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Nodal flow and *Situs inversus*: A literature review

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ABSTRACT

Variations of situs solitus totalis, the normal organ disposition pattern, involve dextrocardia, situs inversus, situs inversus totalis and situs ambiguous. These laterality defects have been documented for more than 400 years, and a connection to ciliary abnormalities has been elucidated by observing cilia from the primitive node, which can promote "nodal flow", possibly necessary for the development of left-right asymmetry. The aim of this work was to perform a literature review, showing variations in the usual anatomy and the connection between nodal flow and normal asymmetric pattern. A narrative review was carried out with articles from the MEDLINE/PubMed, SciELO and LILACS databases and with books relevant to the theme. The initial embryonic development comprises the formation of the organizing structure, a primitive node, and its cilia are responsible for the nodal flow, determinant in many vertebrates in the breaking of the embryonic bilateral symmetry. The formation of asymmetry is associated with morphogenic gradients to the left of the node, the model of two cilia and the mechanism of nodal vesicular plots, until it results in organogenesis. Ciliary abnormalities configure clinical manifestations in disorders such as Primary Ciliary Dyskinesia and Kartagener syndrome. The nodal flow, in fact, is important in normal anatomical standardization, and the cilia are necessary for the configuration of situs solitus totalis.

Keywords: Nodal flow, Situs Inversus, Laterality defects, Primary Ciliary Dyskinesia, Kartagener syndrome.

LIST OF ABBREVIATIONS AND ACRONYMS

Cerl2 - Cerberus-like 2
FGF8 - Fibroblast Growth Factor 8
LPM - Mesoderm of the lateral plate
NVP - Nodal vesicular portion
PCP - Planar cell polarity
SHH - Sonic Hedgehog
RA - Retinoic Acid
TGF-β - Transforming Growth Factor Beta
TIF - Intraflagellar transport

1 INTRODUCTION

The normal anatomical pattern, with all structures in their usual position, is termed *situs solitus totalis*. There are variations of this standardization, which may or may not be associated with important functional changes: *situs solitus* with dextrocardia, in which the abnormality is the presence of the cardiac apex on the right side; *situs inversus*, with the organs located in an inverted position, but

without dextrocardia; *situs inversus totalis*, with all organs inverted, and *Situs ambiguous*, or heterotaxy, in which the thoracoabdominal organs do not follow a normal pattern of disposition, may be partially inverted, arranged in the midline, or symmetrical bilaterally. Heterotaxy, which often causes serious health problems, is divided into two categories: *situs ambiguous* with polysplenia, or left isomerism, with a variable number of bilaterally bilobated spleens and lungs, and *Situs ambiguous* .com asplenia, or right isomerism, with absence of spleen and bilaterally trilobed lungs. Patients with these changes are likely to have other malformations, especially heart defects [1-3].

In the general anatomical context, considering in addition to the variations in cardiac morphology, known laterality defects, characterized by the abnormal disposition of structures and organs, have been documented for more than 400 years, having in many reports, however, unknown underlying causes. Girolamo Fabrizio was the first to describe such abnormalities, around 1600, followed by Marco Aurelio Severino, who in 1643 documented a case of human dextrocardia, and Matthew Baillie, responsible for describing a complete reversal of the thoracoabdominal organs in 1788 [4, 5].

In 1995, the published Afzelius study demonstrated a connection between changes in ciliary structures and the *situs inversus*, one of the existing defects of laterality of the general anatomy [6]. This correlation can be elucidated with the evidence of studies on the gastrulation of mouse embryos, which demonstrated that the primitive node, characterized as an organizing structure, consists of single-cilium presenting cells, that is, each cell has a monocilium [7].

In the fully developed primitive node, there are a total of 200 to 300 lashes, each of these measuring 7 to 10 micrometers in length and being spaced by intervals of 5 to 10 micrometers. Central nodal cells contain monocilia that exhibit motility, while peripheral nodal cells (crown cells) have immobile monocilia. The central cilia are thus able to produce a rotation and generate a directed flow of extra-embryonic fluid to the left through the node. This fact supports the existence of a "nodal flow" model, which would be necessary for the development of the left-right asymmetry of the embryo and, therefore, for a normal arrangement of the anatomical structures [7, 8].

Knowledge about the mechanisms involved in the determination of physiological left-right asymmetry is fundamental for understanding the causality of several laterality defects. The study of these abnormal anatomical states in characteristic disorders or in isolation allows to elucidate not only the triggering factor of the problem, but to establish the correlation between the embryonic abnormality involved and the clinical one presented. From this perspective, the work aims to present a review of the existing literature in this sphere, evidencing the variations of the habitual *situs* in a general anatomical context – without being limited to the nomeclatures of the cardiac context – and the link between the known nodal flow and the standardization of normal asymmetry.

2 METHODOLOGY

The present work consists of a narrative review of the literature, based on scientific articles selected from the databases MEDLINE/PubMed, SciELO (Scientific Electronic Library Online) and LILACS (Latin American and Caribbean Literature in Health Sciences), as well as important books for the explanation of the theme. Data and pertinent information were collected, with the search for studies using descriptors related to the subjects treated and without a pre-established publication period.

3 DEVELOPMENT

3.1 EARLY EMBRYONIC DEVELOPMENT

During the first week of human development, at the stage of blastogenesis, the conceptus – formed by the embryo and its membranes – gives rise to the blastocyst. In this, the separation of the blastomeres by liquid in the blastocystic cavity configures the trophoblast and the embryoblast, which constitute, respectively, the formation of its wall and a projection into the blastocystic cavity. With the gradual degeneration of the zona pellucida, the blastocyst grows rapidly and is adhered to the endometrial epithelium. From this moment, the trophoblast proliferates and differentiates into two layers: the cytotrophoblast (inner layer) and the syncytiotrophoblast (outer layer) which, with its enzymatic synthesis, enables the effective implantation of the blastocyst in the endometrium [9, 10].

With the continuation of the progress of implantation of the blastocyst, in the second week of development, morphological changes in the embryoblast, responsible for the formation of the embryo, lead to the generation of the bilaminar embryonic disc, consisting of the epiblast and the hypoblast. The epiblast contributes to the formation of the amniotic cavity, and the hypoblast to that of the exocoelomic cavity. The formation of the bilaminar disc thus defines the primitive dorsoventral axis of the embryo. In the third week of development, the bilaminar embryonic disc finally becomes a trilaminar embryonic disc, characterizing the gastrulation event. The three germ layers (ectoderm, embryonic endoderm and mesoderm) formed during the stage give rise to specific organs and tissues, constituting the principle of morphogenesis [7, 9, 11].

Gastrulation begins with the formation of a longitudinal structure in the midline of the epiblast surface: the primitive line, close to the caudal region of the embryonic disc, resulting from an induction of epiblastic cells by the extraembryonic region. This formation defines the main body axes: craniocaudal, mediolateral, dorsoventral and left-right axes. The cephalic end of the primitive line forms the primitive node, considered an organizing structure; This contains a circular depression called the primitive pit, which is caudally continuous with the primitive groove, a depression developed in the line. The pit and groove are the result of an invagination of cells of the primitive line. Some of these cells, which migrate to the interior of the embryonic disc, invade the hypoblast, forming a

definitive endoderm layer. Others migrate bilaterally, between the endoderm and epiblast, to form the intra-embryonic mesoderm. The remaining epiblastic cells form the embryonic ectoderm [10, 12].

3.2 CILIARY STRUCTURE

Cilia can be defined as slender extensions that protrude from the surface of different cells. They consist of a cytosolic axis – the ciliary matrix – surrounded by a prolongation of the plasma membrane; Inside, this matrix comprises the axoneme, a filamentous scaffold that follows the longitudinal axis of the ciliary structure and is formed by microtubules. These microtubules consist of protofilaments and sprout from the basal corpuscle (or basal body), which is located inside the cell, under the plasma membrane, and anchors the cilia. In addition, they have a positive end and a negative end, the latter facing the corpuscle. The axoneme and basal body have doubles (doublets) and trios of microtubules, respectively, and are connected by a transition zone. The microtubules A and B of the axoneme doubles are continuous with the microtubules A and B of the cracks of the basal corpuscle [13, 14].

The processes of formation and maintenance of cilia and their counterparts, the flagella, occur through the intraflagellar transport system (TIF), with motor proteins associated with the microtubules - kinesin and cytoplasmic dynein - involved. Under a different system, the fundamental process for moving structures requires a motor protein of the distinct dynein class: axonemal dynein [15], whose driving activity, as well as that of TIF proteins, also requires the hydrolysis of ATP as an energy source [16]. The axonemal dynein contains internal and external arms that protrude from the microtubule A of the axoneme doublets and interact with the microtubule B of the adjacent doublets, forming dynein bridges; through its ATPase activity, then, the portions of the protein associated with microtubule B slide along it toward the negative end. As an effect, motor proteins promote the sliding of one doublet in relation to the other and induce a ciliary curvature, thus characterizing the dynamics of the beating of cilia and flagella [13, 17].

There are three basic categories of eyelashes: mobile, primal, and nodal. The motile cilia, commonly found in epithelia responsible for the transport of secretions, and the flagella, considerably longer and unique by cells, have an axonemal organization 9 + 2, that is, they have 9 doublets of microtubules surrounding 2 central microtubules; Microtubules are associated with motor proteins necessary for ciliary motility. Primary cilia are immobile cellular projections in an arrangement of 9+0 microtubules (9 microtubule doublets, without a central pair), with no associated motor proteins. They are found in cells such as those of the renal ducts and in the epithelium of the bile ducts, and bend passively due to the flow of fluids; thus, they are able to act as sensory antennas and to generate and transmit signals to the intracellular environment in response to extracellular events. In addition, primary cilia are also involved in the transduction of the Hedgehog signal active in the early development of vertebrates. Finally, the nodal cilia, found in the embryo, have an axonemal

organization similar to that of the primary cilia; however, they are holders of associated motor proteins, having the ability to perform active movement. The absence of the central pairs of microtubules is possibly responsible for their rotational movement in full cone trajectory, contrasting with the movable cilia 9 + 2, whose trajectory is in half cone [18, 19].

The nodal cilia with axonemal architecture 9 + 0, then, are responsible for the dynamics of the nodal flow, which occurs at a speed of 15 to 20 μm/s, specifically between the stages of one to two somites and that of six somites [20]. Still, the rotational movement is established clockwise, unlike the commonly found eyelashes [21]. Studies in vertebrates also demonstrate a posterior positioning of these cilia at a time coinciding with the onset of flow specifically to the left. Initially, they are projected into the center of cells, but apparently become posterior by movement from the basal body to the posterior cell pole [22]. This positioning of monocilia is determined by signaling of the PCP (Planar Cell Polarity) pathway in some vertebrates [23].

In addition, the dominant unidirectional character of the flow to the left becomes feasible by the also posterior inclination of these cilia from a vertical angle, with the trajectory of its tip displaced posteriorly when compared to its root; With this configuration, the cilia perform a swing to the left, away from the surface of the cell, and a movement to the right, towards the surface of the cell. Thus, a movement of fluid directed specifically to the left occurs, since, according to hydrodynamics, the surface of the cell is stationary and slows the movement of fluid by the resistance to shear and, as an effect, the sweep to the right is less effective than the movement to the left [22, 24].

3.3 DETERMINATION OF THE LEFT-RIGHT PLANE

In all vertebrates, despite the noticeable external symmetry between the right and left sides, there are processes that trigger an asymmetrical pattern of organization of internal structures and organs, as well as the vascular network [25]. The fundamental processes for generating asymmetry require distinct mechanisms. Initially, a mechanism is needed for the breaking of bilateral symmetry of the embryo, such as a molecular asymmetry. Subsequently, the signaling of this asymmetry generated on a small scale must be established in larger regions of the embryo and ultimately result in an asymmetric organogenesis. Thus, the normal development of the left-right configuration results in the so-called *Solitus site* [8]. The left-right axis is possibly determined in relation to the anteroposterior and dorsoventral axes, these being the axes along which embryonic morphogenesis occurs [26].

The initiation of the asymmetric left-right configuration in vertebrates is a controversial process. In mice, however, experiments suggest that symmetry breaking begins with the generation of leftward extra-embryonic fluid flow: the so-called nodal flow, which occurs through the primitive node [20]. The node is described as a transient structure of the midline, holding a "ciliated well" on the ventral surface, which carries important activity for the determination of left-right asymmetry. This activity

can be proven by the role of the monocilia of the nodal cells, which with their rotational movements, are responsible for generating this nodal flow. Analyses of experiments with fluorescent spheres added to the liquid in the node region demonstrated the activity of the cilia in the nodal flow: in normal wild embryos, these spheres moved unidirectionally to the left, but in mutant embryos with absence of the motor proteins KIF3A and KIF3B, belonging to the kinesin superfamily, they showed Brownian (random) motion [24].

The application of artificial flow in embryos of cultured mice also evidenced the relationship between nodal flow and the beginning of the asymmetry process. It was observed that a rapid flow directed to the right resulted in a reversal of the normal flow to the left and in a reverse left-right development. On the other hand, the application of a flow directed to the left in embryos with mutations affecting inversin – a protein with ankyrin repeats –, which had slow nodal flow, and the left-right axonemal dynein, which had monociliary paralysis, was sufficient to rescue the normal asymmetric pattern. Several mutations are related to the impairment of left-right development, and some of the genes involved are responsible for the formation of the embryonic node itself [8].

Hirokawa *et al* [24] consider an absence of asymmetric event prior to nodal flow in mice. Thus, they discuss the possibility that flow has become the main mechanism of asymmetry with the emergence of viviparous and that it is sufficient for the determination of laterality in mammals. Dasgupta and Amack [22] report that many vertebrate embryos have ciliated structures analogous to the primitive mouse knot that are responsible for an asymmetrical flow, such as the Kupffer vesicle in certain fish, the gastrocele roof plate in the frog, and the posterior notochordal plate in the rabbit, characterizing left-right organizers. However, they point out that cilia would not be necessary to generate asymmetry in all vertebrates. The Hensen node, as they exemplify, holds the first asymmetrical gene expression in the chick, however, this structure may not present mobile cilia, since mutants with defective eyelashes present normal development of asymmetry. They also report that the node of the pig embryo does not have cilia, nor is it exposed to an extra-embryonic liquid – a factor also present in the cow embryo – unlike what occurs in the mouse embryo. Thus, they demonstrate that some vertebrates, and among them mammals, can use mechanisms independent of cilia in the development of asymmetry, unlike the idea that nodal flow, *for itself*, be sufficient.

Considering an asymmetric information generation based on the nodal flow mechanism, this can be elucidated, in principle, from two proposed models. The first concerns the formation of a gradient of morphogens on the left side of the node – equivalent to the left-right organizer of the mouse; The chemical morphogens would be secreted into the node and transported to the left through the nodal flow. The second proposes the existence of a physical stimulation generated by the flow, which would be mechanically detected by the immobile cilia of the peripheral region of the node, being called "two-

lash model", since there are distinct cilia responsible for both the generation of the flow and the perception of it [24].

McGrath e Brueckner [8] clarify that, in fact, the flow may be responsible for generating a morphogenic gradient on the left side of the node; The morphogenic molecule would then be able to set up an asymmetric gene expression cascade on the left. Many molecules found in the structure, such as the Nodal, perform an activity in left-right development, thus being candidates for the role of morphogen. Genes expressed on a single side of the embryonic midline employ mechanisms to propagate signals between cell subpopulations, and this occasionally results in the asymmetric morphogenesis of specific structures. In general, the gene products responsible for the asymmetry are not involved solely in this process, and some of them are not even present in an asymmetric way [26].

Nodal is a signaling molecule belonging to the transforming growth factor beta (TGF-β) family of proteins, initially expressed bilaterally in the crown cells of the mouse organizer. The protein produced in the node can migrate to the mesoderm of the left side plate (LPM) and thus activate the expression of the gene itself *Nodal*; the asymmetric manifestation in the LPM begins at the two-somite stage and ends at the six-somite stage, leading to the conclusion that nodal flow can occur specifically to initiate expression *Nodal* on the left side. The cells of the left LPM that received the Nodal signaling then contribute to the specific morphological characteristics of the left side, while those of the right side, which did not receive them, contribute to the characteristics of the right side [20, 27].

The fibroblast growth factor 8 (FGF8) transported by nodal flow also seems necessary for left-right determination by some mechanism, since mice deficient in *Fgf8* have no expression *Nodal* in the LPM. In mammals, Nodal signaling also induces the expression of Lefty genes: *Lefty1*, predominantly in the midline of the embryo, and *Lefty2*, predominantly in the left LPM. The absence of any of these – *Lefty1*, which acts as a barrier on the midline, or *Lefty2*, which acts as a Nodal feedback inhibitor – leads to a "leakage" of the mesoderm nodal signal from the left side plate to the right side, resulting in expression *Nodal* bilateral and alteration of normal morphology [20, 26].

Beyond genes *Nodal* and *Lefty2*, *Pitx2* it is also expressed in the left LPM, and its asymmetric expression is induced by Nodal signaling. The transcription factor Pitx2 is considered the main regulator of asymmetric organogenesis at the molecular level, but there are mechanisms independent of the factor also operative. Nodal activity, necessary for the cascade of asymmetric expressions, can also be controlled by a protein belonging to the TGF-β family of antagonists, Cerl2 (Cerberus-like 2). At first, the protein is found in the crown cells on both sides of the node, at the early stage of mouse development. Cerl2 then accumulates on the right side and prevents the Nodal from acting on the right LPM. Subsequently, the protein is translocated by the nodal flow to the left, terminating the nodal activity on the left side of the node and on the left LPM in a precise time [20, 28].

In the "two-cilia model", the distinct characteristics of the monocilia of the mouse organizer may provide support for the hypothesis. Monocilia that are mobile, centrally located, contain the motor protein Lrd (left-right dynein) and the protein polycystin-2, a cation-permeable channel; the immobile cilia, located peripherally, lack the Lrd protein, but have polycystin-2. This protein is a calcium-activated channel, which in the primary cilia of the renal epithelium functions as a mechanotransducer, increasing the concentration of intracellular calcium in response to fluid flow. In the nodal structure, it is evident that, while the Lrd-holding monocilia generate the nodal flow, the immobile monocilia, which do not have the Lrd protein, can function as mechanosensory cilia. Thus, they detect the nodal flow to the left, and the existing polycystin-2 leads to an increase in intracellular calcium on the left side of the node, generating asymmetric signaling. The calcium signal resulting from mechanosensitivity, then, can trigger the expression of a specific growth factor to the left of the node [25, 29].

A third mechanism proposed for the generation of asymmetric information by nodal flow – analogous to the first model – concerns nodal vesicular parcels (NVPs). NVPs are materials secreted from the surface of the mouse node that are transported to the left through the flow. These vesicles are made up of lipoprotein particles coated by a membrane; they may also comprise a content of signaling molecules such as Sonic Hedgehog (SHH), Retinoic Acid (RA) and other morphogens. The NVPs apparently fragment on the left side of the node, with the aid of the peripheral cilia, through the interaction of the SHH of the vesicles with its receptor called Smoothened in the cilia, and are absorbed by the nodal surface. Thus, they may be responsible for producing a concentration gradient to the left of the node, and the released molecules may play a role in the left-right determination. In addition, they may also be responsible for an asymmetrical elevation of intracellular calcium through Hedgehog signaling [21, 24].

Shiratori and Hamada [20], however, expose the fact that SHH plays an important role in left-right determination in avian species, but in distinct vertebrates, such as the mouse, it does not appear to be directly involved in asymmetric determination, but rather in the formation of a functional midline. Similarly, AR would be related to the maintenance of bilateral symmetry during the formation of somites, but not to the left-right determination itself. Thus, the authors refute the potential role of the content of NVPs in the establishment, in fact, of the asymmetric configuration in other vertebrates.

Macroscopically, at least three distinct mechanisms are involved in the formation of anatomically asymmetric structures: the first concerns the directional "looping" of initially tubular organs, such as the heart, which go through a series of steps (looping, flexion and rotation) and reach their correct final positioning; the second corresponds to differential branching, in which a pair of symmetrically formed organs, on both sides, acquires differences in their size or branching pattern, as occurs with the lungs and; The third – unilateral regression – is related to the disappearance of one side

of a symmetrical structure, such as a blood vessel [20]. The failure in the processes of establishing adequate left-right asymmetry results in a range of laterality defects, such as absence of bilateral symmetry breaking, independent localization of structures in relation to the *Site* normal and partially or totally abnormal asymmetric orientation [26, 29].

3.4 CILIARY ABNORMALITY

Changes in genes associated with cilia can lead to changes in their structure and affect their signaling mechanisms in various anatomical localities. The gene *PKHD1* (Polycystic Kidney and Hepatic Disease 1), for example, encodes the protein fibrocystin, or polyductin, expressed in the axoneme and basal body of primary cilia. Mutations in the gene lead to autosomal recessive polycystic kidney disease, a severe form of pediatric cystic kidney disease [30]. Genes *PKD1* (Polycystic Kidney Disease 1) e *PKD2* (Polycystic Kidney Disease 2) encode polycystin proteins, which are also located in the primary cilia and which play a necessary role for kidney function. Mutations involving these genes are responsible for the ciliopathy of autosomal dominant polycystic kidney disease, or adult polycystic kidney disease [31, 32].

Ciliary genes *BBS* (Bardet-Biedl Syndrome) mutants cause Bardet-Biedl syndrome, a rare autosomal recessive disorder that leads to varied manifestations such as obesity, renal abnormalities, hypogonadism, and retinopathy [33]. It is also characterized by involvement of the primary cilia, since the normally encoded BBS proteins are located in the basal body and ciliary axoneme and contribute to the formation of a complex active in the intraflagellar transport system [34].

Other mutations that lead to the absence or immotility of the cilia can cause abnormalities in the left-right organization, confirming their essential role in generating the directed nodal flow and, consequently, the initial process of symmetry breaking. Mice with the gene *Dvl* mutant – gene responsible for coding Dishevelled, cytoplasmic protein of the PCP pathway – present interruption of the posterior positioning of the basal bodies and randomization of the flow, which also determines a defective left-right standardization [35].

3.4.1 Changes in the laterality of the heart

Cardiac anatomy also involves the characterization of *Situs* and its complexities. Or *situs solitus* atrial corresponds to the usual arrangement of the atrium morphologically right to the right and left to the left, while the mirror image of this situation corresponds to the *situs inversus*. The presence of atrial isomerism, with the two atria presenting the right or left morphology, constitutes the *Situs ambiguous*. Also, the atrioventricular connection is characterized as concordant, when the right atrium is connected to the right ventricle and the left atrium to the left ventricle, and discordant, when the right atrium connects to the left ventricle and the left atrium to the right ventricle; When the atria are connected to

a single ventricular chamber, the univentricular connection is configured. The venoatrial connection is also analyzed, observing how the systemic and pulmonary veins are connected to the atrial chambers. The ventriculoarterial connection, finally, is classified as: concordant, when the aorta emerges from the left ventricle and the pulmonary trunk from the right ventricle; discordant, when the opposite occurs; double outflow tract, when the two vessels emerge from the same ventricle; and single outflow tract, when only one arterial vessel emerges from the heart or there is aortic or pulmonary atresia [36, 37].

3.5 IN THE MEDICAL CLINIC

3.5.1 Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is a classically autosomal recessive disorder, rarely linked to the X chromosome, caused by mutations in more than 40 genes responsible for coding proteins necessary for the proper assembly and functioning of mobile cilia. Distinct mutant genes determine different severities of symptomatology, which is dependent on the extent of ciliary structural and functional changes. Mutations in the gene *DNAH5* (Dynein Axonemal Heavy Chain 5), for example, which encodes the heavy chain of the outer arm of the dynein of the proximal and distal zones of the cilium, lead to ciliary imotility, while mutations in the gene *DNAH9* (Dynein Axonemal Heavy Chain 9), which encodes the heavy chain of the outer arm of the dynein of the distal zone, lead only to a reduced curvature of the distal ciliary portion and do not alter the beating frequency [38, 39].

The disorder is characterized by a generalized ciliary dysfunction and, therefore, there are ciliary involvements in distinct anatomical regions, such as in the epithelium of the respiratory tract, in the epithelium of the vas deferens, in the fallopian tubes and in the ependyma, as well as there is involvement of the spermatozoa. Thus, the disease predisposes to dysfunctional mucociliary clearance and recurrent respiratory infections, and it is important to consider it as a differential diagnosis in cases of chronic infections of the respiratory system, and is also often associated with infertility and hydrocephalus, in addition to other clinical manifestations (TAB 1). The presence of other clinical indicators also raises the suspicion of PCD, including a family or personal history of ciliopathies and the existence of laterality disorders, since the cilia that present motility play an essential role in the left-right pattern [40, 41].

TABLE 1 - Clinical manifestations of primary ciliary dyskinesia

Lung	Respiratory distress syndrome, pneumonia, atelectasis (neonatal period)
	Chronic productive cough
	Bronchrhea
	Recurrent episodes of pneumonia
	Severe and/or atypical "bronchial asthma" with no response to conventional therapy
	Bronchiectasis
	Digital hypocratism
Middle ear	Chronic serous otitis media
	Transmission hypoacusis
	Persistent otorrhea after tympanostomy
Paranasal sinuses and nasal cavities	Chronic rhinosinusitis
	Nasal polyposis
	Continuous mucopurulent rhinorrhea (early neonatal period)
Lateralization of organs	*Situs inversus* total
	Heterotaxy - left isomerism (polysplenia) - Right isomerism (asplenia)
Fertility	Male infertility
	Decreased female fertility and ectopic pregnancy
Associated diseases	Complex congenital heart disease
	Polycystic renal and/or hepatic disease
	Biliary atresia
	Esophageal atresia
	Severe gastroesophageal reflux
	Hydrocephalus
	Retinitis pigmentosa

Source: Adapted from Fermeiro *et al* [42]

The definitive diagnosis is established when at least three of the following phenotypic manifestations coexist: difficulty breathing in term infants, chronic nasal congestion and chronic productive cough throughout the year, chronic otitis media with effusion for more than 6 months, chronic pansinusitis, bronchiectasis and other recurrent lower respiratory tract infections and, finally, male infertility, laterality defects and family history of the disease. When there is a simultaneous presence of bronchiectasis, pansinusitis and *situs inversus totalis*, one of the existing laterality disorders, the diagnosis is Kartagener syndrome [41, 42].

3.5.2 Kartagener syndrome

Kartagener syndrome is a subgroup of primary ciliary dyskinesia, being the most severe clinical form of the disorder and being present in 50% of cases. It is related to the deficiency of ciliary dynein, protein responsible for the generation of mechanical force in the movement of the eyelashes. It is also known as Immobile Cilia syndrome, associated with the absence of frontal sinuses, chronic rhinosinusitis and bronchiectasis, with recurrent respiratory infections and airway damage. The spermatozoa are immobile, being the male carrier, infertile [40, 43].

The main picture of a child born with the disorder is based on pulmonary symptoms, which become evident within 24 hours after birth and which, in a significant number of cases, cause neonatal respiratory distress syndrome. Other typical symptoms associated with seeking medical help refer to recurrent ear infections, persistent wet cough, nasal congestion, and chronic wheezing. In accordance with the abnormalities that accompany PCD, Kartagener syndrome also presents conditions justified by ciliary changes, which lead to abnormal positioning of certain organs and changes in the function of other structures. Approximately 20% of patients with *situs inversus* belong to the group with Kartagener syndrome [44, 45].

3.5.3 *Situs Inversus*

Or *situs inversus* It is characterized as a mirror image arrangement of the abdominal organs, but with a *Situs* normal cardiac, that is, the apex is located in the left hemithorax, and therefore there is a levocardia. Already the *situs inversus totalis* It is characterized by the mirror image of the *situs solitus totalis*, including abdominal viscera in reverse location, bilobate right lung and trilobed left lung and dextrocardia. Thus, the individual exhibits completely inverted left-right asymmetry, with transposition of the viscera in the chest and abdomen [46, 47]. The abnormality is typically asymptomatic and is not considered a premalignant condition, however, a small number of cancer cases have been reported, as well as cardiovascular malformations, intestinal anomalies, and respiratory comorbidities [48].

This abnormality is inherited as an autosomal recessive genetic trait and may occur in combination with primary ciliary dyskinesia. However, some genes are responsible for the occurrence of *situs inversus* and are not associated with the occurrence of PCD, and the mechanisms involved in this alteration of isolated laterality are based on the role of some of these genes in the coding of ciliary proteins. Mutations in the gene *CFAP52* (Cilia and Flagella Associated Protein 52), for example, which encodes a protein active in the processes of ciliary signal transduction, establish causality with the occurrence of *situs inversus* and heterotaxy in patients without dyskinesia [49, 50].

In the United States, an average distribution of 1 per 10,000 individuals was observed, however, the discovery of such a morphological alteration does not depend on symptoms resulting from the

inversion of the organs themselves, often being discovered in the research of unrelated health alterations, or even at the time of necropsy. This fact can be observed with the American patient Rose Marie Bentley, who died of natural causes, but as soon as her body was donated for research to a university in Portland, Oregon, the students of an anatomy class realized that many of her organs were mirrored [51].

4 FINAL CONSIDERATIONS

The scientific evidence based on the analyzed studies denotes the role that, in fact, the nodal flow can play in the establishment of normal left-right asymmetry in certain vertebrates and, therefore, in the usual anatomical pattern. Additionally, the clinic presented by patients with ciliary disorders reaffirms the conception that cilia are involved in the definition of a pattern of *situs solitus totalis*. As an effect, the laterality defects that initially had an undefined causality can be progressively understood and, thus, the need for a constant acquisition of knowledge in the scope of embryology is remarkable, aiming to elucidate the various abnormalities that involve human development and the mechanisms involved in their occurrence.

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