INTRODUCTION

Primary ciliary dyskinesia (PCD) is a chronic, congenital pathology due to an alteration of the structure and/or functioning of the cilia present on the surface of the respiratory cells of the bronchial tree. This leads to an alteration of mucociliary clearance, with accumulation of secretions and recurrent respiratory infections that usually results in bronchiectasis and chronic lung damage.

It is inherited in an autosomal recessive manner, meaning that 25% of the offspring of two carriers will present the disease. It is estimated that the prevalence is 1 in 10,000 newborns, with a significant under-diagnosis of the disease (1).

CLINICAL PICTURE

PCD is a systemic disease because there is an alteration of the cilia present in different parts of the body, not only at the bronchial level but also at the nasal-sinus level, middle ear, flagellum, fallopian tubes and nodal cilia in the embryo. Thus, the clinical presentation is very heterogeneous and varies with the age of the patient.

Newborn

Respiratory distress, without apparent cause in a full term newborn should make us suspect the presence of PCD. This characteristic occurs in 60% of cases. This respiratory distress characteristically is of later onset and is associated with longer use of oxygen and atelectasis. At this age the presence of wet cough and rhinorrhea also begins, which becomes persistent over time (2,3). During the newborn period, a defect in laterality can also be investigated due to impaired functioning of the nodal cilia in the embryonic period. This normally controls the left-right asymmetry in the distribution of organs. Its malfunction accounts for the presence of situs inversus totalis in about 50% of patients with PCD, while 6 to 12% may present situs ambiguous, which is often associated with congenital heart disease (1).

Preschoolers and schoolchildren

During this period, ENT problems are frequent. In a systematic review and meta-analysis conducted on 1970 patients, persistent rhinorrhea was observed in 75% of the cases, recurrent sinusitis in 69% and chronic otitis media with effusion in 74%, which leads to conduction hearing loss in 36% of cases (4). Otitis media with effusion is more frequent in children under 11 years of age, reaching 83%, observing that the associated hypoacusis improves many times with the insertion of ventilation tubes (5). In all ages persistent wet cough is a characteristic that remains, resulting in frequent infections to the lower respiratory tract, such as pneumonia (4). Bronchial obstructive pathology is also prevalent among patients with PCD, often presented as recurrent wheezing in preschoolers or asthma difficult to control in school children (2). The natural evolution of the disease is towards chronic lung damage with the appearance of bronchiectasis, present in 96% of adults with PCD, according to a study published in the United Kingdom (6). Characteristically, computed tomography shows that patients with PCD have bronchiectasis in the middle lobe, lingula, and lower lobes (7). It is also common to find images of bronchial tree-in-bud, mucous plugs and atelectasis (7).
Diagnosis of primary ciliary dyskenesia

Adult

In this age group, fertility problems are added to the symptoms previously described. In males due to reduced sperm motility and in women due to abnormal motility of the fimbria of the fallopian tubes. In a study that included 49 men and 36 women with PCD it was determined that 75% of men and 61% of women were infertile, however with assisted fertilization techniques conception was achieved in 50% of men and in 30% of women (8).

DIAGNOSTIC METHODS

Nasal Nitric Oxide

It has been shown in multiple studies that nasal nitric oxide levels in patients with PCD are significantly lower than in healthy controls. The reason for this lower production of nitric oxide in patients with PCD remains unclear. In a meta-analysis that included 11 studies, it was observed that the average nasal nitric oxide in patients with PCD was 19 nL · min⁻¹, whereas in healthy controls it was 265 nL · min⁻¹ (9). This marked difference makes nasal nitric oxide a good biomarker for identifying patients with PCD. Patients with compatible history and low levels of nasal nitric oxide should be subjected to other studies such as high-speed video microscopy, electron microscopy or genetic study to confirm the diagnosis. However, normal levels of nasal nitric oxide do not exclude the diagnosis of PCD since it has been found that in some genetic variants, nasal nitric oxide levels are normal. Therefore, if the clinical history is very suggestive despite having normal levels of nasal nitric oxide, other tests should be performed to rule out the diagnosis (10). The measurement of nasal nitric oxide requires patient collaboration, so in general this test is performed after 5 years of age (Figure 1).

Nasal biopsy

Most tests to establish the diagnosis of PCD require a sample of ciliated respiratory epithelium, which extends from the inferior nasal turbinates to the terminal bronchioles. Usually, the sample is taken by nasal brushing since this is a faster and less invasive procedure, but the sample can also be taken at the bronchial level by means of a fibrobronchoscopy. The nasal sample is done using a very fine brush, smaller in diameter than a phosphorous. The brush is inserted into the nostril and the inner turbinate is brushed, rotating the brush on its major axis. The sample is then introduced into a culture medium and taken to the laboratory to be analyzed in fresh. The procedure is fast, it is performed in the examination room and does not require sedation or analgesia (Figure 2). This procedure can be done at any age.

It has been estimated that up to a quarter of patients will require a second biopsy due to inconclusive results (11). This is more frequent when the patient has had a recent acute respiratory infection. For this reason, to minimize findings compatible with secondary ciliary dyskinesia, it is recommended to not take the sample before 4 weeks of an acute respiratory infection.

High speed video microscopy (HSVA)

The sample of ciliated cells of the respiratory epithelium is deposited in a cell culture solution and is observed directly through a special microscope that has a high-speed camera attached, that allows to record ciliary movement and analyze it (Figure 3). This examination allows to observe the ciliary movement pattern and calculate the beat frequency. Both the beat pattern and the frequency are important when analyzing the HSVA. Normal ciliary movement consists of two phases, the first is a fast forward movement, like a whiplash and then the recovery phase comes. Typical PCD findings through HSVA are: complete ciliary immotility, rotational movement of the cilium on its own axis and vibratory movement in which the cilia are seen rigid without folding on their axis, as normally happens (11). HSVA is a test with a sensitivity of 96 to 100% and specificity of 93 to 95%, with a very good interobserver agreement (kappa 0.7) (12, 13, 14). Despite the above, this procedure also has some drawbacks such as the difficulty in differentiating between PCD and ciliary dyskinesia by-product of an infectious process. This difficulty...
Diagnosis of primary ciliary dyskinesia could be overcome by repeating the procedure at another time and evaluating whether the findings are repeated. Another option available at international centers is to perform an air-liquid cell culture from the cells taken by nasal brushing until a differentiated epithelium is obtained and then observe if the alteration observed initially in the first sample is repeated, confirming the diagnosis. However, this type of culture is very laborious and difficult to carry out.

Furthermore, HSVA is a procedure that requires experience to be able to interpret the findings, there is still no consensus or standardization regarding the way to report it and subtle defects can go unnoticed.

Electron microscopy (EM)

In this case, the respiratory epithelium sample is fixed in a glutaraldehyde solution and included in a paraffin cylinder, then cut into very thin sections and stained with heavy metals. With this technique it is possible to appreciate the ciliary ultrastructure. Normally, in a cross section of cillum, the classic 9 + 2 structure can be seen; 9 pairs of peripheral microtubules and a central pair. The peripheral doublets are linked together by the nexin and are linked to the central pair by the radial spokes. The peripheral microtubules also contain inner and outer dynein arms, which are rich in ATP and act as a true motor that allows normal movement of the cilia (Figure 4). There are three characteristic defects of the ciliary structure confirming the diagnosis: absence of outer dynein arms, absence of inner and outer dynein arms and microtubular disorganization with absence of inner dynein arms (11). The absence of inner dynein arms is not enough to diagnosis PCD since this alteration is often due to secondary changes. Electron microscopy has a sensitivity that varies between 71 to 96% depending on the series, with a specificity of 100% (10). It is important to note that this high specificity, reported in literature, comes from reference centers with extensive experience in the analysis of electron microscopy, which may vary depending on the center since the interpretation of the examination is highly operator dependent. Despite the above, EM, like other PCD diagnostic methods, has some drawbacks. Some ultrastructural defects by-product of infection, inflammation or problems with the processing of the sample, can be found, so it is very important that the test is performed by someone experienced. Currently, there is a lack of standardization in the analysis of the sample, it is not clear the number of cuts that should be observed nor the proportion of alterations necessary to make the diagnosis. Until a few years ago, EM was the gold standard for PCD diagnosis, but this has changed, due to the fact that up to 30% of cases can present normal ultrastructure.

Genetics

PCD genetics is quite complex. Cilium is composed of 250 proteins, which is why there are many genes that may be involved. The first mutation described was in the year 1999 and from then, new variants have appeared. Currently, there are 40 mutations identified, which in patients with high clinical suspicion, are capable of discovering 50 to 75% of PCD cases (10). The most frequently found variants are DNAH5 and DNAH11 that cause defects in the outer dynein arms and CCDC39 and CCDC40 that cause microtubular disorganization and absence of inner dynein arm (15). The sensitivity of genetics as a first-line diagnostic test is unknown at present, but it is presumed to be low (10). With the identification of more mutations through new sequencing techniques, it is likely that genetics will be considered in the future as a gold standard. For the moment, genetics is useful when diagnostic confirmation is difficult by other methods.

Immunofluorescence

Immunofluorescence is a relatively new method. This technique uses a sample of respiratory epithelium which is fixed and incubated with antibodies marked with fluorescence, which are directed against the most important ciliary proteins (11). If the protein of interest is present then fluorescence appears and the opposite happens if it is absent,
Diagnosis of primary ciliary dyskenesia as it occurs in patients with PCD (Figure 5). Some of the available antibodies are directed against the outer dynein arm (DNAH5), inner dynein arm (DNAHI1), radial spokes (RSPH4A) and nexin (GAS8). Immunofluorescence helps identify almost all the ultrastructural alterations detectable by the EM and also some cases of PCD where EM is normal. One of the drawbacks of this technique is that currently available antibodies do not detect all the proteins that are part of the ciliary structure. There is still no clarity regarding its clinical usefulness in the diagnosis of the disease, however recent studies show that it would have a sensitivity and specificity similar to the EM when using a panel of 6 antibodies (16).

Diagnostic algorithm (Figure 6)

It is important to bear in mind that all exams available for the diagnose of PCD have their limitations, currently there is no "gold standard" reference test. The European consensus for the diagnosis of PCD establishes three diagnostic categories: positive diagnosis, highly probable diagnosis and highly unlikely diagnosis (10). Positive diagnosis is made when classic ultrastructural alterations are investigated in the EM or presence of a biallelic mutation in one of the PCD causing genes is found in the genetic tests. The diagnosis is highly probable if the patient has a very suggestive history of the disease plus significantly lower levels of nasal nitric oxide and an altered video-microscopy. Furthermore, diagnosis is highly unlikely if the clinical history is modest and the patient has normal or high levels of nasal nitric oxide and a normal HSVA. According to the diagnostic algorithm proposed by this consensus, the testing should start with the measurement of nasal nitric oxide and HSVA. If these tests are normal, diagnosis is highly unlikely, so further diagnostic tests would not be necessary. If the clinical presentation is very suggestive and both tests are altered, diagnosis is then highly probable. In this case, if other confirmatory tests cannot be performed, the patient should be treated for PCD. If the clinical presentation is very suggestive but these two tests are

Figure 5. Immunofluorescence technique. From left to right, a respiratory cell of a healthy patient is described in panel A. In the first box, a green stain can be seen that corresponds to antibodies directed against the tubulin that is part of the ciliary structure, in the second one, the antibodies directed against the outer dynein arm are red. In the third box, when the image converges due to the presence of both proteins, yellow appears. Panel B shows the cell of a patient with ciliary dyskinesia in which the green corresponding to the presence of tubulin can be seen but the red corresponding to the outer dynein arm is not seen, that is why when the image converges it is still green and not yellow as it should be when there is presence of both proteins. The cell nucleus is stained blue.

Figure 6. Diagnostic algorithm for primary ciliary dyskinesia (1). PCD: primary ciliary dyskinesia, NNO: nasal nitric oxide, HSVA: high-speed video-microscopy, EM: electron microscopy
normal or inconclusive, diagnostic testing should be continued with either EM or genetic testing.

In our country we now recently have the possibility to perform measurement of nasal nitric oxide and HSVA that complements the EM. It is important to be sensitive to the diagnosis of this disease and to start actively looking for it as we do now with cystic fibrosis. Early diagnosis of PCD will allow us to better manage our patients, delaying or avoiding the appearance of complications and allowing for a better evolution and quality of life.

REFERENCES


