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DIAGNOSING CYSTIC FIBROSIS IN NEWBORN SCREENING IN POLAND – 15 YEARS OF EXPERIENCE

ROZPOZNANIE MUKOWISCYDOZY W PRZESIEWIE NOWORODKOWYM W POLSCE – 15 LAT DOŚWIADCZEŃ

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Abstract

Early diagnosis of cystic fibrosis (CF) made by the introduction of CF NBS (Cystic Fibrosis Newborn Screening) provides the opportunity to undertake preventive measures and provide treatment before the development of irreversible changes in the respiratory tract and other complications. CF NBS was conducted as a pilot programme in four Polish districts in the period 1999-2003. In 2006 CF NBS started again and was gradually extended across the country.

The aim of this study was to show the evolution of the Polish CF NBS strategies and assess the diagnostic consequences of this programme.

Material and methods: The study involved children diagnosed and treated only in the IMiD Centre. The strategy in Polish CF NBS was modified over time. Firstly, the model IRT/IRT and IRT/IRT/DNA with one mutation was implemented, which was followed by IRT/DNA with a gradually expanding number of CFTR mutations (tab. I). Newborns with positive results of CF NBS were called to the CF IMiD Centre, and sweat tests were performed. The children diagnosed and children with mutations in both alleles of the CFTR gene (even if at least one of them had undefined pathogenicity) were taken under IMiD Centre care. Sensitivity, specificity and positive predictive values during subsequent stages of CF NBS were calculated (tab. III).

Results: During the 1999-2003 pilot study 444 063 newborns underwent CF NBS and in 74 cases CF was diagnosed. 582 693 newborns were screened from September 2006 to December 2011 in four regions and 100 children were diagnosed with CF. The frequencies of CF in the Polish population in both screening periods were 1:5767 and 1:5712 respectively. Firstly, the IRT/IRT model was implemented, but the number of newborns called to the CF Centre was high - the PPV was 7.6%. In the next step CF NBS DNA analysis was used. Here sensitivity and specificity were high – nearly 100%. In the following years the number of mutations detected was expanded (including 16 most common ones in the Polish population). Due to the panel changes, the number of calls declined and the PPV (predictive positive value) improved (to 26.1%) after the application of expanded genetic analysis. Expanding the panel of mutations resulted in an increased number of carriers and observational subjects.

Conclusions: IRT/DNA strategy with expanded DNA analysis provides the opportunity for earlier CF diagnosis even in children with normal sweat test values. However, this model caused frequent carrier detection and inconclusive diagnosis in comparison to IRT/IRT or IRT/IRT/DNA with a limited number of mutations. Further research and changes in Polish CF NBS are needed to increase the PPV, while preserving high sensitivity and specificity.

Key words: diagnostics, newborn screening, cystic fibrosis

Streszczenie

Wczesne rozpoznanie mukowiscydozy dzięki wprowadzeniu CF NBS (Cystic Fibrosis Newborn Screening) daje możliwość podjęcia działań profilaktycznych i leczniczych przed powstaniem nieodwracalnych zmian w układzie oddechowym oraz innych powikłań. W latach 1999-2003 prowadzono pilotażowy CF NBS na terenie czterech polskich województw. W 2006 roku wznowiono program na tym samym obszarze, a od czerwca 2009 roku jest on prowadzony w całym kraju.

Celem pracy było przedstawienie ewolucji algorytmów diagnostycznych, według których prowadzono w Polsce badanie przesiewowe noworodków w kierunku mukowiscydozy oraz ich przydatności diagnostycznej.

Materiał i metody: Praca dotyczy dzieci, u których diagnostyka i leczenie prowadzone było w Instytucie Matki i Dziecka. W trakcie prowadzenia polskiego CF NBS algorytm diagnostyczny ulegał modyfikacji. Początkowo wdrożono schemat IRT/IRT oraz IRT/IRT/DNA z oznaczeniem jednej mutacji. Następnie wprowadzono schemat IRT/DNA ze stopniowo rozszerzanym panelem mutacji genu CFTR (tab. I). Dzieci z nieprawidłowym wynikiem CF NBS wzywane były na wizytę weryfikacyjną, podczas której wykonywano testy potowe. Dzieci, u których potwierdzono chorobę lub gdy stwierdzano mutacje w obu allelach genu CFTR (nawet jeśli przynajmniej jedna z nich była mutacją o nieznanym lub niejednoznacznych konsekwencjach klinicznych), pozostawały pod opieką IMiD. Czulość, swoistość oraz wartość predykcyjną dodatnią (PPV) obliczono dla poszczególnych etapów prowadzenia CF NBS (tab. III).

Wyniki: W okresie prowadzenia badania pilotażowego (1999-2003) zbadano 444 063 noworodków, u 74 rozpoznano CF. W latach 2006-2011 na terenie województw mazowieckiego, warmińsko-mazurskiego, podlaskiego i lubelskiego zbadano 582 693 noworodki i rozpoznano CF u 100 dzieci. Częstość występowania mukowiscydozy w populacji polskiej w obu okresach, w których prowadzony był przesiew, wynosiła odpowiednio 1:5767 oraz 1:5712.

Początkowo stosowano schemat IRT/IRT. Skutkiem była duża liczba wezwań na wizyty weryfikacyjne i niskie PPV – 7,6%. Dołączenie analizy DNA do CF NBS pozwoliło na uzyskanie wysokiej czulości i swoistości bliskiej 100%. W kolejnych latach prowadzenia CF NBS poszerzono panel analizy DNA uwzględniający wykrywanie 16 najczęściej występujących w polskiej populacji mutacji genu CFTR, stopniowo zwiększając liczbę mutacji rzadziej występujących. Dzięki temu zmniejszyła się liczba wezwań do ośrodka na wizyty weryfikacyjne. Najwyższe PPV – 26,1% uzyskano po zastosowaniu rozszerzonej analizy genetycznej. Rozszerzanie panelu analizy molekularnej przyczyniło się do wzrostu liczby przypadków obserwowanych.

Wnioski: Na podstawie przedstawionych wyników można wnioskować, że zastosowanie schematu IRT/DNA z rozszerzonym panelem analizy DNA daje możliwość wczesnego rozpoznania CF nawet u dzieci z prawidłowymi wartościami testów potowych. Jednakże zastosowanie tego modelu powoduje zwiększenie liczby przypadków wymagających obserwacji w porównaniu do używanego uprzednio schematu IRT/IRT lub IRT/IRT/DNA z ograniczonym panelem mutacji.

Potrzebne są dalsze badania oraz wprowadzenie zmian w polskim CF NBS, tak aby zwiększyć PPV utrzymując wysoką czulość i swoistość.

Słowa kluczowe: diagnostyka, przesiew noworodkowy, mukowiscydoza

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INTRODUCTION

Cystic fibrosis is a serious congenital chronic disease. Clinical symptoms result from pathogenic mutations in both alleles of the *CFTR* gene (cystic fibrosis transmembrane conductance regulator gene) and are connected with the dysfunction of many systems and organs.

Due to the severity of the symptoms, as well as the decrease of life quality and length, early diagnosis of the disease, before irreversible lesions in the respiratory tract, malnutrition and other CF characteristic complications set in, is a high priority issue. Cystic Fibrosis Newborn Screening (CF NBS) is today an optimum method to diagnose the disease in the neonatal period. As a disease, cystic fibrosis complies with the terms of running the screening for a congenital disease which were formulated in 1968 and approved by the World Health Organization [1, 2]. CF is a serious health and social problem with a natural course and asymptomatic onset. The rules and strategy of therapeutic procedures are well known, so

positive modification of the natural course of the disease is possible. Fully accepted screening tests, as well as examinations verifying the diagnosis are available as well. Demonstration of increased immunoreactive trypsinogen (IRT) in CF newborns in 1979 and the definition of the *CFTR* gene ten years later were a real breakthrough in CF NBS, making it possible to define the concentration of IRT and DNA analysis of dried blood spots (DBS) on sampling paper [3, 4, 5, 6]. At present, the majority of programmes are based on these two determinations. In many countries they are included in a national programme and in some of them the tests are carried out in some regions only. However, over 60% of European newborns are included in CF NBS [7, 8].

THE AIM OF THE STUDY

The aim of the study was to present the evolution of diagnostic algorithms used in CF NBS in Poland and assess the diagnostic consequences of this programme.

MATERIAL AND METHODS

CF NBS stages

In 1999, CF NBS was started in Poland. The test was joined to the NBS for phenylketonuria and hypothyreosis and functioned as a pilot programme. It was implemented in four Polish districts: the Masovian, Warmian-Masurian, Podlaskie and Lublin Voivodeships. The programme was completed in August 2003 and in September 2006 it was restarted in the same regions. In subsequent years, its range was successively enlarged and in June 2009 it encompassed the whole country. At present CF NBS in IMiD covers about 25% of live births in Poland.

Patients

The study comprised the patients in whom CF NBS diagnostics was carried out in the Institute of Mother and Child (IMiD) born from 01.01.1999 till 31.12.2011.

Data collection

CF NBS was based on the organizational structure, documentation and sampling paper with biological material used in the screening tests for phenylketonuria and hypothyreosis. It was controlled by the Neobase computer system. All NBS stages were supervised from the time of material collection till the moment of diagnosis. There was a set of three identical bar code copies. One copy was stuck to the sampling paper, the second one to the child's health certificate and the third one to the hospital documentation. The data of all the neonates enrolled into the NBS programme were collected and stored in the Newborn Screening Department of the IMiD. Neonates' blood samples were collected onto Schleicher&Schuell 9229 sampling paper. IRT was determined using the colorimetric method with the use of *Immunoreactive Trypsin Neonatal Screening ELISA colorimetric assay* (IBL International).

CF NBS protocols

During the pilot study, carried out from 01.01.1999 till 30.06.2000, IRT/IRT strategy was introduced (Table I). If IRT concentration in the blood sample collected during the 1st week of life (sampling paper I) was between 99.4-99.7 percentile, IRT concentration was observed in the 4th week of life on "sampling paper II". Children were called to IMiD for a verifying visit if the concentration value on sampling paper I exceeded 99.7 percentile or if IRT concentration on sampling paper II exceeded 98.7 percentile.

From 01.07.2000 till the end of the pilot study in August 2003, CF NBS was carried out according to the IRT/IRT/DNA strategy. In the case of increased IRT concentration on sampling paper I, the presence of one – the most common mutation of the *CFTR* gene – i.e. F508del, was examined. The previous scheme of proceeding was preserved in the case of IRT concentration exceeding threshold values. In this scheme, children with at least one mutation of the *CFTR* gene were also called for a verifying visit.

In 2006 CF NBS was repeated in the region where the pilot study had been carried out earlier. IRT definition

on sampling paper I was performed after the material collection on the 3rd - 5th day of life. If the value exceeded 99.4 percentile DNA molecular analysis was made and IRT was defined on the basis of sampling paper II on which the blood was collected on the 21st-28th day of the neonate's life.

Since 2007 the *CFTR* gene mutation panel was gradually broadened within the framework of the IRT/IRT/DNA scheme. The use of the micro matrix by Asper Biotech made it possible to detect 47 mutations. Then, the sequencing method was applied, which allowed the detection of 570 mutations (Table I). Genomic DNA was elicited from sampling paper I by cutting out circles which were 3 mm in diameter and processing them with the Extract Blood PCR Kit (Sigma Aldrich, St Louis Mo, USA). Molecular analysis of DNA was performed in the Genetics Dept of IMiD and since 01.10.2010 this task was taken over by Genomed – a Private Healthcare Clinic.

Since November 2009, IRT definition on the basis of filtration paper II has been given up and the IRT/DNA scheme was introduced. In this strategy, after exceeding the IRT threshold value of 99.4 percentile, DNA analysis was performed, which enabled the detection of 570 mutations of the *CFTR* gene.

Since September 2011 the expanded genetic analysis (EGA) analysis panel has been used and it comprises 95% of the mutated alleles in the Polish population.

Sensitivity, specificity and positive predictive value (PPV) with 95% exact confidence intervals (95% CI) during subsequent stages of CF NBS were calculated on the assumption that in all the false-negative cases screened by IMiD till the end of 2011, the correct diagnosis of CF had already been made either by IMiD or by the other centre and reported to ours.

RESULTS

During the pilot studies, 444 063 neonates were examined and in 74 of them CF was diagnosed during the first verifying visit (frequency of CF diagnosis 1:5767). Four children remained under the supervision of the medical centre and in 3 of them the diagnosis was confirmed. In the years 2006-2011, 582 693 children were examined in the area under the supervision of the IMiD, and in 100 of them CF diagnosis was made (1:5712). At present 19 children remain under the care of the centre as observational subjects.

Table II presents data concerning the population examined, verifying visit calls as well as the number of diagnoses and diagnostic cases. Table III presents the sensitivity, specificity and the predictive positive value (PPV) of the studies in subsequent stages.

False-negative cases

False-negative cases were reported to the IMiD by specialised CF centres, where they were referred.

In one case, that of a boy who underwent CF NBS with an IRT negative result, the disease was diagnosed at the age of 5 years. The reason for initiating the diagnostic process was the CF positive diagnosis in his younger brother. In the five-year-old boy the diagnosis revealed

Table I. Stages of CF NBS in Poland.

Tabela I. Etapy CF NBS w Polsce.

Protocol Protokół		Analysis of CFTR gene Analiza genu CFTR	Period Okres
Pilot study Pilotaż	IRT/IRT	-	01.01.1999-30.06.2000
	IRT/IRT/DNA	1 mutation 1 mutacja	01.07.2000-02.08.2003
Current NBS Aktualny NBS		47-570 mutations 47-570 mutacji	01.09.2006-31.12.2006
	01.01.2007-17.10.2009		
	IRT/DNA	570 mutations 570 mutacji	18.10.2009-21.09.2011
		95% of the mutated alleles 95% zmutowanych alleli	22.09.2011-31.12.2011

Table II. Calls, CF, and observational cases.

Tabela II. Wezwania, rozpoznania, i przypadki obserwacyjne podczas kolejnych etapów CF NBS.

Protocol CF NBS Protokół CF NBS	Total screened Populacja badana	Screened positive Wezwania	Total CF Rozpoznania	Observation Obserwacje	Frequency Częstość
IRT/IRT 01.01.1999-30.06.2000	146531	314	24 +2	1	1:5635
IRT/IRT/DNA F508del 01.07.2000-02.08.2003	297532	576	50+1 (47)	0 (3)	1:5833
IRT/IRT/DNA F508del 01.09.2006-31.01.2006	34057	116	9	1	1:3784
IRT/IRT/DNA * 01.01.2007-17.10.2009	309772	293	56+2	8	1:5340
IRT/DNA # 18.10.2009- 21.09.2011	209971	141	29	4	1:7240
IRT/DNA (EGA)† 22.09.2011-31.12.2011	28893	23	6	6	1:4815

*gradually extended panel of mutations from 47 to 570

panel mutacji stopniowo poszerzany od 47 do około 570

#panel of mutations about 570

panel mutacji około 570

†panel of mutations about 95% of the mutated alleles in the Polish population

panel mutacji około 95% zmutowanych alleli populacji polskiej

(..)CF diagnoses and observations at NBS initial stage

rozpoznania CF i obserwacje w początkowym okresie NBS

+Frequency calculations include false negative cases

W obliczeniach częstości uwzględniono przypadki fałszywie ujemne

Table III. Sensitivity, specificity and positive predictive value (PPV) during subsequent stages of CF NBS.

Tabela III. Czułość, swoistość oraz wartość predykcyjna dodatnia (PPV) na poszczególnych etapach prowadzenia CF NBS.

Protocol CF NBS Protokół CF NBS	Sensitivity Czułość % (95%CI)	Specificity Swoistość % (95%CI)	PPV % (95%CI)	False negative cases Przypadki nierozpoznane
IRT/IRT 01.01.1999-30.06.2000	92.31 (74.87-99.05)	99.80 (99.78-99.82)	7.64 (4.96-11.16)	2
IRT/IRT/DNA F508del 01.07.2000-02.08.2003	98.04 (89.55-99.95)	99.82 (99.81-99.84)	8.68 (6.51-11.28)	1
IRT/IRT/DNA F508del 01.09.2006-31.12.2006	100.00 (66.37-100)	99.68 (99.62-99.74)	7.76 (3.61-14.22)	0
IRT/IRT/DNA * 01.01.2007-17.10.2009	96.55 (88.09-99.58)	99.92 (99.91-99.93)	19.11 (14.77-24.09)	2
IRT/DNA # 18.10.2009- 21.09.2011	100.00 (88.06-100)	99.95 (99.94-99.96)	20.57 (14.23-28.18)	0
IRT/DNA (EGA)† 22.09.2011-31.12.2011	100.00 (54.07-100)	99.94 (99.91-99.97)	26.09 (10.23-48.41)	0

*gradually extended panel of mutations from 47 to 570

panel mutacji stopniowo poszerzany od 47 do około 570

#panel of about 570 mutations

panel około 570 mutacji

†panel of mutations covering about 95% of the mutated alleles in the Polish population

panel mutacji pokrywający około 95% zmutowanych alleli populacji polskiej

Calculations included patients with meconium ileus (MI)

W obliczeniach uwzględniono pacjentów z niedrożnością smółkową (MI).

not only typically high electrolyte concentration in the sweat and the presence of pathogenic mutations in both alleles of the *CFTR* gene but also body mass deficiency resulting from pancreatic failure, bronchopulmonary disease and thoracic abnormalities in the radiogram.

In the second case, CF was diagnosed at the age of 9 months. However, in the course of NBS, increased IRT was not observed. In this child there were findings of body mass insufficiency, as well as recurrent infections of the respiratory tract. The study revealed abnormal values of sweat tests and the presence of two pathogenic mutations.

In the remaining 3 CF patients (1 from the pilot study and 2 in the present NBS), increased IRT was not observed. In these neonates meconium ileus (MI) was diagnosed. This fact was not reported to the screening centre (Table IV). Out of these three cases, there was only one case in which MI was the reason for beginning the procedure of CF diagnosis.

Observational subjects

In some situations it was not possible to establish the diagnosis during the first visit in the diagnostic centre. In such cases, further observation was necessary and in 3 patients such a procedure made the diagnosis possible. The children were examined at the time of the application of the IRT/IRT/DNA scheme, when the determination of only one mutation, F508del (most common in Poland), was possible.

In the first case, increased IRT determined in the 1st and 4th week in a neonate was observed. The presence of the F508del mutation was found in one allele of the *CFTR* gene and sweat test results were normal. The clinical image did not allow diagnosis of the disease and suggested carrier status. However, due to the slight decrease of body mass, the child remained under IMiD observation. Finally, the diagnosis was established after extending the DNA analysis.

The patient's younger sister underwent CF NBS at the time when the analysis panel was extended. In both girls, apart from F508del mutations, the presence of 3849+10kbC >T was detected in the second allele. Similarly, increased IRT mutation in one allele of the *CFTR* gene and initial boundary values of sweat tests did not allow earlier diagnosis in another neonate. After the extended DNA analysis, the F508del/3849+10kbC>T genotype was confirmed also in this case.

In the third child, borderline values of sweat tests were observed and the F508del mutation was found in one allele. In subsequent months, typical symptoms concerning the respiratory system were observed without pancreatic failure and the R117H mutation was observed in the second allele of the *CFTR* gene.

Twenty other children remain under the care of the medical centre. These are patients with mutations in both alleles of the *CFTR* gene in whom at least one abnormality has unknown or ambiguous clinical consequences. Cl⁻ concentration in sweat defined during the verifying visit

Table IV. False negative cases.

Tabela IV. Przypadki fałszywie ujemne.

Case Patient	Genotype Genotyp	Age of diagnosis [months] Wiek rozpoznania [miesiące]	Cause of diagnostics Przyczyna diagnostyki	Clinical symptoms Objawy kliniczne
1	F508del/R347P	63	sibling with positive CF NBS (rodzeństwo z CF rozpoznaną w NBS)	cough (<i>kaszel</i>) diarrhoea (<i>biegunka</i>) low body mass (<i>niska masa ciała</i>) digital clubbing (<i>palce pałeczkowate</i>)
2	N1303K/del2,3(21kb)	9	respiratory infections (<i>zakażenia układu oddechowego</i>) low body mass (<i>niska masa ciała</i>)	respiratory infections (<i>zakażenia układu oddechowego</i>) cough (<i>kaszel</i>) diarrhoea (<i>biegunka</i>) low body mass (<i>niska masa ciała</i>)
3	F508del/2143delT	3	low body mass (<i>niska masa ciała</i>)	MI (<i>niedrożność smółkowa</i>) Cough (<i>kaszel</i>) low body mass (<i>niska masa ciała</i>)
4	F508del/F508del	0.5	MI (<i>niedrożność smółkowa</i>)	MI (<i>niedrożność smółkowa</i>) low body mass (<i>niska masa ciała</i>) cholestasis (<i>cholestaza</i>)
5	F508del/F508del	2	low body mass (<i>niska masa ciała</i>)	MI (<i>niedrożność smółkowa</i>) low body mass (<i>niska masa ciała</i>)

using the quantitative pilocarpine ionophoresis method amounted to 14.5–49.4 mmol/l and when measured using the conductometric method it was 24–79 mmol/l. All these children are characteristic for normal somatic development and preserved pancreatic function and they do not reveal any abnormalities of the respiratory tract (Table V).

DISCUSSION

The Polish experience in CF NBS started 15 years ago. In this time, very many strategies and modifications have been applied not only in Poland. Countries and even regions differ from one another concerning schemes and the number of determined mutations of the *CFTR* gene. This situation results from the differences in the prevalence of particular mutations, economic conditioning and the development of medical knowledge [7]. In the past, there were trials to apply the test of albumin concentration in meconium, however it turned out not to be sensitive enough [9]. Due to the fact that IRT concentration in blood is characteristic for its low specificity, CF NBS based on single determination of IRT resulted in a high number of calls with PPV of 3%, so this model has been given up [7, 10].

At present, the IRT concentration test is repeated, or IRT concentration measurement is combined with DNA

analysis. Both in Europe and in Australia, pancreatitis associated protein (PAP) programmes are carried out in the case of IRT abnormal concentration. In accordance with other schemes of CF NBS, apart from IRT concentration and PAP testing, DNA analysis is made at subsequent stages [11, 12, 13, 14].

At the initial stage of the Polish CF NBS, the IRT double determination model was applied. The scheme was characteristic for high sensitivity – 92.3%, which resulted in a high number of calls for verifying examinations with the PPV of 7.65%. The diagnosis was established in 1 out of 13 children called and this scheme did not provide carrier detection. NBS programme modifications aimed to minimize the number of false-positive diagnoses but not decrease the test's sensitivity. In the parents of children who revealed increased IRT and were called for verifying examinations, the fear level increased and depressive behaviour appeared. In some parents these symptoms persisted in spite of normal results of sweat tests [15, 16]. In order to decrease the number of children called, another scheme was implemented in July 2000 and September 2006 in the case of an IRT abnormal result which included DNA analysis determining the most frequent mutation, F508del. The PPV was slightly higher (8.7 and 7.8% respectively), however it did not reach the value of 15.55, which was elicited in the survey carried out in Wisconsin [17]. Application of the IRT/

Table V. Mutations causing unproven or uncertain clinical consequences in children remaining under IMiD care.

Tabela V. Mutacje o niejasnych lub nieznanym konsekwencjach klinicznych u dzieci pozostających pod obserwacją w IMiD.

Mutation Mutacja	Number of detected alleles Liczba stwierdzonych alleli
R117H	7
IVS 8-5T+(TG)11	3
F1052V	2
D1152H	2
D537N	1
P731L	1
V938G	1
G1069R	1
Q1352H	1
R1162L	1
D727Y	1
D537N	1

/IRT/DNA strategy in Poland made it possible to achieve sensitivity close to 100%, despite including MI children in whom IRT concentration may be normal [18]. Due to the heterogenic character of the Polish population, NBS subsequent stages consisted in extending the panel of mutations of the *CFTR* gene examined. The panel comprised 16 of the most common defects in the Polish population and gradually increased to include a number of rarer abnormalities. It caused an increasingly higher PPV, the maximum reaching 26.1% regarding sensitivity, and specificity close to 100%. The PPV of 16.1% was elicited in the Czech Republic with the use of the DNA analysis panel, which made it possible to examine 50 mutations of the *CFTR* gene [11]. The highest PPV in the Polish NBS programme amounted to 26.1% and it was elicited with the use of a broad panel DNA-EGA analysis which allowed the detection of 95% of the mutated alleles in the Polish population. Similarly, PPV of 27.1% was characteristic for the French scheme of IRT/DNA with the analysis of 30 mutations [13].

Since 2009, after giving up IRT determination in the 4th week of life, decreased sensitivity and specificity have not been observed.

Three children included in the NBS pilot programme and two more in a later period were diagnosed positive on the basis of clinical symptoms. About 4-5% of the children who have undergone CF NBS may not be diagnosed in the course of the investigation but on the basis of clinical symptoms [19]. False-negative results were observed in children who revealed normal IRT. In three of them, MI was observed and in such a case, IRT concentration may prove normal. Diagnosis was established in them between the 2nd week and the 4th month of life. Hence it is important to carry out diagnostic investigations

despite negative CF NBS, when MI or CF suggestive symptoms appear. Each case of MI should be reported to the NBS centre by means of an appropriate note on sampling paper or by phone.

The consequence of CF NBS schemes with DNA analysis is the detection of carriers. This procedure is strictly connected with the stigmatisation of children and the parents' stress. In every case of detecting the carrier status in a child, a consultation visit in the Genetic Clinic is recommended. This problem's positive aspect is the fact that DNA analysis of the parents makes it possible to identify couples in whom both partners are carriers, as it may largely influence their procreative decisions.

Another problem which appeared while carrying out the CF NBS programme in Poland was the detection of observational subjects – children who during the pilot programme revealed normal or borderline values of sweat tests and one mutant allele in the *CFTR* gene. CF diagnosis in these patients was established only after extending the DNA analysis panel. In two cases the F508del/3849+10kbC>T genotype was found. If the CF NBS scheme had been applied in those children, including the investigation of the extended mutation panel with 16 of the mutations that are most characteristic for the Polish population (with 3849+10kbC>T mutation), the diagnosis would have been established during the verifying visit. Such a model has been applied since 2007 and it helped to diagnose one of the discussed children's sister [20]. It is possible that during the application of the IRT/IRT or IRT/DNA schemes determining only the F508del abnormality, the diagnosis of patients with mutation 3849+10kbC>T might not have been established if their sweat tests had been normal.

In the third observational subject from the pilot CF NBS programme – a boy with the F508del/R117H genotype, CF clinical symptoms were observed. In the remaining cases children remained under the care of the centre without a CF diagnosis. The number of observational subjects increased along with extending the DNA analysis panel. There were children who did not meet CF diagnosis criteria. In those patients, it was impossible to confirm the *CFTR* gene dysfunction in a laboratory, as at most one mutation was of confirmed pathogenicity and chloride concentration in the sweat was normal or borderline. In such situations, CF was neither confirmed nor excluded. Genetic consultation was suggested for all the families of children from the observational group. Prognosis for those children is uncertain. Some of them may develop the symptoms of benign CF, in some of them a *CFTR* dependent disease may occur and others may never suffer from any clinical symptoms typical of *CFTR* dysfunction. The children selected in the course of CF NBS in whom CF is neither confirmed nor excluded were classified as CRMS (*CFTR*-related metabolic syndrome)-[21]. Children with a CF NBS ambiguous result are a challenge for specialists dealing with CF diagnostics and treatment, while for their parents they are a source of fear and anxiety.

Each of the methods that has been incorporated has its advantages and disadvantages. The advantages of the IRT/IRT scheme is low cost and its non-detection of carriers, whereas the disadvantages are the large number of children called for consultation visits, which is connected with the parents' stress and the large number of sweat tests performed. DNA analysis with the determination of one mutation reduces the number of false-positive cases, increases sensitivity, implies the detection of a small number of carriers, however it increases the costs of screening tests, does not allow CF diagnosis in the case of a mutation which is characteristic for normal sweat tests and requires the parents' consent for DNA tests. The extension of the *CFTR* gene mutation panel decreases the number of false-positive cases (sensitivity and specificity are very high), enables CF diagnosis of sweat tests yielding normal results but increases NBS costs and reveals patients with mutations of uncertain prognosis or unknown clinical consequences [22]. NBS diagnostic schemes undergo constant modifications. There are trials to form a strategy with the use of techniques as efficient as those incorporating DNA. However, the strategies should avoid carrier detection. PAP concentration measurement allows another screening strategy. In CF neonates, PAP increased level in blood is observed. Isolated increased PAP is of similar diagnostic value as increased IRT. In neonates undergoing CF NBS, a combination of IRT concentration and PAP is assessed. Programmes incorporating this technique were carried out in several European countries. In France, the studies began at the end of the 1990s and have been continued up to now [13, 23]. The examination model was based on determining two threshold values for each of the parameters examined – IRT and PAP. In accordance with the surveys carried out by Sarles J et.al. in the years 2002-2003, IRT +PAP strategy was found to be sensitive and specific enough

to apply. This solution does not imply the requirement of obtaining the parents' consent, enables CF diagnosis without either the disclosure of the *CFTR* gene mutation carrier or forms of unclear clinical consequences. This model of CF NBS is widely used in countries where legal aspects impair the application of the DNA molecular analysis [24]. Besides, another advantage seem to be its lower costs than in the case of the IRT/DNA model [25]. Centres dealing with complex treatment of CF patients in the Czech Republic and Germany carry out surveys concerning PAP application. According to scientists from Prague, the IRT+PAP strategy characteristically results in a higher number of false-positive results than the parallel application of the IRT/DNA/IRT [11]. The advantage of the IRT+PAP model in comparison with the IRT/IRT model is that there is no need to collect the neonate's blood twice on the filtration paper, which decreases the parents' fear. The IRT/PAP/DNA/sequencing model was examined by Dutch specialists and it proved to combine IRT concentration, PAP assessment and the examination of a 35 mutation panel with *CFTR* gene sequencing at the last stage. This scheme is characteristic for high PPV value (87.5%), however such a test's sensitivity is lower than in the case of IRT/DNA application [12]. The assessment of implementing this technique into national programmes requires further studies.

CONCLUSIONS

Regardless of the protocol applied in a particular region, screening tests make it possible to diagnose the disease at an earlier stage of its pathological stages than the clinical image, which results in numerous health benefits. The application of the IRT/DNA scheme with an extended panel of DNA analysis allows CF early diagnosis even in children with normal values of sweat tests. However, it reveals more observational subjects than the restricted panel scheme. Further investigation, as well as changes in the Polish CF NBS, are required to increase the PPV while preserving high sensitivity and specificity.

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Conflicts of interest/Konflikt interesu

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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.