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Multidisciplinary approach to patients with manifestations and pulmonary complications of cystic fibrosis

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Abstract

Cystic fibrosis (CF) is a genetic disease, with autosomal recessive transmission, multisystemic, characterized by a remarkable clinical polymorphism and significant lethal prospective. Respiratory manifestations dominate the clinical picture, being present in all patients. The aim of the paper was to analyze the incidence of clinical manifestations, especially respiratory ones, as well as the contribution of interdisciplinary consultations to the positive diagnosis of CF, in a group of 16 patients who were hospitalized and treated in the IInd Pediatric Clinic and IInd Medical Clinic of the Emergency County Hospital, Craiova, Romania, in a period of 20 years. The 16 patients diagnosed with and treated of CF had all shown increased values of sweat chloride concentration of over 60 mmol/L. The main symptoms and clinical signs encountered in these patients were cough (75%), sputum (62.5%), dyspnea (50%), wheezing (50%), stature hypotrophy (100%), pallor (37.5%), cyanosis (25%). All 16 patients had an acute exacerbation of chronic pulmonary disease. Of the total hospitalizations, the death was recorded only in the case of one female patient. The association of some clinical aspects specific with a positive result of the sweat test or the presence of the two pathological alleles made room for determining a positive diagnosis. The multisystemic nature of this disease requires a multidisciplinary approach to these patients. Histopathologically, there was a correspondence between lung morphological lesions and the results of imaging investigations.

Keywords: cystic fibrosis, genetic disease, pulmonary manifestations, multisystemic approach.

Introduction

Cystic fibrosis (CF) is an inherited autosomal recessive disorder, with a significant clinical heterogeneity, that requires from a complex treatment [1]. In Romania, the frequency of the disease is estimated in approximately one in 2500 newborns, with a prevalence of about 1.5 cases per 100 000 inhabitants and a carrier frequency (heterozygotes) of one in 25 [2]. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on the long arm of chromosome 7 (7q31), which encodes a transmembrane conductance regulator protein, CFTR, acting as a chloride channel at the apical membrane of epithelial cells [3]. The phenotypic features result from the absence or inappropriate function of the chloride channels at the cellular level, which involves the presence of viscous secretions adherent to the epithelium of the excretory ducts difficult to discharge as well as to their accumulation and the alteration of the

function and destruction of various organs (lungs, pancreas, liver, intestine, and reproductive organs) [4]. The diagnosis of CF is based on specific clinical symptoms and anamnestic data and then it is confirmed by the sweat test or the molecular genetic testing [5].

A CF test must be conducted for every child presenting with severe respiratory distress associated with a chronic pulmonary disease (chronic cough, mucopurulent or purulent sputum, expiratory dyspnea, wheezing, pulmonary hyperinflation) or a bronchial obstruction with modified values in the spirometry tests, specific circumstances for a persistent pulmonary colonization with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, persistent pulmonary radiological modifications, nasal polyposis, sinusitis or chronic ethmoiditis [6], but can be found and associated with common gastrointestinal and nutritional manifestations: meconium ileus, rectal prolapse, pancreatic insufficiency with malabsorption and steatorrhea, recurrent acute pancreatitis or chronic

pancreatitis, persistent neonatal jaundice, distal intestinal obstruction syndrome, chronic hepatopathy clinically and biologically manifested or on ultrasonographic and/or histological assessment by having the aspect of a focal or lobular biliary cirrhosis, protein-calorie malnutrition resulting in hypoproteinemic edema, as well as secondary complications to liposoluble vitamin deficiency (skin or mucous membrane hemorrhages, rickets, and nocturnal cecity) [7].

There are other specific manifestations of this disease such as salt-wasting syndrome, hypochloremic metabolic alkalosis with hyponatremic dehydration, increased levels of serum trypsinogen in newborn screening and obstructive azoospermia in case of congenital absence of *vas deferens* [8].

The diagnosis of CF can be determined in the presence of one or multiple phenotypic traits of the above-mentioned CF, in a patient that have the proof of the abnormal function of *CFTR* by detecting two abnormal values of sweat Cl^- by quantitative pilocarpine iontophoresis test or identification of diallelic *CFTR* pathogenic variants [9]. The sweat test remains the gold standard test for diagnosing the disease and assessing the concentrations of Cl^- and Na^+ ions in the sweat. A sweat Cl^- concentration greater than 60 mmol/L, in two different assessments, can determine the correct diagnosis [10]. Molecular genetic testing approaches *CFTR* mutation panel that includes the most common pathogenic variants followed by extended *CFTR* gene sequencing and evaluation for deletions or duplications if only one or no pathogenic variant is found [11]. The diagnostic role of histopathological (HP) investigations was secondary, confirming the morphological substrate of the clinical picture in patients who die during hospitalization.

Aim

The aim of the paper is to demonstrate that due to the multisystemic nature of this disease, a multidisciplinary approach of patients is required, in order to early diagnose the complications of the disease and administer appropriate therapy, so as to increase quality of life and survival.

☐ Patients, Materials and Methods

We have realized a retrospective clinical study of 16 patients who were hospitalized and diagnosed with CF in the IInd Pediatric Clinic and IInd Medical Clinic of the Emergency County Hospital, Craiova, Romania, from 2000 to 2020. The diagnosis of CF was achieved based on the anamnesis and the clinical evaluation of children, correlated with the paraclinical investigations in infants and children and confirmed by the sweat test or genetic testing of these patients. Patients were included in the study group according to high levels of Cl^- ions concentration in the sweat after stimulating it with quantitative pilocarpine (a sweat Cl^- concentration greater than 60 mmol/L). The sweat test measures the concentration of Cl^- and Na^+ ions in the sweat test. A sweat Cl^- concentration greater than 60 mmol/L, in two different assessments, confirmed the diagnosis. When a patient with hyponatremia and hypochloremia is clinically suspected of having CF, the sweat test was delayed until an electrolytic balance has been restored. The normal values of electrolytes in sweat are

less than 40 mmol/L. Positive values were observed in children >60 mmol/L, while adolescents and teenagers registered >70 mmol/L; values considered equivocal, between 40 and 60 mmol/L required repeating the investigation after a while. Genetic testing was performed in two Genetics Laboratories (Bucharest and Timișoara). Peripheral blood samples were collected from each child testing positive for the sweat test and for healthy controls, in ethylenediaminetetraacetic acid (EDTA) tubes. The deoxyribonucleic acid (DNA) extraction was performed by QIAmp DNA Blood Mini Kit (QIAGEN). All samples (patients and controls) were analyzed for the 29 common mutations by Elucigene™ CF29 multiplex ARMS kit (Tepnel Diagnostics Ltd, UK), as a rapid screening according to the manufacturer's instructions. To evaluate the frequency of the 5T allele of intron 8, amplification and sequencing of the polypyrimidine tract in front of exon 9 was performed using primers 9i-5 and 9i-3 [12]. Imagistic investigations (radiography, tomography, ultrasound exam) were made with the equipment used by the Laboratory of Radiology and Medical Imaging, Emergency County Hospital of Craiova. Laboratory from samples involving hematology, biochemistry, immunology, bacteriology, and gasometric methods) were done in the Clinical Laboratory of the Hospital, using the system known as Celltac Nihon Khoden and Vitros 250 dry chemistry analyzers. The HP investigations were performed on the specimens harvested from the patient who died because of the evolution of this disease. The necropsy fragments of the lungs were processed by the classic histological technique with 24 hours routine 4% neutral buffered formalin fixation, and paraffin embedding in the Laboratory of Pathology from the Emergency County Hospital of Craiova. Four μm -thick sections from the resulting paraffin blocks were cut at the microtome, and then stained with Hematoxylin–Eosin (HE). The stained sections were examined under a Nikon Eclipse 55i microscope by two pathologists highlighting the lesions characteristic of CF. The statistical analysis was performed using the Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM Statistical Package for the Social Sciences (SPSS) Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for data processing. Because the numerical variables investigated had a normal data distribution, globally or in each studied group, we were allowed to use the parametric statistical tests (*e.g.*, Student's *t*-test) and the results were summarized as the mean value \pm standard deviation (SD). For all statistical tests, *p*-values less than 0.05 were considered significant. All imagistic, hematological, biochemical, immunological, bacteriological, functional, and genetic investigations were made after obtaining the informed consent from the tutor of the hospitalized child or the diagnosed teenager, which guarantees the fundamental rights of the patient.

☐ Results

The 16 patients suffering from CF (13 children and three adults) diagnosed and treated in the Emergency County Hospital of Craiova, from 2000 to 2020. Patients were between four and 27 years old, with an average age of 12.31 ± 6.27 years. Most patients were male, 12 (75%) patients, while the minority was female, four (25%) patients.

The patients included in the study came from Oltenia Region, as follows: 10 (62.5%) patients from Dolj County, four (25%) patients from Olt County, and two (12.5%) patients from Vâlcea County. The age of mothers when they gave birth was between 22 and 31 years old, with an average age of 25.42 ± 5.74 years. The outstanding family antecedents of the patients were found in the brother's case who manifested CF in two (12.5%) cases. The kids showed first symptoms at an age between one day and seven months of life, with an average age of debut of 3.088 ± 2.87 months. The symptoms of debut were respiratory symptoms in four (25%) patients, digestive signs and symptoms in six (37.5%) patients or association of wheezing with chronic diarrhea in four (25%) patients, and retarded growth associated with coughing and diarrhea in two (12.5%) patients. The average age of the patients at the time of diagnosis was 3.63 ± 2.72 years, with limits between 0 and 12 years. The average body mass index (BMI) value was 14.24 ± 1.65 kg/m², with limits between 12.26 and 16.49 kg/m².

Clinical manifestations among CF patients hospitalized and under treatment are shown in the Table 1.

Table 1 – Incidence of clinical manifestations

Symptoms	No. of patients/ incidence (%)	Clinical signs	No. of patients/ incidence (%)
Cough	12 (75%)	Stature hypotrophy	16 (100%)
Expectoration	10 (62.5%)	Paleness	6 (37.5%)
Dyspnea	8 (50%)	Cyanosis	4 (25%)
Wheezing	8 (50%)	Jaundice	2 (12.5%)
Fever	8 (50%)	Persistent skin crease	8 (50%)
Diarrhea	10 (67.5%)	Clubbing fingers	3 (18.75%)
Constipation	2 (12.5%)	Orthopnea	2 (12.5%)
Loss of appetite	8 (50%)	Bronchial rales	4 (25%)
Weight loss	12 (75%)	Bronchoalveolar rales	2 (12.5%)
Abdominal pain	6 (37.5%)	Abdominal distension	10 (62.5%)
Nausea	4 (25%)	Hepatomegaly	4 (25%)
Vomiting	2 (12.5%)	Splenomegaly	2 (12.5%)
Oliguria	8 (50%)	Alteration of consciousness	1 (6.25%)

All 16 patients had exacerbations of chronic bronchopulmonary disease. The number of infectious exacerbations registered in these patients was 49 per year, with an average value of 3.06 ± 0.46 acute symptoms/patient/year. The total number of hospitalizations of the patients included in the present study per year was 67, which represents an average value of 4.18 ± 1.24 hospitalizations/patient/year. There was only one reported death, a 10-year-old girl, whose leading cause of death was a cardiorespiratory arrest resulting from a staphylococcal pneumonia with acute chronic respiratory failure. All hospitalized patients showed high levels of Cl⁻ concentration in the sweat test, over 60 mmol/L, the average value registered with these patients being 114.75 ± 18.07 mmol/L, with limits between 80 and 193 mmol/L in the sweat test. Genetic analysis was performed on DNA samples obtained from 14 (87.5%) patients. Two mutations were found in all patients. Six different *CFTR* mutations were detected: *ΔF508* at 18 (53.57%) out of 28 *CFTR* alleles tested, *N1303K* at four

(14.28%) out of 28 *CFTR* alleles, *G85E* at three (10.71%) out of 28 *CFTR* alleles, *394delTT* and *2184delA* in only one (3.57%) out of 28 *CFTR* alleles and *5T* polymorphism in one (3.57%) patient. Most patients, nine (56.25%) out of 14 patients, were compound heterozygous, and five (31.25%) were homozygous. In other two patients, who did not undergo genetic testing, the sweat test was used to help diagnose CF.

Modifications present in pulmonary radiography and computed tomography (CT) are shown in the Table 2.

Table 2 – Incidence of imagistic examination modifications

Radiological features (16 patients)	No. of patients/ incidence (%)	CT features (five from 16 patients)	No. of patients/ incidence (%)
Accentuation of the peribronchovascular pulmonary interstitium	12/16 (75%)	Bronchiectasis of bilateral upper lobes	3/5 (60%)
Apical bilateral bronchial dilataions	4/16 (25%)	Disseminated bronchiectasis of both lungs	2/5 (40%)
Pulmonary opacity with the appearance of pulmonary condensation	2/16 (12.5%)	Peribronchovascular interstitial pulmonary fibrosis	5/5 (100%)
Pulmonary hyperinflation	2/16 (12.5%)	Pneumonic-type condensation	2/5 (40%)
Normal lung radiograph appearance	2/16 (12.5%)	Lung distention	1/5 (20%)

CT: Computed tomography.

On chest radiographs, the earliest modification was accentuation of the peribronchovascular pulmonary interstitium (Figure 1).

On CT images, the most common changes were lung hyperinflation, pulmonary hypertension, and bronchiectasis (Figures 2 and 3).

Spirometry was performed for 12 patients and allowed for a registered forced vital capacity, both with an absolute and a relative (percentage) value, the forced expiratory volume in the first second (FEV1) and the index of bronchial permeability. Low values of forced vital capacity (FVC) were registered in the investigated patients, less than 80% of standard values were calculated in three (25%) patients, low levels of FEV1 were less than 80% of standard values calculated in five (41.66%) patients, low levels of the index of bronchial permeability less than 70% in three (25%) patients. Germs involved in the production of infectious exacerbations (identified in pathological products of tracheobronchial tree), which complicated the evolution of the disease, were: Methicillin-sensitive *S. aureus* (MSSA) 20 (40.81%) infectious episodes, *P. aeruginosa* 12 (24.48%) infectious episodes, Methicillin-resistant *S. aureus* (MRSA) six (12.24%) infectious episodes, *Klebsiella pneumoniae* four (8.16%) infectious episodes, *Burkholderia cepacia* two (4.08%) infectious episodes, and *Serratia liquefaciens* one infectious episode (2.04%). There were four (7.27%) mixed infectious episodes with *P. aeruginosa* and MRSA.

The average, minimum and maximum values of hematological and biochemical parameters are shown in the Table 3.

The average, minimum and maximum values of liver and pancreatic laboratory tests are shown in the Table 4.

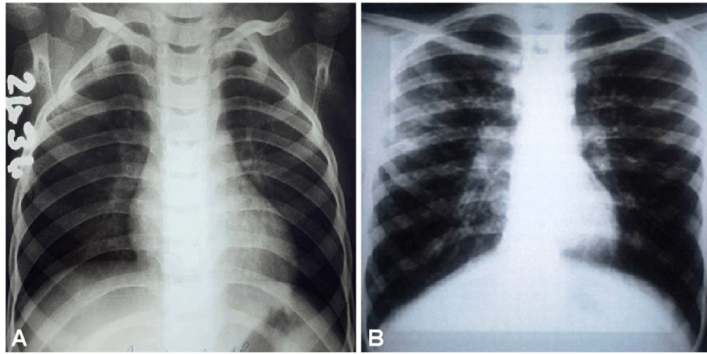


Figure 1 – Radiological aspects in CF. Chest radiographs in postero-anterior incidence. (A) Four-year-old child diagnosed with CF. Pulmonary hyperinflation. Pulmonary hypertension – vascular enlargement of pulmonary hilum. Accentuation of the apical and perihilar pulmonary interstitium, bilateral. (B) Eighteen-year-old adolescent with CF. Pulmonary hyperinflation. Pulmonary hypertension – vascular enlargement of pulmonary hilum. Bronchial dilatations with a predominant cylindrical appearance, in the upper half of the bilateral lung areas. CF: Cystic fibrosis.

Figure 2 – CT scans in CF. Axial CT images. (A) Pulmonary hyperinflation. Pulmonary hypertension – vascular enlargement of pulmonary hilum. Bronchi with thickened walls. Cylindrical bronchiectasis (1). (B) Cystic dilated bronchi (1) in an 18-year-old adolescent patient. The “signet ring of bronchiectasis” (2), with the cystic dilated bronchus representing the “ring” and the adjacent smaller artery representing the “jewel” on the ring. CT: Computed tomography; CF: Cystic fibrosis.

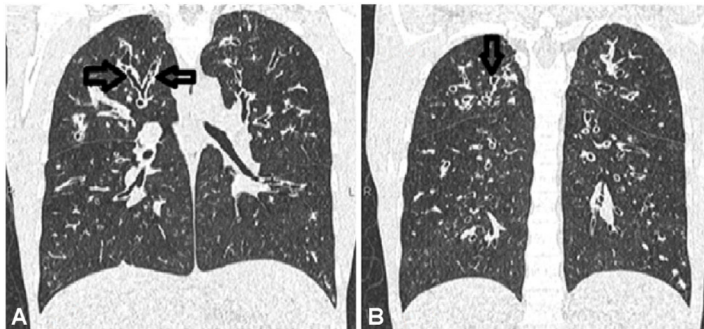
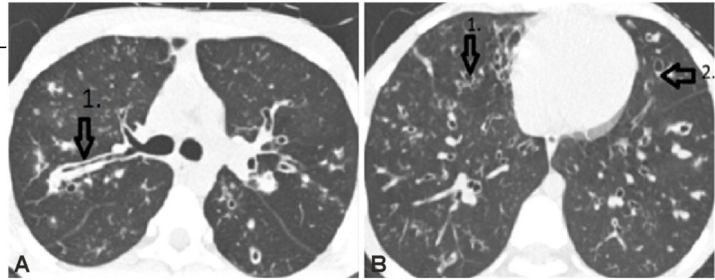


Figure 3 – Coronal reformatted CT image in CF. CT images shows corresponding dilated bronchi with thickened walls and dilated bronchi: (A) Cylindrical bronchiectasis in a 14-year-old patient; (B) Cystic bronchiectasis in an 18-year-old patient. CT: Computed tomography; CF: Cystic fibrosis.

Table 3 – The average, minimum and maximum values of the hematological and biochemical parameters

Parameter	Average value, minimum and maximum values	Parameter	Average value, minimum and maximum values
Hb [g/dL]	11.43±3.43 (7.7–14.5)	γ-Globulin [g%]	15.87±6.91 (6–34)
MEV [fL]	83.8±14.68 (66–105)	pH	7.42±0.05 (7.35–7.51)
MCH [pg/mL]	25.4±3.51 (20–29)	PaO ₂ [mmHg]	72.8±22.88 (37–99)
Leukocyte count [1/mm ³]	11550.63±5290.67 (7500–28 000)	PaCO ₂ [mmHg]	33.6±5.22 (27–36)
Platelet count [1/mm ³]	387 540.43±112 400.62 (180 000–540 000)	HCO ₃ ⁻ [mEq/L]	33.6±5.22 (27–36)
Fe [µg/dL]	48.5±22.98 (12–113)	D-dimers [µg/mL]	1.24±0.48 (0.5–1.8)
ESR [mm/1 h]	26.56±11.07 (5–52)	NT-proBNP [pg/mL]	54.42±23.45 (30–120)
Fb [mg/dL]	435.07±113.14 (250–750)	Urea [mg/dL]	28.5±14.45 (13–98)
CRP [mg/L]	2.975±1.78 (0.3–9)	Uric acid [mg/dL]	0.46±0.12 (0.3–0.9)

Hb: Hemoglobin; MEV: Mean erythrocyte volume; MCH: Mean corpuscular hemoglobin; Fe: Serum iron; ESR: Erythrocyte sedimentation rate; Fb: Fibrinogen; CRP: C-reactive protein; PaO₂: Partial pressure of oxygen; PaCO₂: Partial pressure of carbon dioxide; HCO₃⁻: Serum bicarbonate; NT-proBNP: N-terminal pro-brain natriuretic peptide.

Table 4 – The average, minimum and maximum values of liver and pancreatic laboratory tests

Parameter	Average value, minimum and maximum values	Parameter	Average value, minimum and maximum values
TBIL [mg%]	1.31±1.13 (0.5–6.8)	Am [U/L]	42.8±28.72 (20–97)
DBIL [mg%]	0.63±0.37 (0.2–2.73)	Lip [U/L]	30.66±10.14 (23–45)
ALP [U/L]	286.46±148.76 (125–576)	Ca [mg/dL]	8.36±0.84 (7.4–10.6)
GGT [U/L]	77.78±14.06 (12–477)	Ca ²⁺ [mg/dL]	3.32±0.78 (1.2–4.4)
ALT [U/L]	42.5±28.55 (13–100)	Mg [mg/dL]	1.75±0.26 (1.4–2.1)
AST [U/L]	42.8±28.72 (20–197)	VitD3 [U/L]	38.8±13.91 (24–54)
TP [g%]	6.29±1.35 (4.4–9.5)	Na [mEq/dL]	130.22±8.8 (118–137)
S-alb [g%]	3.72±0.65 (2.9–4.8)	K [mEq/dL]	4.3±0.9 (3.1–5.1)
PT [%]	77.93±11.66 (68–100)	Cl [mEq/dL]	95.87±9.94 (76–104)

TBIL: Total bilirubin; DBIL: Direct bilirubin; ALP: Alkaline phosphatase; GGT: Gamma-glutamyltransferase; ALT: Alanine transaminase; AST: Aspartate transaminase; TP: Total proteins; S-alb: Serum albumin; PT: Prothrombin time; Am: Amylasemia; Lip: Lipasemia; Ca: Serum calcium; Ca²⁺: Ionized calcium; Mg: Magnesium; VitD3: Vitamin D3; Na: Sodium; K: Potassium; Cl: Chloride.

The modifications resulted from the stool test were as follows: presence of drops of undigested fats in eight (50%)

patients, presence of undigested fat drops and muscular fibers in four (25%) patients, presence of fat drops and undigested starch in three (18.75%) patients. The average value of the fecal pancreatic elastase performed on six patients was 78.42 ± 43.36 , with limits between 29–100 mg/dL. Abdominal ultrasound exam performed on the tested patients revealed: hepatomegaly in four (25%) patients, modifications of hepatic echogenicity in six (37.5%) patients, modifications of hepatic structure in six (37.5%) patients, modifications of hepatic contour in one (6.25%) patient, caudate lobe hypertrophy in one (6.25%) patient, increase in portal vein diameter in one (6.25%) patient, splenomegaly in three (18.75%) patients and an increase in the splenic vein diameter in one (6.25%) patient. The endocrinology exam performed on these children confirmed the following results: postural hypotrophy in 16 (100%) patients, osteopenia in four (25%) patients, and delayed menarche in two (12.5%) patients. The ear, nose, and throat (ENT) exam performed on most hospitalized patients

described acute nasopharyngitis in four (25%) patients, acute tonsillitis in four (25%) patients, nasal polyposis in four (25%) patients, acute otitis in three (18.75%) patients, acute rhino-adenoiditis in two (12.5%) patients, chronic tonsillitis in six (37.5%) patients, and chronic rhinosinusitis in four (25%) patients.

HP examination of pulmonary necropsy fragments revealed the presence of a large lesion diversity. Thus, the predominant lesions have been hypertrophy of the bronchial glands, mucous cell hyperplasia of the trachea and main bronchi (Figure 4A), the presence of intraluminal mucous plugs, acute and chronic inflammation of bronchi, with the presence of mucopurulent exudate with microbial colonization in the lumen of lower respiratory airways and the presence of a diverse inflammatory cell population with neutrophils, histiocytes, lymphocytes, and plasma cells (Figure 4B). Others associated lesions were the ulcerations of the bronchial and bronchiolar mucosa, endobronchial abscesses, bronchiectasis, and atelectasis.

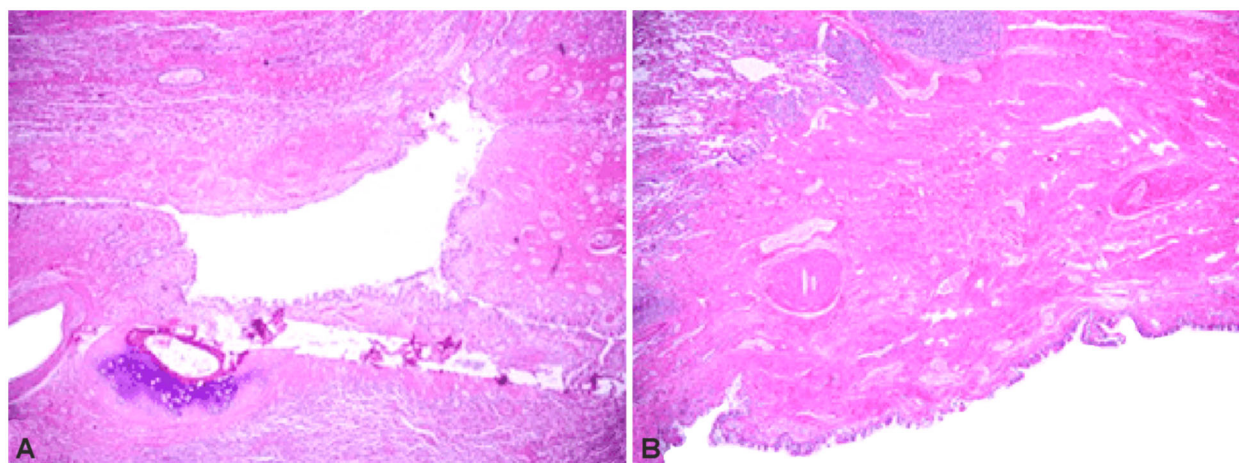


Figure 4 – Lung necropsy fragments (HE staining, $\times 40$): (A) A main bronchus with mucous cell hyperplasia, mucous gland hypertrophy, fibrosis predominantly in the submucosa and in the intima of the pulmonary arterial branches; (B) A bronchiole with ulcerated areas of the epithelium, extensive fibrosis starting from the basement membrane of the bronchial epithelium and extending into the pulmonary interstitium associated with fibrosis in the intima and hypertrophy of the middle layer of the branches of the pulmonary artery. HE: Hematoxylin–Eosin.

☒ Discussions

Amongst the patients hospitalized in the IInd Pediatric Clinic and IInd Medical Clinic of the Emergency County Hospital of Craiova and diagnosed with CF, the most majority were males, the male/female ratio being 3:1, which clearly shows a male genetic predisposition to this disease, an aspect which has been also highlighted by the specialists [13]. The average age of the investigated patients was 12.31 ± 6.27 years, with limits between four and 27 years [13]. The reported average age of the onset of the disease was 3.088 ± 2.87 months, with value limits of one day and seven months [14]. The onset symptoms of the disease were respiratory manifestations in four (25%) patients, digestive symptoms and signs in six (37.5%) patients or the association of wheezing with chronic diarrhea in four (25%) patients, and the growth retardation with cough and diarrhea in two (12.5%) patients [14]. The average age of the patients at the time of diagnosis of the disease was 3.63 ± 2.72 years, with limits in between zero and 12 years. The incidence of the main symptoms and clinical signs

recorded in these children was cough in 12 (75%) patients, expectoration in 10 (62.5%) patients, dyspnea in eight (50%) patients, wheezing in eight (50%) patients, diarrhea in 10 (62.5%) patients, loss of appetite in eight (50%) patients, growth retardation in 12 (75%) patients, abdominal pain in six (37.5%) patients, postural hypotrophy in 16 (100%) patients, pallor in six (37.5%) patients, cyanosis in four (25%) patients, persistent skin fold in eight (50%) patients, mucosal dryness in eight (50%) patients, bronchial rales in four (25%) patients, tachycardia in eight (50%) patients, abdominal distension in 10 (62.5%) patients and hepatomegaly in four (25%) patients.

Iontophoresis (sweat test) was the basic exploration for the diagnosis of mucoviscidosis. All patients presented sweat Cl^- concentration values of over 60 mmol/L, the average value recorded in these patients being of 114.75 ± 18.07 mmol/L, with limits in between 80 and 193 mmol/L [3]. These results were in accordance with the genotyping tests, the patients showing genetic changes specific to this disease [15]. Genetic analysis performed in 14 patients revealed six allelic mutations, each patient analyzed had

two different alleles on chromosome 7 q31.2 locus. The frequency of mutations found was: *ΔF508* in 18 (53.57%) situations, *N1303K* mutation in four (14.28%) situations, *G85E* mutation found in three (10.71%) situations, *5T* polymorphism in one situation (7.14%), deletion *394TT* in one case (3.57%), and deletion *2184A* in another situation (3.57%), frequency similar to that found in a study conducted on 777 samples collected from patients [16]. Most patients, nine (56.25%) patients, presented with a heterozygous status, while a homozygous status was identified in five (31.25%) patients. In our study, in two (12.5%) patients who did not undergo genetic testing, the sweat test was used to help diagnose CF.

Conventional chest radiographs are usually adequate to detect the salient radiographic features of CF and provide objective parameters for longitudinal disease progression [17]. On the radiographs, the earliest change is the accentuation of the pulmonary interstitium. Also, pulmonary hypertransparency is a change that occurs quite early. Changes in lung imaging are due to thickening of the bronchial walls by infiltration of the bronchial submucosa with lymphocytes and plasma cells. In many cases, recurrent pneumonia, cylindrical or cystic bronchiectasis and multiple small abscesses developed in the bronchiolar walls appear [18]. Advanced complications of CF are atelectasis, pneumothorax, pneumomediastinum, pulmonary hemorrhage, cardiomegaly, and dilation of the pulmonary artery with the appearance of radiographic signs of chronic pulmonary heart [17]. High-resolution computed tomography (HRCT) is more sensitive and secure than standard chest radiography in establishment the presence and severity of bronchiectasis and other parenchymal and airway changes in CF [17]. There are scoring systems using HRCT to assess bronchiectasis severity in CF. These systems rely on a subjective evaluation of the presence, extent, and severity of different lesions of the CF, including bronchial wall thickening, bronchiectasis, mucous plugging, and emphysema [19]. Pulmonary function measurements showed low values of FVC less than 80% of the standard values calculated in three (25%) patients, low values of FEV1 less than 80% of the standard values calculated in five (41.66%) patients, low values of bronchial permeability index less than 70% in three (25%) patients. The analysis of the values of the spirometric indices obtained at the pulmonary function tests allowed the description of the obstructive ventilatory dysfunction syndrome in three (25%) patients and mixed in two (12.5%) patients. The chronic bronchial infection (colonization) and a persistent inflammatory response leading to the appearance of progressive bronchiectasis and obstructive and restrictive lung disease [20]. The measurement of FEV1 by spirometry is currently the essential parameter for monitoring lung function, assessing its severity and progression [21]. Lung function tests play an important role in the management of CF patients of all ages. We must be aware of the reference values used for spirometry since according to the chosen one, the respiratory functional diagnosis can be variable [22]. The bacteriological examination of the pathological respiratory disorders should occur whenever the patients present an inflammatory process of the respiratory symptomatology (intense coughing, increasing thickness and quantity of sputa, which intermingles with

mucopurulent matter, the occurrence or disappearance of dyspnea), considering the risk of pulmonary colonization of these patients, mainly with extremely pathogenic, antibiotic resistant stems. Early sustained therapy with antibiotics of respiratory infections contributed to the longevity of patients with CF [23].

The low hemoglobin values, found in seven (43.75%) patients could be explained by malabsorption syndrome, acute or chronic infectious phenomena, immunological phenomena recorded in patients with hypergammaglobulinemia [24]. The increased values of erythrocyte sedimentation rate (ESR) recorded in 14 (87.5%) children were the consequence of acute inflammatory processes caused by infection, which complicated the evolution of the disease of these patients, the highest values being observed in bacterial lung infections (pneumonia) [24]. High γ -globulin levels in two (12.5%) patients suffering from CF can be explained *via* the chronic infectious disease, which can cause local immunological reaction followed by a systemic one from the immunity system. Under these circumstances, a larger number of immunological tests is needed in order to be able to exclude an autoimmune pathology or a simultaneous hematological one [25]. High levels of pH determined in four patients (25%), associated with low levels of electrolytes in blood (Na^+ and Cl^-) and high bicarbonate levels determined the suspicion and diagnosis of CF, based on hypochloremic and hyponatremic metabolic alkalosis, mainly in patients who, at the debut, presented episodes of diarrhea associated with dehydration with hydroelectrolytic imbalance and acid-base disorders [26]. Low levels of partial pressure of oxygen (PaO_2) in six (37.5%) patients affected by advanced bronchopulmonary disease (especially those suffering from bronchiectasis) determined chronic hypoxemia as well as the risks it generates, which necessitates a plan completion for caring and treating these patients [27]. Low levels of partial pressure of carbon dioxide (PaCO_2) (hypocapnea) were present in those four (25%) patients diagnosed with metabolic alkalosis, attributed to chronic diarrhea, which caused the suspicion and early diagnosis of CF. High levels of PaCO_2 (hypercapnea) correctly defined the type of hypercapnic respiratory insufficiency manifested in two (12.5%) of the hospitalized patients, with the occasion of infectious exacerbations of the chronic bronchopulmonary disease and helped to set up an appropriate treatment [28]. Unfortunately, this type of respiratory insufficiency which complicated the evolution of the disease in the deceased child could not be corrected and was noted down as the immediate cause of death. The biochemical evaluation of the myocardial function, by determining the serum *N*-terminal pro-brain natriuretic peptide (NT-proBNP), is useful for early diagnosis of the contractile dysfunction of the right ventricular myocardium, when the completion of the therapeutic plan is indicated [29]. Renal functional exploration, along with an assessment of the water-electrolyte imbalance and acid-base disorders highlighted electrolyte imbalances in patients manifesting dehydration, eight (50%) patients and the one suffering from acute pyelonephritis in one (6.25%) patient, which allowed the implementation of a correct therapeutic

approach. The functional exploration of the liver, which was of major importance in these patients revealed biochemical modifications specific for cytolysis in five (31.25%) patients and intrahepatic cholestasis in four (25%) patients, pathological aspects common in such cases [30]. These modifications must be highlighted any time they appear, since in patients who are hospitalized for infectious exacerbations of mucoviscidosis and who need antibiotherapy, the antibacterial drugs must be chosen according to the level of hepatotoxicity. The evaluation of the exocrine pancreatic function was necessary both for the diagnosis of pancreatic insufficiency, but mostly for establishing an appropriate therapeutic care. In these patients, the medication for pancreatic substitution must replace the nutritional deficiency of patients, while it is acknowledged that protein-calorie malnutrition has its own supplementary contribution to the alteration of the pulmonary function [31]. Establishing the levels of albuminemia and oligoelements (calcium, iron, and magnesium blood levels) had a significant meaning in the therapeutic management of these patients, knowing that an early improvement in the manifestations of the syndrome of malabsorption will prevent metabolic and hematological disorders from occurring, since these had a negative impact on the evolution of the disease [25]. The investigation of the function of the endocrine pancreas is indicated by the glucose tolerance test, especially in patients with a history of prolonged pulmonary diseases. It is necessary and useful for the early diagnosis of diabetes associated with CF, knowing that a delay in the introduction of the antidiabetic treatment will result in irreversible modifications at the level of small and large vessels [32].

CF is an autosomal recessive disorder with a varied penetrance and wide clinical variability. Interdisciplinary examinations were extremely important in these patients given the multisystemic nature of the disease, the role of the pediatrician being the most important, because he must recognize all the manifestations of the disease and request these examinations at the right time [33]. The neonatologist was requested whenever the newborn presented either respiratory distress at birth that required oxygen supply, or delayed appearance of meconium. The collaboration of the neonatologist with the pediatric surgeon was necessary and lifesaving in the case of newborns with meconium ileus, who did not respond to drug therapy or in the case of the newborn with jejunal atresia. In such cases, early indication for sweat test was beneficial to diagnose the disease since the first days of life [34]. ENT examination was indicated and provided useful information if the child had specific manifestations of acute adenoiditis, nasal polyposis, ethmoiditis, and acute/chronic sinusitis. These diseases required immediate performance of the sweat test and early diagnosis of this disease [35]. The pneumological examination helped establish the correct type and degree of respiratory failure and established, together with the pediatrician, the therapeutic conduct in the transition of childhood to adulthood [36]. Cardiological examination was required in all children diagnosed with CF who showed manifestations of severe respiratory failure and who were hospitalized for clinical and biological evaluation. It is necessary to

develop a periodic cardiological exploration plan that allows the early detection of secondary pulmonary hypertension, which will indicate the establishment of new therapeutic measures particularly useful in preventing the installation of chronic pulmonary heart disease [37]. Periodic pediatric examination with clinical–biological evaluation will help for early diagnosis of malabsorption syndrome, establishing the degree of pancreatic damage by correct morpho-functional examination of the pancreas, early identification of hepatobiliary suffering and the establishment of appropriate hepatoprotective and anti-cholestatic therapy for this type of disease [38]. Urological examination in adulthood will be required whenever a young man with CF will want to start a family. Infertility induced by the absence of vas deferens can be overcome by intracytoplasmic injection of sperm after transcutaneous harvesting the sperm from the epididymis [39]. Neurological examination proved to be particularly necessary in agitated patients, in exacerbation of chronic respiratory failure, in whom hypoxic, but especially hypercapnic cerebral distress, required the exclusion of an organic neurological pathology [40]. Endocrinological examination was practiced in most children, given the endocrine and metabolic manifestations of malabsorption syndrome. The endocrinologist will establish the therapeutic conduct in girls who have delayed menarche, but especially the counseling of young man with azoospermia [41]. Psychological examination should not be missing from the exploration plan of these young patients, considering the special psychological problems that occur during the diagnosis of this chronic disease, and especially for the family of the patient in time of the transition of the patient to adulthood [42]. Genetic counseling is very important for families where one of the children has been diagnosed with CF, or if a patient with CF wants to start a family [43]. Screening for carrier status and prenatal testing for pregnancies at increased risk are possible.

The HP examination highlighted the morphological substrate of the clinical and imaging manifestations of the patients investigated by us, emphasizing the existence of a correspondence between the imaging picture and the HP one. Most HP lung lesions detected by us in the child who died of CF were mucous cell hyperplasia, mucosal gland hypertrophy and acute and chronic inflammation of the lower respiratory tract. Changes such as bronchiectasis, atelectasis, emphysema, and pulmonary fibrosis are more the prerogative of older children and young people affected by this disease. Many authors have shown that the lungs are normal at birth [44], but that even before the infectious clinical picture became evident in the airways were observed: hypertrophy of the submucosal glands, obstruction of the excretory ducts, hyperplasia of mucous cells in the trachea and large bronchi and mucus hypersecretion [45]. In overt clinical forms of bronchiolitis, the airway lumen is filled with mucopurulent secretions and bacterial colonies, and the respiratory epithelium proliferates endoluminal as papillary projections. Over time, reinfections change the morphopathological picture to a chronic bronchial type with a mixed inflammatory infiltrate, which includes neutrophils and histiocytes, lymphocytes and plasma cells, and quite often is associated with follicular bronchiolitis

[46]. The formation of endobronchial abscesses is the first step towards the development of bronchiectasis, their number increasing with age. They are more numerous in the proximal pulmonary airways, affecting especially the upper lobes, the middle lobe of the right lung, and the lower lobes [45, 46]. In children, atelectatic lesions are also quite common, being the result of obstructive mucus plugs and lymphoid follicle hyperplasia adjacent to the bronchi [47]. In contrast, emphysematous lesions appear to develop at older ages, so that they become present in 41% of patients aged between 10–24 years old [45]. At the same time, if in young children the HP picture is dominated by acute pneumonia, in the older children and youths the morphopathological picture is dominated by appearance of chronic inflammatory interstitial infiltrate, lymphocytes and plasma cell, interstitial fibrosis and the presence of lung cystic structures. It is discussed the existence of four types of pulmonary cystic structures developed in the CF evolution, respectively: (i) bronchiectatic type, the most common, in which there is a direct communication with the bronchi, (ii) interstitial type, located in the visceral pleura and interlobular septa, frequently associated with pneumothorax, (iii) pneumatocele type, and (iv) emphysematous type [46]. In older patients, with the increase of bronchiectasis number, and as well as due to fibrous obliterative bronchiolitis, was noticed a decrease in the density of small airways [48]. At the same time, it seems that there is a prevalence of lesions such as pulmonary atelectasis, bronchial obliteration with mucus plugs and air cysts for the upper lung lobes [49, 50]. Over time, pulmonary hypertension develops, due to the medial hypertrophy and intimal fibrosis of the pulmonary artery branches, which is often incriminated in the death of some of these patients due to rupture of dilated arteries and bronchial veins in the airway walls or bronchiectasis [51]. The severity of lung lesions increases with age, with emphysematous changes, pneumothorax, chronic pulmonary arterial hypertension, and severe chronic respiratory failure on the foreground [52]. Pulmonary amyloidosis with a diffuse interstitial pattern was also found in patients with a longer survival time [53].

☒ Conclusions

The association of some phenotypic aspects specific for CF (recurrent pulmonary infections, MRSA or *P. aeruginosa* respiratory tract colonization, fat soluble vitamin malabsorption, the hyponatremic dehydration syndrome associated with hypochloremic metabolic alkalosis, or the connection between diarrhea and recurrent wheezing) with a positive result of the sweat test or the presence of the two pathological alleles made room for determining a positive diagnosis. The multisystemic character of CF requires a multidisciplinary approach to the problems of the patients involved in research, with a view to early diagnosis and guiding of the appropriate and timely treatment, which will alleviate and improve the quality of our lives and will increase life expectancy. A HP approach to this study revealed a connection between morphological characteristics of pulmonary lesions and the results of the imagistic investigations.

Conflict of interests

The authors declare that they have no conflict of interests.

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