

Katarzyna Walicka-Serzysko, Dorota Sands

THE CLINICAL PRESENTATIONS OF PULMONARY ASPERGILLOSIS IN CHILDREN WITH CYSTIC FIBROSIS – PRELIMINARY REPORT*

PREZENTACJE KLINICZNE ASPERGILOZY PŁUCNEJ U DZIECI Z MUKOWISCYDOZĄ – DONIESIENIE WSTĘPNE

Cystic Fibrosis Centre, Institute of Mother and Child, Warsaw, Poland

Abstract

Pulmonary aspergillosis is a very serious complication in cystic fibrosis (CF) patients due to the great variety of its clinical presentations and the fact that it worsens the prognosis. We can distinguish the following: Aspergillus colonization (AC), Aspergillus infection (AI) and allergic bronchopulmonary aspergillosis (ABPA). Aspergillus colonization (AC) is defined as isolation of Aspergillus spp. from 50% or more sputum samples over six months to one year without observing deterioration in lung function and an increase in such respiratory symptoms as cough. Aspergillus infection (AI) is diagnosed in subjects with Aspergillus colonization and a decline in lung function, respiratory exacerbation with and without cough or with an incomplete response to a 2-4 week course of appropriate broad-spectrum antibiotics. Aspergillus can also cause allergic bronchopulmonary aspergillosis (ABPA). The classic diagnostic criteria of allergic bronchopulmonary aspergillosis in cystic fibrosis have been established during the Cystic Fibrosis Foundation Conference in 2001.

***Aim:** To establish the prevalence of pulmonary aspergillosis in children with cystic fibrosis under the care of our centre and to investigate the potential predisposing factors to Aspergillus infection (AI) and allergic bronchopulmonary aspergillosis (ABPA).*

***Material and methods:** An analysis was conducted of the medical documentation of 374 children aged 0-18 years monitored regularly in the Cystic Fibrosis Centre of the Institute of Mother and Child in Warsaw from 01.01.2010 to 31.08.2014. We selected 13 patients who presented an evidently worsening clinical status and course of the bronchopulmonary disease (decline in lung function parameters, respiratory exacerbations with increased cough, new or recent abnormalities in chest imaging) despite standard treatment with a high calorie diet, supplementation of pancreatic enzymes and vitamins, dornase alpha, inhaled and/or oral antibiotics, inhaled or oral corticosteroids, bronchodilators, physiotherapy. In this group of 13 CF children Aspergillus fumigatus was isolated from sputum. They represented 3.5% of the patients treated in our centre. Pulmonary aspergillosis was analyzed in relation to the age, sex, genotype, exocrine pancreatic insufficiency, body mass index, pulmonary function, microbiological examination of sputum, pulmonary complications and therapies. The mean age was 10.7 years (range 4.5-16.3). Only one child was under the age of six years. Patients were divided into 3 groups: patients with Aspergillus infection (AI), patients with allergic bronchopulmonary aspergillosis (ABPA), and a patient with Aspergillus infection and bronchopulmonary aspergillosis.*

***Results:** Aspergillus infection (AI) was diagnosed in 9 cases (2.4%) and allergic bronchopulmona-ry aspergillosis (ABPA) in 3 (0.8%). One patient was treated with corticosteroids, because of allergic bronchopulmonary aspergillosis (ABPA) and after 8 months he developed Aspergillus infection (AI).*

Most of the children were homo- or heterozygous for mutation F508del. Pancreatic insufficiency was recognized in all the children with ABPA, most of those with AI (8/9) and in one boy with ABPA and AI. Most of the patients had chronic respiratory colonization of Staphylococcus aureus and Pseudomonas aeruginosa. Children with AI were older (mean age:12.4), had a worse nutritional status (three of them had a BMI <3rd percentile), poorer lung function (five had severe lung disease (FEV1 <40%), complications

*Publication of CT images with the consent of the head of the Department of Diagnostic Imaging Dr hab .n. med. Monika Bekiesińska-Figatowska Prof. of the Institute of Mother and Child in Warsaw.

Publikacja zdjęć CT za zgodą kierownika Zakładu Diagnostyki Obrazowej dr hab.n.med. Moniki Bekiesińskiej-Figatowskiej Prof. Instytutu Matki i Dziecka w Warszawie

occurred in one of the underlying diseases (haemoptysis, CFRD – Cystic Fibrosis Related Diabetes), two of them had vascuport inserted due to the need for frequent intravenous antibiotic therapy. All the patients received inhaled antibiotics. A long-term oral azithromycin regime was applied in all the children with allergic bronchopulmonary aspergillosis, in most of those with Aspergillus infection (6/9) and in one boy with ABPA and AI. In three patients diagnosed with Aspergillus infection, antifungal treatment did not give any clinical or radiological improvement. They underwent surgical resection in the Department of Thoracic Surgery in Rabka (Poland). One patient had pneumonectomy and two underwent lobectomies. One boy had lung transplantation in Rigshospitalet in Copenhagen nine months after being diagnosed with Aspergillus infection.

Conclusions: *Since pulmonary aspergillosis is a very serious complication in CF children, it seems reasonable to include screening for early detection of Aspergillus colonization in the annual assessment of CF patients who are over 6 years old. Due to the small sample size and retrospective design of our analysis, the identification of risk factors of pulmonary aspergillosis in CF children require further prospective studies.*

Key words: cystic fibrosis (CF), *Aspergillus fumigatus*, allergic bronchopulmonary aspergillosis (ABPA), *Aspergillus* infection (AI)

Streszczenie

Aspergiloza płucna jest bardzo ciężkim powikłaniem u pacjentów z mukowiscydozą, pogorszającym rokowanie. Rozróżniamy: kolonizację Aspergillus (AC), zakażenie Aspergillus (AI) i alergiczną aspergilozę oskrzelowo-płucną (ABPA). Kolonizację Aspergillus (AC) definiuje się jako izolację Aspergillus (kropidlak) z 50% lub większej liczby próbek plwociny badanych w czasie sześciu miesięcy do jednego roku, bez pogorszenia czynności płuc i nasilenia objawów oddechowych między innymi takich jak kaszel. U osób z kolonizacją Aspergillus i pogorszeniem czynności płuc, zaostrzeniem zmian oskrzelowo-płucnych przebiegającym z kaszlem, a także z brakiem lub niepełną odpowiedzią na trwającą dwa do czterech tygodni antybiotykoterapię o odpowiednio szerokim spektrum, możemy rozpoznać zakażenie Aspergillus (AI). Aspergillus może również powodować alergiczną aspergilozę oskrzelowo-płucną (ABPA). Klasyczne kryteria diagnostyczne alergicznej aspergilozy oskrzelowo-płucnej w przebiegu mukowiscydozy zostały ustalone podczas w konferencji Cystic Fibrosis Foundation w 2001 roku.

Cel: *Określenie częstości występowania aspergilozy płucnej u dzieci z mukowiscydozą uczęszczających do naszego ośrodka oraz zbadanie potencjalnych czynników predysponujących do zakażenia Aspergillus i alergicznej aspergilozy oskrzelowo-płucnej (ABPA).*

Materiał i metody: *Przeanalizowano dokumentację medyczną 374 dzieci w wieku 0-18 lat, regularnie zgłaszających się do Zakładu Mukowiscydozy Instytutu Matki i Dziecka w Warszawie od 01.01.2010 do 31.08.2014. Wyłoniono grupę 13 pacjentów, którzy byli w gorszym stanie klinicznym i mieli gorszy przebieg choroby oskrzelowo-płucnej (obniżenie wskaźników funkcji płuc, zaostrzenia zmian oskrzelowo-płucnych z nasileniem kaszlu, nowe nieprawidłowości płucne w badaniach obrazowych klatki piersiowej) pomimo standardowego leczenia obejmującego: dietę wysokokaloryczną, suplementację enzymów trzustkowych i witamin, dornazę alfa, wziewne i /lub doustne antybiotyki, doustne lub wziewne kortykosteroidy, leki rozszerzające oskrzela, fizjoterapię. Aspergillus fumigatus wyhodowano z plwociny 13 dzieci z CF. Grupa ta stanowiła 3,5% dzieci pozostających pod naszą opieką. Aspergilozę płucną analizowano uwzględniając wiek, płeć, genotyp, zewnątrzwydzielniczą niewydolność trzustki, wskaźniki masy ciała, testy czynnościowe płuc, wyniki badania mikrobiologicznego plwociny, powikłania płucne oraz stosowane leczenie. Średnia wieku wynosiła 10,7 lat (zakres 4,5-16,3). Tylko jedno dziecko było w wieku poniżej sześciu lat. Pacjentów podzielono na 3 grupy: pacjentów z zakażeniem Aspergillus (AI), pacjentów z alergiczną aspergilozą oskrzelowo-płucną (ABPA), pacjenta z zakażeniem Aspergillus i aspergilozą oskrzelowo-płucną.*

Wyniki: *Zakażenie Aspergillus (AI)rozpoznano w 9 przypadkach (2,4%), a alergiczną aspergilozę oskrzelowo-płucną w 3 (0,8%). Jeden pacjent był leczony kortykosteroidami ze względu na alergiczną aspergilozę oskrzelowo-płucną (ABPA) i po 8 miesiącach rozwinęło się u niego zakażenie Aspergillus (AI). Większość badanych dzieci była homo- lub heterozygotami mutacji F508del. Niewydolność zewnątrzwydzielniczą trzustki rozpoznano u wszystkich pacjentów z ABPA i u większości z AI (8/9) oraz u chłopca z ABPA i AI. Większość dzieci w naszym badaniu miało stwierdzaną przewlekłą kolonizację dróg oddechowych Staphylococcus aureus i Pseudomonas aeruginosa. Dzieci z AI były starsze (średnia wieku: 12,4), miały gorszy stan odżywienia (troje z nich miało BMI<3 centyla), gorszą funkcję płuc (pięcioro miało ciężką chorobę płuc (FEV1<40%), występowały u nich powikłania choroby podstawowej (krwioplucie, CFRD), dwoje z nich miało założony vascuport ze względu na konieczność częstych antybiotykoterapii*

dożylnych. Wszyscy pacjenci otrzymywali antybiotyki wziewne. Przewlekłe leczenie azytromycyną stosowano u wszystkich dzieci z alergiczną aspergilozą oskrzelowo-płucną, u większości z zakażeniem Aspergillus (6/9) oraz u chłopca z ABPA i AI. U trzech pacjentów z rozpoznanym zakażeniem Aspergillus leczenie przeciwgrzybicze nie spowodowało klinicznej ani radiologicznej poprawy. Wykonano u nich resekcję chirurgiczną w Klinice Chirurgii Klatki Piersiowej w Rabce: u jednego – pneumonectomię, a dwóch – lobektomię. Jeden chłopiec dziewięć miesięcy po rozpoznaniu zakażenia Aspergillus przeszedł transplantację płuc w Rigshospitalet w Kopenhadze.

Wnioski: *Ponieważ aspergiloza płucna jest bardzo poważnym powikłaniem u dzieci z mukowiscydozą, zasadne wydaje się włączenie badań przesiewowych dla wczesnego wykrywania kolonizacji Aspergillus do corocznych badań bilansowych u pacjentów z CF powyżej 6 rż. Ze względu na małą liczebność i retrospektywny charakter naszej analizy określenie czynników ryzyka aspergilozy płucnej u dzieci z CF wymaga dalszych badań prospektywnych.*

Słowa kluczowe: mukowiscydoza, *Aspergillus fumigatus* (kropidlak popielaty), alergiczna aspergiloza oskrzelowo-płucna, zakażenie *Aspergillus*

DEV PERIOD MED. 2015;XIX,1:66-79

INTRODUCTION

Aspergillus is a ubiquitous spore-bearing fungus found worldwide in the soil, water, food, dust and decomposing organic matter. This thermotolerant mould can grow at body temperature. The genus *Aspergillus spp* comprises approximately two hundred species, but pulmonary disease is caused mainly by *Aspergillus fumigatus*. The small *Aspergillus* spores (2-3 μm) may easily become airborne and, when inhaled, deposit in the distal airways in humans. In healthy individuals, spores are quickly cleared by the mucociliary escalator and phagocytosed by immune system cells, such as macrophages and neutrophils [1].

Spores can easily persist in the respiratory tracts of patients with chronic lung diseases or immunocompromised ones, including transplant recipients. They are able to non-invasively colonise the bronchial tree but they can also cause infections. The large spectrum of clinical syndromes of aspergillus infections depends on the host's immune status, or the severity of the kind of bronchopulmonary disease [2, 3]. *Aspergillus* is the only organism that can cause both invasive, life-threatening disease and allergic disease in humans [4, 5].

Patients with cystic fibrosis are especially prone to fungi respiratory infections which lead to clinical deterioration and worsening of the lung disease. Due to increased mucus viscosity in CF airways, inhaled spores are "trapped". Impaired mucociliary clearance and mucus plugging in the bronchial tree, the ability of *Aspergillus spp*. to evade or interfere with phagolysosome fusion and complement fixation can lead to locally invasive or disseminated infection [1, 3]. The continued presence of the allergen leads to persistent airway inflammation and subsequent lung damage [3].

Aspergillus fumigatus is the most common mould in the sputum of CF patients [1, 6].

The isolation rate varies widely from 6% to 58% in the reported literature from different geographical areas [7-14]. In Canadian CF patients the prevalence of *A. fumigatus* isolated from sputum rose from 8% in 2001 to 18% in 2009 [11]. There are differences in the prevalence of fungal colonization in children with CF, due to local clinical and microbiological practices, varying techniques of obtaining respiratory samples and laboratory protocols. The methods of fungi detection may influence the detection rate as well [1, 9, 10, 13, 14].

The altered airway surface fluid thickness and composition in the respiratory tract of CF patients represents a favorable environment for the development of pulmonary aspergillosis. In these individuals, inhalation of *Aspergillus* conidia can mainly result in three clinical scenarios: *Aspergillus* colonization (AC), *Aspergillus* infection (AI) or allergic bronchopulmonary aspergillosis (ABPA) [5]. *Aspergillus* colonization (AC) is defined as isolation of *Aspergillus spp.* from 50% or more sputum samples over six months to one year without deterioration in lung function and an increase in respiratory symptoms like cough (Table I). *Aspergillus* infection (AI) is diagnosed in subjects with *Aspergillus* colonization and a decline in lung function, respiratory exacerbation with cough and incomplete response to a two to four week course of appropriate broad-spectrum therapy with antibiotics. The classic diagnostic criteria of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis have been established during the Cystic Fibrosis Foundation Conference in 2001 (Table II). They include: acute or subacute clinical deterioration not attributable to another etiology, total serum IgE concentration greater than 1000 IU/mL (unless the patient is receiving corticosteroid therapy), immediate cutaneous reactivity to *Aspergillus* (while the patient is not being treated with antihistamines) or *in vitro* presence of serum IgE antibodies to *A. fumigatus,* precipitating antibodies or serum IgG antibodies to *A. fumigatus,* new or recent abnormalities on chest radiography or chest CT that have not cleared with antibiotics and standard physiotherapy (Table II).

Table I. Definitions for *Aspergillus spp.* colonisation and infection [1].
Tabela I. Definicje kolonizacji i zakażenia Aspergillus spp. [1].

Aspergillus spp.* colonization** ***Kolonizacja Aspergillus spp.	***Aspergillus spp.* Infection** ***Zakażenie Aspergillus spp.***
Isolation of *Aspergillus spp.* from 50% or more sputum samples over six months to one year *Izolacja Aspergillus z 50% lub większej liczby próbek plwociny w ciągu 6 miesięcy do 1 roku* No deterioration in lung function *Bez pogorszenia funkcji płuc* No increase in respiratory symptoms like coughing *Bez nasilenia objawów z układu oddechowego, takich jak kaszel*	Isolation of *Aspergillus spp.* from 50% or more sputum samples over six months to one year *Izolacja Aspergillus z 50% lub większej liczby próbek plwociny w ciągu 6 miesięcy do 1 roku* Decline in lung function parameters *Pogorszenie funkcji płuc* Respiratory exacerbation with (increased) coughing *Zaostrzenie zmian płucnych z (nasilonym) kaszlem* *Aspergillus spp.* the only organism isolated from repeated sputum samples *Aspergillus spp. to jedyny organizm izolowany z powtarzanych próbek plwociny* No or incomplete response to a two to four week course of appropriate broad spectrum antibiotics *Brak lub niepełna odpowiedź na trwającą 2-4 tygodni antybiotykoterapię o odpowiednio szerokim spektrum*

Table II. Criteria for diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis [5, 16].
Tabela II. Kryteria diagnostyczne alergicznej aspergilozy oskrzelowo-płucnej w mukowiscydozie [5, 16].

Classic Diagnostic Criteria of ABPA in cystic fibrosis (Cystic Fibrosis Foundation Consensus) ***Klasyczne Kryteria Diagnostyczne ABPA w mukowiscydozie (Konsensus Cystic Fibrosis Foundation)***
Acute or subacute clinical deterioration not attributable to another etiology *Ostre lub podostre pogorszenie kliniczne nie związane z inną przyczyną*
Total serum IgE concentration greater than 1000 IU/mL unless patient is receiving corticosteroid therapy *Stężenie całkowitej IgE w surowicy powyżej 1000 jm./mL, chyba że pacjent otrzymuje leczenie kortykosteroidami*
Immediate cutaneous reactivity to *Aspergillus fumigatus* while the patient is not being treated with antihistamines or in vitro presence of serum IgE antibody to *A. fumigatus* *Dodatnia natychmiastowa reakcja skórna z Aspergillus fumigatus jeśli pacjent nie jest leczony lekami przeciwhistaminowymi lub obecność in vitro surowiczych przeciwciał IgE p/A. fumigatus*
Precipitating antibodies or serum IgG antibody to *A. fumigatus* *Obecność precypityn lub surowiczych przeciwciał IgG p/A. fumigatus*
New or recent abnormalities on chest radiography or chest CT that have not cleared with antibiotics and standard physiotherapy *Nowe lub ostatnio występujące nieprawidłowości w radiogramach klatki piersiowej lub w KT, które nie ustępują pomimo antybiotykoterapii i standardowej fizjoterapii.*

The diagnosis of pulmonary aspergillosis is difficult in patients with CF because of overlapping clinical, radiological, microbiological and immunological features [15]. Both imaging and functional signs required for the diagnosis of AI or ABPA are also usual symptoms of CF.

AIM

The aim of this study was:

- to establish the prevalence of pulmonary aspergillosis in children with cystic fibrosis treated at our centre;

- to investigate the potential predisposing factors to *Aspergillus* infection (AI) and allergic bronchopulmonary aspergillosis (ABPA).

MATERIAL AND METHODS

According to the Polish Cystic Fibrosis Patient Registry, 374 patients remained under the care of the Institute of Mother and Child in Warsaw as of 31st August 2014. Most of them inhabited central and eastern regions of Poland. Analysis was conducted of the database of 374 children aged from 0 to 18 years regularly monitored in the Cystic Fibrosis Centre of the Institute of Mother and Child in Warsaw from 01.01.2010 to 31.08.2014. Their cystic fibrosis treatment included mainly: a high calorie diet, supplementation of pancreatic enzymes and vitamins, dornase alpha, inhaled and/or oral antibiotics, inhaled or oral corticosteroid, bronchodilators, physiotherapy.

A group of 13 patients was selected who presented worsening clinical status and a deterioration in the course of the bronchopulmonary disease (decline in lung function parameters, respiratory exacerbations with increased cough, new or recent abnormalities on chest imaging) despite the routine treatment. In this group pulmonary aspergillosis was diagnosed. The children examined were divided into the following groups: 1 – patients with *Aspergillus* infection (AI), 2 – patients with allergic bronchopulmonary aspergillosis (ABPA), 3 – patients with *Aspergillus* infection and bronchopulmonary aspergillosis (Table III).

The diagnosis of AI and ABPA were defined according to the recommendations explained below. Based on the literature reviewed, we used the criteria described by Liu et al. for *Aspergillus spp.* colonization (AC) and infection which we have summarized in Table I [1]. The diagnosis of ABPA was based on the criteria from the 2001 consensus conference (Table II) [5, 16].

This group represented 3.5% of the children under the care of the Cystic Fibrosis Centre (Figure 1). In this group *Aspergillus* infection was diagnosed in 9 children (2,4%), allergic bronchopulmonary aspergillosis in 3 (0,8%), and in 1 boy ABPA and AI after 8 months of corticosteroids therapy (Table III). The mean age of the pulmonary aspergillosis patients was 10.7 years (range 4.5-16.3), for AI it was 12.4 years (range 4.5-16.3) and for ABPA 9.3 years (range 6-13.7) (Table III). The boy with ABPA and AI was 8.75 years old. The following data were collected and analyzed: age, gender, *CFTR* genotype(cystic fibrosis transmembrane conductance regulator), exocrine pancreatic insufficiency (confirmed by determining the concentration of elastase in the feces), body mass index (BMI), microbiological examination of sputum, chest X-rays or chest-CT, blood tests, particularly *Aspergillus*-related tests, and respiratory function tests. The forced expiratory volume in one second (FEV1) was calculated as percentages of the predicted values and used to determine the severity of the disease in patients ≥6yrs of age. The intention was to model the FEV1 percent predicted value for a given age using a severity score (severe: FEV1<40%; moderate FEV1 40-69%; mild FEV1 ≥70%), age groups (<6 yrs, 6-12 yrs, 13-18 yrs) and association with AI or ABPA.

We also analyzed pulmonary complications (haemoptysis, pneumothorax) and therapies recommended in CF including: dornase alfa, inhaled antibiotics, corticosteroids and bronchodilators, a long-term azithromycin regimen, the number of antibiotic therapies (oral or intravenous) for bronchopulmonary infection during the six months before the recognition of pulmonary aspergillosis and lung transplantation.

In our study all the patients with pulmonary aspergillosis received dornase alfa therapy and inhaled antibiotics (Table V). A long-term and low-dose azithromycin regimen was reported in the boy with ABPA and AI and in all the children with ABPA. Most patients with AI (6/9) also received long-term therapy with azithromycin. Patients with ABPA were effectively treated with corticosteroids or with a combination of corticosteroids and a systemic antifungal drug (itraconazol). Subjects with AI received antifungal therapy (itraconazol or voriconazole for a period of 4-24 weeks).

RESULTS

Pulmonary aspergillosis was diagnosed in only one patient <6 years old (1/13), but more often in children >6 years (Table III). Details about the relationship between CF patient characteristics and the occurrence of AI or ABPA was shown in Table III.The oldest children were in the group with AI. The mean age in this group was 12.4 (range 4.5-16.3). In the group with ABPA, the average age was 9.3 (range 6-13.7). The boy with ABPA and AI was 8.75 years old. Sex differences were observed. Both ABPA were mostly prevalent in males (2/3), as was AI (6/9).

Most of the children with ABPA or AI were homo- or heterozygous for the mutation F508del. The boy with ABPA and AI, two patients with ABPA (2/3) and four with AI (4/9) were homozygous for F508del. In the group with ABPA one child had the F508del/K710X mutation. In the group with AI four patients were homozygous for F508del (F508del/W1310X, F508del/2184insA, F508del/3849+kbCT, F508del/dele2,3(21kb)) and one patient had other mutations (N1303K/230000del/G).

When we analyzed and compared the clinical data of the patients from the 3 groups, it was found that the patients with AI were in a worse condition and had a worse clinical course of the bronchopulmonary disease and complications. Pancreatic insufficiency was recognized in all the individuals with ABPA and in most of those with AI (8/9). The boy with ABPA and AI also had exocrine pancreatic insufficiency. He was in a good nutritional status (BMI>3rd). Generally the body weight was lower in the group of AI than in the ABPA group. Three of the nine children with AI had BMI <3rd percentile but those with ABPA were in a good nutritional status.

Patients with AI also had lower FEV1% predicted values than those with ABPA. The prevalence of severe or moderate lung disease (FEV1<40% and 40-70%) in individuals with AI was reported in 6 of the 7 cases. Two patients from the group with ABPA had mild lung

Table III. Relationship between CF patient characteristics and the occurrence of *Aspergillus* infection or ABPA.
Tabela III. Zależność pomiędzy charakterystyką pacjentów z CF a występowaniem infekcji Aspergillus lub ABPA.

	Aspergillus* infection (AI)** ***Zakażenie Aspergillus	**Allergic bronchopulmonary aspergillosis (ABPA)** ***Alergiczna aspergiloza oskrzelowo-płucna***	**Allergic bronchopulmonary aspergillosis (ABPA) and *Aspergillus* infection (AI)** ***Alergiczna aspergiloza oskrzelowo-płucna (ABPA) i zakażenie Aspergillus (AI)***
N	9	3	1
Age (years)-mean (range) *Wiek (lata)-średnia (zakres)*	12.4 (4.5-16.3)	9.3 (6-13.7)	8.75
n<6 yrs of age n/N *n<6 rż n/N*	1/9	-	-
n= 6-12 yrs of age n/N *n=6-12 rż n/N*	2/9	2/3	1/1
n=13-18 yrs of age n/N *n=13-18 rż n/N*	6/9	1/3	-
Male n/N *Płeć męska n/N*	6/9	2/3	1/1
CFTR genotype (genotyp) F508del/F508del n/N	4/9	2/3	1/1
F508del/other n/N	4/9	1/3	-
other/other n/N *inna/inna (N1303K/230000del/G)*	1/9	-	-
Pancreatic insufficiency *Zewnątrzwydzielnicza niewydolność trzustki*	8/9	3/3	1/1
BMI <3rd centile n/N	3/9	0	-
FEV1% <70% n/N	6/7 (2- not performed *niewykonano)*	0	-
FEV1% < 40% n/N (Severe lung disease) *(Ciężka choroba oskrzelowo-płucna)*	5/7	-	-
FEV1% (40%-70%) n/N (Moderate lung disease) *(Umiarkowana choroba oskrzelowo-płucna)*	1/7	-	-
FEV1% >70% n/N (Mild lung disease) *(Łagodna choroba oskrzelowo-płucna)*	1/7	2/3 (1-not performed *niewykonano)*	1/1
Haemoptysis n/N *Krwioplucie*	3/9	0	1/1
Pneumothorax n/N *Odma opłucnowa*	-	-	-
CFRD n/N *Cukrzyca w przebiegu CF*	2/9	-	-
Vascuport n/N	2/9	1/3	-

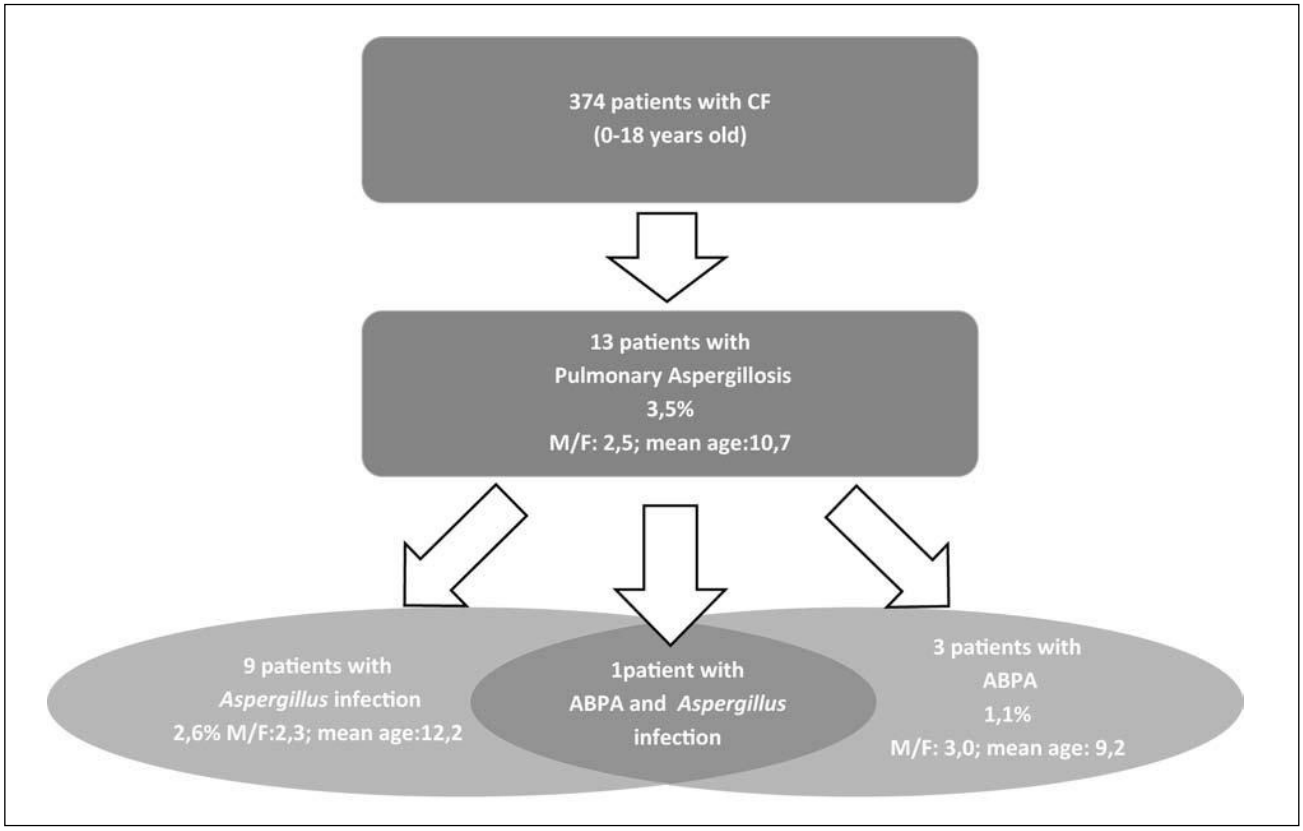

Fig. 1. Patient flow chart.

Ryc. 1. Schemat pacjentów.

disease (FEV1>70%). For two children with AI and one with ABPA it was not possible to perform functional respiratory tests due to their age. The boy with ABPA and AI had a mild lung disease (FEV1>70%).

Haemoptysis was observed in 3 patients from the group with AI and in the boy with AI and ABPA. Nobody from the group with ABPA had hemoptysis. The complication of pneumothorax occurred in none of the patients studied. Cystic Fibrosis Related Diabetes (CFRD) was diagnosed in 2 patients with AI (2/9). In this group two patients (2/9) and one child from the group with ABPA (1/3) had vascuport due to the need of frequent intravenous antibiotic therapies.

Most of the patients in our study had chronic respiratory colonization of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Details about the chronic respiratory colonization in CF of patients with AI and ABPA are presented in Table IV. Six patients from the group with AI, two patients from the group with ABPA and the boy with ABPA and AI had *Staphylococcus aureus* in the sputum. In the group with AI one child had MRSA and another *Stenotrophomonas maltophilia* chronic colonization.

Pseudomonas aeruginosa was isolated in the sputum culture of eight patients with AI and in one with ABPA , as well as in the boy with ABPA and AI. *Achromobacter xylosoxidans* colonization occurred in one subject with AI and one with ABPA. During our studies no growth of *Burkholderia cepacia complex* from the sputum in any patient occurred. In all the cases, both AI as well as ABPA, *Aspergillus fumigatus* was isolated from the sputum.

The treatment of CF patients with AI and ABPA is shown in Table V. Bronchodilators were received by 5 patients with AI (5/9), all the patients with ABPA (3/3) and the boy with ABPA and AI. In the group with AI five subjects (5/9) used inhaled corticosteroids and one oral corticosteroids (1/9). All the individuals received dornase alfa and inhaled antibiotics. An azithromycin long-term regimen was used in six of the nine patients with AI and all the patients with ABPA and in the boy with ABPA and AI. Antibiotic courses due to pulmonary exacerbation in the past 6 months before diagnosis were received accordingly: 3.7; 2.5 and 3 times respectively.

We performed imaging studies to confirm the diagnosis of pulmonary aspergillosis. In the group of patients with ABPA pulmonary infiltrates (Figure 3) and central bronchiectases were observed on chest radiographs. The chest HRCT of patients with AI showed a focus of massive nodular parenchymal changes with surrounding halos (Figure 4), patchy consolidation (Fig ure 5), cavity lesion and pleural thickening in the upper lobe (Figure 6).

A complete response to antifungal therapy and improvement was seen in 10 of the 13 children with pulmonary aspergillosis. Three patients in the group with AI had a residual, localized but active disease, despite adequate antifungal therapy. They underwent surgical resection: one patient – pneumonectomy and two – lobectomy (Figure 2). They had a good response and surgery was not associated with significant complications. One patient underwent lung transplantation nine months after the diagnosis of AI.

Table IV. Chronic respiratory colonization in CF patients with AI or ABPA.
Tabela IV. Przewlekła kolonizacja dróg oddechowych u pacjentów z mukowiscydozą z rozpoznaniem AI lub ABPA.

	Aspergillus* infection (AI)** ***Zakażenie Aspergillus	**Allergic bronchopulmonary aspergillosis (ABPA)** ***Alergiczna aspergiloza oskrzelowo-płucna***	**Allergic bronchopulmonary aspergillosis (ABPA) and *Aspergillus* infection (AI)** ***Alergiczna aspergiloza oskrzelowo-płucna i zakażenie Aspergillus***
Staphylococcus aureus (n/N)	6/9	2/3	1/1
MRSA (n/N)	1/9	-	-
Pseudomonas aeruginosa (n/N)	8/9	1/3	1/1
Stenotrophomonas maltophilia (n/N)	1/9	-	-
Achromobacter xylosoxidans (n/N)	1/9	1/3	-
Burkholderia cepacia (n/N)	-	-	-
Aspergillus spp (n/N)	9/9	3/3	1/1

Table V. Cystic fibrosis treatment and development of *Aspegillus* infection or ABPA.
Tabela V. Leczenie mukowiscydozy a rozwój zakażenia Aspergillus lub ABPA.

	Aspergillus* infection (AI)** ***Zakażenie Aspergillus	**Allergic bronchopulmonary aspergillosis (ABPA)** ***Alergiczna aspergiloza oskrzelowo-płucna***	**Allergic bronchopulmonary aspergillosis (ABPA) and *Aspergillus* infection (AI)** ***Alergiczna aspergiloza oskrzelowo-płucna i zakażenie Aspergillus***
Bronchodilators n/N *Leki rozszerzające oskrzela*	5/9	3/3	1/1
Corticosteroids (inhaled) n/N *Kortykosteroidy (wziewne)*	5/9	-	-
Corticosteroid (oral) n/N *Kortykosteroidy (doustne)*	1/9	-	-
Dornase alfa n/N	9/9	3/3	1/1
Inhaled antibiotics n/N *Antybiotyki wziewne*	9/9	3/3	1/1
Azithromycin n/N	6/9	3/3	1/1
Numbers of antibiotics courses because of PEX/past 6 months (range) *Liczba kursów antybiotykoterapii z powodu zaostrzenia zmian oskrzelowo-płucnych w ciągu ostatnich 6 miesięcy (zakres)*	3.7 (2-5)	2.5 (2-3)	3
Surgery treatment n/N *Leczenie chirurgiczne*	3/9	-	-
Lung transplant n/N *Transplantacja płuc*	1/9	-	-

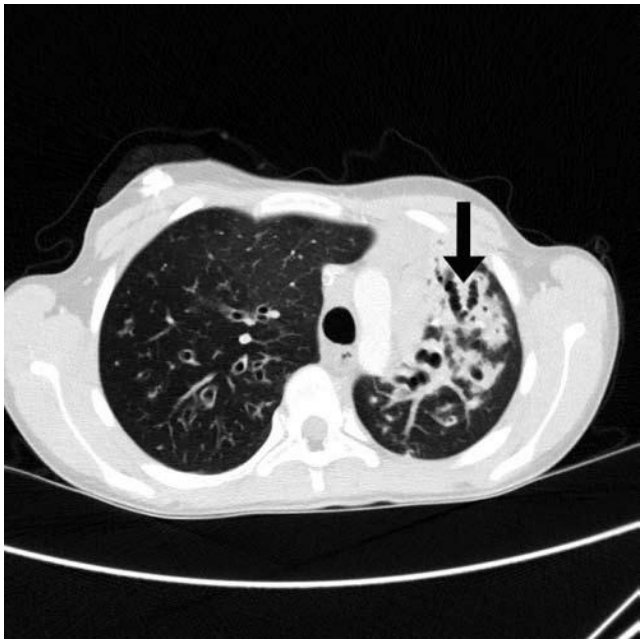

Fig. 2. Chest CT scan of a 13.5-year old girl with AI and CF shows significant retraction of the left lung, severe cirrhotic changes and bronchiectasis in the upper lobe. Saccular bronchiectasis seen in the left upper lobe – denoted by arrow. The displacement of the mediastinum to the left, with herniation of the right lung to the left. Patient after an ineffective antifungal treatment underwent a left pneumonectomy. (CT was performed in the Department of Diagnostic Imaging of the Institute of Mother and Child in Warsaw).

Ryc. 2. Tomografia komputerowa klatki piersiowej 13,5-letniej dziewczynki z AI i CF pokazuje znaczną retrakcję płuca lewego, nasilone zmiany marskie oraz rozstrzenia oskrzeli w płacie górnym. Rozstrzenia workowate zaznaczono strzałką. Przemieszczenie śródpiersia na stronę lewą, z przepukliną płuca prawego na lewo. Pacjentka po nieefektywnym leczeniu p-grzybiczym miała wykonaną pneumonektomię lewostronną. (TK wykonano w Zakładzie Diagnostyki Obrazowej Instytutu Matki i Dziecka w Warszawie).

DISCUSSION

To our knowledge this was the first study to establish the prevalence of pulmonary aspergillosis in children with cystic fibrosis in Poland and to analyze the risk factors for both AI and ABPA. The main limitation of our work was the small sample of patients and its retrospective design. All the patients were under the care of our Centre and the data were thus relatively restricted. However, we indicated problems concerning pulmonary aspergillosis in our patient population, which presented distinct characteristics. In our Cystic Fibrosis Centre diagnostic tests for fungal infections were not routinely performed. Such tests were performed inter alia for pulmonary aspergillosis only in patients who presented clinical and functional deterioration of the lung disease despite standard therapy. When we analyzed the medical documentation of 374 children aged 0-18 years regularly monitored in our Centre from 01.01.2010 to 31.08.2014, we found that *Aspergillus fumigatus* was isolated from the sputum of 13 patients whose clinical condition deteriorated despite routine treatment. This group represented 3.5% of the children under the care of the Cystic Fibrosis Centre (Figure 1).

We were aware that *Aspergillus* can occasionally contaminate sputum cultures and in these cases might not be associated with clinical significance. Our study, however, consists of retrospectively analyzed results of patients, whose clinical condition deteriorated despite appropriate antibiotic treatment. We were, therefore, able to evaluate the incidence of pulmonary aspergillosis. Due to the lack of routine testing of sputum for fungi we did not have data to assess the prevalence of colonization of *Aspergillus* in our patients.

We used the criteria described by Liu et al [1] to diagnose *Aspergillus* infection (Table I) and the criteria from the Cystic Fibrosis Foundation Conference to diagnose ABPA (Table II) [5, 16]. In our study both AI as ABPA, *Aspergillus fumigatus* was isolated from sputum samples in all the cases. Although the mere presence of *A. fumigatus* is not associated with ABPA, *Aspergillus* infection was diagnosed in 9 children (2.4%) and allergic bronchopulmonary aspergillosis in 3 (0.8%). One patient was treated with corticosteroids because of allergic bronchopulmonary aspergillosis (ABPA) and after 8 months he developed the *Aspergillus* infection (AI).

Aspergillus is a ubiquitous environment pathogen; environmental exposure, rather than patient-to-patient contact is responsible for *Aspergillus*-related conditions in CF. It is recognized that *A. fumigatus* is found in all domestic environments, including ventilation systems, or dust; it is to be found on false ceilings, on plants and in animals' bedding. In various environments there are different levels of airborne spores of *A. fumigatus,* with a difference manifested e.g.in rural areas. Most of our patients with pulmonary aspergillosis lived in the countryside, they had different kinds of animals (e.g. hamsters). The parents of the boy with ABPA and AI had a piggery, which could have been be a source of *Aspergillus* spores.

In the current analysis, the prevalence of pulmonary aspergillosis was 3.5%, AI was 2.4% and ABPA 0.8% (Figure 1). This was much lower than assessed in the studies of other authors. Data from the Epidemiologic Registry of Cystic Fibrosis (ERCF) conducted on 12,447 CF patients gathered from 224 CF centres in nine European countries showed that the overall prevalence of ABPA in the ERCF population was 7.8% (range: 2.1% in Sweden to 13.6% in Belgium). The prevalence was dependent on the distinct characteristics of the patient population [17].

Over the last decade an increase in the annual prevalence of aspergillosis in children with CF has been described. Between 1998 and 2002, the annual prevalence of *Aspergillus* colonization (AC) increased from 7.4% to 18.8% and ABPA increased from 0.3% to 4% in children and teenagers with CF [18]. The prevalence of ABPA ranges from 6-25% in patients with CF, and occurs primarily in older children and adults [19]. The number of isolates of fungi increases with age in CF patients. Changes in the airway microbiome over time, with *Aspergillus spp.* filling the empty seats, a changing *milieu* at the level of the airway surface but also the frequency, type and administration route of antibiotics and increased

exposure over time, have all been suggested as potential factors in the increase of *Aspergillus spp.* colonization with increasing age [1, 7, 14]. Similarly to our study in data from ERCF, the prevalence was low in children<6 years of age but almost a constant 10% thereafter. No sex differences were observed. ABPA affected 8.0% of the patients with a F508del/F508del genotype and 5-6% with F508del/G551D, F508del/G542X and F508del/N1303K genotypes. ABPA patients presented a lower forced expiratory volume in one second (FEV1) than those without ABPA at any age [17]. A recent large retrospective cohort study in Canadian children also reported a lower FEV1% predicted of children with persistent *Aspergillus spp.* infection compared to uninfected children. In our study most of the individuals (6/7) with AI had severe or moderate lung disease (FEV1<40% and 40-70%).

Children with *Aspergillus spp.* infection faced the greatest risk of pulmonary exacerbations requiring hospitalization. Amin et al. have shown a deleterious effect of AC for baseline pulmonary function, of the association of chronic *A. fumigatus* infection with pulmonary exacerbation requiring hospitalization. There was a very strong association between *Aspergillus spp.* infection and *Pseudomonas* isolation in the study group [11]. In the North American Epidemiologic Study of Cystic Fibrosis (ESCF) and the European Epidemiologic Registry of Cystic Fibrosis (ERCF), there was increased prevalence of ABPA in those over 6 years of age, adolescents, those with impaired lung function, wheezes, and microbial chronic infection with *Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia* and *Candida albicans* [17, 20]. In our study most patients with pulmonary aspergillosis had chronic respiratory infection with *Pseudomonas aeruginosa* and one child with *Stenotrophomonas maltophilia* (Table II).

In the CF lung, thick, viscous secretions and impaired mucociliary clearance causes the trapping of mucus, and colonisation with common respiratory bacteria such as *Haemophilus influenzae, Staphylococcus aureus* and *Pseudomonas aeruginosa*, and a persistent inflammatory response in the airways. Widespread use of broad-spectrum antibiotics has favored the selection of fungi [21].Some studies investigatedthe association between *Pseudomonas* and *Aspergillus spp.* colonisation and infection[11,14]. A recent large retrospective review in Canadian children with CF found a significant link between *P.aeruginosa* and *Aspergillus spp.*respiratory infection [11]. There is in vitro evidence that on the molecular level *P. aeruginosa* produces proteases that directly affect and damage the respiratory epithelium and it has been suggested that this may promote *A. fumigatus* sensitization [22]. The interaction between respiratory pathogens like *P. aeruginosa* and fungi is likely to be a complex one and this merits further study. Whether the presence of both microorganisms simultaneously has a cumulative effect of damaging respiratory epithelia in CF patients over time remains to be shown [1].

Due to acute or chronic bacterial respiratory infection, CF patients frequently receive broad-spectrum antibacterials via a variety of routes including oral, intravenous and increasingly nebulised ones, either as prophylaxis or for treatment of respiratory exacerbations. Several studies have highlighted the association between nebulized antibacterials (especially tobramycin) and *Aspergillus spp.* isolation from the airway [8, 14, 23]. In our analysis all the patients with pulmonary aspergillosis received inhaled antibiotics (Table III). Most of them were treated with colistin. In Poland inhaled tobramycin was very expensive, so no patients from our study received such a treatment.

The selective pressure of antibacterials may reduce competition and leave an unfilled niche for *Aspergillus spp.* colonisation and infection. The type of antibacterial and the method of administration may have different effects on *Aspergillus spp.* acquisition [1]. From the available evidence, it does appear that inhaled antibiotic therapy, particularly tobramycin, may be associated with an increase in AC. It has to be stressed, however, that any potential clinical implications of AC are largely unknown and would need to be balanced with the beneficial effects of antibiotic therapy [1].

Likewise azithromycin might facilitate *Aspergillus* colonization [24]. In our study, a long-term azithromycin regimen was reported in all the children with allergic bronchopulmonary aspergillosis and in most with an *Aspergillus* infection (Table III). Jubin et al reported that long-term and low-dose azithromycin therapy was significantly associated with AC. Patients treated with long-term azithromycin were more frequently colonized with *P. aeruginosa* than untreated patients (60% vs.28,6%).This association might be explained by the inhibitory effect of azithromycin on both the recruitment and the activation of neutrophils, which represent the first-line defenses against *Aspergillus*. Multivariate analysis confirmed that long-term azithromycin therapy was the only measured variable independently associated with AC [12].

In patients with persistent respiratory exacerbations who do not respond to two or more courses of appropriate oral or intravenous antibiotics and in whom no other organisms are isolated from respiratory secretions, the clinical relevance of fungi and whether antifungal therapy should be started is a dilemma for the CF physician. In our analysis patients with pulmonary aspergillosis received antibiotic courses due to pulmonary exacerbation in the past 6 months before diagnosingAI or ABPA 3.7 and 2.5 times respectively (Table V).

There are a lot of difficulties in recognizing pulmonary aspergillosis in the context of CF, because of overlapping clinical, radiological, microbiological and immunological features. Clinical, imaging, and functional signs required for diagnosis of pulmonary aspergillosis are usual symptoms of CF. The recognition of ABPA in CF remains difficult due to the poor specificity of the clinical manifestations, such as bronchoconstrictions and pulmonary infiltrates. Pulmonary infiltrates (Figure 3) and central bronchiectases on chest radiographs, usually suggestive of ABPA, are commonly encountered in CF as a result of chronic bacterial infection and should be viewed with caution as selective criteria for diagnosis. The recommended combination of immunological evidence and clinical manifestations is important. Positive immune parameters and elevated total serum

IgE levels in patients experiencing increased coughing or wheezing, pulmonary infiltrates, or a decrease in pulmonary function that are unresponsive to aggressive therapy (increased antibiotics and bronchodilators) may suggest the diagnosis of ABPA [17].

Similarly patients with AI frequently complain of constitutional symptoms which can occur during cystic fibrosis, such as fever, malaise, fatigue, weight loss, in addition to chronic productive cough and haemoptysis. Imaging studies, such as chest radiographs and chest CT scans, usually show different kinds of consolidation, pleural thickening and cavity lesion in the upper lung lobes [25]. We confirmed this in our studies analyzing chest HRCT which showed a focus of massive nodular parenchymal changes with surrounding halos (Figure 4), patchy consolidation (Figure 5), cavity lesion and pleural thickening in the upper lobe (Figure 6).

The connection of characteristic clinical and radiological findings and either serological results positive for *Aspergillus,* or the isolation of *Aspergillus* from respiratory samples is highly indicative of pulmonary aspergillosis [25]. The combination of specific immunological criteria in conjunction with clinical deterioration and the newly-reported appearance of radiological shadowing in patients poorly responding to antibiotics underlie the diagnosis of pulmonary aspergillosis.

If pulmonary aspergillosis is diagnosed early and treated promptly, respiratory symptoms may be decreased, lung function improved and antibiotic treatments, such as intravenous antibiotics, reduced. This may also

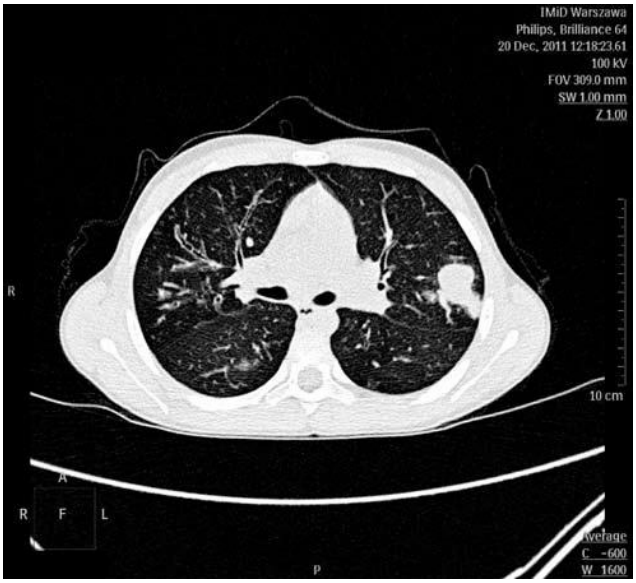

Fig. 4. An eight-year-old boy's chest HRCT scan with AI and CF shows a focus of massive nodular parenchymal changes with surrounding halos in the 3rd segment of the left lung; bronchial wall thickening, bronchiectasis mediocre degree. (CT was performed in the Department of Diagnostic Imaging of Institute of Mother and Child in Warsaw).

Ryc. 4. HRCT klatki piersiowej 8-letniego chłopca z AI i CF pokazuje ognisko masywnych guzowatych zmian miąższowych z otaczającym "halo" w 3 segmencie lewego płuca; pogrubienie ścian oskrzeli, rozstrzenia oskrzeli miernego stopnia.(TK wykonano w Zakładzie Diagnostyki Obrazowej Instytutu Matki i Dziecka w Warszawie).

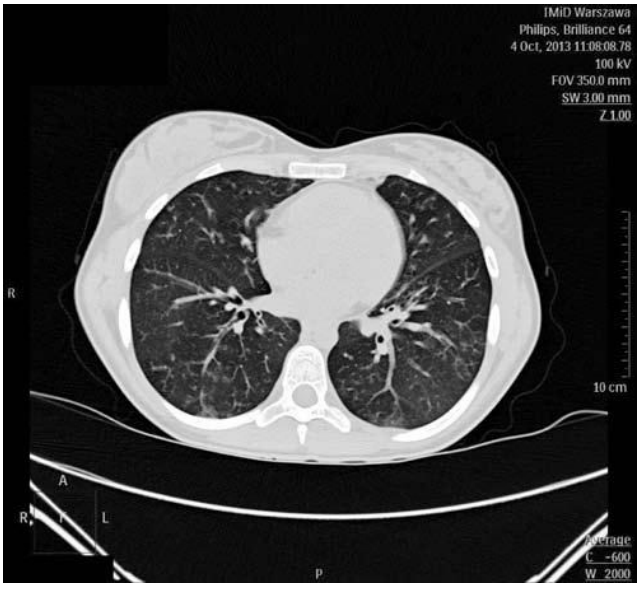

Fig. 3. Chest CT scan of a 14-year-old girl with ABPA and CF shows ethereal infiltrates most severe in 6 segments of both lungs. (CT was performed in the Department of Diagnostic Imaging of the Institute of Mother and Child in Warsaw).

Ryc. 3. Tomografia klatki piersiowej 14-letniej dziewczynki z ABPA i CF przedstawia zwiewne nacieki najbardziej nasilone w segmentach 6 obu płuc. (TK wykonano w Zakładzie Diagnostyki Obrazowej Instytutu Matki i Dziecka w Warszawie).

prevent long-term damage, such as bronchiectases and fibrosis. However, due to clinical and radiological features of pulmonary aspergillosis overlapping with those of infective exacerbations in CF, diagnosis can be difficult. Maintaining a high level of clinical suspicion and then investigating for ABPA and AI is important. The Cystic Fibrosis Foundation Consensus conference made suggestions for screening for ABPA in CF [5] (Table VI). The physician's suspicion of ABPA and AI based on multiple clinical, radiological and additional immunological conditions was considered a reasonable compromise. The diagnosis should be suspected if there is a poor response to intravenous antibiotics, a markedly increased or new-onset wheeze, or pleuritic chest pain. Diagnosis needs to be confirmed by radiological and serological testing [3].

Another clinical problem is that *Aspergillus* syndromes may co-exist or may progress from one entity to another [2]. AI in patients with ABPA, like in the case of our patient, has been sporadically reported in the literature. In some studies, the invasion by the *Aspergillus* species is restricted to the tissues surrounding the bronchiectatic segments with a granulomatous reaction [26]. This local invasion by *Aspergillus* probably develops as a result of chronic immunosuppression associated with corticosteroids and/or the presence of underlying chronic lung disease. Disseminated AI has rarely been described in patients

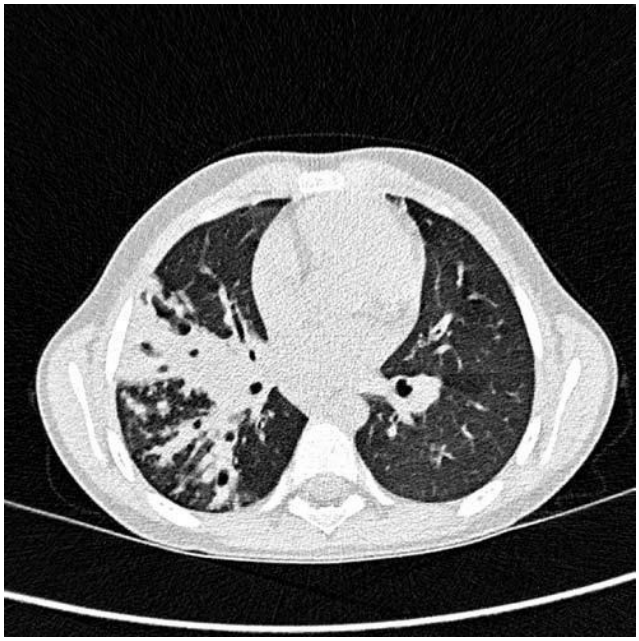

Fig. 5. Chest HRCT scan of an 7-year-old-boy with AI and CF shows patchy consolidation in segments 4 and 6 of the right lung and bronchiectasis with mucous plugs. After ineffective antifungal treatment the patient had lower right bilobectomy performed. (CT was performed in the Department of Diagnostic Imaging of Institute of Mother and Child in Warsaw).

Ryc. 5. CT klatki piersiowej 7-letniego chłopca z AI and CF przedstawia zmiany marskie w segmencie 4 i 6 płuca prawego oraz rozstrzenia oskrzeli z korkami śluzowymi. Po nieefektywnym leczeniu przeciwgrzybiczym pacjent miał wykonaną bilobektomię dolną prawą. (TK wykonano w Zakładzie Diagnostyki Obrazowej Instytutu Matki i Dziecka w Warszawie).

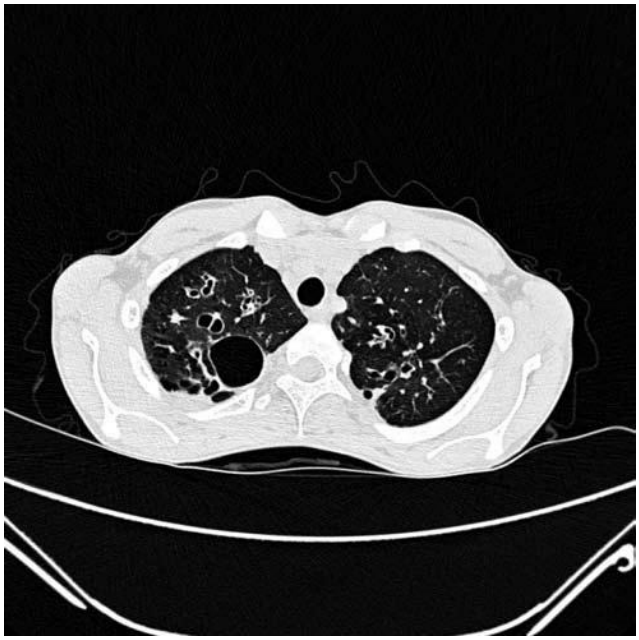

Fig. 6. Chest HRCT scan of a 15.5-year old boy with AI and CF shows cavity lesion, pleural thickening in the right upper lobe, bronchiectasis on both sides. (CT was performed in the Department of Diagnostic Imaging of the Institute of Mother and Child in Warsaw).

Ryc. 6. HRCT klatki piersiowej 15,5-letniego chłopca z AI i CF przedstawia jamy oraz zgrubienie opłucnej w szczycie płuca prawego, rozstrzenia oskrzeli obustronnie. (TK wykonano w Zakładzie Diagnostyki Obrazowej Instytutu Matki i Dziecka w Warszawie).

Table VI. Cystic Fibrosis Foundation Consensus suggestions for screening for ABPA in CF [5].

Tabela VI. Propozycje konsensusu Cystic Fibrosis Foundation badań przesiewowych w kierunku ABPA w CF [5].

Suggestions for screening for ABPA in CF: ***Sugestie skriningu w kierunku ABPA w CF:***
1. Maintain a high level of suspicion for ABPA in patients >6 years of age. *Należy utrzymać wysoką czujność odnośnie możliwości wystąpienia ABPA u pacjentów>6 rż.*
2. Determine the total serum IgE concentration annually. If the total serum IgE concentration is 1500 IU/mL, determine immediate cutaneous reactivity to A. fumigatus or use an in vitro test for IgE antibody to A. fumigatus. If results are positive, consider diagnosis on the basis of minimal criteria. *Oznaczanie jeden raz w roku stężenia IgE całkowitej w surowicy. Jeżeli stężenie IgE całkowiej w surowicy jest >500 jm/mL, należy wykonać test natychmiastowej reakcji skórnej z A. fumigatus lub test in vitro na oznaczenie przeciwciał IgE p/A. fumigatus. Jeśli wyniki są dodatnie należy rozważyć możliwość rozpoznania ABPA na podstawie minimalnych kryteriów*
3. If the total serum IgE concentration is 200-500 IU/mL, repeat the measurement if there is increased suspicion for ABPA, such as by disease exacerbation, and perform further diagnostic tests (immediate skin test reactivity to *A. fumigatus*, *in vitro* test for IgE antibody to *A. fumigatus*, *A. fumigatus precipitins*, or serum IgG antibody to A. fumigatus, and chest radiography). Jeżeli stężenie całkowitej IgE w surowicy wynosi 200-500 jm/mL, należy powtórzyć oznaczenie jeżeli istnieje zwiększone podejrzenie ABPA na podstawie zaostrzenia choroby i przeprowadzić dalsze badania diagnostyczne (test natychmiastowej reakcji skórnej z *A. fumigatus*, *in vitro* oznaczenie przeciwciał IgE p/*A. fumigatus*, oznaczenie precypityn p/*A. fumigatus* lub IgG p/*A. fumigatus* w surowicy, rtg klatki piersiowej).

with ABPA. Few case reports describe patients with cystic fibrosis on high-dose corticosteroids who acquire a viral infection, like influenza, predisposing them to AI [27, 28]. The proposed mechanisms for the development of *Aspergillus* overlap with syndromes that include coincidence, the presence of severe underlying lung disease, corticosteroid therapy (AI in a patient with ABPA), or *Aspergillus* fungal exposition. It is also possible that genetic factors may predispose patients to progress from one form of aspergillosis to another [2]. For example, *CFTR* gene mutations may result in AI. Viral illnesses in a patient with ABPA have rarely been reported as a risk factor for AI [2]. However, given the paucity of literature about these cases, there are no validated predictors for the development of AI in patients with ABPA.

Summarizing, we would like to emphasize that pulmonary aspergillosis is a serious complication in cystic fibrosis patients. We confirmed that it was associated with older age and the use of inhaled antibiotics and also prophylactic oral antibiotics (azithromycin). In our study risk factors include: the age over 6 years, duration of morbidity, chronic respiratory infection of *Pseudomonas aeruginosa*, long-term treatment with azythromycin, inhaled antibiotics, FEV1<40%, the F508del mutation (the most common in our population), exocrine pancreatic insufficiency. As our CF patients become older and as chronic inhaled antibiotics become pervasive, the prevalence of AI and ABPA are increasing. AI and ABPA were found in patients with a more severe disease and treatment burden. The clinician should maintain a high level of suspicion and then examine CF patients to avoid the deterioration of pulmonary function and a faster progression of the lung disease due to pulmonary aspergillosis.

CONCLUSIONS

Since pulmonary aspergillosis is a very serious complication in CF children, it seems reasonable to include screening for early detection of *Aspergillus* colonization in the annual assessment of CF patients over 6 years old. Due to the small size of the sample included in the study and the retrospective design of our analysis, the identification of the risk factors of pulmonary aspergillosis in CF children requires further prospective studies.

REFERENCES

1. Liu JC, Modha DE, Gaillard EA. What is the clinical significance of filamentous fungi positive sputum cultures in patients with cystic fibrosis? J Cyst.Fibros. 2013;12:187-193.
2. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. Eur Respir Rev. 2011;20,121:156-174.
3. Thia LP, Balfour Lynn IM. Diagnosing allegic bronchopulmonary aspergillosis in children with cystic fibrosis. Paediatr Resp Rev. 2009;10:37-42.
4. Casadevall A, Pirofski LA. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Immun. 1999;67:3703-3713.
5. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis – state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis. 2003;37 Suppl 3:S225-S264.
6. de Valk HA, Klaassen CH, Yntema JB, Hebestreit A, Seidler M, Haase G, *et al* Molecular typing and colonization patterns of A. fumigatus in patients with cystic fibrosis. J Cyst. Fibros. 2009;8:110-114.
7. Saunders R, Modha D, Claydon A, Gaillard E. Chronic A. fumigatus colonisation of the cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. J Cyst Fibros. 2011;10(Suppl. 1):S37.
8. Bargon J, Dauletbaev N, Kohler B, Wolf M, Posselt HG, Wagner TO. Prophylactic antibiotic therapy is associated with an increased prevalence of Aspergillus colonization in adult cystic fibrosis patients. Respir Med. 1999;93:835-838.
9. Bakare N, Rickerts V, Bargon J, Just-Nubling G. Prevalence of Aspergillus fumigatus and other fungal species in the sputum of adult patients with cystic fibrosis. Mycoses. 2003;46:19-23.
10. Valenza G, Tappe D, Turnwald D, Frosch M, Koning C, Hebestreit H, *et al.* Prevalence and antimicrobial susceptibility of microorganisms isolatedfrom sputa of patients with cystic fibrosis. J Cyst Fibros. 2008;7:123-127.
11. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with A. fumigatus on lung function and hospitalization in patients with cystic fibrosis. Chest 2010;137:171-176.
12. Jubin V, Ranque S, Stremler Le Bel N, Sarles J, Dubus JC. Risk factors for Aspergillus colonization and allegic bronchopulmonary aspergillosis in children with cystic fibrosis. Pediatr Pulmonol. 2010;45:764-771.
13. Paugam A, Baixench MT, Demazes-Dufeu N, Burgel PR, Sauter E, Kanaan R, *et al.* Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. Med Mycol. 2010;48(Suppl. 1):S32-36.
14. de Vrankrijker AM, Van Der Ent CK, Van Berkhout FT, Stellato RK, Willems RJ, Bonten MJ, *et al.* A. fumigatus colonisation in cystic fibrosis: implications for lung function? Clin Microbiol Infect. 2011;17:1381-1386.
15. Popiel A. Alergiczna aspergilloza oskrzelowo-płucna u chorych na mukowiscydozę. Klin Pediatr. 2010;18 (4):443-452.
16. Knutsen AP, Slavin RG. Allergic Bronchopulmonary Aspergillosis in Asthma and Cystic Fibrosis. Clin Dev Immunol. 2011;2011:843763. doi: 10.1155/2011/843763. Epub 2011 Apr 5.
17. Mastella G, Rainisio M, Harms HK, Hodson ME, Koch C, Navarro J, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Eur Respir J. 2000;16:464-471.
18. Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. Respir Med. 2005;99:887-893.
19. de Almeida MB, Bussamra MHF, Rodrigues JC. Allergic bronchopulmonary aspergillosis in pediatric cystic fibrosis patients. Paediatr Resp Rev. 2006;7:67-72.
20. Geller DE, Kaplowitz H, Light MJ, Colin AA. Alergicbronchopulmonary aspergillosis in cystic fibrosis; reported prevalence, regional distribution, and patient characteristics. Scientific Advisory Group, Investigators, and Coordinators of the Epidemiologic. Study of Cystic Fibrosis Chest 1999;116:639--646.

21. Report of the UK Cystic Fibrosis Trust Antibiotic Working Group. Antibiotic treatment for cystic fibrosis. The Cystic Fibrosis Trust. 3rd ed.; May 2009. Available from: http://www.cftrust.org.uk/aboutcf/publications/consensusdoc/Antibiotic_treatment_for_Cystic_Fibrosis.pdf.
22. Amitani R, Murayama T, Nawada R, Lee WJ, Niimi A, Suzuki K, *et al.* Aspergillus spp. culture filtrates and sputum sols from patients with pulmonary aspergillosis cause damage to human respiratory ciliated epithelium in vitro. Eur Respir J. 1995;8:1681-1687.
23. Hodson ME, Gallagher CG, Govan JRW. A randomized clinical trial of nebulised tobramycin or colistin in cystic fibrosis. Eur Respir J. 2002;20:658-664.
24. Verleden GM, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med. 2006;174:566-570.
25. Denning DW, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavity and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. Clin Infect Dis. 2003;37:Suppl. 3:S265-S280.
26. Ganassini A, Cazzadori A. Invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis. Respir Med. 1995;89:143-145.
27. Brown K, Rosenthal M, Bush A. Fataal invasive aspergillosis in a adolescent with cystic fibrosis. Pediatr Pulmonol. 1999;27:130-133.
28. Massam J, Bitnum A, Solomon M, Somers GR, Guerguerian AM, Wylick R, Waters V. Invasive aspergillosis in cystic fibrosis. A fatal case in an adolescent and review of the literature. Pediatr Infect Dis J. 2011;30(2);178-180.

Conflicts of interest/Konflikt interesu
The Authors declare no conflict of interest.
Autorzy pracy nie zgłaszają konfliktu interesów.

Received/Nadesłano: 08.10.2014 r.
Accepted/Zaakceptowano: 09.12.2014 r.

Published online/Dostępne online

Address for correspondence:
Katarzyna Walicka-Serzysko
Cystic Fibrosis Centre, Institute of Mother and Child
Kasprzaka Street 17A, 01-211 Warsaw, Poland
tel. (+48 22)-32-77-455
e-mail: katarzyna.walicka@wp.pl