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Review

Biochemical and genetic tools to predict the progression to Cystic Fibrosis in CRMS/CFSPID subjects: A systematic review

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EDUCATIONAL AIMS

The reader will come to appreciate:

- An analysis of the characteristics of CFSPID individuals who evolve into CF.
- That the presence of one CF-causing CFTR variant, an initial sweat chloride (SC) $\geq$ 40 mmol/L or an increase of SC $>$ 2.5 mmol/L/year could allow identification of subjects at risk of progression to CF.
- That CFSPID individuals with a CF causing variant/VVCC genotype and first SC in the higher borderline range may require more frequent and prolonged clinical follow-up.

ABSTRACT

Objectives: Aim of this study was to identify risk factors for a progression to cystic fibrosis (CF) in individuals detected as CF Screening Positive, Inconclusive Diagnosis (CFSPID).
Methods: This is a systematic review through literature databases (2015–2023). Blood immunoreactive trypsinogen (b-IRT) values, *CFTR* genotype, sweat chloride (SC) values, isolation of *Pseudomonas aeruginosa* (Pa) from respiratory samples, Lung Clearance Index (LCI) values in CFSPIDs who converted to CF (CFSPID > CF) and age at CF transition were assessed.

Abbreviations: b-IRT, Blood-Immuno Reactive Trypsinogen; CF, Cystic Fibrosis; CRMS, CFTR-related metabolic syndrome; CFTR, cystic fibrosis transmembrane conductance regulator; CFTR-RD, CFTR-related disorder; CFSPID, cystic fibrosis screening positive inconclusive diagnosis; CFSPIDs, CFSPID subjects; CFSPID>CF, CFSPID converting to CF; CFSPID-P, CFSPID persistent; CFSPID>CFTR-RD, CFSPID converting to a CFTR-RD; LCI, Lung Clearance Index; NBS, newborn screening; NGS, next generation sequencing; Pa, Pseudomonas aeruginosa; $ppFEV_1$, percent predicted FEV1; SC, sweat chloride; ST, sweat test; VVCC, variant with varying clinical consequences.

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Results: Percentage of CFSPID > CF varies from 5.3 % to 44 %. Presence of one CF-causing *CFTR* variant in trans with a variant with variable clinical consequences (VVCC), an initial SC ≥ 40 mmol/L, an increase of SC > 2.5 mmol/L/year and recurrent isolation of pseudomonas aeruginosa (Pa) from airway samples could allow identification of subjects at risk of progression to CF.
Conclusions: CFSPIDs with CF causing variant/VVCC genotype and first SC in the higher borderline range may require more frequent and prolonged clinical follow-up.

Introduction

Cystic Fibrosis (CF), the most common life-threatening autosomal recessive and multisystemic disease, is due to alterations in CF Transmembrane Conductance Regulator (*CFTR*) gene that encodes a membrane glycoprotein [1]. Such protein acts as a transmembrane channel by regulating chloride and sodium transport and its alteration or reduction leads to the production of thick secretions within the affected organs, causing progressive lung damage, pancreatic injury and multiorgan involvement [2]. Furthermore, there is a growing number of individuals diagnosed as CFTR-related disorders (CFTR-RD), a clinical condition with evidence of *CFTR* protein dysfunction that does not fulfil the diagnostic criteria for CF [3,4].

The introduction of newborn screening (NBS) allowed early diagnosis of CF and, consequently, the opportunity to commence specific treatments with an improvement of clinical outcome, quality of life and survival [5]. Different NBS protocols are used in different countries even within the same country [6,7]. All protocols start with the measurement of blood immunoreactive trypsinogen (b-IRT) on dried blood spots at 49–72 h after birth. The second level may include either molecular analysis of *CFTR* with techniques that explore a limited panel of variants or with the whole gene scanning by next generation sequencing (NGS), or a repeated measurement of b-IRT concentration at the age of 4–6 weeks [7,8] followed or not by molecular analysis. The sweat test (ST), the gold standard for the diagnosis of CF, is offered to all children positive for NBS and it is considered pathological for levels of sweat chloride (SC) ≥ 60 mmol/L [9,10]. In most patients with CF the SC is pathological and *CFTR* gene analysis shows two *CFTR* causing variants (https://cftr2.org/), however such genotype may be observed even in patients with a SC value in the intermediate (30–59 mmol/l) or normal (<30 mmol/L) range [11].

Following the enhancement of diagnostic techniques, particularly NGS for *CFTR* gene scanning, and the increase of the number of subjects screened, a growing and variable number of positive NBS subjects with an inconclusive diagnosis of CF has been identified over years [12,13]. This cluster of asymptomatic subjects, firstly designated in USA as CFTR-related metabolic syndrome (CRMS), [14] and then in Europe as CF Screen Positive, Inconclusive Diagnosis (CFSPID) [7], shows elevated b-IRT with persistently intermediate SC levels and fewer than 2 CF causing *CFTR* variants; or normal SC concentration (<30 mmol/L) and 2 *CFTR* variants with 0 to 1 known to be CF-causing [15].

The two terms have been harmonised introducing the definition of CRMS/CFSPID to improve indefinite diagnosis, international communications, and analysis of clinical outcomes [15]. Here, we preferred the shorter term CFSPID throughout the rest of the paper. The number of such subjects (and the ratio between CF and CFSPID cases) revealed by NBS is widely different between countries depending on the different protocols used for NBS and on genetic differences between populations [16,17]. Over time, a variable percentage of CFSPIDs will be diagnosed as CF owing to a positive ST, a re-classification of *CFTR* variants as CF causing, or onset of CF-related symptoms (CFSPID > CF) [17]. However, the most probable risk for these children seems the evolution in the CFTR-RD label [3,4]. Even with the published revised guidance from the European Cystic Fibrosis Society (ECFS) neonatal screening working group [17], one of most important aspect concerning management and monitoring of CFSPIDs is to early identify those at greatest risk of transitioning in CF. This could avoid overmedicalization of healthy subjects or healthy carriers and properly provide more information to the families of these children, as persistence of an inconclusive diagnosis may cause a negative psychological impact for families, increasing parents' distress [18–20].

Here, we performed a systematic literature review to investigate possible biochemical, genetic and microbiological criteria, which could early identify CFSPIDs who deserve a closer follow-up for the high chance of developing a CF phenotype.

Material and Methods

Literature review

We performed this systematic literature review according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) [21], including papers published between January 2015 and December 2022, and using a protocol registered with the International Prospective Register of Systematic Reviews (PROSPERO CRD42023398840).

Two reviewers (SM, FDA) independently conducted searches on electronic databases, including PubMed, Global Health, and EMBASE. The search strategy of each reviewer is detailed in Search Strategy (Appendix 1). Manual searches of the current literature were also performed by referring to Web of Science, Google Scholar, and BMJ Best Practice. The following variations and terms were used: for "cystic fibrosis", "CF Screen Positive, Inconclusive Diagnosis", "CFSPID", "CFTR-related metabolic syndrome", "CRMS", "immune-reactive trypsinogen", "IRT analysis", "sweat chloride test", "colonization", "Pseudomonas aeruginosa" (Pa), "Lung Clearance Index" (LCI), "LCI", "infants", "children", "adolescent", and "adult". Lastly, selected references of included papers were searched to find any other relevant documents in accordance with the inclusion criteria.

Inclusion and exclusion criteria

Inclusion criteria were publication in peer reviewed journals, written in any language and including children and/or adults who have been diagnosed with CF. The included publication types were guidelines, *meta*-analysis and systematic reviews, narrative reviews, original articles, case series, case reports, and letters.

Exclusion criteria were publications not focusing on CRMS/CFSPID in CF paediatric and adult populations. The first screening of the retrieved publications was made according to the title and the abstract.

Results

Included manuscripts' characteristics

The electronic search resulted in 85 articles that were reduced by 63 after duplicates were removed. Therefore, a total of 22 studies focusing on CFSPID who progressed to CF (CFSPID > CF) were included in this review (Appendix 2, Fig. 1, Table 1): 10/22 multicentre retrospective studies, 4/22 multicentre prospective studies, 2/22 monocentre retrospective studies, 1/22 monocentre prospective studies, 3/22 retrospective cross-sectional studies, 2/22 case series. 19/22 studies were performed on children and 3/22 both children and adults. 11/22 papers focused on CFSPID and IRT values (Table 3); 17/22 studies on CFSPID and *CFTR* gene analysis (Table 4); 16/22 studies on CFSPID and SC values (Table 5); 9/22 studies on CFSPID and *Pseudomonas aeruginosa* (Pa) isolation (Table 6); 4/22 studies on CFSPID and lung clearance index (LCI) (Table 7) and 5/21 on CFSPID follow-up.

Transition from CFSPID to CF

Among CFSPID infants, the percentage of subjects who converted to a CF diagnosis varies in the different case series from 5.3 % found in an Italian population, to 48 % described in a CF screening positive population from Australia, with F508del/other mutation and borderline sweat test.

Table 2 shows the different included studies.

CFSPID and b-IRT analysis

Since the late 1970 s, b-IRT, a pancreatic enzyme precursor, has been measured using dried blood spots collected from newborns to select infants with elevated levels as they are at risk of CF [22]. Despite lacking sensitivity and specificity as markers for CF, b-IRT is universally used as the initial test for CF NBS programs.

Given the strong correlation between *CFTR* dysfunction, b-IRT levels, and severity of pancreatic disease, it is reasonable to speculate on a possible correlation between b-IRT levels and evolution from CFSPID to CF [23,24]. Some authors [25–29] highlighted that b-IRT values were significantly higher in CFSPID > CF than in those who remained with an inconclusive diagnosis (CFSPID-P): moreover, CFSPID > CF seemed to have b-IRT levels more like those of CF patients. Ooi CY et al, in a multicentric (Canada-Italy) prospective study, evaluated b-IRT in CF, in CFSPID > CF and in CFSPID-P. As expected, significantly higher b-IRT concentrations (ng/ml) were present in CF than CFSPID infants (P <

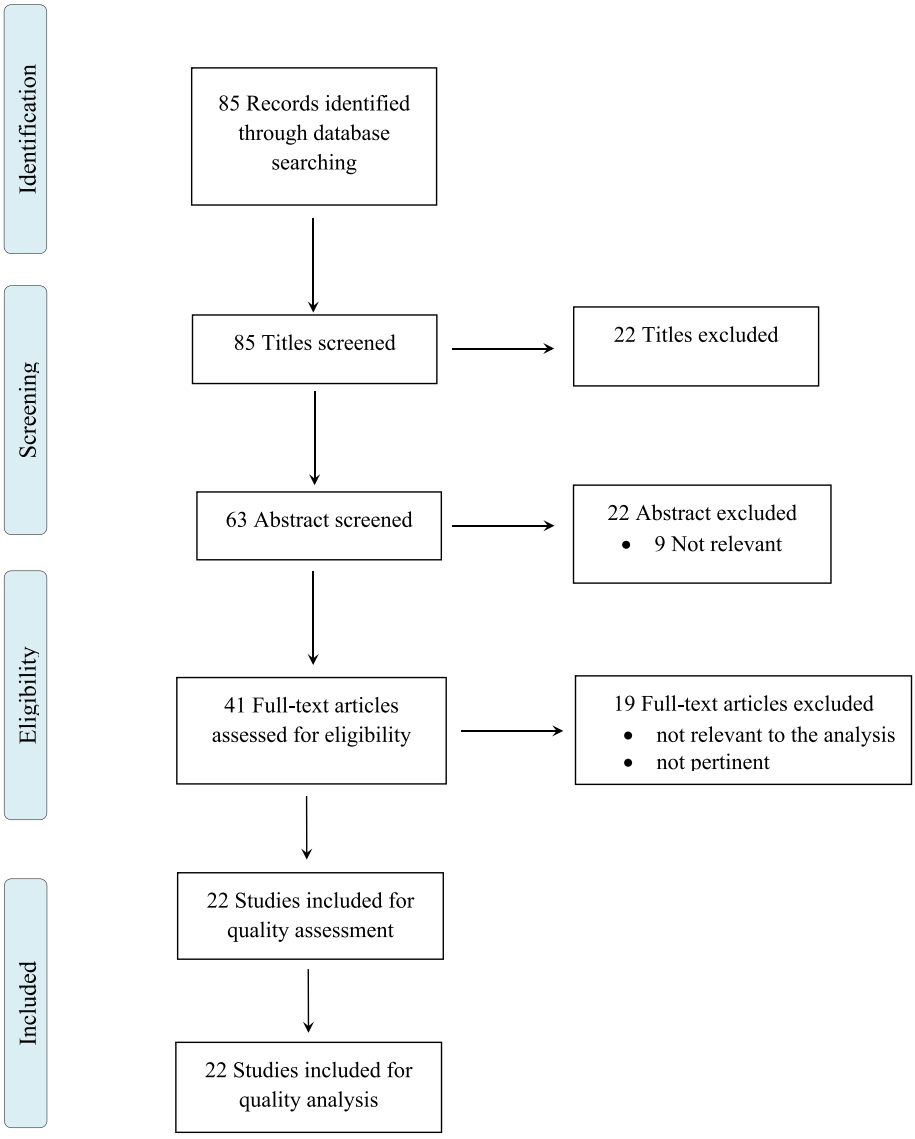

Fig. 1. Flow chart of the literature research for two independent reviewers.

Table 1
Population and number of CF, CFSPID patients included in the selected papers.

Authors	Year	Enrolled population	Nr. CF	Nr. CFSPIDs	Ref.
Ren CL et al. §	2015	1.962	1.540	309	[52]
Ooi et al.	2015	162	80	82	[25]
Groves T et al.	2015	29	0	29	[54]
Levy et al.	2016	376	300	57	[31]
Şaşihüseyinoğlu1 et al.	2019	66	12	54	[27]
Munck A et al.	2019	126	63	63	[30]
Ooi et al.	2019	218	120	98	[26]
Terlizzi V et al.	2019	82	32	50	[42]
Terlizzi V et al.	2020	43	0	43	[32]
Kasi AS et al.	2020	54	19	17	[58]
Terlizzi V et al.	2020	19	0	19	[44]
Terlizzi V et al.	2021	593	257	336	[16]
Ginsburg D et al.	2021	10	0	10	[41]
Hatton A et al.	2021	23	0	23	[45]
Bauer SE et al.	2021	2.613	45	145	[40]
Gonska T et al.	2021	115	0	115	[28]
Dolce D et al.	2022	217	0	217	[53]
Tosco A et al.	2022	129	30	58	[35]
McGarry M et al. §	2022	51.941	46.729	5.212	[60]
Fingerhut R et al.	2022	815.899	232	27	[29]
Salinas DB et al.	2022	112	53	59	[37]
Gunnett MA et al.	2023	1.346	129	63	[35]

§ Data from US CFF Registry.

Table 2
Percentage of CFSPID > CF/CFSPID in the different populations.

Authors	Year	CFSPID > CF/CFSPID	%	Ref.
Terlizzi V et al.	2021	18/336	5.3 %	[16]
Tosco A et al.	2022	6/58	10.3 %	[35]
Ooi CY et al.	2015	11/82	11.0 %	[25]
Ooi CY et al.§	2019	14/98	14.3 %	[26]
Gunnett MA et al.	2023	11/63	17.5 %	[34]
Salinas DB et al.	2022	12/59	20.3 %	[37]
Gonska T et al.	2021	24/115	21.0 %	[28]
Munck A et al.	2020	28/63	44.0 %	[30]
Groves T et al.	2015	14/29	48.0 %	[54]

§ also includes patients from the same authors' 2015 paper.

0.0001). Moreover CFSPID > CF infants (n = 14) had significantly higher b- IRT concentrations (ng/ml) than CFSPID-P (n = 83), (P = 0.02). This study shows a gradation of elevated b-IRT concentrations among diagnostic cohorts, and authors concluded that b-IRT, in concert with other factors, may have the potential to predict the likelihood of CF in CFSPIDs [26]. However, to date, it has been impossible to identify any b-IRT cut-off level to predict a later CF diagnosis in CFSPIDs [28].

In a more recent French multicentre cohort study, enrolling 63 CFSPIDs, authors showed lower b-IRT and SC values than in CF patients [30]. Accordingly, Terlizzi et al. noticed in a large Italian population, including 336 infants, that b-IRT levels were significantly lower in CFSPIDs compared to CF infants. Moreover, 18 CFSPID > CF (5.3 %) showed levels of b-IRT similar to CFSPID-P [16].

To explain this discrepancy in results, a role of *CFTR* variants has been hypothesized since only some genotypes, such as D1152H/CF-causing variant and R117H (p.Arg117His) variant, have been associated with higher b-IRT levels in CFSPIDs [31,32]. The complete list of papers focusing on b-IRT in CFSPIDs is reported in Table 3.

CFTR genotype of CFSPID subjects transitioned to CF

One of the major challenges in the management of CFSPIDs is to identify early on those with a higher or lower risk of later progression to a clinical diagnosis of CF based on *CFTR* genotype. The genetic background of the several cohorts described are very different, based on the geographical context and regional or population-specific genetics factors [33]. In 2015 Ooi et al. reported, in a prospective multicenter study, a diagnosis of CF in nine of 82 (11 %) CFSPIDs: the reason for the change in diagnosis was an abnormal repeat ST and updated functional variant analysis by CFTR2 (https://cftr2.org/), which identified two disease-causing variants. All nine subjects had two *CFTR* variants, the second of which (as for L206W or R117C) was later labeled as CF causing [25].

In a French multicenter study, Munch et al. matched CFSPIDs (n = 63) and CF patients (n = 63) diagnosed on NBS: 28 cases (44 %) converted to CF diagnosis based on genotype (44 %), SC (28 %) or both (28 %). Interestingly five of 28 (18 %) cases had at least one R117H;7T *CFTR* complex allele, and all but six showed clinical features suggestive of CF [30]. According to Ooi's study [26], all CFSPID > CF carried two *CFTR* variants. Unlike R117H;5T, which is a CF-causing variant, R117H;7T is a variant with varying clinical consequences (VVCC), ranging from isolated infertility in males to severe pulmonary disease, typical of the French population and less common in other cohorts of CFSPIDs [16,25,34,35]. However, similar results have been described more recently by Gonska et al. in a prospective, longitudinal, multicenter, Canada-wide cohort study on 115 children with CFSPID [28]. Twenty-four subjects (21 %) met CF diagnostic criteria during the seven years of follow-up. For 12 of 24 children, the CF diagnosis was based on reinterpretation of their second *CFTR* gene variant as a CF-causing allele and again the most frequently genotypes were F508del in trans with VVCC such as R117H;7T.

In an Italian retrospective multicenter study on 336 infants with a CFSPID designation, 18 (5.3 %) converted or were reclassified to a diagnosis of CF, and all but two were asymptomatic babies with a SC increased over time up to pathological values [16]. Again, all CFSPID > CF had two *CFTR* variants, in most cases a CF causing variant in trans with a VVCC. However, two CFSPID > CF carried also two VVCC (S1455X/5T; TG13) or a VVCC (Q1476X) in trans with a variant not reported in CFTR2 database (3272-26A > G). The same authors further confirmed the role of the genetic profile in predicting the risk of disease progression in CFSPIDs in other two papers: the first on 43 CFSPIDs carrying D1152H *CFTR* variant [32]. Those with D1152H in trans with a *CFTR* variant classified as CF-causing had a higher risk to evolve to CF and more frequently developed episodes of pancreatitis, isolation of Pa and respiratory exacerbations in the first year of life, also requiring respiratory physiotherapy, radiological examinations, or saline supplementation. In another paper on 129 Italian patients with F508del/5T; TG12 (including 58 CFSPIDs), after a median follow-up of 6.7 years, 6 (10.3 %) out of 58 CFSPIDs transitioned to CF [36], a higher percentage than that previously reported on Italian CFSPIDs [16]. Conversely only one of 18 CFSPIDs with VVCC/5T; TG12 genotype converted to CF after mean follow up 4.0 ± 3 years [37]. Similar outcomes were also in CFSPIDs from California: 12 (20.3 %) out of 59 were reclassified to CF, all with two *CFTR* variants and eight (66.7 %) of 12 carrying 5 T; TG12, 5 T; TG13 or D1152H [38].

The complete list of papers focusing on genetic profile of CFSPID > CF is reported in Table 4.

CFSPID and sweat test

With the aim to identify subjects early on at risk of evolving into CF, and therefore move them into CF care pathways, due to the relationship between SC levels, genotype, severity of the disease and treatment [39,40], it seemed reasonable to consider whether the initial SC values after NBS could be higher in CFSPID > CF than in subjects who will remain with an inconclusive diagnosis.

In a 10-year retrospective study performed in Indiana, USA, enrolling 2613 infants NBS positive plus one *CFTR* variant, Bauer SE et al. [41] reported that no infants with an initial SC of 30–39 mmol/L were subsequently diagnosed with CF and only one out the 31 infants with an initial SC of 40–49 mmol/L, was subsequently diagnosed as CF. In contrast, 61 % of those with SCs of 50–59 mmol/L were later diagnosed

Table 3
List of the papers focusing on b-IRT in CF, CFSPID > CF or CFSPID-P.

Authors	Year	Study	Nr. CFSPID	Aim	Main findings	Ref.
Ooi et al.	2015	Multicenter prospective case control	82	To identify CFSPIDs and evaluate outcomes	b-IRT value was significantly higher in CF than CFSPIDs	[25]
Levy et al.	2016	Two-center retrospective cross sectional	57	To evaluate the concordance between physician diagnoses and consensus guidelines	CF and CFSPIDs significantly differed in b-IRT levels	[31]
Ooi CY et al. *	2019	Multicenter Prospective study	98	To identify CFSPIDs at risk of developing CF	Infants CFSPID > CF had significantly higher b-IRT levels than CFSPID-P	[26]
Munck A et al.	2019	Prospective study	63	To characterize the genotypic expression of CFSPIDs	No differences in b-IRT value between CFSPID > CF and CFSPID-P	[30]
Terlizzi V et al.	2020	Multicenter retrospective study	43	To define the role of the second *CFTR* variant as a predictive factor of CF evolution in CFSPIDs carrying the D1152H variant	IRT values were higher in CFSPIDs with D1152H/CF-causing genotypes	[32]
Terlizzi V et al.	2021	Multicenter retrospective study	336	To evaluate the prevalence, clinical data, management, and outcome for Italian CFSPIDs	No differences in b-IRT value between CFSPIDs > CF and CFSPID-P	[16]
Gonska T et al.	2021	Multicenter prospectivelongitudinal study	115	To describe the clinical course of CFSPIDs	No differences in b-IRT value between CFSPIDs > CF and CFSPID-P	[28]
Fingerhut R et al.	2022	Multicenter retrospective study	27	To compare b-IRT levels between healthy newborns, CF and CFSPIDs	No evaluation of b-IRT levels in CFSPID > CF	[29]
Salinas DB et al.	2022	Multicenter retrospective study	59	To describe the progression to a CF diagnosis in CFSPIDs	No differences in b-IRT value between CFSPIDs > CF and CFSPID-P	[37]

CF = Cystic Fibrosis; CFSPID = Cystic Fibrosis Screen Positive Inconclusive Diagnosis; CFSPID > CF = subjects who progressed to CF; CFSPID > P = subjects who remained CFSPID b- IRT = Immunoreactive Trypsinogen.

with CF. These results suggest that infants with a positive CF-NBS and one *CFTR* variant whose initial SC concentration is 50–59 mmol/L need to be monitored more closely for CF with strong consideration for earlier repeat ST and immediate genotyping.

In a recent CFSPID cohort of Alabama [35], 11 out of 63 (17.5 %) children progressed to CF: none had an initial SC value less than 30 mmol/L, 12 % with an initial SC between 30 and 39 mmol/L converted to CF (4 out of 34), 4/8 (50 %) with an initial SC value ranging 40–49 mmol/L and 3/5 (60 %) with an initial SC value ranging between 50 and 59 mmol/L received a CF diagnosis during follow up.

In an Italian multicentre study in 336 CFSPID infants [16], among infants with an initial SC lower than 30 mmol/L only two out of 139 (2.1 %) converted to CF, while 8 % (16/197) of infants with an initial SC in the borderline range (30–59 mmol/L) did.

A Canadian study followed a cohort of 103 CFSPID infants [28] evaluating if their initial SC level could be considered as a predictive biomarker for a CF diagnosis. 12 % of CFSPID converted to CF during the follow-up period due to a SC level above 60 mmol/l. Authors showed that the first sweat chloride value could be predictive of a conversion to a CF diagnosis. Children with an initial value >40 mmol/L chloride had a 10 times higher hazard of having a CF-converting ST of >60 mmol/l chloride later in life, compared with those with an initial value <40 mmol/L (hazard ratio: 12.1 [95 % CI 2.6 to 55.6]; P = 0.001). Of the 10 children with CFSPID with a ST > 40 mmol/L, nine converted to a CF diagnosis between the ages of 2.8 and 4.4 years.

On the contrary, Ginsburg et al. [42] reported a series of seven CFSPID who progressed to CF, despite an initial SC lower than 30 mmol/L.

Indeed, during the initial follow up, a gradual increase of SC in CFSPIDs may be observed both in those who will evolve to CF and, in subjects who will not progress [38]. This may be due to ongoing maturation of sweat gland, changes in the innervation or hormonal levels, or other yet to be identified factors. Therefore, attention could be directed to the trend of increasing chloride value that CFSPIDs could present in the first years of life, wondering if subjects who progressed to CF showed a different trend of increase in the chloride value compared to CFSPID-P.

In this regard, some authors have evaluated the trend of variation of SC value during CFSPIDs follow up [43].

Ooi et al. presented a series of 82 CFSPIDs, among them nine (11 %) subjects fulfilled the diagnostic criteria for CF during the follow-up period based on genotype and/or abnormal SC [25]. As expected CFSPID > CF individuals had a significantly higher SC values than did CFSPID-P ($p < 0.001$), moreover, the study showed a significant difference in the longitudinal SC trajectories between the two groups of subjects.

Also, in their multicenter Italian study, Terlizzi et al. [16] showed a different trend in increasing SC values during follow up between CFSPID > CF and those CFSPID-P.

Ginsburg et al. [42] reported a series of 10 children reclassified as CF, who presented an increase in SC concentration of 5.8 mmol/L/year. These authors suggested that the rate of SC increase, together with genotype, clinical features and *CFTR* functional assay, could potentially be used as prognostic tools in CFSPIDs.

A Californian CFSPID population was studied and described by Salinas et al. [38] with the aim to identify risk factors for reclassification from CFSPID to a CF diagnosis. Analyzing repeated values of SC over time showed distinct trajectories between children CFSPID > CF compared to CFSPID-P (p-value for difference between slopes = 0.013). CFSPID > CF children presented an increase of 4.71 (95 % confidence interval [CI]: 2.45–6.97) mmol/l/year of SC, compared to 1.21 (95 % CI: 0.4–2.02) mmol/l/year among those who did not progress to CF. Moreover, the initial SC values were also significantly different between the two groups ($p < 0.0001$).

Regarding the correlation between genotypes and SC levels [44], as also seen in CF patients (37), some CFSPID genotypes could have a more significant correlation with high SC levels during follow-up, like the F508del/R117H;7T; F508del/D1152H, F508del/5T;TG12 or F508del/5T;TG13 [39,45,46].

Other researchers have showed normal SC levels in CFSPIDs bearing a *CFTR* variant of unknown significance (VUS), such as D537N, T582I, M952T, in trans to a CF-causing variant [27,31].

In conclusion, a greater risk of evolution in CF seems to be in CFSPIDs with first SC in the higher borderline range and increasing trend of chloride value (>2.45 mmol/l/year) as per Salinas's study [38].

Nevertheless, a recent retrospective study, conducted on the series of

Table 4
List of the papers focusing on CFSPIDs and *CFTR* gene analysis.

Authors	Year	Type of study	CFSPID > CF	Aims	*CFTR* genetic profile of CFSPID > CF	Length of follow up (months)	Ref.
Groves et al.	2015	Retrospective case control	14	To describe the clinical course of CFSPIDs with intermediate sweat chloride values	7/14: F508del/unknown variant; 4/14: F508del/R117H	14	[54]
Ooi CY et al.	2015	Multicenter prospective case control	9	To identify and evaluate infants with CFSPID	most frequent genotypes were CF causing/R117C or L206W	36	[25]
Munck A et al.	2019	Prospective study	63	To characterize the genotypic expression of children with CFSPID	5/28 had at least one R117H;7T *CFTR* complex allele	90	[30]
Ooi C Y et al.	2019	Prospective study	98	To define a correlation between CF level and the degree of *CFTR* dysfunction to identify CFSPID at risk of developing CF	9/14 reassigned according to genotype; 2/14 had R117H/7T	120	[26]
Terlizzi V et al.	2019	Monocenter retrospective study	50	To evaluate prevalence and clinical outcome of CFSPID infants	all with CF causing variant/ VVCC or S737F, variant typical in Tuscany region	6.6	[42]
Terlizzi V et al.	2020	Retrospective analysis	19	To illustrate prevalence, SC trend and outcome of patient with VVCC	all with CF causing variant/ R117H/7T; D1152H or 5 T; TG12	3.1	[44]
Ginsburg D et al.	2021	Case series	10	To illustrate evolution from CFSPID to CF	7/10 had CF causing variant/ 5 T;TG12, 5 T;TG13 or D1152H	NS	[41]
Terlizzi V et al.	2020	Multicenter retrospective study	43	To define the role of the second *CFTR* variant as a predictive factor of disease evolution in CFSPID carrying the D1152H variant	all with D1152H/CF causing variant	40.6	[32]
Terlizzi V et al.	2021	Multicenter retrospective study	336	To evaluate the prevalence, management and outcome of Italian CFSPID subjects	16/18 had CF causing/VVCC, such as 5 T;TG12 or D1152H	40	[16]
Hatton A et al.	2021	Case series	23	To describe a CFSPID population performing in vivo and in vitro functional studies	4/23 (17.4 %) CFSPID > CF; three with CF causing variant/ D1152H; one 5 T;TG12/5T;TG13	84.7	[45]
Gonska T et al.	2021	Multicenter prospective, longitudinal study	115	To describe the clinical course of CFSPID	12/24 reassigned according to genotype; most frequent genotypes were F508del/VVCC, such as R117H;7T or poly T tract	84.7	[28]
Tosco A et al.	2022	Multicenter retrospective study	58	To describe the progression to a CF diagnosis for subjects with F508del/5T;TG12	6/58 (10.3 %) CFSPID > CF	72.7	[35]
Salinas DB et al.	2022	Multicenter retrospective study	59	To describe the progression to a CF diagnosis	8/12 with one causing variant/ 5T;TG12, 5 T;TG13 or D1152H	NS	[37]
Gunnett MA et al.	2023	Multicenter retrospective study	63	To identify features of progression from CFSPID to CF	2/11 reassigned according to genotype; 2/11 with CF causing/R117H/ 7T	NS	[34]

Abbreviations: CF = Cystic Fibrosis; CFSPID = Cystic Fibrosis Screen Positive Inconclusive Diagnosis; CFTR = Cystic Fibrosis Transmembrane Conductance Regulator; CFTR-RD = CFTR related disorders; VVCC = Variants of varying clinical consequence; SC = Sweat Chloride; NS = Not Stated.

Table 5
List of the papers focusing on CFSPID and sweat chloride test.

Authors	Year	Nr CFSPID	Length of follow up (months)	60 mmol/L at end of follow up	Percentage (%)	Nation	Ref.
Ooi CY et al.	2015	82	36	11	11.0	Canada, Italy	[25]
Munck A et al.	2019	63	90	28	17.64	France	[30]
Ooi CY et al.	2019	98	120	10	10.2	Canada, Italy	[26]
Munk A et al.	2019	63	90	28	44.0	France	[30]
Terlizzi V et al.	2020	19	3.1	8	42.1	Italy	[44]
Gonska T et al.	2021	115	84.7	12	10.43	Canada	[28]
Hatton A et al.	2021	23	84.7	2	8.69	Poland	[45]
Terlizzi V et al.	2021	336	40	18	5.3	Italy	[16]
Gonska T et al.	2021	115	84.7	24	21	Canada	[28]
Tosco A et al.	2022	58	72.7	6	10.3	Italy	[35]
Salinas DB et al.	2022	12	NS	59	20.3	USA	[37]

CF = Cystic Fibrosis; CFSPID = Cystic Fibrosis Screen Positive Inconclusive Diagnosis; CFTR = Cystic Fibrosis Transmembrane Conductance Regulator; CFTR-RD = CFTR related disorders; FEV1: Forced Expiratory Volume in 1 s; IRT = Immunoreactive Trypsinogen; LCI = Lung Clereance Index; MVCC = Mutations of varying clinical consequence; NBS = Newborn screening; PA = Pseudomonas Aeruginosa; SC = Sweat Chloride; SCT = Sweat Chloride Test: NS = Not Stated.

a single center [47] draws attention to the great intra-individual variability of the SC values recorded in CFSPIDs, calling for caution in diagnosing CF in asymptomatic children with pathological ST.

Furthermore, the sweat collection method affected chloride values in CFSPIDs and, therefore, the definitive diagnostic category. As recently reported, the mean values of SC measured by the Gibson–Cooke method are significantly higher than those using the Macroduct system-based method, and this makes the different cohorts described harder to compare [48].

The complete list of papers focusing SC in CFSPIDs is reported in Table 5.

Table 6
List of the papers focusing on CFSPID and *Pseudomonas aeruginosa* (Pa) isolation.

Authors/year	Year	Type of the study	Nr CFSPID	Ref.
Groves T et al.	2015	Retrospective	14	[54]
Ooi CY et al.	2015	Prospective	82	[25]
Ren CL et al.	2015	Retrospective	309	[52]
Levy et al.	2016	Prospective	57	[31]
Munck A et al.	2019	Prospective	63	[30]
Terlizzi V et al.	2021	Multicenter retrospective study	336	[16]
Ginsburg D et al.	2021	Case series	10	[41]
Dolce D et al.	2022	Multicenter retrospective study	217	[53]
Salinas DB et al.	2022	Multicenter retrospective study	59	[37]

CF = Cystic Fibrosis; CFSPID = Cystic Fibrosis Screen Positive Inconclusive Diagnosis; CFSPID > CF = CFSPID who converted to CF; CFSPID > P = CFSPID persistent; PA = Pseudomonas Aeruginosa.

CFSPID and Pseudomonas aeruginosa isolation

It is well known that the isolation of Pa from airways of CF subjects is a typical feature of CF pulmonary disease, and chronic endobronchial Pa infection is correlated to decline in lung function [49]. Then, it is reasonable to hypothesize that acquiring Pa could be a risk factor for CFSPID > CF.

The percentage of Pa isolation in CFSPIDs varies from 11 % [50] to 39 % [31] in different series (Table 6).

In an American retrospective study of 309 CFSPIDs cohort, 11 % of infants had a positive Pa respiratory tract culture in their first year of life [50]. A similar percentage (12 %) was reported in a prospective multicentre (Canada, Italy) study enrolling eighty-two CFSPIDs [25]. Higher percentages in Pa isolation were found in a Californian case series (20.5 %) [38], and in a French one (24 %) [30]. The highest percentage was reported in a cross-sectional study in CFSPIDs from Wisconsin where 22/57 (39 %) had a positive bacterial culture for Pa [31].

The two studies [30,38] evaluated if Pa chronic isolation was found in CFSPIDs during follow up, assess that none of the enrolled subjects developed it. CFSPID > CF may present Pa isolation as CFSPID-P in the Ooi et al.'s study on 82 subjects [25], while significantly higher rates of respiratory cultures positive for Pa in CFSPID > CF was reported in the Salinas' study on 59 subjects [38].

Due to the correlation between Pa isolation and lung function deterioration in CF subjects, a risk of overtreatment in CFSPIDs may be present. To date, the clinical consequences of early isolation of Pa and its outcome (both in the presence and absence of therapy) in CFSPIDs have not been described in large case series, therefore no univocal therapeutic approach is advised.

However, although several authors investigated the incidence of Pa isolation in CFSPIDs, and some evaluated the possibility of spontaneous clearance, there is a need for further studies that evaluate any differences in Pa isolation between CFSPID > CF and CFSPID-P.

As no evidence supports scheduled routine microbiological studies in CFSPIDs, ECFS recommendations stated that respiratory cultures should be performed when clinically indicated [17].

Neither chronic colonization, nor lung function deterioration were reported in CFSPIDs with isolated Pa [51,52], while a spontaneous clearance of Pa was observed. Specifically, of 217 CFSPIDs 44 (20.3 %) had a respiratory culture positive for nonmucoid Pa, sensitive to antibiotics, and probably not acquired in the hospital setting. After a median follow-up of 6.2 years, Pa clearance occurred in 22/24 (91.6 %) individuals treated *vs* spontaneous clearance in 16/19 (84.2 %) untreated patients (chi-square, 0.5737; p = 0.44878) [51].

Although Pa isolation alone does not seem to represent a signal alarm for a following CF diagnosis, the question arises whether high values of b-IRT and SC, a genotype as CF-causing variant/ VVCC, and recurrent Pa isolation, could be all together considered a clinical risk factor of evolution into CF disease.

The complete list of papers focusing on Pa isolation in CFSPIDs is reported in Table 6.

CFSPID and LCI

The LCI is a quantitative measure of ventilation inhomogeneity within the lungs, assessed using the multiple breath washout (MBW) technique. In CF, mucus accumulation and inflammation in the airways cause uneven distribution of ventilation. Regions of the lung with blocked airways are poorly ventilated and contribute to increased LCI values. The LCI provides a sensitive marker for early lung abnormalities, even before traditional spirometry measures like percent predicted forced expiratory volume in one second (ppFEV1) [53–55].

The applicability of LCI in detecting early lung disease and predicting the progression of disease in CFSPIDs has been recently investigated.

In an observational study [56] enrolling pre-school children (19 CF,

Table 7
List of the papers focusing on CFSPID and LCI.

Authors	Year	Type of Study	CFSPID > CF	Aim	Results	Conclusions	Ref.
Kasi AS et al.	2020	Observational	17	To evaluate the LCI in detecting early lung disease in CFSPIDs. To compare LCI to spirometry in detecting early lung disease in CFSPIDs	LCI in CFSPIDs was not statistically different from healthy control but significantly different when compared to CF patients. Mean ppFEV$_1$was not statistically different between CFSPID and CF	LCI can potentially detect early lung disease in CFSPIDs as part of assessing their risk for reclassification to CF diagnosis in addition to spirometry	[58]
Gonska T et al.	2021	Multicenter prospective, longitudinal	115	To describe the clinical course of CFSPIDs	LCI was evaluated in 17 CFSPIDs; average LCI was 6.92 (95 % CI 6.62 to 7.22), at a mean age of 7.99 (95 % CI 6.88 to 9.1)	LCI did not differ between CFSPIDs and healthy controls. No evaluation of LCI in CFSPID > CF	[28]
Salinas D B et al.	2022	Multicenter retrospective	112	To describe the progression to a CF diagnosis	No statistical difference in distribution between the above and below LCI cut-off among CF, CFSPIDs, and CF patients (p = 0.20 and p = 0.55, p = 48, respectively)	LCI did not differ between CFSPID > CF and CFSPIDs	[37]
Terlizzi V et al.	2022	Prospective	42	To assess the value of the LCI in correctly predicting the progression of CFSPID > CF	The mean LCI value for patients with CF (7.39; 5.98–10.24) was statistically higher compared to both the mean LCI in the CFSPID > CF (6.62; 5.69–7.58) and in CFSPID-P (6.56; 5.64–7.21).	Most of asymptomatic CFSPIDs or progressed to CF had normal LCI	[56]

CF = Cystic Fibrosis; CFSPID = Cystic Fibrosis Screen Positive Inconclusive Diagnosis; FEV_1 = Forced Expiratory Volume in 1 s; LCI = Lung Clearance Index; CFSPID > CF: CFSPID who progressed to CF; CFSPID-P: CFSPID persistent.

17 CFSPID and 18 healthy controls), Kasi et al. reported that mean LCI from the CFSPID group was not statistically different from healthy controls ($p = 0.49$), but significantly different when compared to CF ($p = 0.04$). LCI values appeared abnormal in two CFSPID children carrying potentially deleterious *CFTR* variants. On the contrary, $ppFEV_1$ detected no difference between CF and CFSPID.

In a prospective, multicenter, Canadian cohort study on CFSPIDs monitored for seven years, LCI values in a subgroup of children were like those of healthy controls [28].

The most interesting studies are those that evaluated LCI in CFSPID > CF. In this context we find two very recent studies. Salinas et al. evaluated a population of 112 children, 59 CFSPID and 53 CF. Among the latter, 12 were children CFSPID > CF: authors found no statistically different values between them and CFSPID-P [38].

Similarly, Terlizzi et al. evaluated LCI in 42 children. Of these, 26 were CFs, eight were CFSPID > CF, and eight were CFSPID-P. They pointed out that LCI could be normal in many asymptomatic CFSPID or CFSPID > CF with a significant difference with the CF's LCI values [54].

From these studies we can draw the conclusions that CFSPID > CF subjects likely maintain normal LCI values, probably because lung damage might develop later with age. Certainly, further prospective studies are needed to clarify this finding. Since LCI is a non-invasive test, it seems advisable to perform this test over time in CFSPIDs. Further data and for a longer follow up will help to understand if CFSPIDs with abnormal LCI may be at greater risk of evolution into CF disease.

The complete list of papers focusing on LCI in CFSPIDs is reported in Table 7.

CFSPID follow-up

One of the crucial points in the follow-up of CFSPIDs is to define how long it is indicated to continue follow-up in CF centres. However, because CFSPID label only began in 2015, studies report outcomes in the pre- or school age [16,28,57], whereas the long-term outcomes of CFSPIDs, both CFSPID > CF and CFSPID-P, remain unknown.

Current evidence suggests CFSPIDs should pursue a specialized follow-up for the first six years of life [30] and children who remain healthy with normal SC, growth, and lung function/ imaging at 6 years of age are considered unlikely to progress to CF.

A multicentre prospective study by Gonska et al. [28] followed 115 CFSPID for a mean period of 7.7 years (7.1–8.4). In the 91 remaining CFSPID-P normal growth and lung function were maintained until school age, as Munck et al. [30] and Salinas et al. [57] had shown as well in previous studies.

Hatton et al. [46] described 23 CFSPIDs with a seven-year follow-up (range 4–13 years): all remained pancreatic sufficient, even though five out of 23 converted to CF.

Therefore, the first six years of CFSPID life seem be crucial for a re-diagnosis of CF, whereas there is no information on any conversion to CF or CFTR-RD at older ages.

Future perspectives

While the ST remains the gold standard for the diagnosis of CF, recently new tools have been proposed that are more sensitive in distinguishing CF, CFTR-RD or CFSPID subjects.

Image-Sensor measurements of beta-Adrenergic Sweat Secretion Test (BAST) determines in vivo CFTR function as reflected by β-adrenergically stimulated eccrine sweat secretion, it is comparable to evaporimetry, which evaluates sweat secretion stimulated trough beta-adrenergic pathways [58]. It is reliable, safe, rapid and easy to be performed, since it avoids the analysis of SC. Preliminary studies describe its higher diagnostic sensitivity in discriminating between CF, CFTR-RD, CFSPID and healthy subjects as compared to classic ST [59,60]. Therefore, BAST seems to be more accurate than ST for excluding CF in CFSPIDs.

However, despite such encouraging results, BAST lacks standardization either in the procedures and in the collection of reference values on a large population. Thus, further studies are needed to establish reference ranges for BAST at different ages, also considering the lower β-adrenergic sweat secretion in children compared to adults [61].

The combination of clinical follow up, *CFTR* gene analysis, and biomarkers allowing the evaluation in vivo and in vitro of the CFTR protein function in each individual (i.e., BAST, epithelial samples from nasal brushing and/or in vivo intestinal current measurement), could open up a promising perspective in the predictive evaluation of subjects with inconclusive diagnosis [38,47].

Thus, a diagnostic use of organoids [62], now tested to predict possible clinical response to *CFTR* modulators in individuals bearing *CFTR* variants of unknown clinical significance might help in elucidating the risk of progression to CF disease in CFSPID infants.

Conclusion

Individuals with an inconclusive CF diagnosis after a positive NBS pose several challenges to clinicians in terms of correct communication to parents about prognosis, management of diagnostic and therapeutic tools, and duration of follow up in a specialized CF Centre. Conversely, there is a risk of overmedicalization in children who may remain with an inconclusive diagnosis for years.

The knowledge about the clinical features and outcomes of CFSPIDs has been increasing since 2015 and we have so far learned that a number will change their classification to CF in the pre-school years.

Our review attempted to highlight the risk factors for CFSPID > CF evolution: however, to date, none of the biomarkers or clinical features studied (i.e., IRT level at birth, *CFTR* genotype, initial SC value or its increasing trend, occurrence of Pa, functional respiratory indices) seems effective alone in predicting the risk of a future CF diagnosis, despite the expanding reports. This also depends on the wide differences in the experimental design of the studies that have addressed this issue (number of cases, genetic background, duration and frequency of follow-up, biomarkers considered, etc.).

Really, the simultaneous presence of some events could be considered as a risk factor for a CF reclassification: a) the presence of one CF-causing *CFTR* variant in trans with a VVCC, as D1152H, 5 T;TG12, R117H-7 T [30,32,37,46]; b) an initial SC ≥ 40 mmol/L [28]; c) an increase of SC > 2.5 mmol/L/years [38]; and d) recurrent isolation of Pa from airways could allow identification of subjects who may require more frequent and prolonged clinical follow up.

As regards genotypes, few data are available on the long-term evolution of lung disease in subjects with VVCC or VUS and cohorts described so far are too young to provide adequate long-term information. Adults with CFTR-RD show a high frequency of *CFTR* variants that occur commonly in infants with CFSPID [9]. Furthermore, retrospective data from CF patients enrolled in the U.S. CF Foundation patient registry and with at least one variant typically found in CFSPIDs have a milder phenotype, with lower prevalence of pancreatic insufficiency and higher median $ppFEV_1$ [63]. While these retrospective data may be useful in genetic counseling, further studies are needed to properly inform families and guide the management of these children.

Nevertheless, results from registry data must be taken with caution, since CFSPIDs entered in the registry with a clinical diagnosis of CF may be represent possible misclassifications [51,64].

Since unequivocal conclusions cannot be achieved, it is reasonable that CFSPID infants carrying VVCC *CFTR* variant in trans with a CF-causing variant should be tested with SC test frequently, at least with the aim to identify and discharge healthy infants as soon as possible [65,66] or follow children at greater risk of evolution in CF even after six years of age.

As regards follow up duration at CF specialized clinics [67], although a longer follow-up will provide more information on CFSPID > CF rate of conversion over time, on clinical features of both CFSPID > CF and

CFSPID-P, it should also be taken into consideration that CFSPIDs' families could be challenged by years of diagnostic uncertainty, with unpredictable psychological consequence, risk of overmedication.

The establishment of an international registry collecting data of subjects labeled CFSPID, would be able to answer in the future the several questions remaining open on prognosis of these children.

Future research directions

- The combination of clinical follow up, CFTR gene analysis, and biomarkers allowing the evaluation in vivo and in vitro of the CFTR protein function in each individual could open up a promising perspective in the predictive evaluation of subjects with inconclusive diagnosis.
- Studies on adolescents or adults previously CFSPID are needed to better identify early factors of evolution in disease.
- A dedicated registry collecting longitudinal data on CFSPIDs would be able to clarify long-term prognosis of these children.

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Appendix A. Supplementary data

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References

[1] Rafeeq MM, Murad HAS. Cystic fibrosis: current therapeutic targets and future approaches. J Transl Med 2017;15(1):84. https://doi.org/10.1186/s12967-017-1193-9.
[2] Dayasiri K, Hull J, Rao S. NICE guidance on diagnosis and management of cystic fibrosis. Arch Dis Child Educ Pract Ed 2021;106(1):31–4. https://doi.org/10.1136/archdischild-2019-316882.
[3] Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et al. Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros 2011;10(Suppl 2):S86–. https://doi.org/10.1016/S1569-1993(11)60014-3.
[4] Castellani C, De Boeck K, De Wachter E, Sermet-Gaudelus I, Simmonds NJ, Southern KW, et al. ECFS Diagnostic Network Working Group. ECFS standards of care on CFTR-related disorders: Updated diagnostic criteria. J Cyst Fibros 2022;21(6):908–21. https://doi.org/10.1016/j.jcf.2022.09.011.
[5] Dijk FN, McKay K, Barzi F, Gaskin KJ, Fitzgerald DA. Improved survival in cystic fibrosis patients diagnosed by newborn screening compared to a historical cohort from the same centre. Arch Dis Child 2011;96(12):1118–23. https://doi.org/10.1136/archdischild-2011-300449.
[6] Rehani MR, Marcus MS, Harris AB, Farrell PM, Ren CL. Variation in cystic fibrosis newborn screening algorithms in the United States. Pediatr Pulmonol 2023;58(3):927–33. https://doi.org/10.1002/ppul.26279.
[7] Munck A, Berger DO, Southern KW, Carducci C, de Winter-de Groot KM, Gartner S, et al. European CF Society Neonatal Screening Working Group (ECFS NSWG). European survey of newborn bloodspot screening for CF: opportunity to address challenges and improve performance. J Cyst Fibros 2023;22(3):484–95. https://doi.org/10.1016/j.jcf.2022.09.012.
[8] Barben J, Castellani C, Dankert-Roelse J, Gartner S, Kashirskaya N, Linnane B, et al. The expansion and performance of national newborn screening programmes for cystic fibrosis in Europe. J Cyst Fibros 2017;16(2):207–13. https://doi.org/10.1016/j.jcf.2016.12.012.
[9] Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros 2009;8(3):153–73. https://doi.org/10.1016/j.jcf.2009.01.004.
[10] Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 1959;23(3):545–9.
[11] Farrell PM, White TB, Howenstine MS, Munck A, Parad RB, Rosenfeld M, et al. Diagnosis of Cystic Fibrosis in Screened Populations. J Pediatr 2017;181S:S33–S44.e2.. https://doi.org/10.1016/j.jpeds.2016.09.065.
[12] Padoan R, Bassotti A, Seia M, Corbetta C. Negative sweat test in hypertrypsinaemic infants with cystic fibrosis carrying rare CFTR mutations. Eur J Pediatr 2002;161(4):212–5. https://doi.org/10.1007/s00431-001-0910-8.
[13] Parad RB, Comeau AM. Diagnostic dilemmas resulting from the immunoreactive trypsinogen/DNA cystic fibrosis newborn screening algorithm. J Pediatr 2005;147(3 Suppl):S78–82. https://doi.org/10.1016/j.jpeds.2005.08.017.
[14] C. Fibrosis Foundation; Borowitz D, Parad RB, Sharp JK, Sabadosa KA, Robinson KA, et al. Cystic Fibrosis Foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first two years of life and beyond J Pediatr. 155 6 Suppl 2009 S106 S116 10.1016/j.jpeds.2009.09.003.
[15] Ren CL, Borowitz DS, Gonska T, Howenstine MS, Levy H, Massie J, et al. Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis. 181S:S45–S51.e1 J Pediatr 2017. https://doi.org/10.1016/j.jpeds.2016.09.066.
[16] Terlizzi V, Claut L, Tosco A, Colombo C, Raia V, Fabrizzi B, et al. A survey of the prevalence, management and outcome of infants with an inconclusive diagnosis following newborn bloodspot screening for cystic fibrosis (CRMS/CFSPID) in six Italian centres. J Cyst Fibros 2021;20(5):828–34. https://doi.org/10.1016/j.jcf.2021.03.015.
[17] Barben J, Castellani C, Munck A, Davies JC, de Winter-de Groot KM, Gartner S, et al. European CF Society Neonatal Screening Working Group (ECFS NSWG). Updated guidance on the management of children with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID). J Cyst Fibros 2021;20(5):810–9. https://doi.org/10.1016/j.jcf.2020.11.006.
[18] Tosco A, Marino D, Polizzi S, Tradati V, Padoan R, Giust C, et al. A Multicentre Italian Study on the Psychological Impact of an Inconclusive Cystic Fibrosis Diagnosis after Positive Neonatal Screening. Children (Basel) 2023;10(2):177. https://doi.org/10.3390/children10020177.
[19] Ginsburg DK, Salinas DB, Cosanella TM, Wee CP, Saeed MM, Keens TG, et al. High rates of anxiety detected in mothers of children with inconclusive cystic fibrosis screening results. J Cyst Fibros 2023;22(3):420–6. https://doi.org/10.1016/j.jcf.2022.12.002.
[20] Johnson F, Southern KW, Ulph F. Psychological Impact on Parents of an Inconclusive Diagnosis Following Newborn Bloodspot Screening for Cystic Fibrosis: A Qualitative Study. Int J Neonatal Screen 2019;5(2):23. https://doi.org/10.3390/ijns5020023.
[21] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews BMJ 2021; 372 :n71 doi:10.1136/bmj.n71.
[22] Crossley JR, Elliott RB, Smith PA. Dried-blood spot screening for cystic fibrosis in the newborn. Lancet 1979;1(8114):472–4. https://doi.org/10.1016/s0140-6736(79)90825-0.
[23] Scotet V, Gutierrez H, Farrell PM. Newborn Screening for CF across the Globe-Where Is It Worthwhile? Int J Neonatal Screen 2020;6(1):18. https://doi.org/10.3390/ijns6010018.
[24] Sontag MK, Corey M, Hokanson JE, Marshall JA, Sommer SS, Zerbe GO, et al. Genetic and physiologic correlates of longitudinal immunoreactive trypsinogen decline in infants with cystic fibrosis identified through newborn screening. J Pediatr 2006;149(5):650–7. https://doi.org/10.1016/j.jpeds.2006.07.026.
[25] Ooi CY, Castellani C, Keenan K, Avolio J, Volpi S, Boland M, et al. Inconclusive diagnosis of cystic fibrosis after newborn screening. Pediatrics 2015;135(6):e1377–85. https://doi.org/10.1542/peds.2014-2081.
[26] Ooi CY, Sutherland R, Castellani C, Keenan K, Boland M, Reisman J, et al. Immunoreactive trypsinogen levels in newborn screened infants with an inconclusive diagnosis of cystic fibrosis. BMC Pediatr 2019;19(1):369. https://doi.org/10.1186/s12887-019-1756-4.
[27] Şaşihüseyinoğlu AŞ, Altıntaş DU, Bişgin A, Doğruel D, Yılmaz M, Serbes M. Two years of newborn screening for cystic fibrosis in Turkey: Çukurova experience. Turk J Pediatr 2019;61(4):505–12. https://doi.org/10.24953/turkjped.2019.04.006.
[28] Gonska T, Keenan K, Au J, Dupuis A, Chilvers MA, Burgess C, et al. Outcomes of Cystic Fibrosis Screening-Positive Infants With Inconclusive Diagnosis at School Age. Pediatrics 2021;148(6). https://doi.org/10.1542/peds.2021-051740.e2021051740.
[29] Fingerhut R, Rueegg CS, Imahorn O, Pedersen ESL, Kuehni CE, Gallati S, et al. Immunoreactive trypsinogen in healthy newborns and infants with cystic fibrosis. Arch Dis Child Fetal Neonatal Ed 2023;108(2):176–81. https://doi.org/10.1136/archdischild-2021-323549.
[30] Munck A, Bourmaud A, Bellon G, Picq P, Farrell PM, DPAM Study Group. Phenotype of children with inconclusive cystic fibrosis diagnosis after newborn screening. Pediatr Pulmonol 2020;55(4):918–28. https://doi.org/10.1002/ppul.24634.
[31] Levy H, Nugent M, Schneck K, Stachiw-Hietpas D, Laxova A, Lakser O, et al. Refining the continuum of CFTR-associated disorders in the era of newborn screening. Clin Genet 2016;89(5):539–49. https://doi.org/10.1111/cge.12711.

[32] Terlizzi V, Padoan R, Claut L, Colombo C, Fabrizzi B, Lucarelli M, et al. Subjects Carrying D1152H CFTR Variant: Can the Second Variant Be a Predictor of Disease Development? Diagnostics (Basel) 2020;10(12):1080. https://doi.org/10.3390/diagnostics10121080.

[33] Castellani C, Cuppens H, Macek Jr M, Cassiman JJ, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7(3):179–96. https://doi.org/10.1016/j.jcf.2008.03.009.

[34] Keenan K, Dupuis A, Griffin K, Castellani C, Tullis E, Gonska T. Phenotypic spectrum of patients with cystic fibrosis and cystic fibrosis-related disease carrying p.Arg117His. J Cyst Fibros 2019;18(2):265–70. https://doi.org/10.1016/j.jcf.2018.09.002.

[35] Gunnett MA, Baker E, Mims C, Self ST, Gutierrez HH, Guimbellot JS. Outcomes of children with cystic fibrosis screen positive, inconclusive diagnosis/CFTR related metabolic syndrome. Front Pediatr 2023;11:1127659. https://doi.org/10.3389/fped.2023.1127659.

[36] Tosco A, Castaldo A, Colombo C, Claut L, Carnovale V, Iacotucci P, et al. Clinical outcomes of a large cohort of individuals with the F508del/5T;TG12 CFTR genotype. J Cyst Fibros 2022;21(5):850–5. https://doi.org/10.1016/j.jcf.2022.04.020.

[37] Tosco A, Carnovale V, Claut L, Fabrizzi B, Majo F, Castellani C, et al. Clinical outcome of individuals carrying 5T;TG12 in trans with CFTR variants with varying clinical consequences. Pediatr Pulmonol 2023;58(4):1253–5. https://doi.org/10.1002/ppul.26323.

[38] Salinas DB, Ginsburg DK, Wee CP, Saeed MM, Brewington JJ. Gradual increase in sweat chloride concentration is associated with a higher risk of CRMS/CFSPID to CF reclassification. Pediatr Pulmonol 2023;58(4):1074–84. https://doi.org/10.1002/ppul.26296.

[39] Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, et al. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 1995;127(5):705–10. https://doi.org/10.1016/s0022-3476(95)70157-5.

[40] Accurso FJ, Van Goor F, Zha J, Stone AJ, Dong Q, Ordonez CL, et al. Sweat chloride as a biomarker of CFTR activity: proof of concept and ivacaftor clinical trial data. J Cyst Fibros 2014;13(2):139–47. https://doi.org/10.1016/j.jcf.2013.09.007.

[41] Bauer SE, Wesson M, Oles SK, Ren CL. Outcomes of repeat sweat testing in cystic fibrosis newborn screen positive infants. Pediatr Pulmonol 2021;56(6):1521–6. https://doi.org/10.1002/ppul.25296.

[42] Ginsburg D, Wee CP, Reyes MC, Brewington JJ, Salinas DB. When CFSPID becomes CF. J Cyst Fibros 2022;21(1):e23–7. https://doi.org/10.1016/j.jcf.2021.06.012.

[43] Terlizzi V, Mergni G, Buzzetti R, Centrone C, Zavataro L, Braggion C. Cystic fibrosis screen positive inconclusive diagnosis (CFSPID): Experience in Tuscany. Italy J Cyst Fibros 2019;18(4):484–90. https://doi.org/10.1016/j.jcf.2019.04.002.

[44] Espel JC, Palac HL, Bharat A, Cullina J, Prickett M, Sala M, et al. The relationship between sweat chloride levels and mortality in cystic fibrosis varies by individual genotype. J Cyst Fibros 2018;17(1):34–42. https://doi.org/10.1016/j.jcf.2017.11.002.

[45] Terlizzi V, Mergni G, Centrone C, Festini F, Taccetti G. Trend of sweat chloride values in a cohort of patients carrying CFTR mutations of varying clinical consequence: Is there a risk of increasing sweat chloride over time? Pediatr Pulmonol 2020;55(5):1089–93. https://doi.org/10.1002/ppul.24721.

[46] Hatton A, Bergougnoux A, Zybert K, Chevalier B, Mesbahi M, Altéri JP, et al. Reclassifying inconclusive diagnosis after newborn screening for cystic fibrosis. Moving forward J Cyst Fibros 2022;21(3):448–55. https://doi.org/10.1016/j.jcf.2021.12.010.

[47] Terlizzi V, Dolce D. Variability of the sweat test in children with Cystic Fibrosis previously CRMS/CFSPID: A retrospective monocenter experience. J Cyst Fibros 2023;22(3):496–8. https://doi.org/10.1016/j.jcf.2023.04.018.

[48] Dolce D, Fevola C, Camera E, Orioli T, Lucenteforte E, Malanima MA, et al. Comparison between Gibson-Cooke and Macroduct Methods in the Cystic Fibrosis Neonatal Screening Program and in Subjects Who Are Cystic Fibrosis Screen-Positive with an Inconclusive Diagnosis. Int J Neonatal Screen 2023;9(3):41. https://doi.org/10.3390/ijns9030041.

[49] Svedberg M, Gustafsson P, Tiddens H, Imberg H, Pivodic A, Lindblad A. Risk factors for progression of structural lung disease in school-age children with cystic fibrosis. J Cyst Fibros 2020;19(6):910–6. https://doi.org/10.1016/j.jcf.2019.10.014.

[50] Ren CL, Fink AK, Petren K, Borowitz DS, McColley SA, Sanders DB, et al. Outcomes of infants with indeterminate diagnosis detected by cystic fibrosis newborn screening. Pediatrics 2015;135(6):e1386–92. https://doi.org/10.1542/peds.2014-3698.

[51] Dolce D, Claut L, Colombo C, Tosco A, Castaldo A, Padoan R, et al. Different management approaches and outcome for infants with an inconclusive diagnosis following newborn screening for cystic fibrosis (CRMS/CFSPID) and Pseudomonas aeruginosa isolation. J Cyst Fibros 2023;22(1):73–8. https://doi.org/10.1016/j.jcf.2022.07.007.

[52] Groves T, Robinson P, Wiley V, Fitzgerald DA. Long-term outcomes of children with intermediate sweat chloride values in infancy. J Pediatr 2015;166(6).

[53] McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. Eur Clin Respir J 2014:1. https://doi.org/10.3402/ecrj.v1.25898.

[54] Terlizzi V, Parisi GF, Ferrari B, Castellani C, Manti S, Leonardi S, et al. Effect of Dornase Alfa on the Lung Clearance Index in Children with Cystic Fibrosis: A Lesson from a Case Series. Children (Basel) 2022;9(11):1625. https://doi.org/10.3390/children9111625.

[55] Parisi GF, Cannata E, Manti S, Papale M, Meli M, Russo G, et al. Lung clearance index: A new measure of late lung complications of cancer therapy in children. Pediatr Pulmonol 2020;55(12):3450–6. https://doi.org/10.1002/ppul.25071.

[56] Kasi AS, Wee CP, Keens TG, Salinas DB. Abnormal Lung Clearance Index in Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID) Children with Otherwise Normal FEV1. Lung 2020;198(1):163–7. https://doi.org/10.1007/s00408-019-00307-3.

[57] Salinas DB, Sosnay PR, Azen C, Young S, Raraigh KS, Keens TG, et al. Benign outcome among positive cystic fibrosis newborn screen children with non-CF-causing variants. J Cyst Fibros 2015;14(6):714–9. https://doi.org/10.1016/j.jcf.2015.03.006.

[58] Quinton P, Molyneux L, Ip W, Dupuis A, Avolio J, Tullis E, et al. β-adrenergic sweat secretion as a diagnostic test for cystic fibrosis. Am J Respir Crit Care Med 2012 Oct 15;186(8):732–9. https://doi.org/10.1164/rccm.201205-0922OC.

[59] Salinas DB, Peng YH, Horwich B, Wee CP, Frisbee E, Maarek JM. Image-based β-adrenergic sweat rate assay captures minimal cystic fibrosis transmembrane conductance regulator function. Pediatr Res 2020;87(1):137–45. https://doi.org/10.1038/s41390-019-0503-8.

[60] Nguyen-Khoa T, Nguyen-Khoa T, Hatton A, Drummond D, Aoust L, Schlatter J, et al. Reclassifying inconclusive diagnosis for cystic fibrosis with new generation sweat test. Eur Respir J 2022;60(2):2200209.

[61] Zampoli M, Verstraete J, Nguyen-Khoa T, Sermet-Gaudelus I, Zar HJ, Gonska T, et al. β-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: Do we need new reference ranges? Pediatr Pulmonol 2023;58(1):187–96. https://doi.org/10.1002/ppul.26179.

[62] Twynam-Perkins J, Fall A, Lefferts JW, Urquhart DS. An innovative strategy for personalised medicine in a CFSPID case that evolved with time. Paediatr Respir Rev 2023;47:23–6. https://doi.org/10.1016/j.prrv.2023.06.001.

[63] McGarry ME, Ren CL, Wu R, Farrell PM, McColley SA. Detection of disease-causing CFTR variants in state newborn screening programs. Pediatr Pulmonol 2023;58(2):465–74. https://doi.org/10.1002/ppul.26209.

[64] Terlizzi V, Padoan R, Amato A, Campagna G, Castellani C, Salvatore M. Hidden CFSPID in CF patient registries? The Italian CF Registry experience. S1569-1993(23)00867-6 J Cyst Fibros 2023. https://doi.org/10.1016/j.jcf.2023.07.010.

[65] Terlizzi V, Claut L, Colombo C, Tosco A, et al. Outcomes of early repeat sweat testing in infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/CF screen-positive, inconclusive diagnosis. Pediatr Pulmonol 2021;56(12):3785–91. https://doi.org/10.1002/ppul.25683.

[66] Fevola C, Dolce D, Tosco A, aò.. Risk of CFTR-related disorders and cystic fibrosis in an Italian cohort of CRMS/CFSPID subjects in preschool and school age. Eur J Pediatr 2023. https://doi.org/10.1007/s00431-023-05359-5.

[67] Sermet-Gaudelus I, Brouard J, Audrézet MP, Couderc Kohen L, Weiss L, Wizla N, et al. Guidelines for the clinical management and follow-up of infants with inconclusive cystic fibrosis diagnosis through newborn screening. Arch Pediatr 2017;24(12):e1–14. https://doi.org/10.1016/j.arcped.2017.07.015.