

CASE REPORT

Primary ciliary dyskinesia diagnosed by electron microscopy in one case of Kartagener syndrome

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Abstract

Primary ciliary dyskinesia (PCD) is associated with abnormalities in the structure of a function of motile cilia, causing impairment of mucociliary clearance, with bacterial overinfection of the upper and lower respiratory tract (chronic oto-sino-pulmonary disease), heterotaxia (situs abnormalities), with/without congenital heart disease, abnormal sperm motility with male infertility, higher frequency of ectopic pregnancy and female subfertility. The presence of recurrent respiratory tract infections in the pediatric age requires differentiation between primary immunodeficiency, diseases with abnormal mucus (e.g., cystic fibrosis) and abnormal ciliary diseases. This case was hospitalized for recurrent respiratory tract infections and total situs inversus at the age of five years, which has enabled the diagnosis of Kartagener syndrome. The PCD confirmation was performed by electron microscopy examination of nasal mucosa cells through which were confirmed dynein arms abnormalities. The diagnosis and early treatment of childhood PCD allows a positive development and a good prognosis, thus improving the quality of life.

Keywords: Kartagener syndrome, dextrocardia, situs inversus, bronchiectasis, primary ciliary dyskinesia.

Introduction

Primary ciliary dyskinesia (PCD), known as immotile ciliary syndrome [1] comprises a small group of human genetic autosomal recessive disorders, caused by malfunctioning organelles, analogous to the mitochondrial, lysosomal or peroxisomal diseases [2].

Ciliary dysfunction associated with total situs inversus (Kartagener syndrome) represents 50% of the cases [3], but only 25% of patients with situs inversus have PCD. Also, ciliary malfunction is involved in other diseases such as polycystic liver and kidney disease, central nervous system disorders (retinopathy, hydrocephalus) and biliary atresia [4]. It is assumed that the normal rotation of the viscera depends on the movement of the bowel ciliated cells, early in embryo-fetal development [5].

Clinical symptoms in PCD varies, some may begin with neonatal respiratory distress, or later develop chronic productive cough, due to bronchiectasis, non-responsive to treatment "atypical asthma", chronic rhinosinusitis and otitis, ectopic pregnancy and subfertility in women, or male infertility. Confirmation of PCD consists in demonstrating abnormal ciliary motility and ultrastructural defects of the respiratory epithelial cilia [6].

The authors present a rare autosomal recessive disorder consisting in the triad of chronic sinusitis, bronchiectasis and situs inversus, which was confirmed by electron microscopy of the nasal mucosa as PCD.

Patient, Methods and Results

A 5-year-old boy, coming from urban areas is admitted to "St. Mary" Clinical Emergency Hospital for

Children, Iassy, Romania, for expiratory dyspnea, wheezing, fever, cyanosis, productive cough, asthenia and malaise. In the personal history it is noted neonatal respiratory distress (respiratory and metabolic reanimated for three days) and many upper and lower respiratory infections, chronic productive cough that required antibiotics, adenoidectomy at age of 4, followed by chronic rhinosinusitis with bilateral serous otitis media. On examination he presented hypotrophy, septic fever, facial dysmorphism without clubbing, SpO₂ 92%, mixed dyspnea, lower intercostal and subcostal retractions, tachypnea (40 breaths/minute), postero-basal bilateral crepitation; the apical impulse to the right midclavicular line, in the 5th intercostal space and palpation of the liver in the left upper quadrant raises the suspicion of situs inversus. The biological tests describe neutrophilic leukocytosis, inflammatory syndrome (ESR 64 mm/1 h, CRP >6 mg/dL, fibrinogen 502 mg/dL), hepatic and renal tests, immunogram, electrolytes and bicarbonate were normal. Blood cultures were negative, and sputum culture shows *Streptococcus pneumoniae* and *Haemophilus influenzae* infection. Initially cystic fibrosis was suspected, and the Pilocarpin iontophoresis was normal on two determinations (44 mmol/L NaCl and 55 mmol/L NaCl) and two measurements were pathological (79 mmol/L NaCl and 74 mmol/L NaCl). The tuberculin skin test was negative. Chest radiograph (Figure 1) describes hilio-basal emphasized bilateral lung interstitium and dextrocardia.

The electrocardiogram (Figure 2) shows: right axis deviation, negative P wave, QRS complex and T wave in DI, positive QRS complex in aVR, the absence of R-wave progression in precordial leads.

Echocardiography describes dextrocardia.



Figure 1 – Chest radiographs with dextrocardia.

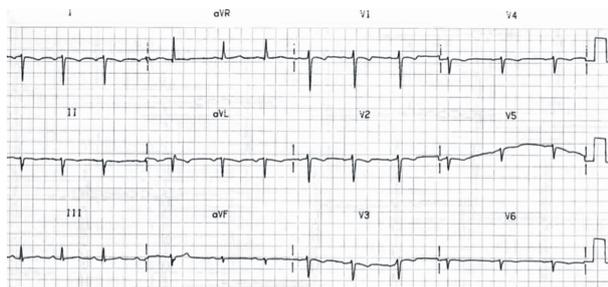


Figure 2 – ECG with typical changes of dextrocardia.

Abdominal ultrasound as well as abdominal CT-scan (Figure 3) confirms total situs inversus with the liver located in the left upper quadrant, spleen in the right upper quadrant and kidney of normal appearance.

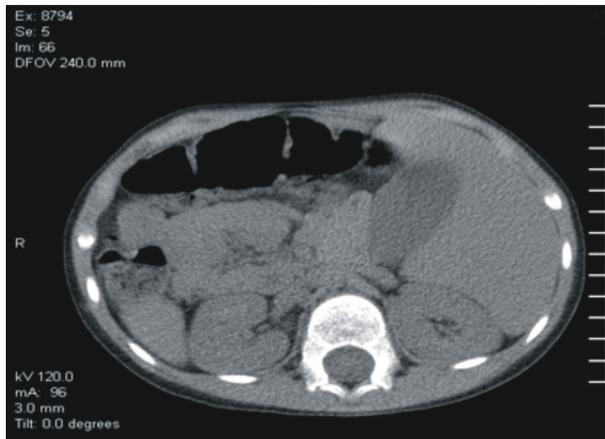


Figure 3 – Abdominal CT with situs inversus.

ENT examination describes chronic rhinosinusitis and bilateral chronic serous otitis. Tympanogram confirmed conductive hearing loss.

The cranio-cerebral CT-scan shows complete obliteration by thickening with mucus and secretions of the paranasal sinuses, mastoid cells and both middle ear, small air-liquid levels visible in the left maxillary sinus and in some of the ethmoid cells.

The chest CT (Figure 4) shows cylindrical bronchiectasis, interesting small subsegmental bronchi, in the anteromedian segments of the middle lobe and lingular lower lobe, their wall being thickened and around these dilated bronchi many micronoduli that creates the appearance of “budding tree” can be seen.

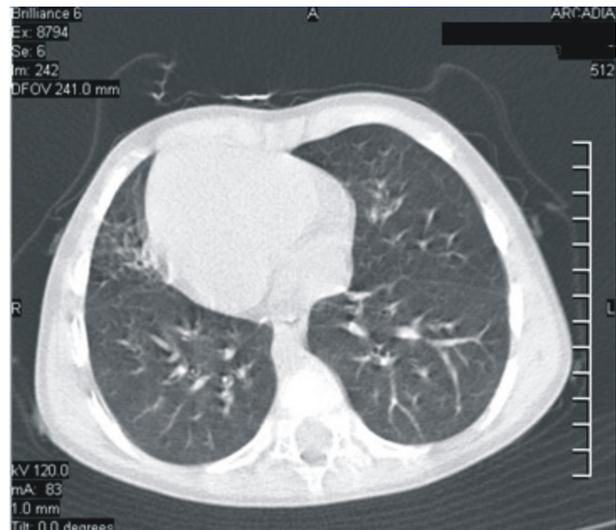


Figure 4 – Chest CT with bilateral cylindrical bronchiectasis.

The association between total situs inversus, chronic pansinusitis, chronic otomastoiditis and bilateral bronchiectasis confirms Kartagener syndrome. He received treatment with oxygen, postural drainage, broad-spectrum antibiotics, NSAIDs, Dornase alfa (Pulmozyme) and saline aerosols, inhaled corticosteroid Fluticasone propionate. Because in our hospital is not performed histological examination of the nasal mucosa by electron microscopy family addresses U.O. Pediatria Medica, Florence, Italy (Dr. Massimo Resti), where nasal lavage is performed for ultrastructural examination of the respiratory epithelium (Dr. Margherita de Santi). Many ciliar well-preserved cells were examined in which both of dynein arms are missing from all examined axonemes (>100) – PCD characteristic features (Figure 5). The iontophoresis was normal.

Diagnosis of PCD requires ciliate epithelial cells in order to assess function and ultrastructure. Further investigation, e.g., following primary cell culture, can assist diagnosis. There is no single gold standard test and diagnosis should be made in a specialty center.

Ciliate epithelium is easily obtained by simple nasal brushing, using a bronchoscopic brush and/or bronchoscopy with flexible endoscope. In some centers, curettage of nasal epithelium is used to obtain ciliate tissue. Electron microscopy (EM) is important in the diagnosis of PCD. However, specialist knowledge is required in order to interpret the various ultrastructural defects responsible for PCD. Inner dynein arm defects, in particular, are difficult to determine since they are less electron dense and less frequent along the ciliary axoneme. In addition, it has been shown that dynein motor protein composition varies along the ciliary length, meaning that ultrastructural defects can be missed using EM, depending upon the site of the ciliary cross-section. Therefore, other techniques have been proposed for a complete diagnosis such as analysis of beat pattern and frequency analysis using video recording, cell culture with redifferentiation of the ciliate epithelium and analysis of intracellular localization of dynein protein (DNAH5) by immunofluorescence microscopy.

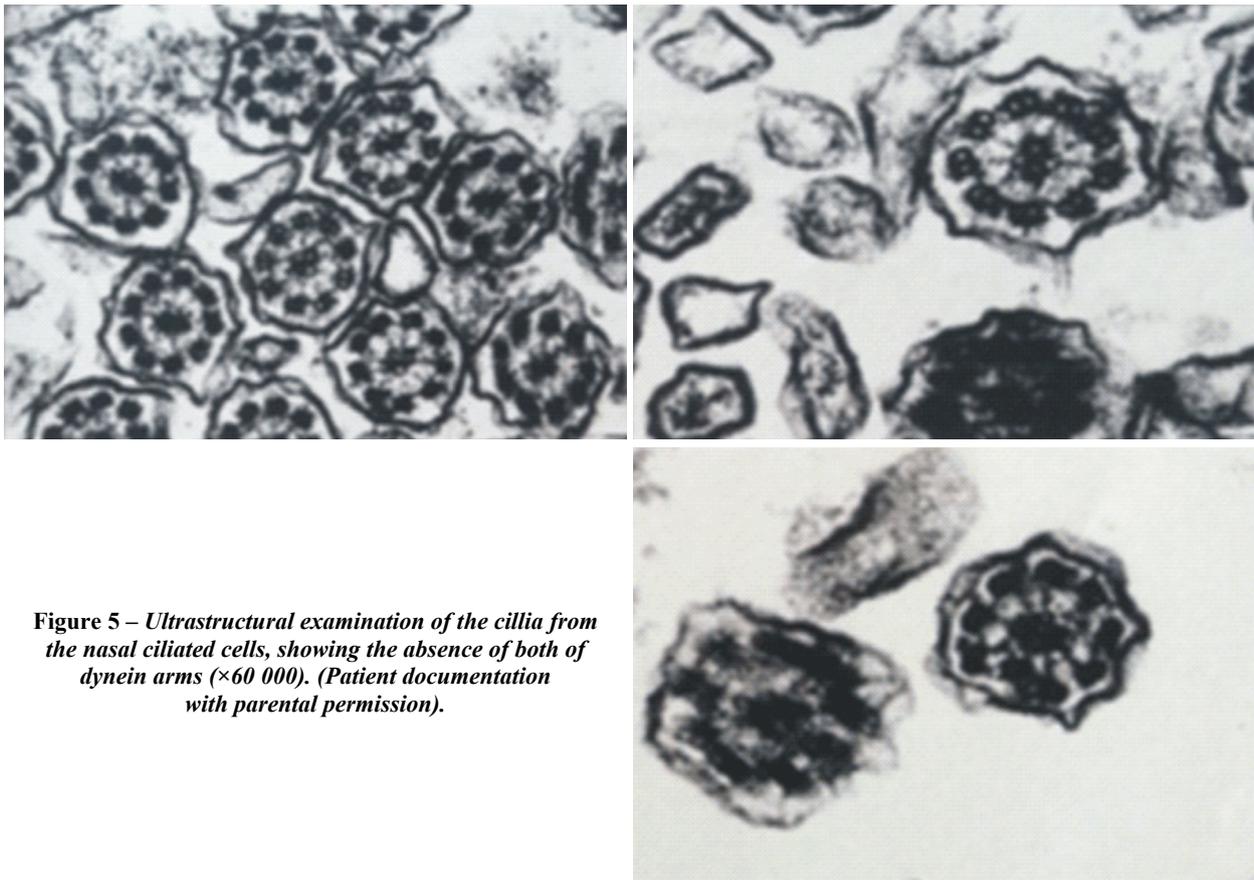


Figure 5 – Ultrastructural examination of the cilia from the nasal ciliated cells, showing the absence of both of dynein arms ($\times 60\ 000$). (Patient documentation with parental permission).

Discussion

Kartagener syndrome (KS) described by Manes Kartagener in 1933 is an autosomal recessive disorder characterized by dextrocardia, bronchiectasis and chronic sinusitis [7]. Ciliary structural abnormalities (immobility/reduced mobility) of airway epithelial cells, spermatozoa or other ciliated cells in the body causes a defective muco-ciliary clearance, leading to abscesses and necrotizing bronchitis and bronchiectasis, as well as chronic rhinitis, serous otitis media, male infertility, female subfertility, corneal abnormalities, headache and reduced olfactory acuity [8]. KS is a subtype of PCD (50% of cases), in which the affected gene was located on chromosome 15q24–25 [9], and the most common ciliary ultrastructural changes are the anomalies of dynein arms, along with radial spoke deficiency, transposition of microtubules or abnormalities with nexin links. The first author who suggested PCD as the cause of KS was Canner *et al.* in 1975 (quoted by Afzelius).

So far, these abnormalities of immotile cilia were insufficiently studied in children, most of the therapeutic strategies being derived from protocols used in cystic fibrosis. Early identification of the disease allows prophylactic treatment, which reduces the severity of bronchiectasis and the evolution to chronic sinusitis. Other authors define PCD as a ciliopathy, along with other genetic syndromes, such as Bardet–Biedl syndrome, polycystic liver and kidney disease, nephronophthisis, Alström syndrome, Meckel–Gruber syndrome and some forms of retinal degeneration [10].

Children with PCD have a history of neonatal

respiratory distress in term neonates, or upper and/or lower respiratory infections, manifested by chronic cough, recurrent wheezing or wheezing with exertional dyspnea. The presented clinical case had neonatal respiratory distress, which required oxygen for three days followed by repeated infections of upper and lower respiratory tract, adenoidectomy at the age of four years, recurrent serous otitis and the diagnosis of KS at the age of five years. The clinical manifestation that helps to distinguish between PCD and cystic fibrosis is chronic serous otitis requiring implantation of trans-tympanic aerator to prevent hearing loss, an event that was present in this case. Kennedy *et al.* research [11] shows that more than 6% of patients with PCD have heterotaxia, as normal ciliary motility is needed to develop left-right asymmetry. Ciliary immotility leads to a various range of positioning of the heart from situs solitus, where all the organs are normally positioned to situs inversus, in which there is a mirror image of all organs [12]. This case presents total situs inversus, not associated with intracardiac anatomical defects, unlike heterotaxia, that includes cardiac malformations in more than 3% of the cases. There are a number of recognized subtypes of heterotaxia, which are determined based on the anatomy of cardiac atria: left isomerism (polysplenia) and right isomerism (asplenia) [13]. Currently, about 50% of patients with left isomerism and congenital heart disease survive to age 15. The study published by Kennedy MP [11] points to a 200-fold higher prevalence of congenital heart diseases in relation to heterotaxia in children with PCD (1 in 50 patients), while PCD is not routinely cited as a cause of congenital heart disease [14].

Recognition and identification of bronchiectasis allow early therapeutic measures that delay the evolution to chronic obstructive pulmonary disease and death. In the presented case, dilated bronchi were revealed at CT-scan and not at standard chest radiographs. Consequently, in 2009, Barbato *et al.* [15, 16] published a consensus of the *European Respiratory Society* (ERS) for the diagnosis and treatment of childhood PCD summing 36 recommendations. Confirmation of the diagnosis of PCD in this KS case was performed by analysis by EM of nasal ciliated epithelial cells obtained by nasal brushing using a bronchoscopic brush in a specialized center in Florence, Italy. Inner or outer dynein arms defects are difficult to determine because they are less dense in electrons and less frequent along the ciliary axoneme. In addition, it has been shown that dynein motor proteins have variable composition during ciliary length, which means that ultrastructural defects may be lost, depending on the ciliary cross section site. Moreover, there are cases in which defects affecting ciliary function cannot be visualized with EM and for which were proposed ciliary pulse pattern and frequency analysis through the use of video recording, cell culture, dynein protein localization analysis (DNAH5) by immunofluorescence microscopy and genetic analysis. The genetic basis of PCD is gradually revealed. The *ERS Task Force Committee* [17] does not recommend it in the initial diagnosis of the disease. After clinical diagnosis, genetic testing can be targeted to the specific version of PCD (*e.g.*, DNAI1 and DNAH5 tests in PCD patients with defects of the outer dynein arm, or DNAH11 test for a particular functional defect) [18, 19]. The molecular complexity of respiratory ciliary cells and spermatozoa axoneme involves a large number of potential candidate genes. Mutations in two genes encoding dynein were confirmed in PCD: DNAH5 located on chromosome 5p15.2 encoding the dynein heavy chain and DNAI1 located on chromosome 9p13.3, which encodes the dynein intermediary chain. Homozygous mutation of the DNAH11 gene on chromosome 7 encoding axonemal heavy chain can cause situs inversus without ciliary affection [20, 21]. The difficulty and high cost of these determinations turned towards the use of screening tests that can precede ciliary function analysis and for pediatric age nasal nitric oxide measurement, as well as exhaled nitric oxide levels, which are low represent a strong recommendation [22–24].

Treatment of the patient with KS and PCD was similar to the treatment of cystic fibrosis: antibiotics according to the antibiogram of bacterial isolates from sputum culture, Pulmozyme nebulization, hypertonic saline aerosols, N-acetylcysteine, adenoidectomy, trans-tympanic aerators, postural drainage and respiratory kinotherapy. The use of prophylactic oral antibiotics is not recommended, but the use of prophylactic antibiotic and corticosteroid inhaler for the *Pseudomonas aeruginosa* and mycobacteria non-tuberculosis infection is used in some centers. The children with PCD can be immunized. The surgical treatment includes lobectomy in forms with localized bronchiectasis or lung transplantation in severe forms.

✚ Conclusions

Ciliopathy is a multisystemic disease, in which PCD is an important subgroup. PCD must be studied by electron microscopy of nasal ciliated epithelium in all cases with KS. Patients with situs inversus, heterotaxia and congenital heart disease require screening tests for PCD. Adults with male infertility and females with recurrent ectopic pregnancies, also female subfertility requires exploration for PCD. Studies of gene mutation correlated with PCD phenotypes will enable these families prenatal advice.

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