European Journal of Pediatrics (2025) 184:38
https://doi.org/10.1007/s00431-024-05845-4

BRIEF REPORT

Four-year evaluation of neonatal cystic fibrosis screening in Southern Belgium

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Received: 3 August 2024 / Revised: 13 October 2024 / Accepted: 25 October 2024

Abstract

Newborn screening for cystic fibrosis (CF-NBS) using an IRT-DNA algorithm with a 12 CFTR-variant panel and an IRT/IRT failsafe was officially implemented in the French-speaking Community of Belgium in January 2020. This screening protocol was evaluated after 4 years according to the criteria defined by the European Cystic Fibrosis Society's working group on neonatal screening. Immunoreactive trypsinogen concentration (IRT) was measured on dried blood spots collected between the second and the fourth day of life. *CFTR* variants genotyping was initiated when IRT ≥ 99th percentile (P99). If at least 1 variant was identified, the child was referred to a CF center for a sweat test (ST). If IRT ≥ 99.9th percentile with no variant identified, a failsafe was provided with IRT repeated on day 21 and subsequent ST in case of persistent IRT > P99. Extensive Gene Analysis was initiated if sweat chloride level was ≥ 30 mmol/L. Over a period of 4 years, 212.979 newborns were screened. Forty-two were diagnosed with CF: 34 by CF-NBS, 3 following a meconium ileus, 3 by family history and 2 missed cases. Additionally, 112 healthy carriers and 14 CFSPID were identified. The median age at the first consultation was 23 days. The sensitivity of our CF-NBS is 95% (target ≥ 95%), the positive predictive value 18% (target ≥ 30%) and CF/CFSPID ratio 2.8. *Conclusion*: Although follow-up is limited, this first evaluation demonstrates encouraging sensitivity and early management before one month of age.

What is Known:
- *Newborn screening for cystic fibrosis improves the prognosis of patients.*

What is New:
- *This article presents an initial assessment of our screening after 4 years.*

Keywords Cystic fibrosis · Newborn screening · Immunoreactive trypsinogen

Communicated by Peter de Winter

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Published online: 21 November 2024

Abbreviations

CF	Cystic Fibrosis
CFSPID	Cystic Fibrosis Screening Positive Inconclusive Diagnosis
CFTR	Cystic Fibrosis Transmembrane conductance Regulator
Cl-	Chloride
ECFS NSWG	European Cystic Fibrosis Society Neonatal Screening Working Group
EGA	Extensive Gene Analysis
FH	Family history
IRT	Immunoreactive trypsinogen
MC	missed cases
MI	Meconium ileus
NBS	Newborn screening
PAP	pancreatic associated protein
P99	99th percentile
PPV	positive predictive value
ST	Sweat test

Introduction

Newborn screening for cystic fibrosis (CF-NBS) is essential for early diagnosis of CF, as it improves the patient's prognosis and quality of life. This is especially important now that current treatments targeting the basic defect can lead to a near-normal life but only if they are initiated early in life [1]. This has led to the widespread implementation of CF-NBS programs in several countries in Europe. While these programs vary by country, they all rely on the measurement of immunoreactive trypsinogen (IRT) from a blood sample taken around the third day of life as the initial screening step. From this initial step, different second-tier testing approaches are used to assess samples with elevated IRT levels. These include measuring pancreatitis-associated protein (PAP), conducting a second IRT measurement or performing DNA analysis with a panel of selected Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) variants (4 to 680 variants depending of the country) [2].

CF-NBS was implemented in the Belgian French Community in January 2020. This screening process is based on an IRT-DNA algorithm including genotyping of 12 selected *CFTR* pathogenic variants covering 81.2% of the variants identified in the Belgian CF patients and an IRT failsafe [3, 4]. The main objective of this study was to evaluate the performance of this new algorithm, according to the quality criteria defined by the European Cystic Fibrosis Society Neonatal Screening Working Group (ECFS NSWG) [5, 6], and to compare it with available data from European CF-NBS programs [2].

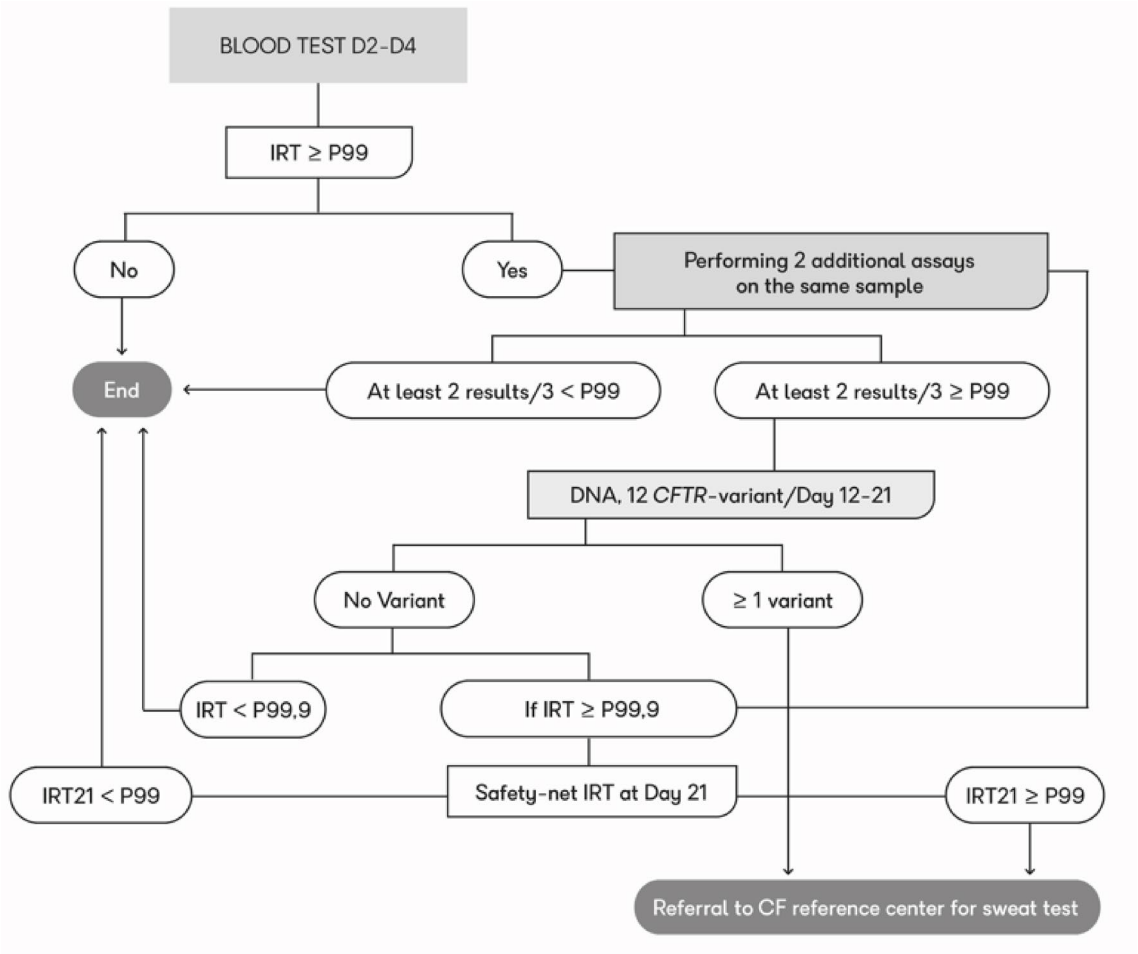
Methods

The data were based on a retrospective multicentric study conducted between January 2020 and December 2023. The data were encoded in an Excel file (Microsoft 2021) and were analyzed between March and June 2024. CF-NBS was carried out in three laboratories (Cliniques Universitaires Saint-Luc (CUSL), Cliniques Universitaires de Bruxelles (CUB), and Centre Hospitalier Universitaire de Liège (CHU Liège)) and covered 43 maternity centers. IRT concentration was measured on dried blood spots collected on a filter paper card between the second and fourth day of life (NEONATAL IRT Screening ELISA® (Zentech/Lacar) in CUSL and CHU Liège, GSP Neonatal IRT kit® (Revvity) in CUB). Genotyping of the 12 pathogenic variants causing CF (Elucigene CFEU2v1®) was initiated when the concentration of IRT was ≥ 99th percentile (P99). If one or two variants were identified, the child was referred to one of the three CF Reference Centers for a sweat test (ST). If no variant was detected but the IRT concentration was ≥ 99.9th centile (P99.9), a second IRT was collected on the 21st day of life (IRT21). If the IRT21 levels remained > P99, the child was referred for a ST (Fig. 1). Extensive Gene Analysis (EGA) was initiated if sweat chloride (Cl-) level was ≥ 30 mmol/L. Meconium ileus (MI) was an indication for an immediate ST.

A CF-NBS was considered positive when it led to a ST [6]. CF was confirmed if sweat chloride (Cl^-) concentration was ≥ 60mmol/L or if two pathogenic variants causing CF were detected. The diagnosis of Cystic Fibrosis Screening Positive Inconclusive Diagnosis (CFSPID) was made in the presence of a CF-causing variant associated or not with a non-CF causing variant, and/or with intermediate sweat chloride value (30-59mmol/L). Sensitivity, positive predictive value (PPV) and CF/CFSPID ratio were calculated according to ECFS NSWG recommendations, excluding MI [6]. A CF-NBS report is drawn up annually and several meetings between the screening laboratories and the CF reference centers have been held to analyze the results and verify the data.

Results

A total of 212.979 newborns were screened. Among these, 2,469 newborns (1.16%) had an IRT ≥ P99, including 370 (0.17%) with IRT ≥ P99.9. Molecular analysis was carried out on 2468 newborns. Molecular analysis in a child with an IRT ≥ P99 who died rapidly could not be carried out due to a technical problem with DNA extraction. CF was confirmed in 42 newborns: 34 were identified by CF-NBS, 3



CF: Cystic Fibrosis, CF-NBS: Cystic Fibrosis Neonatal Screening, D: Day, IRT: Immunoreactive Trypsinogen, P: Percentile

Fig. 1 Algorithm of CF-NBS in Belgium

were referred for MI, 3 were identified by familial history (FH), and 2 were missed cases (MC) on IRT screening. All 6 children with MI/FH underwent CF-NBS and had an IRT ≥ P99 (Table 1). They were included in the analysis. The incidence of CF in our population was 1/5071.

Regarding CF-NBS, an IRT ≥ P99 associated with at least one of the 12 pathogenic variants led to the diagnosis of CF in 37 newborns, and the failsafe (IRT21) allowed the diagnosis of 3 additional patients (Table 2). The program identified 112 healthy carriers (*F508del*: 92, *G542X*: 4, *N1303K*: 5, *3272–26 A-> G*: 1, *S1251N*: 2, *2789 + 5G-> A*: 2, *R553X*: 3, *3849 + 10kbC > T*: 1, *R1162X*: 2) and 14 CFSPIDs (Table 3). A ST was performed in 203 infants. Of these, 24 underwent ST following IRT21 > P99 and 12 were lost to follow-up for IRT21. One CF patient died due to prematurity before ST. The median age at the first consultation was 23 days [9–31]. Fifteen infants (0.007%) were lost to follow-up (Table 1).

Sensitivity was 95%, PPV 18% and CF/CFSPID ratio was 2.8. For one patient with CF, only one variant was identified despite sequencing of all exons and exon/intron boundaries of the *CFTR* gene. However, both ST showed a Cl- level > 60mmol/L, and he was therefore considered as a CF patient. In all other patients, two CF-causing alleles were identified.

Two missed cases were diagnosed. The first patient presented recurrent atelectasis in the right upper and middle lobes at the age of one month. Bronchoscopy revealed viscous mucus impaction. IRT was 63.9 µg/L (P98.4). Sweat Cl- was 72mmol/L, and EGA revealed two CF-causing variants (*2183AA > G/V232D*) [7]. The second patient presented in utero signs of intestinal hyperechogenicity. The parents refused to perform an amniocentesis. At birth, IRT was 58.2 µg/L (P96). However, the ST was pathological and the child was homozygous for the *F508del* variant.

Table 1 CF-NBS detailed by year

	2020	2021	2022	2023	Total
Total number of screened newborns	54 035	54 822	53 438	50 684	**212,979**
n ≥ P99	653	555	629	632	**2469**
n ≥ P99.9	92	94	78	106	**370**
Genetic testing	653	555	628	632	**2468**
Total number of sweat tests	42	63	49	49	**203**
Total number of sweat tests for CF, CFSPID and carrier	39	41	44	43	**167**
Total number of sweat tests fort elevated IRT21 or missing IRT21	3	22	5	6	**36**
Total number of cystic fibrosis cases	10	12	10	10	**42**
Total Number of cystic fibrosis cases diagnosed through NBS	9	10	7	8	**34**
Total number of cystic fibrosis cases diagnosed through FH	1	1	1	0	**3**
Total number of cystic fibrosis cases diagnosed through MI	0	1	1	1	**3**
Missed cases0	0	0	1	1	**2**
Healthy carriers	25	24	31	32	**112**
CFSPID	3	4	4	3	**14**
Number of infants with IRT ≥ P99 lost to follow-up	3	4	3	5	**15**

CF Cystic Fibrosis, *CF-NBS* Cystic Fibrosis Neonatal Screening, *CFSPID* Cystic Fibrosis Screening Positive Inconclusive Diagnosis, *FH* Familial History, *IRT* Immunoreactive Trypsinogen, *P* Percentile

All CFSPID patients were seen during a CF specialized consultation to receive information about potential complications. To date, none of them became CF.

Discussion

While CF-NBS was already implemented in many European countries, in Belgium, CF-NBS was officially implemented in Flanders in 2019 and in 2020 in the French Community. It is essential to regularly evaluate the CF-NBS algorithm and assess its performance. Quality criteria have been established by the ECFS NSWG, including a percentage of children screened positive < 0.5%, PPV > 30%, sensitivity ≥ 95%, realization of the sweat test on the same day as the consultation, and age for first consultation < day 35 [6]. The initial results from Flanders using the same algorithm, were published after 3 years, showing a sensitivity of 100%, a PPV of 31%, and a CF/CFSPID ratio of 8 [8]. Our analysis after 4 years shows a sensitivity of 95%, a PPV of 18%, and a CF/CFSPID ratio of 2.8. Further analysis is underway to understand these differences and it is also essential that missed cases are documented in accordance with the recommendations of the ECFS NSWG [9].

Following a survey conducted in 2022, 22 national programs and 34 regional programs were identified across Europe. The CF-NBS algorithm is specific to each country. The first step in CF-NBS is the IRT assay performed around the 3rd day of life and all missed cases were identified in children whose IRT had been considered normal. The threshold can be fixed, as it is in France (initial threshold 65 µg/L), or floating, as it is in our country (≥ P99) [2]. Given seasonal and batch-specific variations, a floating threshold seems more appropriate [10]. The genetic test used for the second-tier varies widely from country to country and influences the sensitivity of NBS. Four programs also measured PAP. However, when comparing the IRT/DNA and IRT/PAP strategies in terms of PPV and time to management, the IRT/DNA and IRT/PAP/DNA strategies seem more favorable [11]. Four programs (Denmark, Netherlands, Norway and Poland) used EGA as a third or fourth tier of testing after a positive result from initial limited DNA testing, with a sensitivity varying between 97 and 100%. Six national et and 11 regional programs did not use DNA analysis [2].

Fourteen CFSPIDs were identified through our CF-NBS. A distinction is made between CFSPIDs with Cl- levels < 30 mmol/L and 2 *CFTR* variants with 0 or 1 CF-causing, and those with intermediate Cl- levels (30–59 mmol/L) with 0, 1 or 2 *CFTR* variants. Given that all of our CFSPID had intermediate sweat chloride levels due to our CF-NBS algorithm and that 2 variants were found, it is recommended that these children be followed up at a CF consultation and assessed at the age of 6 for the risk of developing CF. If, after the age of 6, the child remains asymptomatic and the ST normalizes, the risk of developing CF is low [12].

This retrospective study has certain limitations. Due to the small number of patients, confidence intervals could not be calculated. Additionally, the short follow-up period raises the possibility that MC may be diagnosed later, potentially lowering sensitivity.

Table 2 Genotypes of CF patients

CF	IRT (µg/L)	≥P99	≥P99,9	Panel 12 variants (F508del, G542X, N1303K, 3272–26 A-> G, S1251N, 1717-1G-> A, A455E, 2789 + 5G-> A, R553X, 3849 + 10kbC-> T, W1282X, R1162X)	Extensive Gene Analysis	Sweat Cl- (mmol/L)
1	234	x	x	*F508del / F508del*	*F508del / F508del*	83
2	166,6	x	x	*F508del / F508del*	*F508del / F508del*	97
3	195,4	x	x	*F508del / F508del*	*F508del / F508del*	102
4	133,4	x	x	*F508del / N1303K*	*F508del / N1303K*	99
5	131	x	x	*N / N*	*c.579 + 1G > T/c.2738 A > G*	93
6	145,2	x	x	*F508del / G542X*	*F508del/G542X*	117
7	99,5	x	x	*F508del /N*	*F508del /W882X*	127
8	201	x	x	*F508del / F508del*	*F508del /DF508*	132
9	110	x	x	*N /N*	*ARG792/ARG792*	99
10	192,4	x	x	*F508del / F508del*	*F508del / F508del*	89
11	132,3	x	x	*F508del / F508del*	*F508del/F508del*	100
12	156,8	x	x	*F508del /N*	*F508del /N*	69
13	240,9	x	x	*F508del /N1303K*	*F508del /N1303K*	103
14	99,8	x		*F508del / A455E*	*F508del/A455E*	68,3
15	146,1	x	x	*F508del / N1303K*	*F508del/N1303K*	104
16	129	x		*S125N-N1303K*	*S125N/N1303K*	72
17	88	x		*F508del / F508del*	*F508del / F508del*	85
18	174,4	x	x	*N1303K / N1303K*	*N1303K/N1303K*	95
19	130,3	x	x	*N/N*	*1677DelTA/1677DelTA*	88
20	191	x	x	*F508del / S1251N*	*F508del/S1251N*	69
21	176	x	x	*R1162X/R1162X*	*R1162X/R1162X*	74
22	73	x		*F508del /3849 + 10kbC > T*	*F508del /3849 + 10kbC > T*	44
23	63,9			*N/N*	*2183AA > G/V232D*	70
24	137	x	x	*F508del / F508del*	*F508del / F508del*	94
25	167	x	x	*F508del /N1303K*	*F508del /N1303K*	170
26	229	x	x	*F508del /G542X*	*F508del /G542X*	108
27	79,8	x		*F508del / 3849 + 10 kb C > T*	*F508del/3849 + 10 kb C > T*	33
28	106,69	x		*F508del / F508del*	*F508del / F508del*	85
29	139,17	x	x	*F508del / F508del*	*F508del / F508del*	94
30	188	x	x	*F508del / F508del*	*F508del / F508del*	107
31	87,2	x		*F508del / F508del*	*F508del / F508del*	102
32	118	x	x	*F508del / N*	*F508del/Tyr1092**	96
33	163.2	x	x	*F508del/F508del*	*F508del/F508del*	98
34	155,1	x	x	*F508del /N*	*F508del /Y192X*	97
35	101.6	x	x	*F508del/F508del*	*F508del/F508del*	84
36	58.2			*F508del/F508del*	*F508del/F508del*	90
37	192	x	x	*F508del / F508del*	*F508del / F508del*	-
38	134.8	x	x	*F508del/F508del*	*F508del/F508del*	97
39	230	x	x	*F508del /G542X*	*F508del /G542X*	122
40	172.23	x	x	*F508del/F508del*	*F508del/F508del*	94
41	134,9	x	x	*F508del /N*	*F508del /G178R*	83
42	199.5	x	x	*F508del/2789 + 5G > A*	*F508del/2789 + 5G > A*	87

CF Cystic Fibrosis, *Cl-* Chloride, *IRT* Immunoreactive Trypsinogen, *N* No variant, *P* Percentile

Table 3 Genotype of the CFSPID patients

CFSPID	IRT (µgg/L)	≥P99	≥P99,9	Panel 12 variants (F508del, G542X, N1303K, 3272–26 A-> G, S1251N, 1717-1G-> A, A455E, 2789 + 5G-> A, R553X, 3849 + 10kbC-> T, W1282X, R1162X)	Extensive Gene Analysis	Sweat Cl- (mmol/L)
1	62,9	x		*R1162X/N*	*R1162X/[Arg74 TRP;270Asn]*	57
2	76	x		*S1251N/N*	*S1251N/V201M*	36
3	105,9	x	x	*F508del /N*	*F508del /R117H- 7T*	33
4	72,5	x		*F508del /N*	*F508del /5T*	56
5	90	x		*F508del /N*	*F508del /R117H- 7T*	35
6	60,8	x		*R553X/N*	*R553X/L997F*	34
7	58,7	x		*F508del /N*	*F508del /A349V*	35
8	107,4	x		*F508del / N*	*F508del / R117H -7T*	32
9	64,2	x		*F508del /N*	*F508del /5T*	48
10	75,1	x		*F508del/N*	*F508del / Ile444Thr*	35
11	104	x	x	*F508del /N*	*F508del /5T TG12*	35
12	77.5	x		*F508del /N*	*F508del /5T-11TG*	33
13	68,4	x		*F508del /N*	*F508del /5T-11TG*	38
14	118	x	x	*F508del /N*	*F508del /exon 12, 13, 14 del*	50

CFSPID Cystic Fibrosis Screening Positive Inconclusive Diagnosis, *Cl-* Chloride, *IRT* Immunoreactive Trypsinogen, *N* No variant, *P* Percentile

Conclusion

The initial analysis of our CF-NBS in the French-speaking Community of Belgium is encouraging, with a sensitivity of 95%. However, this should be interpreted with caution due to the short time scale. PPV needs improvement. A longer-term analysis of our Belgian CF-NBS would be valuable.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Matthieu Thimmesch. The first draft of the manuscript was written by Matthieu Thimmesch. All authors read and approved the final manuscript."

Funding information Nothing to declare.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

1. Terlizzi V, Farrell PM (2024) Update on advances in cystic fibrosis towards a cure and implications for primary care clinicians. Curr Probl Pediatr Adolesc Health Care 54(6):101637. https://doi.org/10.1016/j.cppeds.2024.101637
2. Munck A, Berger DO, Southern KW, European CF Society Neonatal Screening Working Group (ECFS NSWG) et al (2023) European survey of newborn bloodspot screening for CF: opportunity to address challenges and improve performance. J Cyst Fibros 22(3):484–495. https://doi.org/10.1016/j.jcf.2022.09.012
3. Lebecque P, Lebecque O, Proesmans M, Leal T (2019) Cystic fibrosis newborn screening implemented in Belgium in 2019. Louvain Med 138(09):509–518
4. Annual Data Report Belgian Cystic Fibrosis Registry (BCFR) (2021) Brussels, Belgium. Version publique: Annual report Belgian Cystic Fibrosis Registry 2021 | sciensano.be (Last accessed september 2024)
5. Munck A, Southern KW, Castellani C, European CF Society Neonatal Screening Working Group (ECFS NSWG) et al (2021) Defining key outcomes to evaluate performance of newborn screening programmes for cystic fibrosis. J Cyst Fibros 20(5):820–823. https://doi.org/10.1016/j.jcf.2021.02.006
6. Castellani C, Duff AJA, Bell SC et al (2018) ECFS best practice guidelines: the 2018 revision. J Cyst Fibros 17(2):153–178
7. Thimmesch M, Tuerlinckx D, Habay M, Lebecque P (2023) Recurrent atelectasis in an infant: about one case of false-negative newborn screening for cystic fibrosis. Rev Med Liege 78(7–8):436–440
8. Proesmans M, Regal L, Eyskens F et al (2022) P001 cystic fibrosis newborn screening (CF-NBS) start-up in Flanders (Belgium): report of first evaluation after 3 years. J Cyst Fibros 18(1):S61
9. Munck A, Southern KW, Murphy J, European CF Society Neonatal Screening Working Group (ECFS NSWG) et al (2023) Cystic fibrosis cases missed by newborn bloodspot screening-towards a consistent definition and data acquisition. Int J Neonatal Screen 9(4). https://doi.org/10.3390/ijns9040065
10. Kloosterboer M, Hoffman G, Rock M et al (2009) Clarification of laboratory and clinical variables that influence cystic fibrosis newborn screening with initial analysis of immunoreactive trypsinogen. Pediatrics 123:e338–e346
11. Sommerburg O, Hammermann J (2020) Pancreatitis-Associated protein in neonatal screening for cystic fibrosis: strengths and weaknesses. Int J Neonatal Screen 6(2):28. https://doi.org/10.3390/ijns6020028

12. Terlizzi V, Manti S, D'Amico F et al (2024) Biochemical and genetic tools to predict the progression to cystic fibrosis in CRMS/CFSPID subjects: a systematic review. Paediatr Respir Rev 51:46–55. https://doi.org/10.1016/j.prrv.2024.01.001