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## **DIAGNOSTIC PROBLEMS IN CYSTIC FIBROSIS – SPECIFIC CHARACTERISTICS OF A GROUP OF INFANTS AND YOUNG CHILDREN DIAGNOSED POSITIVE THROUGH NEONATAL SCREENING, IN WHOM CYSTIC FIBROSIS HAD NOT BEEN DIAGNOSED**

### **TRUDNOŚCI W DIAGNOSTYCE MUKOWISCYDOZY – CHARAKTERYSTYKA NIEMOWLĄT I MAŁYCH DZIECI Z DODATNIM WYNIKIEM PRZESIEWU NOWORODKOWEGO, U KTÓRYCH NIE ROZPOZNANO MUKOWISCYDOZY**

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#### **Abstract**

**Introduction:** Neonatal cystic fibrosis screening contributes to an early diagnosis of cystic fibrosis and to implementing appropriate therapeutic management. Long-standing screening tests have made it possible to identify a group of newborns in whom the diagnosis was ambiguous and required further specialised tests.

**Aim:** The aim is to present cases of patients with a positive result of newborn screening for cystic fibrosis who were found to be carriers of the mutation in both alleles, however the lack of clinical symptoms and correct sweat testing values did not lead doctors to diagnosing cystic fibrosis and by the same token implementing the treatment.

**Material and methods:** The analysis encompassed a group of 22 infants and children 3 months to 3 years of age, in whom, in spite of a positive result of newborn screening for cystic fibrosis and the presence of 2 mutations in the CFTR gene, the diagnosis of cystic fibrosis was not made, and appropriate treatment was not administered because of diagnostic doubts (due to correct concentration of chlorides in sweat, correct IRT level and lack of clinical signs of cystic fibrosis). The control group consisted of 55 children treated in our centre, in whom neonatal screening for cystic fibrosis was positive and the diagnosis was confirmed by genetic testing, sweat chloride testing and IRT concentration.

**Results:** There were no differences in birth body weight between the groups. The differences in chloride ion levels in sweat secretion tests and mean IRT values were statistically significant and were: 97.5 for the control group and 26.4 for the test group. At the present time there are no clinical symptoms to give a diagnosis of cystic fibrosis and start treatment in the test group.

**Conclusions:** Newborn screening contributes not only to an early diagnosis of cystic fibrosis but also to CFTR-related metabolic syndromes (CRMS), which is a phenomenon requiring further observation. This fact constitutes a definite psychological problem for the parents of these patients.

**Key words:** cystic fibrosis, neonatal screening, diagnosis, chlorides

#### **Streszczenie**

**Wstęp:** Badania przesiewowe noworodków w kierunku mukowiscydozy dają możliwość wczesnego rozpoznania choroby i wdrożenia odpowiedniego postępowania terapeutycznego. Długoletnie prowadzenie badań przesiewowych pozwoliło wyodrębnić grupy dzieci, u których postawienie rozpoznania mukowiscydozy nie było jednoznaczne i wymagało dalszych specjalistycznych badań.

**Cel pracy:** Celem pracy jest przedstawienie przypadków pacjentów z dodatnim wynikiem noworodkowych badań przesiewowych w kierunku mukowiscydozy, u których wykryto obecność mutacji genu CFTR w obu allelach, natomiast brak objawów klinicznych oraz prawidłowe wartości testów potowych nie upoważniły do rozpoznania choroby, a tym samym do włączenia leczenia.

**Materiał i metody:** Badaniami objęto 22 niemowląt i dzieci w wieku od 3 miesiąca do 3. roku życia, u których mimo dodatniego wyniku badania przesiewowego w kierunku mukowiscydozy i stwierdzenia w badaniu genetycznym 2 mutacji genu CFTR, nie rozpoznano mukowiscydozy (CF) i nie rozpoczęto leczenia ze względu na wątpliwości diagnostyczne (prawidłowe stężenie chlorków w pocie i prawidłowe stężenie IRT oraz brak klinicznych objawów CF/grupa badana).

Za grupę kontrolną uznano 55 dzieci leczonych w naszym ośrodku, u których test przesiewowy w kierunku CF wypadł dodatnio, a rozpoznanie potwierdzone zostało badaniem genetycznym oraz badaniem stężenia chlorków w pocie i stężeniem IRT.

**Wyniki:** Nie stwierdzono różnic w zakresie masy urodzeniowej ciała między grupą badaną a kontrolną. Natomiast różnice w stężeniu chlorków w pocie i w stężeniu IRT były istotne statystycznie i wynosiły: dla grupy kontrolnej (średnio) – 97,5, a dla grupy badanej 26,4.

W chwili obecnej u dzieci z grupy badanej nie stwierdzono objawów klinicznych dających podstawę do rozpoznania CF i rozpoczęcia leczenia.

**Wnioski:** Badanie przesiewowe noworodków przyczynia się do wczesnego rozpoznania nie tylko mukowiscydozy ale także CFTR-zależnych zespołów metabolicznych wymagających dalszej obserwacji. Fakt ten stanowi poważny problem psychologiczny u rodziców tych dzieci.

**Słowa kluczowe:** mukowiscydoza, badania przesiewowe noworodków, diagnostyka, chlorki

DEV. PERIOD MED. 2015;XIX,1:25-31

## INTRODUCTION

Cystic fibrosis is an autosomal recessive disease of genetic origin associated with the dysfunction of the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator) located on the long arm of the 7th chromosome. The product of the gene is a membrane channel protein, the expression of which is associated with the apical surface of the epithelial cells of the exocrine glands (e.g. the pancreas, gastrointestinal, respiratory and reproductive systems). The mutation of the gene leads to the production of an improper protein.

The incidence of cystic fibrosis in the European population differs significantly between particular countries. Based on neonatal screening, it is currently 1:4394-1:5000 in the Polish population [1].

In mid-2009, all the provinces in Poland were covered by obligatory neonatal screening for cystic fibrosis. According to current standards, the screening is based on the so-called two-tier system employing the measurement of IRT/DNA in a capillary blood sample taken from an infant's heel after 72 hours (3 days) of his/her life. In the event of abnormal results of the immunoreactive trypsinogen (above 99.4 pcn), a baby undergoes a genetic test encompassing a panel of several hundred mutations of the CFTR gene, including the 16 most common ones in the Polish population (2). The total number of the CFTR gene mutations exceeds 1970. Mutations can be divided into 6 main classes: severe (classes I-III) and mild ones (classes IV-VI) depending on the effects of the mutation, the changes in the CFTR protein (production of an abnormal CFTR protein, improper insertion into the apical membrane, etc.),

and the clinical manifestations related to the individual mutations (Table I). Correlation between the class of the mutation and clinical symptoms of cystic fibrosis is incomplete, especially in the range of pulmonary manifestations (Table I, on the basis of 3,4).

The most common pathogenic mutation (i.e. one which is responsible for the occurrence of clinical symptoms), also in the Polish population, is the deletion of three nucleotides that code phenylalanine in the 508 position (the so-called F508del), which belongs to the 2nd class of mutations. It constitutes around 64% of all the CFTR gene mutations in the Polish population. Those are (in order of incidence): 3849+ 10 kbC->T (3%); N1303K (2%), G542X (1.9%), CFTR dele2,3(21kb) (1.7%) (based on the register data given by the Polish Cystic Fibrosis Society, [2]).

An abnormal IRT result or the presence of mutation in at least one of the CFTR gene alleles requires extended diagnostics, which is performed by specialist centres in the second month of a baby's life. For verification purposes, besides physical examination with the assessment of a patient's clinical condition, the infant's sweat chloride ion concentration and IRT level is measured by means of the standard and the conductometric methods (using the Nanoduct-Wescor device). According to the criteria for identifying cystic fibrosis, in order to diagnose infants with a positive result of a screening test, it is necessary to confirm: mutations in the CFTR gene alleles and sweat chloride concentration exceeding 59 mmol/l (in newborns >40 mmol/l), as well as clinical features typical for cystic fibrosis [5]. In the first six months of life, a CF diagnosis with the sweat chloride ion concentration below 30 mmol/l is questionable. Over the age of 6 months, the

Table I. Summary of CFTR gene mutation classes (based on 3,4).  
 Tabela I. Zestawienie klas mutacji genu CFTR (w oparciu o 3,4).

Class <i>Klasa</i>	I	II	III	IV	V	VI
Disorder <i>Zaburzenie</i>	Lack of synthesis of the proper CFTR protein/ <i>Brak syntezy prawidłowego białka CFTR</i>	Disturbed CFTR protein maturation/ <i>Zaburzone dojrzewanie białka CFTR</i>	Deregulation of the chloride channel/ <i>Deregulacja kanału chlorkowego</i>	Disturbed conductivity of the chloride channel/ <i>Zaburzone przewodnictwo kanału chlorkowego</i>	Reduced synthesis of the proper CFTR protein/ <i>Zmniejszona synteza prawidłowego białka CFTR</i>	Decreased stability of the CFTR protein and increased rate of the chloride channel turnover; disturbed interactions between the CFTR channels and other ion channels/ <i>Zmniejszona stabilność białka CFTR i przyspieszony obrót kanału chlorkowego; zaburzenie oddziaływań pomiędzy kanałami CFTR a innymi kanałami jonowymi</i>
Mutation <i>Mutacja</i>	G542X; dele2,3(21kb)	F508del; N1303K	G551D; G1349D	R117H; D1152H	3849+10kbC>T ; A455E	120delI23; N287Y 4279insA
Incidence (%) <i>Częstość (%)</i>	16.4	80	3.9	3.3	3	Isolated cases/Pojedyncze przypadki
Course <i>Przebieg</i>	Classic CF/ <i>Klasyczna CF</i>	Classic CF/ <i>Klasyczna CF</i>	Classic CF/ <i>Klasyczna CF</i>	Mild phenotype of CF/ <i>łagodny fenotyp CF</i>	Mild phenotype of CF/ <i>łagodny fenotyp CF</i>	Usually a milder phenotype of CF/ <i>Zazwyczaj łagodniejszy fenotyp CF</i>

suggested cut-off threshold is the value of 30-40 mmol/l. Infants with a boundary value of the sweat secretion test should undergo a follow-up examination with extended diagnostics at a specialist centre [6, 7].

On the basis of full screening, cystic fibrosis can be either ruled out or confirmed in most of the cases. However, among the infants and babies diagnosed, there is also a group of patients, in the case of whom making a final diagnosis poses a significant problem, primarily due to the detection of a mutation of uncertain/so far unknown clinical prognosis and doubtful results of sweat secretion tests. During the first years of their lives, those babies remain under the supervision of specialist centres to be observed for the potential development of CF-related symptoms or the manifestation of symptoms typical of the CFTR-Related Metabolic Syndrome, which is a condition that does not fulfil the diagnostic criteria for CF diagnosis. The CFTR-related Metabolic Syndrome is diagnosed in infants with hypertrypsinogenemia (IRT>99.4 pcn) found through a screening test, whose sweat chloride concentration level is lower than 60 mmol/l and at least one of the two CFTR-gene allele mutations is not determined to be pathogenic or is not fully clarified. The main diseases referred to as the CFTR-related syndrome include: multiple bronchiectasis, idiopathic pancreatitis and congenital bilateral absence of the vas deferens [8, 9].

### THE AIM

The aim is to present cases of babies with positive results of neonatal screening for cystic fibrosis, who were found to be carriers of the CFTR gene mutation in both alleles, however the lack of clinical symptoms and correct sweat testing values did not provide sufficient grounds for the diagnosis of cystic fibrosis and by the same token lead to implementing the treatment.

### MATERIAL AND METHODS

The analysis encompassed a group of 22 infants and children aged 3 months to 3 years (test group), in whom, in spite of a positive result of newborn screening for cystic fibrosis and the presence of 2 mutations in the CFTR gene, the diagnosis of cystic fibrosis was not made and the appropriate treatment was not implemented, because of diagnostic doubts (correct concentration of chlorides in sweat, correct IRT level and lack of the clinical signs of cystic fibrosis).

The control group consisted of 55 children treated in our centre, in whom neonatal screening for cystic fibrosis was positive and the diagnosis was confirmed by genetic testing, sweat chloride testing and IRT concentration. The measurement of chlorides was made using the classic (mmol/l) and Wescor (mmol) method. Statistical analysis was conducted with the STATISTICA 10 program.

### RESULTS

The variables analysed demonstrated normal distribution. Statistically significant differences ( $p < 0.01$ ) in the range of IRT and chloride ion levels between the groups were observed on the basis of the t-Student test and the Mann-Whitney-Wilcoxon test. Differences between birth body weights in the groups were also noticeable but not statistically significant (Table II).

All the babies under observation report regularly every 3 months in the first year of life, and then 2 times a year. Proper physical and mental development was documented. At the age of twelve months, they reached the proper body weight (8.9 kg-11.5 kg, which remains within the limits of 25-90 pcn for this age). Their medical history indicates that they suffer only from minor infections of the upper respiratory tract. The laboratory tests, including complete blood count, inflammatory markers, hepatic and pancreatic function

Table II. Characteristics of the groups – t-Student test.

Tabela II. Charakterystyka badanych grup – Test t.

Variable Zmienna	Mean c Średnia k	Mean s Średnia b	p	Standard deviation c Odchylenie standardowe k	Standard deviations Odchylenie standardowe b
Birth weight (g)/ Masa ciała ur. (g)	3.1477	3.2241	0.62	0.5848	0.66932
IRT (ug/l)	456.8617	154.6100	<0.01	330.5706	60.63721
Classic sweat analysis (mmol/l) Próba klasyczna (mmol/l)	97.4657	26.4000	<0.01	32.1039	8.30407
Wescor (mmol/)	102.4000	40.3810	<0.01	24.1720	9.42060

c – controlled trial (diagnosed CF)/k – grupa kontrolna (rozpoznanie CF)  
s – study group (under observation)/b – grupa badana (CF w obserwacji)

Table III. Characteristics of the groups – Mann-Whitney-Wilcoxon test.

Tabela III. Charakterystyka badanych grup – Test U Manna-Whitneya-Wilcoxona.

Variable <i>Zmienna</i>	Rank sum c	Rank sum s	p
Birth weight (g)/ <i>Masa ciała ur. (g)</i>	1865	910	0.32
IRT(ug/l)	1966	380	<0.01
Classic sweat analysis (mmol/l)/ <i>Próba klasyczna (mmol/l)</i>	1213	165	<0.01
Wescor(mmol/)	2287.5	268.5	<0.01

c – controlled trial (diagnosed CF)/k – grupa kontrolna (rozpoznanie CF)

s – study group (under observation)/b – grupa badana (CF w obserwacji)

assessment, as well as biochemical nutritional parameters, are within normal range (Table III).

In 10 babies remaining under observation, the most frequent pathogenic mutation was the F508del (constituting 45%), in 3 of the patients – the dele 2,3 (21/kb) (13%), and, in the remaining individuals, one of the following mutations: 3272-26A>G, R553X, K710X, R334Q, G542X, W1282X, Y512N, G1069R and P638S – a potentially pathogenic mutation.

The second prevailing mutation in the babies observed (n=7; 31%) was the IVS8-5T(TG)11 variant, responsible for the occurrence of the CFTR-related syndrome or remaining without clinical significance. Three patients (13%) were found to have the R117H+IVS8-7T mutation, and two (9%) had the D1152H mutation – both mutations were associated with the presence of the CFTR-related syndrome or a mild form of cystic fibrosis. In the other 2 babies (9%) – the R1162L mutation with so far unclear clinical consequences (non-pathogenic or association with the CFTR-related syndrome – the congenital bilateral absence of the vas deferens, pancreatitis) was found. The other mutations detected in the babies during molecular tests include ones associated with the CFTR-related metabolic syndromes or a mild form of cystic fibrosis (variant IVS8-5T(TG)12, with CFTR-related syndromes (M952T – obstruction of spermatic ducts, L967S – idiopathic pancreatitis); with an ambiguous influence (L997F – mild CF, CFTR-related or without any clinical significance; registered yet of unknown influence (P750L and G1343S).

## DISCUSSION

As the panel of mutations examined expands, the number of the so-called observation cases is growing as well. The babies under discussion are ones who do not fulfil the criteria for the diagnosis of cystic fibrosis. Such patients cannot be confirmed to have the CFTR gene dysfunction, because at most one of the revealed mutations had a confirmed pathogenicity, the sweat chloride concentrations (the Wescor test and the standard pilocarpine test) were unremarkable or at most borderline, and there were no clinical symptoms typical of cystic fibrosis. Those babies were qualified as CFTR-related

metabolic syndrome (CRMS) cases. In the research by Zybert and Sands (The Institute of Mother and Child in Warsaw), the observation encompassed 19 babies, which constituted 20.65% of all the babies with a positive result of the screening test. In this group the final diagnosis of cystic fibrosis was not reached either (10).

According to the current procedure, when the diagnosis of cystic fibrosis is questionable, it is recommended to take a wait-and-see attitude in terms of starting conventional treatment. Particular attention must be paid to alarming disease-related symptoms, such as coughing and long-lasting infections (without overlooking the baby's exposure to tobacco smoke), abnormal stools, poor body weight gain, and male infertility at an older age. Regular annual vaccination against influenza is recommended. During observation, apart from the above-mentioned tests, it is recommended to carry out a genetic test in both parents in order to determine the carrier state [11].

The group of babies with uncertain diagnosis constitutes a challenge for CF specialists (both clinicians and geneticists). Those babies are also a major concern for their parents, hence the important role of reassurance and precise explanation of the purposefulness of the observation procedure.

According to the literature data concerning genetic issues in cystic fibrosis, the IVS8-5T(TG)11 mutation found in 6 patients has been described in the literature in connection with the occurrence of nasal polyps. Four p.Q493X heterozygotic variants were identified on the paternal allele, and p.V562I, p.A1006E and (TG)11(T)5 (IVS8-5T) on the maternal allele in the case of cystic fibrosis presenting an isolated nasal polyposis [12]. This mutation may be associated with an increased susceptibility to the mycobacterium avium complex (MAC) infections [13].

According to the data from the CFTR2 base (Clinical and Functional Translation of CFTR – website: <http://www.cftr2.org>), the R1162L mutation occurred in 9 patients and it was not responsible for the presence of clinical symptoms of cystic fibrosis, even if combined with another pathogenic mutation.

793 patients with the R117H mutation have also been registered in the database. This mutation has an

ambiguous influence – in some patients, it may or may not produce CF symptoms, even in combination with another mutation of known pathogenic impact. According to the literature data, it is present in the majority of patients with a stable function of the pancreas. Researchers suggest withdrawing the R117H variant from the panel of screening tests due to the commonly-observed fact that the majority of babies who have been diagnosed with it, have a stable pancreatic function, proper pulmonary function and good nutritional condition [14, 15]. Other data indicate that this particular mutation is associated with pancreatitis, similarly to other variants of the mutation that are not related to the typical form of cystic fibrosis (R74Q, R75Q, R117H, R170H, L967S, L997F, D1152H, S1235R and D1270N(16)).

In the research by Prach et al. describing 55 new CFTR gene mutations not registered to date, ten (10) variants were associated with the clinical manifestation of cystic fibrosis. 58% of the babies in whom those variants had been found, were diagnosed with cystic fibrosis in the first three months of life. In the remaining 42%, the diagnosis was confirmed after a two-year follow-up period. The other 26 variants that had been discovered were present in patients without clinical symptoms of the disease throughout the observation period. They were associated with the CFTR-related metabolic syndrome [17].

The system of neonatal screening for cystic fibrosis, which is currently applicable in Poland, involves the determination of the immunoreactive trypsinogen concentration after an infant is 3 days old. In the event of an abnormal result, a molecular test is performed for the presence of the CFTR gene mutation (the IRT/DNA strategy). The parents are informed only in the event their baby has abnormal results of the above-mentioned tests. In order to make a final diagnosis, an infant requires extended diagnostics, including sweat secretion tests, advanced biochemical tests, and assessment of the general clinical condition. On the basis of own and other authors' studies, it needs to be said that despite the standards of the diagnostic procedure, some cases require many months of observation before a final diagnosis is made. There is a group of babies who do not fulfil all the criteria for CF diagnosis, who, at the same time, are in the risk group requiring further investigation.

The chance to use a different screening strategy is offered by the measurement of the Pancreatitis Associated Protein (PAP) concentration. Currently, the PAP-based method is not a standard testing procedure. According to the literature data, the test has a similar diagnostic value when compared with the IRT measurement. This strategy has high sensitivity and specificity, and is cheaper compared to the IRT/DNA strategy. The IRT/PAP strategy is promising also in cases where for any reason whatsoever genetic testing is difficult to perform [18]. It allows to diagnose cystic fibrosis without detecting the carriers of the CFTR gene mutations, as well as forms of the gene with unclear clinical consequences. The IRT/PAP strategy has a competitive edge, due to the smaller percentage of false-positive results (compared

with the currently used IRT/DNA method). However, when it is necessary to determine the carrier state in parents or when the diagnosis is still ambiguous, the strategy covering the IRT/PAP/DNA marking could be the method of choice [19].

## CONCLUSIONS

Newborn screening not only contributes to an early diagnosis of cystic fibrosis but also to finding CFTR-related metabolic syndromes (CRMS) which require further observation. This fact constitutes a definite psychological problem for the parents of the patients.

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**Conflicts of interest/Konflikt interesu**

The Authors declare no conflict of interest.

Autorzy pracy nie zgłaszają konfliktu interesów.

**Received/Nadesłano:** 16.12.2014 r.

**Accepted/Zaakceptowano:** 10.02.2015 r.

**Published online/Dostępne online**

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