ORIGINAL PAPER

Microbiology characteristics among cystic fibrosis patients in western Romania

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Conflicts of interests

Nothing to declare

Acknowledgements

None

These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Central Eur J Clin Res 2018;1(1):76-82

Received: 15.06.2018, Accepted: 2.08.2018, Published: 1.09.2018

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Abstract

Background: Pulmonary infectious inflammation is a major cause of decline in lung function in patients with cystic fibrosis (CF) marked by exacerbations, consequently, slowing evolution of lung disease is a primary aim in CF management. The objective of the study was to analyze the microbiological spectrum from epidemiological point of view in our patients.

Methods: An observational, cross-sectional transversal study including fifty-seven patients evaluated the prevalence of CF-related microbes in the study group and their pulmonary status.

Results: The most frequent microorganism found in our group, regardless age, was *Staphylococcus aureus*, closely followed by *Pseudomonas aeruginosa*. Bacillus tuberculosis was a rare germ, despite the important frequency in our country. The microbes frequency was different with age groups, thus 3.5% of 1-3 years old children had the methicillin sen-

sitive *Staphylococcus aureus* (MSSA) strain, while for the 6-12 years group, *Pseudomonas aeruginosa* was found in an equal percentage of 14% with MSSA. *Pseudomonas* prevalence was found in 14.0% of adults and the combined infections were diagnosed in about a fifth of our patients.

Conclusions: We concluded that the percentage of respiratory infections with redoubtable microbes is relatively moderate. The presence of underweight among CF patients with severe mutations are risk factor for a worse outcome and measures should be instituted.

Introduction

Cystic fibrosis (CF) is the most common potentially lethal monogenic disease of Caucasian population, autosomal recessive transmitted and manifested by a marked clinical polymorphism [1]. Clinical manifestations include classically: lung disease, expressed by chronic obstructive pulmonary disease associating frequent pulmonary infection with redoubtable germs, exocrine pancreatic insufficiency with secondary steatorrhea or diabetes by endocrine pancreatic insufficiency [2]. Liver disease is an important feature in CF, also nasal polypsis, obstructive azoospermia, meconium ileus, rectal prolapse, chronic sinusitis, salt loss syndrome [3] or bone disease CF associated [4].

Although manifestations are extremely varied, pulmonary disease has a vital importance that determines the prognosis of the disease. Respiratory status is the determining factor in the evolution of the patient with CF, lung infections being the main cause of morbidity and mortality [5]. Besides infections, lung disease's outcome is influenced by other clinical feature like body mass index, the presence of complication like CF related diabetes (CFRD) CF associated liver disease (CFLD) or bone disease [5].

The genetic defect associates a defective mucociliary clearance, which favors pulmonary infection with significant bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia* sp. [6] Because of *P. aeruginosa* multiples "self-protection's" characteristics, like hypermutability and alginate hyperproduction, resistant phenotypes are selected [7], in the meantime, the prevalence of microbes, like *Staphylococcus aureus* are raising also methicillin resistant *S. aureus* (MRSA) in the last decade [8].

The first infection with *P. aeruginosa* can become chronic in 6-12 months [9], during which it is possible to eradicate the infection therapy. It is therefore, extremely important to early di-

agnose *P. aeruginosa* infection, in order to establish eradication therapy and prolong the lives of children with cystic fibrosis [10]. The evolution of chronic infections is marked by frequent exacerbations that lead to loss of lung function and the pulmonary parenchyma over time [11], consequently decelerating the outcome of lung disease in CF.

The aim of the paper is to evaluate our study group from clinical, bacteriological and respiratory functional point of view, studying the actual clinical pulmonary status, the presence of respiratory infections among children with cystic fibrosis and the patient's characteristics like anthropometric measurements, genotypes and lung volumes.

Patients and methods

Patients from western part of our country, attending the annual visit in to the National Cystic Fibrosis Centre Timisoara, were invited to participate in to the study independently to the regular visit, by a doctor not involved in the patient's management. All patients and patient's parents signed the informed consent and the study received the Hospital's Ethics Committee approval.

The observational, cross-sectional transversal study, including patients with typical cystic fibrosis, diagnosed according to ECFS guidelines [12] was designed.

At every visit, three bacteriological samples (sputum sample, cough swab or hypopharyngeal aspirate) were collected, every 3 months, as our national guideline stipulated [13] and supplementary samples were collected during exacerbations. Anthropometric measurement of weight, height and body mass index (BMI) were recorded, besides evaluation of pulmonary function by spirometry, in children older than six years of age.

Isolation on conventional culture media Sheep blood agar, MacConkey agar, Mannitol salt agar (Sanimed-ready plate) and identification of germs were performed at the hospital laboratory. Identification of germs and extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed using the BioMerieux® VITEK 2 automated microbiology system. Leeds criteria [10] were used for classification of the *P. aeruginosa* infection status; first infection was defined by the first detection of the microbe in the culture, intermittent infection by the presence of intermittent positive cultures for a year and chronic infection as persistence of the positivity of *P. aeruginosa* in the cultures.

The data on age-at-CF diagnosis and genetic tests results over ten years retrospectively were obtained from center's database, with patient's agreement. The genetic tests were performed using a 29 mutations kit, using a method based on allele specific amplification technology (ARMS), afterwards the genotypes were classified according to McKone [14]. Pancreatic insufficiency was defined as the use of pancreatic enzymes during the study period and certified by the decrease level of pancreatic faecal elastase-1 below 100 µg/g, specific for exocrine insufficiency, using the ELISA method [15].

Assessment of the pulmonary function was performed by spirometry; forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and maximal expiratory volume at 25-75 percent of FVC (FEF₂₅₋₇₅) were registered as standardized [16]. The values of FEV1, FVC and FEF₂₅₋₇₅ corresponding height and weight were the variables used and expressed as per cent predicted; best FEV1 per year value was registered for reference.

Cystic fibrosis associated liver disease (CFLD) was diagnosed by the presence of at least two of the following features: hepatomegaly+/-splenomegaly, clinically detected and confirmed by ultrasound, persistent elevation of liver function test (more than 2 determinations) and liver parenchyma alteration, detected on ultrasound examination, transient elastography or magnetic resonance imaging, according Colombo criteria [17], for the diagnosis of cystic fibrosis related diabetes (CFRD) the European criteria were used [18].

Statistical analysis

For the description of the continuous variables, we used the mean and the standard deviation and for the description of ordinal and nominal variables, we used frequency and percentage. For statistical analysis, IBM-SPSS v. 18 was used.

Results

The results are presented in two sections: one descriptive part of the patient's characteristics, another part regarding the pulmonary infections epidemiology in our group.

Patient's descriptive characteristics

Fifty-seven patients achieved the inclusion criteria and agreed with the study. Our patients were relatively homogenous regarding

the sex predominance, a slight preponderance of males was found, numbering 57.9%.

As regards the age, we have a relatively young group of patients, described by a median age of 13.02 years ± 6.1 SD, reasonably early diagnosed with CF at the median age-at-diagnosis of 4.5 years ± 4.25 SD, more than a half of them 52.6% being diagnosed before 3 years of age (n=30).

Clinical features

Pancreatic insufficiency (PI), laboratory expressed by low level of pancreatic faecal elastase-1, was found in an important percentage of our patients, 84.22%, most of patients were typical cases characterized by steatorrhea, therefore presumably pancreatic insufficient, while 15.78% patients were pancreatic sufficient at the time of the study.

Concerning the anthropometric characteristics, the study group was characterized by a low median weight of 39.06 kg ± 14.3 SD, but a normal median height of 1.51 meters ± 0.23 SD. On the BMI, the median value was consistent for underweight BMI = 16.45 kilo/m² ± 2.83 SD. An important percentage of 89.5%, were lean, with BMI lower than < 20.0 kg/m², from which 54.9% were males. Cystic fibrosis associated liver disease (CFLD) was diagnosed in 56.1% of our patients, an important prevalence in to a group of cystic fibrosis patients relatively young. The prevalence of cystic fibrosis related diabetes (CFRD) was 10.52%, with a predominance of the female gender (66.6%).

Genotype configurations

As for genotype, the patients were characterized by the predominance of F508del homozygous genotype was the most frequent genotype with a frequency of 49.12%, as expected. 24.56% of patients had a compound genotype, the presence of F508 del mutation, in combination with an unidentified allele (F508del/x) by our testing panel. Compound genotypes like F508del/non F508 were present in 10.52% and the genotype F508del/G542 X found in 5.2% of patients, while 7.01% patients had unknown genotypes.

Pulmonary status

Spirometry data were obtained from 89.4% patients aged over 6 years of age. The lung function were expressed by the value of FEV 1% predicted, in our group of patients, the mean FEV1 was 75.76%; SD=23.201;

median=82.00; range=[23-127], and FVC: mean =88.90; SD=17.675; median=93.00; range=[45-127], FEF 25-75: mean=58.92; SD=27.374; median=56.00; range=[11-107].

Transversal microbial study results

Regarding the distribution of infections on the age group, the infancy period is marked by the presence of MSSA with a prevalence of 3.5%, while in the 6-12 years group, *P.aeruginosa* and MSSA were found in equal percentage of 14.0%.

Figure 1 Bacterial distribution among age groups

Legend: MRSA- Methicillin resistant *Staphylococcus aureus*
 MSSA- Methicillin sensitive *Staphylococcus aureus*
 PSE- *Pseudomonas aeruginosa*

For the older children with the age between 12-18 years, *P.aeruginosa* and MSSA were the most frequent germs, found in an equally prevalence of 8.8% of patients. 14.0% of adults had positive *P.aeruginosa* sputum culture. Eleven patients (19.29%) had coexisting chronic bacterial infection, most frequently with *P.aeruginosa* and *Staphylococcus aureus* (12.28%).

General prevalence of the most significant microorganism, *P.aeruginosa* was substantial, in a percentage of 52.63%, most of them having F508 del homozygous genotypes (93.3%).

In pancreatic sufficient patients, the infections prevalence was low, 0.1% had *P.aeruginosa* compared to patients with insufficiency, where 77.1% had at least one germ at the time of the study.

In patients with associated liver disease, the germs were found in 68.9% and *P.aeruginosa*

prevalence was 34.4%, prevalence was increased compared to patients without this complications who had 38.8% infections and 11.1% *P.aeruginosa*.

Discussion

Regarding age and gender, our group of patients was relatively heterogeneous, with the median age 13.02 years, representative for the interval of time when complications like CFRD and CFLD occur and the frequency of chronic infections increases. Age at CF-diagnosis ranged from 0.2 -17.7 years, with a median age-at-CF diagnosis of 4.5 years, a reasonably early age, due to increase of awareness about CF and subsequently better diagnosis and compared with a previous median of 13.94 years for CF diagnosis a decade ago, reported in our country [19].

As for the anthropometric characteristics of the study group, an alarming feature was the finding of an important percentage of underweighted children (median BMI =16.45 $\text{kg/m}^2 \pm 2.83$ SD.) and the fact that almost all patients had unsatisfactory BMI (89.5 %). Knowing that BMI has a significant consequence on the pulmonary outcome [20], questions on the appropriate nutritional management or pancreatic enzymes supplementation following actual guidelines has to be raised [21].

The most frequent genotype was F508del homozygous genotype found in 49.12% of patients, fact explainable by the inclusion of typical CF forms and increased predominance of F508 del mutation in our population [22]. Another genotype F508del/G542 X, classified as severe [15], was found in 5.2% of patients, counting more than half of patients with severe genotypes and increasing the probability of an unfavorable outcome in our population. One third of patients (31.57%) had undetermined alleles- a significant percentage considering the typical phenotypes included and 7.01% had unknown genotypes, suggesting that an extended genetic panel might be useful.

Pancreatic insufficiency was found in almost all patients, being one of the inclusion criteria based on typical cases; the patients pancreatic sufficient 15.78% were diagnosed by positivity of sweat test and genotyping.

The presence of the CFLD in 56.1% of our patients, showed an important prevalence explicable by the presence of pancreatic insufficiency [23] in almost all patients, and by the sensitive criteria used to detect the liver disease in the initial stages. Moreover, a distinct consideration accorded to this complication as

a result of a previous study [24] which reported an alarming prevalence of 31%. Considering the negative influence of liver failure on the pulmonary outcome in terms of susceptibility to infection [17], lung function deterioration [25] and antibiotics liver toxicity, a special concern should be addressed to early detection of the liver disease [23] and appropriate therapy.

Another complication with an important effect on the lung function [26] and susceptibility to infection is CFRD found in 10.52% of our patients, consistent with data reported by other countries [18] but higher considering the relatively young age of our study population and the fact that CFRD is representative for more older patients.

Transversal microbial study results

In general, the infants diagnosed earlier with CF have fewer bacterial infection [27] with germs like *Staphylococcus aureus* [5], found in our group also, in a small percentage of 3.5%, but an important increasing percentage of 14.0% in the 6-12 years group, where the methicillin-resistant strain MRSA reached a 5.26% prevalence. Fourteen percent of the children with age between 6-12 years had *P.aeruginosa* in the transversal evaluation, a higher percent compared to 12-18 years group, where *P.aeruginosa* was found in 8.8%. A greater percentage would have been expected in this group, because the first positive culture with *P.aeruginosa* was found at 9.4 years in average in the retrospective analysis, and the eradication success rate is considerable low. It would be possible that in this early diagnosed group, the effect of hygiene education to be efficient and the chronic infection expected to decrease in the future [15].

In older children, age between 12-18 years, *P.aeruginosa* and *MSSA* were the most frequent germs, found in an equally prevalence of 8.8% of patients. Chronic infection with *P.aeruginosa* was diagnosed in 14.03% of adults and another 19.29% having a coexisting chronic bacterial infection, most frequently associated *Staphylococcus* sp. (12.28%). A cumulative prevalence of 33.32% of *P.aeruginosa* among adults patient is similar with the other CF centers [28] and data reported in our local intensive therapy units [29]. The prevalence of *Pseudomonas* infection is not as big as expected in a country without newborn screening, but the segregation and non-use of anti-staphylococcal prophylactic antibiotherapy, known to favors the early *Pseudomonas* acquisition [30], might be consistent reasons for it. One contributing factor

could be a false low prevalence because of the low sensitivity of cough swab and hypopharyngeal culture in non-sputum productive children. A better sensitivity would have the detection of microorganism in the bronchoalveolar lavage, but this is an invasive method useful in selected cases or for research purposes.

Our group of children was characterized by a relatively good lung function expressed by a median FEV₁ of 75.76% and FVC mean = 88.9%, means characteristics for a good outcome of a 13 years old patient, which is the median age of the studied patients. The distal obstruction was stated by a FEF₂₅₋₇₅ mean of 58.92% consistent with a mild to moderate distal obstruction, in accord with median values for the age of the group. A better assessment of the lung function would be by using the lung clearance index as a parameter for the lung function evaluation.

Conclusions

Our patients are now in their adolescence, most of them with a relatively good pulmonary function and reasonable percent of chronic pulmonary infection; the prevalence of *Pseudomonas* infection is significant but not massive, considering the delayed diagnosis because of lack of newborn screening missing. Unfortunately, the important percentage of underweighted children is an alarming sign, therefore the necessity of nutritional status improvement raises. An important percent of severe genotypes was found among our group study, also a significant number of unidentified mutations suggest the need for enhancement of the genetic alleles panel for our region. It is understandable that introduction of CF newborn screening would consistently contribute to the increase of diagnosis and a subsequent improved management and life expectancy in CF Romanian children.

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