remission since the first diagnosis.

Myo1g overexpression was partially evident at disease onset but increased upon poor steroid response and remained elevated during the first relapse. Therefore, Myo1g is proposed as a relapse biomarker. These data suggest *de novo* myeloid leukemia after lymphoid leukemia as another differential diagnosis. However, Myo1g was not measured in the second relapse because the parents no longer authorized invasive procedures. Thus, the definitive diagnosis was lineage switch.

In summary, we described a pediatric case of refractory B-ALL with lineage conversion to AML that exhibited alterations in Myosin 1g expression. Further studies are required to investigate the significance of Myo1g expression in leukemia. Finally, targeting multiple antigens on leukemia-initiating cells may be a better strategy to reduce the likelihood of lineage switch.

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 conflicts of interest.

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CLINICAL CASE

Acute lichenoid and varioliform pityriasis in a pediatric patient

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Abstract

Background: *Pityriasis lichenoides et varioliformis acuta (PLEVA) is a rare dermatosis recognized as a benign condition of unknown etiopathogenesis. It is more common in pediatric patients and young adults and is characterized by multiple small or large erythematous plaques spread over the trunk and extremities.* **Case report:** *We describe the case of a 5-year-old male, previously healthy, with multiple erythematous lesions that disappeared leaving hypopigmented macules. The biopsy reported histological changes suggestive of mycosis fungoides. After a second revision of lamellae in this hospital, lymphocytic vasculitis (LV) with focal epidermal necrosis consistent with acute pityriasis lichenoides (PL) was identified.* **Conclusions:** *The existing knowledge about PLEVA lacks a consensus in specifying its classification, etiopathogenesis, diagnosis, and treatment, so this clinical condition represents a medical challenge. The diagnosis is made by clinical suspicion and confirmed by histology. The objective of this article was to report a case of PLEVA with an atypical presentation due to its histopathological findings, being the first report showing LV in children, as well as a review of the literature.*

Keywords: *Lymphocytic vasculitis. Acute lichenoid and varioliform pityriasis. Pityriasis lichenoid.*

Vasculitis linfocítica en pitiriasis liquenoide y varioliforme aguda de un paciente pediátrico

Resumen

Introducción: *La pitiriasis liquenoide y varioliforme aguda (PLEVA) es una dermatosis poco frecuente, de etiopatogenia desconocida y evolución autolimitada. Es más común en pacientes pediátricos y adultos jóvenes, y está caracterizada por la presencia de múltiples placas eritematoescamosas pequeñas o grandes, diseminadas en el tronco y las extremidades.* **Caso clínico:** *Se describe el caso de un escolar de 5 años, de sexo masculino, previamente sano, que presentó múltiples cuadros de lesiones eritematosas que desaparecían dejando máculas hipopigmentadas. La biopsia reportó cambios histológicos sugestivos de micosis fungoide. Se realizó una segunda revisión de laminillas, identificando vasculitis linfocítica con necrosis epidérmica focal, consistente con pitiriasis liquenoide aguda.* **Conclusiones:** *El conocimiento acerca de la PLEVA carece de un consenso que especifique su clasificación, etiopatogenia, diagnóstico y tratamiento, por lo que esta condición clínica representa un desafío médico. El diagnóstico se realiza por sospecha clínica y se confirma por histología. El objetivo de este artículo fue reportar un caso de PLEVA con presentación atípica por los hallazgos histopatológicos, siendo este el primer reporte de vasculitis linfocítica en niños, y además se realiza una revisión de la literatura.*

Palabras clave: *Vasculitis linfocítica. Pitiriasis liquenoide y varioliforme aguda. Pitiriasis liquenoide.*

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Introduction

Pityriasis lichenoides et varioliformis acuta (PLEVA) is a rare inflammatory skin disease of unknown etiopathogenesis. It is characterized by papulosquamous lesions or erythematous-squamous plaques affecting the trunk and extremities; sometimes, it is accompanied by pruritus, or it can also be asymptomatic[1].

PLEVA is characterized by initially presenting papulosquamous lesions or erythematous-squamous plaques of 3-5 mm in diameter, which are usually covered by fine scales and may coalesce to form plaques affecting the trunk and extremities. As the disease progresses, vesicles/pustules appear over the papules, which umbilicate and progress to hemorrhagic necrosis, with purpuric and crusty areas, which, if removed, reveal necrotic ulcers. Necrotic lesions heal in several weeks and leave a varioliform scar, sometimes accompanied by pruritus without any other symptoms[2].

Acute lichenoid and varioliform pityriasis were first described in 1894 by Neisser and Jadassohn. Brocq, in 1902, classified it within parapsoriasis, calling it guttate parapsoriasis. In 1916, the acute variety was described by Mucha, which Habermann soon called PLEVA. Later, in 1966, a variant of PLEVA was described by Degos, which he called ulceronecrotic or hyperthermic[3].

The histopathology of PLEVA is characterized by dermatitis, and perivascular lymphocytic infiltrates, accompanied by hemorrhage. Although the presence of lymphocytic vasculitis (LV) is known, the key finding in its characterization is fibrinoid necrosis of the vessel walls with a predominance of lymphocytic infiltrate; nevertheless, it has rarely been demonstrated[4].

In this article, we present a clinical case of a patient with PLEVA, where histopathologically, we show the presence of LV, which has been rarely reported in the medical literature.

Reports on the racial and geographic predilection of all forms of pityriasis lichenoides (PL) are lacking. Due to its low frequency, its incidence is not well known, but it is estimated to occur in one out of every 2000 inhabitants. It is more frequent in young adults and children, with an average age of presentation of 5 to 10 years. It has a slight predominance in males, although some authors suggest a lack of accurate studies to support this finding[5,6].

Due to the ease with which PL is frequently confused with other conditions, it is necessary to identify it correctly and recognize the varieties that exist using the current classification; by identifying its clinical manifestations, we can guide a diagnosis[1].

Clinical case

We describe the case of a 5-year-old male from the state of Morelos with no important pathologic history. The patient consulted a physician in January 2021 for presenting lesions that were initially erythematous and later became hypopigmented lesions located on the trunk; they were diagnosed as scabies and were managed accordingly. Due to the persistence of the dermatosis, a skin biopsy was performed in the corresponding hospital unit. The biopsy showed histologic changes suggestive of mycosis fungoides (MF), so he was referred to our unit for a comprehensive evaluation. Physical examination revealed disseminated dermatosis on the trunk and extremities, consisting of multiple hypopigmented plaques, some with fine scales on their surface alternating with erythematous papular lesions of scaly appearance and others with central ulceration of varioliform appearance (Fig. 1A-E).

A slide review was performed, identifying a pattern associated with LV with focal epidermal necrosis (Fig. 2A-F). Immunohistochemistry (IMHQ) reported: CD3+, CD2+ weak, CD4+++, CD7 +++, CD8+, CD20 negative. Clinicopathologic correlation concluded the diagnosis of PLEVA (Fig. 3A-E).

Management was started with erythromycin 125 mg every 8 h for 21 days; magistral formula with 15% urea, 20% sunflower oil in cold cream every 6 h all over the body and 1% hydrocortisone cream, application every 12 h for 15 days, and then every 24 h for 15 days with suspension at the end of the scheme, applied in lesions of the abdomen and thighs. With this treatment, a decrease in papules and crust lesions was observed, achieving a partial improvement of the condition.

The patient had a relapse the following month, which motivated the initiation of treatment with PUVA-sol, adapted to our institution. This is formulated with a magistral formula with lime essence oil 40% in liquid petroleum jelly, to be applied at night on the compromised skin surface with morning cleansing and progressive sun exposure at 10 am starting with 10 min and gradually increasing until a tolerance of 30 min is achieved. The response to this management was toward 50% improvement of the papulosquamous lesions over 3 months; currently, residual hypochromic staining persists.

Discussion

PL is characterized by a disseminated dermatosis on the trunk and extremities. In this rash, desquamative

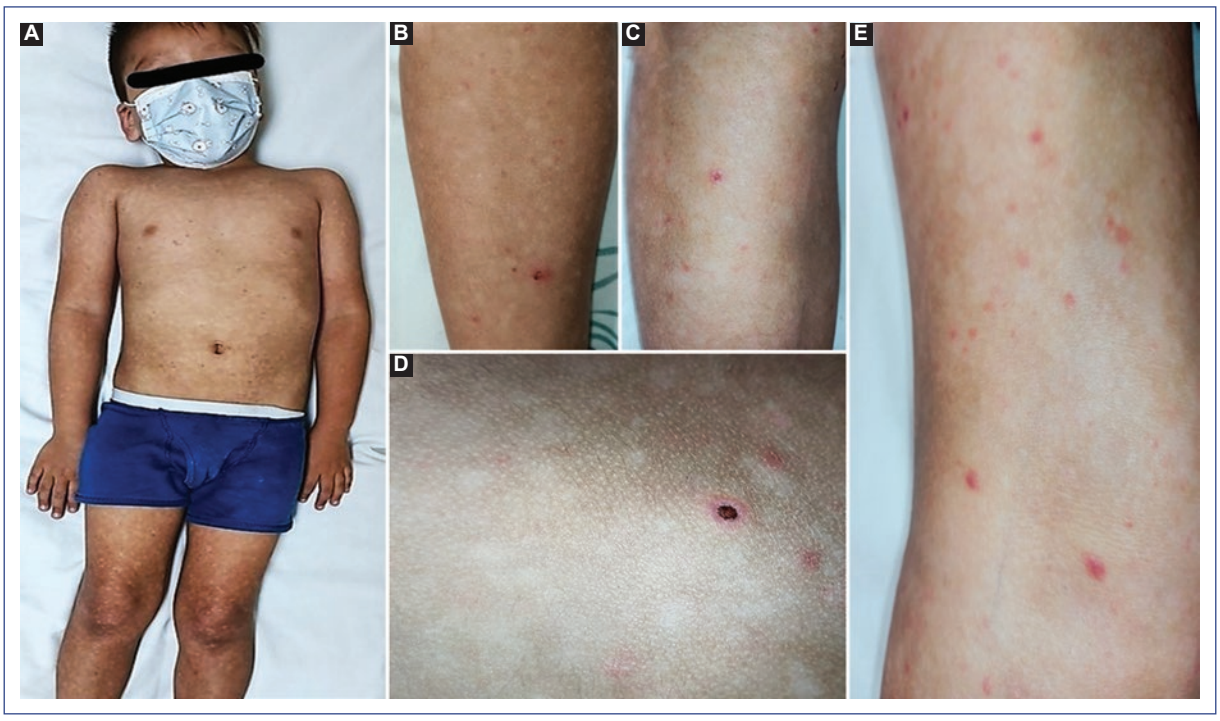

Figure 1. A: disseminated dermatosis characterized by hypochromic lesions and papules. **B-D:** close-ups of the lesions, note the polymorphism of the lesions showing erythematous papules, hypochromic plaques with scaling. **E:** lesions with central ulceration and necrotic crust giving a varioliform appearance.

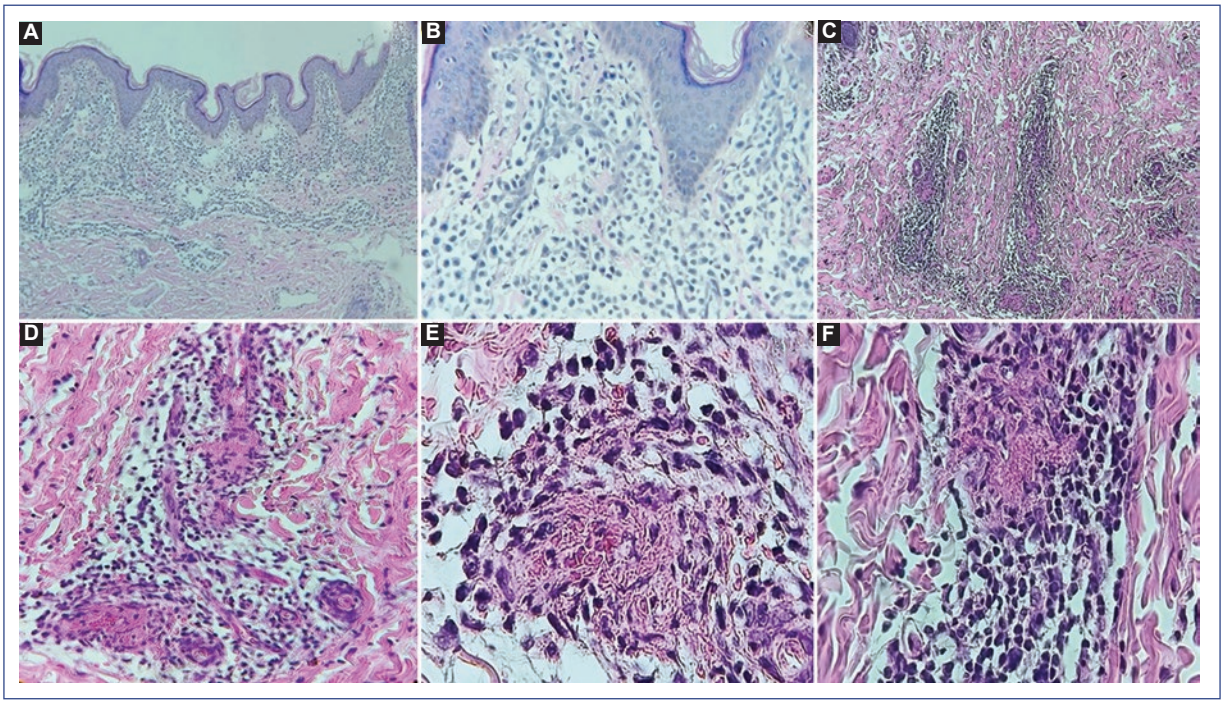

Figure 2. A: hematoxylin and eosin (H&E) stain 4x. Panoramic view identifying preservation of the epidermis with lymphocytic infiltrate with lichenoid distribution. **B:** H&E 10x. Close-up showing the presence of isolated exocytosis and perivascular lymphocytic infiltrate. **C:** H&E 10x. Note the lymphocytic vasculitis in the capillary vessel wall at the level of the mid dermis. **D-F:** H&E 40x. These images demonstrate true vasculitis with the presence of perivascular and invading infiltrate in the vessel wall, fibrinoid necrosis, erythrocyte extravasation, and karyorrhexis.

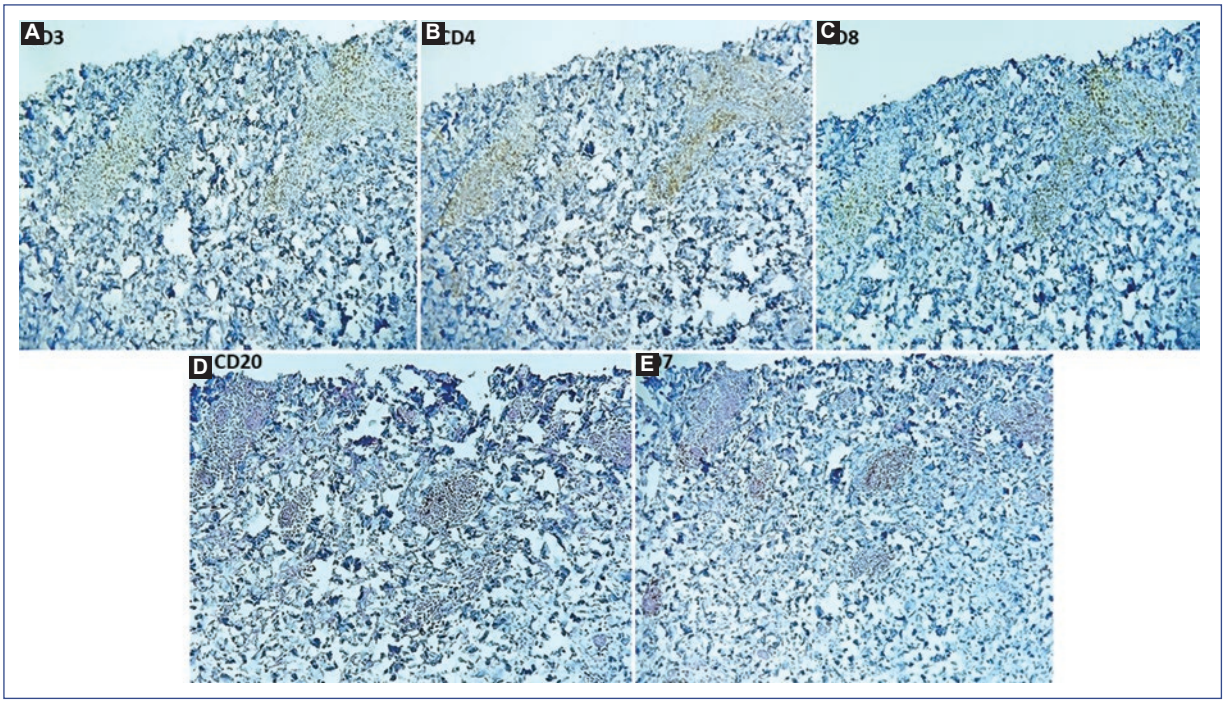

Figure 3. Immunohistochemistry (IMHQ) of vessels with vasculitis showed: **A:** CD3++, **B:** CD4+++, **C:** CD7 +++, **D:** CD8+ and **E:** CD20 negative.

papules are identified, and it has a centripetal distribution, although facial and mucosal involvement is rare. In general, the lesions are asymptomatic, and there is no extracutaneous involvement. However, it is possible to find pruritus, burning lesions, or fever when symptoms are present[7]. Depending on the speed of distribution, appearance, and duration of the lesions, three forms of PL have been classified: acute (PLEVA), chronic (PLC), and the febrile ulceronecrotic variant (FUMHD). The acute and chronic forms represent terminal groups of a continuous spectrum that includes intermediate stages and overlapping forms[6,8].

The etiology of this entity is not yet well established; however, three recognized theories explain its presentation in outbreaks: (1) inflammatory reaction due to infectious agents, (2) inflammatory response secondary to T-cell dyscrasia, and (3) hypersensitivity vasculitis mediated by immunocomplexes[5,6].

In the inflammatory reaction triggered by infectious agents, different pathogens associated with PLEVA, such as toxoplasma gondii, Epstein–Barr virus, Varicella-zoster virus, Parvovirus B19, *Streptococcus*, *Staphylococcus*, *Mycoplasma*, *Cytomegalovirus*, and HIV are recognized[6].

The second theory, which attempts to explain an inflammatory response secondary to T-cell dyscrasia, indicates that PL is a disorder that may precede a T-cell MF arising from a monoclonal lymphocytic proliferation observed in the lesions[6].

Polymerase chain reaction amplification of T-cell receptor genes shows that in some cases of all three PL variants, dominant T-cell clonality is detected within their lesional lymphocytic infiltrates. Clonal T-cell-infiltrating FUMHD deserves special attention due to its destructive nature and neoplastic potential. In general, clonal FUMHD is histologically very similar to epidermotropic cytotoxic T-cell lymphoma and has a poor prognosis, thus perhaps representing a unique form of cutaneous CD8+ T-cell lymphoma. In contrast, in focal T lymphoma, cell clonality is not an absolute sign of underlying malignancy and may represent a benign condition with clonal T-cell proliferation. This condition is seen in cases of repeated exposure to superantigens and other skin disorders[9,10].

The clinical picture of PLEVA is characterized by a sudden eruption consisting of erythematous papules of 3-5 mm in diameter, usually covered by fine scales that may coalesce and form plaques. As the disease progresses, vesicular pustules appear over the papules and umbilicate, progressing to hemorrhagic necrosis with purpuric and crusty lesions, which, when removed,

Table 1. Histopathological characteristics

Area	PLEVA	FUMHD	PLC
Epidermis	Focal and confluent parakeratosis, spongiosis, dyskeratosis, mild to moderate acanthosis, vacuolization of the basal layer with necrotic keratinocytes, occasional intraepidermal vesicles, focal epidermal necrosis; advanced findings: extension of the infiltrate in the epidermis, invasion of erythrocytes, generalized epidermal necrosis, nuclear debris in necrotic areas	Similar to PLEVA but with extensive necrosis	Focal parakeratosis, mild to moderate acanthosis, focal areas of spongiosis, minimal numbers of necrotic keratinocytes, minimal vacuolar degeneration of the basal layer, focal invasion by a small number of lymphocytes and erythrocytes
Dermis	Edema; moderately dense lymphohistiocytic perivascular inflammatory infiltrate, often wedge-shaped and extensible, deep into the reticular dermis as well as diffusely obscuring the dermo-epidermal junction; extravasation of lymphocytes and erythrocytes with epidermal invasion; subepidermal vesicles in later lesions; dermal sclerosis in older lesions	Dense perivascular infiltrate, generally without atypia; otherwise similar to PLEVA	Mild superficial perivascular lymphohistiocytic perivascular edema, infiltrate only focally obscuring the dermo-epidermal junction, occasionally extravasated erythrocytes
Vascular changes	Dilatation and engorgement of blood vessels in the papillary dermis with endothelial proliferation, vascular congestion, occlusion, dermal hemorrhage and erythrocyte extravasation	Similar to PLEVA vascular changes	Dilatation of superficial vessels, without invasion of vessel walls by inflammatory cells
Vasculitis	Invasion of vessel walls by inflammatory cells, rarely fibrin deposits inside vessel walls, very rare leukoplakia	Fibrinoid necrosis of the vessel walls, with leukocytoclastic vasculitis	Not presented

FUMHD: febrile ulceronecrotic Mucha-Habermann disease, PLC: pityriasis lichenoides chronica, PLEVA: *pityriasis lichenoides et varioliformis acuta*. Modified from Bowers and Warshaw[5].

reveal necrotic ulcers. These necrotic lesions heal in several weeks, leaving a varioliform scar[11].

The histology of PLEVA will depend on the clinical form and stage. The most common histopathologic feature is the perivascular mononuclear lymphohistiocytic infiltrate, which primarily surrounds and involves the blood vessels of the superficial dermis[8]. The characteristic histopathologic findings of the PL types are summarized in Table 1.

Particularly, PLEVA with the presence of LV was first reported in 1959. Marks et al. reported that approximately 25% of 102 biopsies of PL showed a degree of vascular damage with inflammation of the vascular endothelium. However, none of the reported cases showed histopathologic evidence of fibrinoid necrosis of the vessel wall[11]. Aydin and Gököz reviewed 127 cases of LV, where LP constituted 13% of the reported cases, the prototype being the lymphocytic lichenoid LV pattern. In addition, there is a study of 12 cases of PLEVA showing intense perivascular lymphocytic infiltration but no evidence of fibrinoid deposition, which concluded that PLEVA is not a true vasculitis[12,13].

In the recent literature, there are no cases of LV in PLEVA in pediatric patients with fibrinoid necrosis confirmed by IMHQ, where the infiltrate cell type can be observed. The finding of fibrinoid necrosis within the vessel wall in confirmed cases of LV is essential to distinguish it from non-vascular inflammatory disorders with a perivascular lymphocytic infiltrate[13].

The Australian College of Dermatology reported the case of an adult patient with LV and PLEVA with IMHQ. This case showed severe angiocentric infiltrate of T lymphocytes and the absence of B-cells and neutrophils in the infiltrate of LV. The results may confirm and explain the role of T lymphocytes in the pathogenesis of vasculitis in PLEVA. The findings support the theory that lymphocytes are capable of damaging endothelial cells and other components of blood vessel walls[14].

LV is a histopathologic finding in various and very heterogeneous dermatoses, such as connective tissue diseases, infections, lichenoid diseases, and drug reactions. However, LV is not well accepted by dermatopathologists as a pathologic mechanism because many inflammatory skin diseases present perivascular

infiltrates of lymphocytes and to apply the term LV to all these conditions would make it meaningless[15].

Some authors state that LV can be defined as "a lymphocyte generally infiltrate perivascular space and located both superficial and deep, accompanied by fibrin in the wall or thrombi within the lumen of the venules." These authors consider it as a "mere epiphenomenon" secondary to a basic pathologic process and not in itself a fundamental pathologic process. While the different types of neutrophilic vasculitis have diagnostic significance for clinicians and histopathologists, LV has none. Arguments against the validity of LV as a meaningful concept include the lack of a disease, in which it is always found. In another article by Lee and collaborators, cases of LV are reported in patients with rickettsiosis and NK cell lymphoma without being demonstrated by IMHQ. In addition, there are case reports where LV has also been found in nodular scabies[13,15].

Therefore, LV can be unequivocally inferred if there are lymphocyte infiltrates in and around the venules' walls, followed by fibrin deposition in their walls or vessels, or both. Diagnosis is problematic if there are no fibrin deposits. To separate such cases from the larger group of perivascular dermatitis, we can look for circumstantial evidence of a delayed hypersensitivity reaction directed against the vessel walls. This evidence may include lamination of the adventitia of venules in the form of concentrically arranged pericytes and basement membrane material, lymphocytic nuclear dust, or subendothelial or intramural infiltration of lymphocytes in arterioles. Supporting evidence of LV may include tissue necrosis from resultant ischemia. The walls of arterioles contain smooth muscle in layers thick enough to prevent diapedesis, so the finding of intramural inflammatory cells alone constitutes evidence of vasculitis[15].

If these criteria are applied and looked for in biopsy specimens of inflammatory skin disease, LV will be found to be uncommon but not rare. All definitions have limitations, and one way ours may fail is the failure to recognize ultrastructural damage to small vessels as evidence of vasculitis[15].

There are three forms of LV: the autodestructive form, which can be seen in lymphoproliferative disorders; lymphocytic endovasculitis, which occurs in thrombosis of obliterative processes; and the lichenoid form, which is seen in inflammatory skin diseases as part of the frequent features of lichenoid vascular change and erythrocyte extravasation[16].

PLEVA has often been considered a LV, perhaps largely due to its clinical appearance. Often, the infiltrate has a wedge-shaped configuration if the sample includes most of a papule. In a small minority (perhaps 5%-10%) of cases, there is a true LV in Mucha-Habermann's disease. It is usually seen as a few tufts of fibrin in the mid or deep dermis vessel walls. In rare cases, large deposits may be present[11,16].

We should consider that other conditions are present along with LV, such as MF, which represents a challenge for its early-stage diagnosis. PL-like MF is a rare subtype of MF that evolves with the characteristic lesions of PL, where the histopathologic findings of MF are epidermal infiltration by lymphoid cells, many with irregular shapes, discrete perivascular infiltrate in the superficial dermis, and numerous lymphocytes with slit nuclei, large atypical, and unclassifiable mononuclear cells. Infiltration of small veins in the deep dermis and subcutaneous fat by atypical cells and fibrinoid necrosis of their walls are also detected[17-20].

Historically, PLEVA has been confused with other dermatoses, some relatively benign and others potentially malignant. Diagnosis is confirmed by biopsy, in which clinical conditions are differentiated in relation to the interpretation of the initial biopsy. The IMHQ shows a cellular infiltrate with a predominance of epidermal T-cells expressing the CD8 marker, in contrast to PLC, where these have a $CD4^5$ phenotype (Table 2).

Various treatments have been used, which should be individualized according to the patient's clinical picture. For example, if a trigger (drugs, infections) is identified, the drug should be withdrawn, and the infection that triggered the condition should be treated[21].

There are no standard guidelines for the treatment of PLEVA in children. According to the literature, suggested therapies include topical corticosteroids and immunomodulators, oral antibiotics, phototherapy, and systemic immunosuppressants. Immunomodulatory therapy with tacrolimus has shown adequate response. Simon et al. reported two patients with PLC with complete resolution of lesions using tacrolimus 0.03%, administered twice daily for 14 and 18 weeks[22].

If the cause is an infectious agent, antibacterials are used, which have shown a good response. Among those used are tetracyclines, erythromycin, azithromycin, and dapsone, which have had acceptable results in some reports and case series. For example, a retrospective study of 24 children with PL treated with oral erythromycin, 30-50 mg/kg/day, showed a good response (> 50% improvement) in 64% of patients after

Table 2. Differential diagnoses of clinical and histological PLEVA

Clinical	Histological
Lymphomatoid papulosis (rare in infancy)	Pityriasis rosea
Chickenpox	Insect bites
Adverse drug reaction	Eczematous dermatitis
Varicelliform rashes due to HSV or enteroviruses	Neurotic excoriation
Varicelliform syphilis	Leukocytoclastic vasculitis
Leukocytoclastic vasculitis	Parapsoriasis gutata
Erythema multiforme	Erythroderma (exfoliative dermatitis)
Dermatitis herpetiformis	Secondary syphilis
	Polymorphic light eruption
	Lymphomatoid papulosis

HSV: herpes simplex virus, PLEVA: *pityriasis lichenoides et varioliformis acuta,* Clinical differential diagnosis was obtained from Zegpi et al.[3] Histological differential diagnosis was obtained from Hood and Mark[4].

1 month of therapy, 73% after 2 months, and 83% after 3 months[23].

In cases resistant to the previously mentioned therapies, methotrexate, acitretin, cyclosporine, and systemic corticosteroids may be used. The response is highly variable, depending on the severity of each condition[23].

According to some authors, phototherapy is an effective therapeutic option, one of the first therapeutic lines. Among them, we find the psoralen plus ultraviolet A (PUVA) or the narrow-band UV-B phototherapy (NB-UVB) especially useful. The treatment consists of a combination of PUVA that causes a photochemical interaction through an oxygen-independent reaction producing the inhibition of deoxyribonucleic acid synthesis and another oxygen-dependent reaction that induces apoptosis by free radicals. Psoralens are tricyclic furocoumarins, among which are methoxypsoralen (8-MOP), bergaptene (5-MOP), and trioxalene (3-MOP). MOP is the most widely used in Mexico[22]. Its action begins orally after 60 minutes; its levels are maximal after 2 h, and its elimination is total after 8 h. Topically, it initiates its action 20 min after application and remains active for approximately 30 min. Although its use is frequent in psoriasis and vitiligo, more than 20 skin conditions respond favorably to PUVA treatment. Unfortunately, the use of phototherapy in our country is limited by the scarcity of centers that offer it. An alternative to this limitation is administering psoralen, as indicated for PUVA, but using sunlight as the source of UVA radiation, known as PUVA-sun[24-26].

In most children, PLEVA follows a benign self-limited course; however, relapses are common, and symptoms may be present for months or years. The duration of the disease ranges from 6 weeks to 31 months. Rare cases of PL progressing to cutaneous T-cell lymphoma have been documented. In 2002, Tomasini et al. reported a patient with a history of pityriasis since the age of 11 years who developed MF[12,19,24].

PLEVA is often misdiagnosed with other more common diseases; therefore, it is important to know this condition to perform an adequate clinical-pathological-evolutionary correlation and implement the appropriate treatment. Furthermore, it is common to confuse it with other relevant conditions due to its poorer prognosis, such as MF. In the pediatric population, its prevalence is infrequent, hence the importance of presenting this case, where in addition to presenting as a LV, it was confirmed by IMHQ.

In general, LV is a poorly sustained histopathologic pattern frequently found in multiple conditions. According to the literature, there are few reports of actual LV within the histopathologic findings of PLEVA, which, in our case, could have an impact on the time course of cutaneous inflammatory activity. Since the finding of LV is unusual, we propose an intentional search in serial sections on papular-necrotic lesions for these changes, mainly in those patients with persistent dermatosis.

In conclusion, we do not know whether LV in PLEVA is a common event or has been missed until now. In the scenario in which cases with this finding present a different evolution, as this particular case could be, questions will arise that will be resolved in subsequent reports. A long-term follow-up will allow to establish its presence with a better therapeutic decision.

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 conflicts of interest.

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