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Original Article

Standards for the care of people with cystic fibrosis (CF): A timely and accurate diagnosis

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ABSTRACT

There is considerable activity with respect to diagnosis in the field of cystic fibrosis (CF). This relates primarily to developments in newborn bloodspot screening (NBS), more extensive gene analysis and improved characterisation of CFTR-related disorder (CFTR-RD). This is particularly pertinent with respect to accessibility to variant-

Abbreviations: CF, Cystic fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; CFSPID, CF Screen Positive, Inconclusive Diagnosis; CRMS, CFTR-Related Metabolic Syndrome; CFTR-RD, CFTR-Related Disorder; ECFS, European Cystic Fibrosis Society; EGA, Extended gene analysis; ICM, Intestinal current measurement; IRT, Immuno-reactive trypsinogen; MI, Meconium ileus; NBS, Newborn bloodspot screening; NPD, Nasal potential difference; NSWG, Neonatal Screening Working Group.

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Extended Gene Analysis (EGA)

specific therapy (VST), a transformational intervention for people with CF with eligible *CFTR* gene variants. This advance reinforces the need for a timely and accurate diagnosis. In the future, there is potential for trials to assess effectiveness of variant-specific therapy for CFTR-RD. The guidance in this paper reaffirms previous standards, clarifies a number of issues, and integrates emerging evidence. Timely and accurate diagnosis has never been more important for people with CF.

1. Introduction

This paper represents an update of previous guidance on diagnostic issues [1,2]. A timely and accurate diagnosis is important to reduce uncertainty and establish people with CF on appropriate care pathways. Although for most people with CF, this is relatively straightforward, some experience delays and it is important that systems are in place for a fast and accurate diagnosis. Expansion of newborn bloodspot screening (NBS) across the globe has facilitated early diagnosis and access to CF care for many infants with CF [3]. The significant positive impact of this public health strategy must be balanced with potential negative outcomes, such as a false positive NBS result, the recognition of carriers and the identification of infants with an unclear or indeterminate diagnosis [4].

NBS programmes employ a wide variety of protocols (laboratory tests), which result in distinct screening outcomes, especially regarding the extent of recognition of infants with an unclear diagnosis. Infants with an unclear or inconclusive diagnosis following a positive NBS test have been given the designation "Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-Related Metabolic Syndrome/CF Screen Positive, Inconclusive Diagnosis (CRMS/CFSPID)." Whilst this term has enabled a global harmonised approach to the reporting of these infants, it is unwieldy and generally is shortened to CFSPID in Europe and CRMS in the US [4]. The ability of an NBS programme to identify infants with CFSPID relates primarily to the extent of DNA analysis that is used in the NBS protocol. Protocols that use extended gene analysis (EGA) are likely to identify relatively more CFSPID infants compared to CF cases. Traditionally, *CFTR* gene analysis in NBS protocols involved a limited panel of known CF-causing variants. Widening availability of sequencing technologies and increasing use of EGA in NBS protocols results in the recognition of *CFTR* gene variants of unknown significance or characterised as resulting in varying clinical consequence [5]. In using a more expansive genetic approach, NBS programmes can choose to remain limited to a panel (albeit larger) of known CF-causing variants, or can report all variants identified, including those with unclear or unknown consequences. It is often difficult to accurately predict through modelling the molecular consequence of a variant of unknown significance [5].

A proportion of infants with a CFSPID designation will later develop disease consistent with a CF diagnosis; others are at increased risk of developing clinical features consistent with a CFTR-related disorder (CFTR-RD) but currently available data suggest the majority will experience no clinical sequelae from this finding. It is more than 10 years since the first description of CFTR-RD as a clinical entity to classify conditions that relate to CFTR dysfunction but do not fulfil a CF diagnosis [6]. The ECFS Standards of Care committee recently updated the definition of CFTR-related disorder [7].

Diagnosing CF is complex and requires clear guidance and pathways to facilitate early appropriate care but avoid unnecessary harm including psychological distress. The diagnostic journey for people with CF is vulnerable as financial constraints impact health services. For example, NBS programmes require regular careful monitoring to assure performance. This is best achieved through a central monitoring facility, but central coordination of results is susceptible to cuts in public health funding. In addition, basic equipment for diagnosis, such as sweat test kits, are often difficult to access in developing CF services and sometimes difficult to procure even in established services. More detailed physiological tests like nasal potential difference (NPD) or intestinal current measurement (ICM), are challenging and can only be reliably undertaken in centres with expertise and resources. Establishing specialist diagnostic hubs is essential to effectively process challenging cases. This is especially important, as new therapies emerge for patients with a clear diagnosis of CF. Variant-specific therapy (such as CFTR modulators) have been transformational for eligible patients, who can access these therapies. Patients with CFTR-RD may also become eligible for certain variant-specific therapy (VST), pending appropriate clinical trials.

A timely and accurate diagnosis remains important for people with CF and key to early access to treatment, at all ages. Here we describe consensus guidelines for achieving this.

2. Methods

At the ECFS Conference in 2022, we invited applications to join the core committee for this project, including representation from the European patient organisation, CF Europe. The multi-disciplinary core committee (listed in Supplementary Table 1) established the framework for this guidance and identified potential authors for each section. The framework was reviewed and ratified by the ECFS Board. Key parameters guiding the recruitment of authors were 1) expertise, 2) geography and 3) inclusivity.

The authors were instructed to follow an evidence-based hierarchy in developing the guidance and to prioritise systematic reviews. Participants were encouraged to reference existing ECFS guidance and to highlight developments since previous guidance [1,3,4,7-9].

For each section, authors were asked to provide two or three statements to highlight key messages, to act as a focus for policymakers. These statements were reviewed using a modified Delphi methodology. Participants from a range of backgrounds (listed in Supplementary Table 1) were asked to review the statements and state if they agreed or disagreed (yes/no/cannot answer). If they disagreed, they were asked to explain why and provide an alternative version. Consensus was achieved when $\geq$80% of contributors agreed with a statement (participants who ticked "cannot answer" were not included in the calculation, as stakeholders previously commented that a "cannot answer" response was not the same as a "disagree"). This threshold of $\geq$80% agreement has been used in previous Delphi exercises in CF [10].

All statements achieved consensus $\geq$90% after one round of consultation (see Supplementary Table 2 for detailed Delphi results). Despite the consensus on all statements, the core committee reviewed all comments on statements and no statements were deemed to require further edits. The consensus statements are presented in Table 1.

3. Standards of care for timely and accurate diagnosis

3.1. Diagnostic definitions and principles

Nicholas J. Simmonds, Olaf Sommerburg, Silvia Gartner

It is important to have a high standard of diagnostic evaluation for CF. Diagnostic confirmation is required for children and adults presenting with suggestive clinical features, but also in specific situations such as asymptomatic infants with a positive NBS test result or family history [8] (Statement 1). Clinical features strongly suggestive of CF include bronchiectasis, positive sputum cultures for a CF-associated pathogen (especially *Pseudomonas aeruginosa*), exocrine pancreatic insufficiency and obstructive azoospermia in males. Less specific, but equally important presentations include a persistent wet cough,

Table 1
Consensus statements. Key statements with >90% agreement achieved through a multi-stakeholder modified Delphi consensus exercise.

1	Individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history (affected sibling), require efficient and accurate diagnostic confirmation.
2	A sweat chloride concentration ≥ 60 mmol/L and/or two CF-causing variants in trans confirm a CF diagnosis.
3	Results supporting a diagnosis of CF should be promptly reported back to the patient and/or their parents/carers, with early CF follow-up arranged. Clear information about the disease and its management should be provided, and genetic counselling offered.
4	A newborn bloodspot screening (NBS) programme for CF should be designed to best address the geography, social health circumstance and ethnicity of the population in that region.
5	The responsible parties for NBS programmes should annually monitor and report their programme's performance using the ECFS-defined key outcome parameters to achieve the ECFS standards (at a minimum).
6	The sweat test remains the diagnostic gold standard for CF and should be performed according to the ECFS standards.
7	In people with CF, the CFTR genotype should always be investigated to determine whether variant-specific therapy may be indicated.
8	If the criteria for CF are not met, and clinical consideration of a diagnosis remains, further CFTR functional tests in a specialist diagnostic hub are required.
9	Infants with a CRMS/CFSPID designation should be evaluated and managed according to ECFS standards [4].
10	Individuals with CFTR-RD should be evaluated and managed according to revised ECFS standards.
11	CF carrier testing and screening should only aim to identify CF-causing variants.
12	Raising concerns about future health risks for *CFTR* carriers may be premature until more consistent data are available.

suboptimal weight gain in childhood, acute recurrent or chronic pancreatitis, chronic rhinosinusitis, nasal polyps, allergic bronchopulmonary aspergillosis, and salt loss (Pseudo-Bartter syndrome). Accurate diagnosis of CF requires high quality sweat testing, *CFTR* gene analysis, and resources to undertake detailed clinical assessment. The clinical assessment should include respiratory culture for CF-specific pathogens, age-appropriate pulmonary function testing, lung imaging, indirect exocrine pancreatic function testing and sperm count/vas deferens evaluation in males [8,9]. CF is confirmed when the sweat chloride concentration is ≥ 60 mmol/L and/or two CF-causing variants are identified (Statement 2). The *CFTR* gene variants should be demonstrated to be on separate chromosomes (*in trans*). If the criteria for CF are not met, further CFTR functional tests should be performed (for example, NPD or ICM) [8].

The previous standards for reporting a confirmed diagnosis remain appropriate, for both symptomatic and asymptomatic patients [1]. These include the prompt reporting of the result to the patient or parents/carers by a CF physician, ideally within 24 h of receiving the confirmatory result [1]. Clear written and verbal information about the disease (including reliable online resources) should be provided and the family/person with CF provided with contact information for the CF team. An early follow-up appointment with the CF team should be arranged, ideally within a week. At this meeting, the model of care should be described, including treatment options such as VST and the potential for involvement in clinical trials. Genetic counselling should be offered to the direct family [11] and information provided for the extended family (Statement 3).

CF teams need to be cognisant of the emotional distress caused by the CF diagnostic journey and should support families accordingly. Providing clear and consistent information in line with ECFS guidance is essential, using multi-media resources when available. Inconsistent advice undermines faith in healthcare systems and workers [12]. Dedicated and regular contact with the CF psychologist is important, as well as access to other members of the CF team to discuss different aspects of CF care. This can provide space and acknowledgement for expressions of grief and disappointment, as well as clarity and education that can help families manage needs and expectations. Genetic counselling plays an important role not just at the time of diagnosis, but also during discussions around future family planning. Health care providers should also consider early routine mental health screening for parents. Given the evidence that caregiver stress can negatively impact many aspects of child development, caregiver emotional health should be addressed as soon as possible [13]. This can lead to greater resilience, effective coping, and ultimately better health outcomes for the whole family. Additionally, ambiguous diagnostic results (for example, evaluation for CRMS/CFSPID and CFTR-RD) and late diagnosis in adult life can cause emotional distress and negative psychological impact. Such patients need similar proactive and consistent treatment protocols as people with CF, and mental health follow-up to ensure their emotional and physical support. There should also be a recognition that sometimes inconclusive situations remain unresolved, even following more extensive testing in a diagnostic hub.

3.2. Screening approaches

Jürg Barben, Silvia Gartner, Nataliya Kashirskaya, Anne Munck, Olaf Sommerburg

NBS for CF is established in many countries and regions, with good evidence of impact on outcomes. The selection of the appropriate NBS protocol for a region or country must reflect the population screened (especially ethnicity and *CFTR* gene variant frequency), geographical circumstances and healthcare systems [3] (Statement 4). The advantages and disadvantages of different NBS protocols must be weighed against each other in each country and region. The selected programme should be equitable and should minimise harms. The measurement of immuno-reactive trypsinogen (IRT) on a dried bloodspot sample in the first week of life remains the initial screening step for all protocols. From there, specificity is improved via a variety of second and third tier testing, for example using DNA analysis for *CFTR* gene variants or measurement of Pancreatitis Associated Protein (PAP), another marker of CF. The introduction of EGA to detect *CFTR* gene variants has improved specificity but also the potential to significantly increase identification of infants with a CRMS/CFSPID designation [3,9].

NBS programmes require regular review of outcomes and performance [14] for quality improvement. Performance should be monitored annually using the key outcome parameters defined by the ECFS Neonatal Screening Working Group (NSWG) to achieve the ECFS standards (as a minimum). These standards remain essentially unchanged with some clarification of definitions, especially for the calculation of sensitivity (Supplementary Table 3) [15] (Statement 5). Strategies for collecting accurate and long-term data relying on ECFS key outcome parameters should be implemented [3].

The complexity and multi-agency nature of NBS for CF can affect timeliness, with programmes vulnerable to a number of factors, including health inequality through poverty, that negatively impact on the processing of a positive result [14,16,17]. It is important that efforts are made to identify systemic problems and address these to ensure that a programme is achieving, as a minimum, ECFS standards on timeliness [1,2]. At the moment many NBS programmes are not [3].

The evaluation of missed cases is important to assess quality and to compare the performance of NBS programmes (Statement 5). There are different approaches to the reporting of missed cases, ideally this should be through a centralised data repository. A recent exercise undertaken by the ECFS NSWG has clarified the definition of a missed case (Supplementary Table 3). Missed cases are children and adolescents with a diagnosis of CF who were tested but not diagnosed by the NBS programme. Missed cases are divided into 1) false negatives cases protocol related (analytical issues), and 2) false negatives cases non-protocol related (pre- and post-analytical issues). A new consideration with respect to missed cases is *in utero* exposure to modulator therapy. Most women with CF will continue modulator therapy through pregnancy. If the baby is affected by CF, the IRT level may be below the cut-off for

second tier testing. This represents a false negative result and could be considered either pre-analytical (as the infants IRT has been lowered by the *in utero* exposure) or analytical (as the IRT measured is falsely lowered). It is apparent that altering IRT cut-off levels is not appropriate on the basis of these rare cases. Women with CF and the Adult CF teams should be aware of this potential situation and not falsely reassured by a negative NBS test result. Linking mother and child with the relevant local Paediatric CF service for a timely assessment is important.

Infants with meconium ileus (MI) diagnosed with CF shortly after birth, who have a false negative NBS result, need to be reported but should be analysed separately. Sensitivity should be calculated from the total number of missed cases (group 1 and 2 above), not including those with MI.

For quality improvement, separate analyses should be undertaken using both NBS protocol related (analytical issues) and NBS non-protocol related (pre- and post-analytical issues) results, to better identify the underlying issues with the programme.

Pre-conceptual or antenatal carrier screening programmes can identify CF cases *in utero*, but availability is limited to just a few regions globally. Such programmes can work alongside NBS programmes.

3.3. Diagnostic standards

Carlo Castellani, Karen Raraigh, Lutz Nährlich, Isabelle Sermet-Gaudelus, Nicholas J. Simmonds

3.3.1. Sweat test standards

The sweat test remains the diagnostic gold standard for CF and should be performed by experienced personnel (who perform ≥80 sweat tests per annum) using equipment approved for diagnostic use and following national or international guidelines, including quality assurance for analytes [9] (Statement 6). Sweat should be collected following quantitative pilocarpine iontophoresis over 30 min for adequate sweat production rate and sufficient quantity (15 μL for the Macroduct™ system). A sweat chloride of ≥ 60 mmol/L is consistent with CF and < 30 mmol/L makes CF unlikely. A sweat chloride of 30–59 mmol/L or sweat conductivity ≥50 mmol/L should trigger further evaluation including *CFTR* gene mutation analysis. Measurement of sweat conductivity is not sufficient as a single test to establish a CF diagnosis, but a normal result may be meaningful to exclude a CF diagnosis (when sweat conductivity < 50 mmol/L) [18].

3.3.2. Genetic testing standards

Recommendations for *CFTR* gene testing in the 2018 ECFS Standards of Care remain valid [1]. Additional recommendations resulting from new evidence, or areas not previously highlighted, are listed below.

In April 2023, the number of *CFTR* gene variants characterised in the CFTR2 database has risen to 804, of which 719 are labelled as CF-causing (www.cftr2.org). Although most of the newly characterised variants are rare, the vast majority of people with CF (>98%) will be able to find information about at least one of their variants on the CFTR2 website. Aggregated data on clinical phenotype are often reported and are informative, but should not be used to predict individual outcomes for people with CF [19]. Identification and characterisation of *CFTR* gene variants in non-Caucasian populations can be more challenging and this area needs further research.

Even though a positive sweat test with concomitant positive NBS or consistent clinical manifestations is sufficient to make a CF diagnosis, the *CFTR* genotype should be investigated to determine whether VST may be indicated [10] and for genetic counselling purposes (Statement 7). Some *CFTR* gene variants, characterised as CF-causing, are associated with intermediate or even normal sweat chloride values.

3.3.3. Further electrophysiological measures

Additional measures of CFTR activity may be informative when there is clinical concern and diagnostic testing is inconclusive (for example, genetic analysis identifies a variant of unknown significance, and/or the sweat chloride concentration is intermediate). A number of CFTR functional tests are available. These include measurement of 1) the transepithelial voltage changes across the nasal epithelium (NPD), 2) the short circuit current of freshly excised rectal tissue (ICM) and, 3) beta-adrenergic sweat secretion [20]. These tests have limited availability and are restricted to specialist diagnostic hubs with appropriate expertise and resources (Statement 8).

3.4. Clarifying unclear situations

Carlo Castellani, Isabelle Sermet-Gaudelus, Nicholas J. Simmonds

3.4.1. CRMS/CFSPID designation; definition, evaluation, and management

CRMS/CFSPID is defined as "an infant with a positive CF NBS result and either a sweat chloride value <30 mmol/L and 2 *CFTR* variants, at least one of which has unclear phenotypic consequences, or a sweat chloride in the 30–59 mmol/L range and one or no CF-causing variants" [21]. Infants with a CRMS/CFSPID designation should be evaluated and managed according to ECFS standards [4] (Statement 9).

These infants are well but may develop clinical features consistent with a diagnosis of CF or may be re-classified following new information on their genotype [7,22]. They must be evaluated and regularly reviewed by CF physicians. The initial assessment should include clinical evaluation, sweat testing, and extended *CFTR* gene analysis [23,24]. In the first two years of life, the frequency of clinical reviews depends on the wellbeing of the infant [23,24] and in pre-school years it should be at least annual. At six years of age, a more extensive evaluation can help determine if the child should continue regular CRMS/CFSPID follow-up or if they can be discharged to primary care with clear information. For children discharged to primary care, a specialist review planned for adolescence (14–16 years) to engage directly with the young person may be appropriate [4].

Families must have clear information on all possible outcomes and be aware of clinical manifestations, such as persistent cough, sino-nasal disease or recurrent abdominal pain which require review by the CF team.

Clear and consistent communication is key to support families of infants with a CRMS/CFSPID designation, especially if the infant converts to diagnosis of CF. In these cases, families should be aware of the possibility of this occurring and given information in a manner that does not make them feel at fault or suggest a diagnosis has been missed by healthcare professionals. In some cases, a reclassification may result from new information being available on a *CFTR* gene variant, which suggests the variant is CF-causing. Again, this needs to be explained clearly with careful use of terms.

It is not appropriate for infants with a CRMS/CFSPID designation to be included on a registry as a CF patient, but it may be possible to use existing CF registry framework to provide a database. A database for infants with a CRMS/CFSPID designation is a key tool to understand better the life journey and outcomes for these children. The ECFS patient registry (ECFSPR) team, in partnership with the NSWG, is currently establishing a CFSPID registry.

3.4.2. CFTR-related disorders; definition, evaluation, and management

CFTR-related disorder (CFTR-RD) is defined as "a clinical entity with features of CF and evidence of CFTR dysfunction but where the diagnostic criteria for CF are not met" [7]. Recent ECFS guidelines provide guidance on diagnosis, evaluation, and management of CFTR-RD [7]

- the mode of inheritance of the disease
- the *a priori* risk for healthy individuals to be CF carriers
- disease spectrum associated with *CFTR* gene variants and the therapeutic options
- the extent of the test offered and its limitation
- the outcomes of a positive and negative genetic test
- the risk of having a child with CF depending on the result, including the residual risk
- the options for reproductive planning, including prenatal and preimplantation diagnosis
- when referral to a CF specialist is appropriate

Fig. 1. Key topics and messages to include when counselling potential CF carriers.

(Statement 10). CFTR dysfunction compatible with a diagnosis of CFTR-RD is defined as 1) Evidence of CFTR dysfunction *in vivo* or *ex vivo* in at least two different CFTR functional tests, OR 2) One *CFTR* variant known to reduce CFTR function and evidence of CFTR dysfunction *in vivo* or *ex vivo* in at least two functional tests, OR 3) Two *CFTR* variants shown to reduce CFTR function, with at most one CF-causing variant.

The level of residual CFTR function required for the diagnosis of CFTR-RD is between 10 and 30% of normal [25]. However differentiating CFTR activity in patients with CFTR-RD from CF and heterozygotes remains a challenge for individual cases. Although VST is not approved for patients with CFTR-RD and there is no evidence of efficacy in this population, research in this area is required as CFTR-RD patients regularly harbour variants that may benefit from VST [10].

3.5. Carrier information

Carlo Castellani, Emmanuelle Girodon and Silvia Gartner

3.5.1. Role of counselling

The role of genetic counselling in CF is to provide non-directive information as well as psychological support, to facilitate informed decision-making, both before and after the genetic testing [11,26-29]. It may be provided by genetic counsellors [11] or healthcare professionals who have skills and training in basic principles of genetics and genetic counselling together with knowledge of the disease. There are essential topics and messages that should be included in information to CF carriers (Fig. 1).

CF carrier testing has long been offered to parents of a child with CF, relatives of any patient (having CF, a CFTR-RD or CFSPID) or individual carrying a CF-causing variant and their partners. Carrier testing is also increasingly part of general preconception screening programmes [30–32]. Unsolicited findings of *CFTR* variants after genome analysis may also be a challenging source of referrals to genetic counselling [33]. Importantly, CF carrier testing/screening should ideally only target CF-causing variants with a high penetrance for CF [34] (Statement 11).

3.5.2. Potential risks associated with carrier status

CF carriers may be detected through NBS, family cascade testing, preconception or prenatal carrier screening or diagnostic workup for CF or CFTR-RD. The high frequency of *CFTR* gene variants in Northern European populations is speculated to be connected with an as yet undetected heterozygote advantage mechanism [35].

Carrier status is associated with an increased risk of having children with CF. Studies identifying carriers have demonstrated slightly increased sweat electrolyte concentrations [36] and higher IRT levels in carrier infants identified from CF NBS tests [37].

Population studies on heterozygotes have reported an increased relative risk to develop a number of conditions, including some cancers, with a low absolute risk for each condition [38–42]. However, these results are partially contradictory, there is a lack of data about *CFTR* gene testing, and some compound heterozygotes could have been missed [40,43,44]. If suggested associations are confirmed, the increased risk might be connected to multifactorial circumstances like influence of other genes and environmental factors [25].

CFTR heterozygotes have so far been considered “healthy carriers”. Regarding them as individuals at increased risk for some, often late onset, disease manifestations would pose societal and ethical challenges [33]. This theme requires further consideration and genetic counselling should be cautious with respect to longer term risks until more data are available (Statement 12).

4. Conclusion

There is considerable activity with respect to diagnosis in the field of CF. This paper collates various ECFS projects, reaffirming recent guidance. The activity relates primarily to developments in NBS, more extensive gene analysis and improved characterisation of CFTR-RD. This is particularly pertinent with respect to VST, which has been transformational for people with CF with eligible *CFTR* gene variants who are able to access these therapies. There is a need for trials to assess effectiveness for CFTR-related disorders.

The guidance in this paper reaffirms previous standards, clarifies a number of issues, and integrates emerging evidence. Timely and accurate diagnosis has never been more important for people with CF.

Author credit

The core committee (listed in Supplementary Table 1) established the framework for the exercise and identified experts to produce each section (highlighted in the paper). All had oversight of the final paper.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.09.008.

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