



Original Article

ECFS standards of care on CFTR-related disorders: Updated diagnostic criteria



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ABSTRACT

This paper is the first in a series providing updated guidance on the definition, evaluation and management of people with a Cystic Fibrosis Transmembrane conductance Regulator (CFTR)-Related Disorder (CFTR-RD). The need for this update relates to more precise characterisation of *CFTR* gene variants and improved assessment of CFTR protein dysfunction. The exercise is co-ordinated by the European CF Society Standards of Care Committee and Diagnostic Network Working Group and involves stakeholder engagement. This first paper was produced by a core group using an extensive literature review and papers graded for their quality. Subsequent wider stakeholder agreement was achieved.

The definition of a CFTR-RD remains "a clinical condition with evidence of CFTR protein dysfunction that does not fulfil the diagnostic criteria for CF". Clearer guidance on CFTR dysfunction and relevant *CFTR* variants will be provided. Thresholds for clinical presentations are presented and the paradigm that pathobiological processes may be evident in more than one organ is agreed. In this paper we reflect on the early patient journey, highlighting that CF specialists as well as other relevant specialists should be involved in the care of people with a CFTR-RD.

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1. Introduction

The concept of a wide range in CFTR (Cystic Fibrosis Transmembrane conductance Regulator) function, from zero to almost normal, is widely accepted [1,2]. This fits our knowledge of more than 2000 different *CFTR* gene variants, many leading to some degree of CFTR protein dysfunction by interfering with the normal route of protein synthesis, folding, trafficking, activation or membrane stability, with possible combinations of these abnormalities [3]. Residual levels of CFTR function are associated with a range of clinical

manifestations which extend beyond what is recognized as the disease cystic fibrosis (CF) [4,5]. At the mild end of the phenotypic spectrum are a group of clinical conditions called CFTR-related disorders (CFTR-RDs) [4,6,7].

CFTR-RDs constitute a small but significant share of the workload in many CF centers. They are probably under-diagnosed, given their non specific symptomatology. In fact, the correct identification and adequate clinical monitoring of patients with CFTR-RD is often limited by clinical practice insufficiently informed by clear guidelines. It is indeed difficult to delineate exactly where CF stops and CFTR-RD begins and not infrequently these patients receive inconsistent messages [6–18].

The European Cystic Fibrosis Society (ECFS) and its Diagnostic Network Working Group assembled a panel of experts to produce standards of care for the diagnosis and management of CFTR-RD. This document is the first of a sequence of four papers dedicated to this topic.

Abbreviations: ABPA, Allergic BronchoPulmonary Aspergillosis; CBAVD, Congenital Bilateral Absence of the Vas Deferens; CFSPID, CF Screen Positive, Inconclusive Diagnosis; CFTR, Cystic Fibrosis Transmembrane conductance Regulator; CFTR-RD, CFTR-Related Disorder; CRMS, CFTR-Related Metabolic Syndrome; ICM, Intestinal Current Measurement; NBS, Newborn Screening; NPd, Nasal Potential Difference; VUS, Variant of Uncertain Significance.

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2. Establishing a name and a clear definition

Since first descriptions of CF [19–21], the awareness of the multi-organ expression of this complex disease has increased and its clinical phenotype meticulously described. In the second half of the last century an increasing number of peculiar cases, usually mild and associated with mono-organ manifestations and inconclusive sweat test results, were reported. This trend increased after the discovery of the *CFTR* gene [22–28] and the identification of the most frequent variant F508del [29]. Indeed, reports of additional often rarer or even unique *CFTR* gene variants followed, many associated with mild disease phenotypes [30]. Evidence was collected of an association between *CFTR* gene variants and Congenital Bilateral Absence of the Vas Deferens (CBAVD), some cases of chronic or acute recurrent pancreatitis and disseminated bronchiectasis [31–45].

In 2000 a WHO statement specified that “where a (*CFTR*) variant is known to be aetiologically related to the disease in an individual, the primary diagnostic code should be assigned from the section which includes CF” [46]. This section was named “Cystic Fibrosis and Related Disorders” and included: classic CF pancreatic insufficient; classic CF pancreatic sufficient; atypical CF; CF other specified; isolated obstructive azoospermia; chronic pancreatitis; allergic bronchopulmonary aspergillosis (ABPA); disseminated bronchiectasis; diffuse panbronchiolitis; sclerosing cholangitis; neonatal hypertrypsinogenemia.

Another significant event was a meeting, organized by the European Cystic Fibrosis Society with the partnership of the European Society of Human Genetics, and the EuroGentest Network of Excellence, with the purpose to provide CF clinicians with information on the optimal use of CF genetic testing [7]. The expert panel agreed to abandon alternative terms like atypical CF or *CFTR*-opathies and proposed the denomination *CFTR*-related disorders, defined as “clinical entities associated with *CFTR* variants, but where a diagnosis of CF cannot be made by the current standard diagnostic criteria”. A more genetically orientated consensus by EuroGentest and the CF Network suggested a very similar definition: “*CFTR*-related disorders ... are clinical entities associated with *CFTR* dysfunction but where the diagnosis of CF cannot be unambiguously established”, which for the first time included the notion of *CFTR* dysfunction [25].

These meetings drove the decision to produce specific *CFTR*-RD recommendations and in 2011 a group of clinicians and geneticists with expertise in CF clinical manifestations, diagnosis and *CFTR* variants published what has since been the reference paper on *CFTR*-RD. They defined the condition as “a clinical entity associated with *CFTR* dysfunction that does not fulfil the diagnostic criteria for CF” [6]. The 2011 recommendations emphasized the relationship between genotype, *CFTR* function and clinical phenotype, in a continuum of clinical presentation from severe disease to milder forms. They validated the use of functional tests when genetic results were not conclusive, indicating that *CFTR*-RD was associated with less *CFTR* dysfunction than CF. They unified so called “atypical”, “borderline”, “non classical”, “mild” forms in clear entities, i.e. CBAVD, disseminated bronchiectasis, chronic pancreatitis. Additionally, they provided algorithms based on genetic and functional testing to guide the clinical diagnosis workflow.

3. The need for updated recommendations

The 2011 document was a major step forward, and augmented awareness of *CFTR*-RD in the medical community, including not only CF specialists, but also general pulmonologists, fertility experts, gastroenterologists and clinical geneticists. Diagnosis rates have steadily increased with some large CF centres, like the Royal

Brompton Adult CF Centre in London, now with dedicated *CFTR*-RD clinics [47].

In the ten years since the publication of the recommendations there have been significant advances in our knowledge of the clinical, functional and molecular aspects of *CFTR*-RD, necessitating updated recommendations.

3.1. The rise of non mutation-specific analysis

Wider availability of Next-Generation Sequencing (NGS) has prompted many clinicians, particularly those who have become increasingly aware of the existence of a *CFTR*-RD population, to ask for *CFTR* exonic sequencing on a routine basis, instead of *CFTR* panels with well-characterized *CFTR* gene variants. This inevitably has revealed a large number of variants of uncertain significance (VUS), which may limit the clinical utility of the genetic information and has resulted in a need for additional *in vitro* characterization of the impact of these variants on *CFTR* function [48–65].

3.2. Databases to assess disease liability of *CFTR* variants

The Clinical and Functional Translation of *CFTR* (*CFTR*-2) project has been created to assess the disease-liability of *CFTR* sequence variations, and to expand the number of known CF-causing variants [5,66,67]. More than 450 variants have been annotated and the clinical and functional data associated with them have been available since 2012 at www.cftr2.org. More than eighty of these variants are of variable or unknown clinical significance. Variants labelled as CF-causing are exclusively associated with CF, whereas those named “variants of varying clinical consequence (VVCC)” can be found either in individuals with CF, *CFTR*-RD or no disease. A third category, the so called “non CF-causing” variants, usually have no clinical consequences, but in some cases may be associated with *CFTR*-RD [5,66,67].

*CFTR*2 collects clinical and genetic data from CF patient registries, which are not expected to include *CFTR*-RD. In contrast, *CFTR*-France, another large database (<https://cftr.iurc.montp.inserm.fr/cftr>), includes genetic records and updated phenotypic information not only from individuals with CF but also from *CFTR*-RD patients, fetuses with bowel anomalies on ultrasound, newborns awaiting clinical diagnosis, and importantly also asymptomatic compound heterozygotes and spouses explored for *in vitro* fertilization. The database contains 906 variants, and classification differs from that used by the *CFTR*2 project [68], going from non disease causing to disease causing and including a 4th class named *CFTR*-RD variant represented by VVCC with an assumed high penetrance for *CFTR*-RD.

3.3. Measures of *CFTR* dysfunction

CFTR dysfunction constitutes an essential component of the definition proposed by the 2011 recommendations [6]. However, the document gives limited information on how such dysfunction can be measured in the individual as well as on the thresholds of *CFTR* activity compatible with a *CFTR*-RD diagnosis. The clinician considering a potential diagnosis of *CFTR*-RD needs to support this with evidence from biomarkers of *CFTR* dysfunction (see below) and must be able to interpret their diagnostic ranges, as well as information on consistent genotypes. Since 2011, our understanding of the mechanisms and levels of dysfunction of many *CFTR* variants has improved and today this information can to a certain extent be provided via the above databases [69–82].

3.4. Inconclusive diagnosis after newborn bloodspot screening for CF

An emerging area of concern is the outcome of infants with an unclear diagnosis following a positive newborn bloodspot screen-

ing (NBS) result. Infants in this situation are given the internationally agreed designation of CFTR-related metabolic syndrome/CF Screen Positive, Inconclusive Diagnosis (CRMS/CFSPID) [83]. These asymptomatic infants often have *CFTR* gene variants of varying clinical consequence that have been associated with CFTR-RDs [83–86]. Additionally, these infants sometimes have an intermediate sweat chloride value on sweat testing, suggestive of some CFTR dysfunction but not fulfilling the diagnostic threshold for CF [83,84,87,88]. Some infants with CRMS/CFSPID will evolve to a diagnosis of CF, as assessed by an increase in the sweat chloride level to greater than 59 mmol/L and the emergence of clinical features consistent with CF [87–95]. Most do not convert to a CF diagnosis and some have a risk to develop CFTR-RD later in life. The level of risk to develop CFTR-RD or CF is currently not known, requires further research and will be considered in more detail in a subsequent paper in the series.

3.5. More CFTR related disorders?

The original CFTR-RD recommendations focus on three clinical entities: congenital bilateral absence of the vas deferens (CBAVD), chronic or acute recurrent pancreatitis and disseminated bronchiectasis [6]. Unusually high levels of sweat chloride and unexpected frequencies of *CFTR* variants have been reported or confirmed in other disorders, including chronic rhinosinusitis [24,88,96–98], ABPA [99–103], non allergic asthma [104], primary sclerosing cholangitis [105–109], aquagenic wrinkling [110–112] and isolated metabolic alkalosis [92,93] and it is presently unclear if these conditions may be considered a CFTR-RD.

These issues underpin the need for updated diagnostic criteria for CFTR-RD [113–117]. Furthermore, some topics which were briefly discussed or not included in the 2011 recommendations pose relevant questions that need to be addressed. Should people with CFTR-RD be followed, and if so by whom, where and how? What treatment if any should be proposed, and is there a role for CF-specific medicines in the treatment of CFTR-RDs? If follow-up and treatment are deemed necessary, how specific for each disorder should they be? This ECFS Standards of Care and Diagnostic Network Working Group project has planned a series of documents that will be developed to meet these needs and answer these questions. This first article is concerned with the diagnosis of CFTR-RD.

4. Development plan of the updated CFTR-RD recommendations

4.1. Participants and output

A six-member committee (CC, EDW, KDB, IS, NJS, KWS) designed the workplan of the guidelines. The committee established workgroups of 46 international experts from 13 countries. Participants were recruited according to their clinical knowledge of specific disorders, experience in CFTR function studies and familiarity with interpretation of clinical liability of *CFTR* variants. Patients with CFTR-RD were also involved, where appropriate. The initial intention to convene a large consensus conference of all those involved had to be abandoned due to inappropriateness of travel and physical meetings during the COVID-19 pandemic.

The plan provides for four documents, of which the present one is the first: 1) diagnosis; 2) biomarkers of CFTR function; 3) specific disorders; 4) objectives for the future.

The second document in this series, an article titled “ECFS standards of care on CFTR-related disorders: diagnostic criteria of CFTR dysfunction”, gives an in-depth review on how biomarkers of CFTR function can be utilized to diagnose CFTR-RD. The biomarkers include CFTR variants, sweat test, nasal potential difference (NPD)

and intestinal current measurements (ICM). Future perspectives for other sweat test techniques and in vitro tests from primary cells are also considered. The third document will discuss diagnosis and management peculiarities of well-established CFTR-RD like CBAVD, disseminated bronchiectases, chronic or acute recurrent pancreatitis. Conditions like ABPA, chronic rhinosinusitis, primary sclerosing cholangitis and aquagenic wrinkling will also be discussed as potential CFTR-RD. The fourth document will consider other important topics related to CFTR-RD, including reproductive risk and genetic counselling, the potential for evolution from CFSPID to CFTR-RD, clinical trials and dedicated registries. An overview of the contents of the documents is shown in table 1.

The drafts of all four papers produced by the CFTR-RD guidelines project are shared amongst the contributors of the papers and members of the ECFS Diagnostic Network Working Group for comments and final assessment. Suggestions considered relevant by the committee were included in the final version of the manuscript.

4.2. Search strategy

To ensure a comprehensive review of relevant literature, a systematic search strategy was undertaken. The search yielded 3140 references, of which 2466 were discarded as not relevant or overlapping. The remaining 674 references were reviewed by the core group and an additional 540 discarded. This resulted in 134 relevant papers. These were independently assessed for quality using a bespoke tool by five members of the core group. Three papers scored inconsistently (> 3 point variance) and adjudication by an independent reviewer (KWS) gave the lowest score. Ten papers were judged high quality (score 8–10) and 31 medium quality [4–7]. Quality rank guided the weight given to each paper in the guidance, although papers were not excluded on the basis of their score (Fig. 1 supplementary material).

5. Defining a CFTR-related disorder

The three components of the definition of CFTR-related disorders are:

- 1) Presence of specific clinical features (phenotypes);
- 2) Exclusion of a diagnosis of CF;
- 3) Evidence of partially functioning CFTR protein, whose lack of activity does not reach CF thresholds [6].

These three attributes delineate the CFTR-RD boundaries, but contain elements of ambiguity and need to be further defined to be used in clinical practice:

5.1. 1a) Presence of specific phenotypes: expansion of CFTR-RD clinical manifestations

The expanding body of phenotypic and genotypic information suggests that the clinical spectrum of diseases potentially associated with CFTR dysfunction might be larger than just CBAVD, acute recurrent or chronic pancreatitis and disseminated bronchiectasis. A raised frequency of *CFTR* variants and results of sweat tests, NPD or ICM outside of normal limits have been reported for other conditions, that may also be found in CF, such as ABPA, chronic rhinosinusitis, primary sclerosing cholangitis and aquagenic wrinkling [8,24,92,93,96–103,105–112]. It seems reasonable to speculate that in selected cases these conditions could be related to CFTR dysfunction, possibly in association with other risk factors. A thorough review of the literature and of available data and clear decision criteria are needed to understand if some individuals with these disorders could have CFTR-RD, which will be examined in the third document of this series.

Table 1
Plan of the CFTR-RD recommendation documents.

Topics	Contents
1) DIAGNOSIS	
Definition of CFTR-RD	Strengths and weaknesses of the current diagnostic criteria Development of the current diagnostic criteria CFTR-RD and multi-system disease
The diagnostic process	The clinical suspicion of CFTR-RD Exclusion of non CFTR related etiologies Who makes the diagnosis Assessment after diagnosis General considerations
Follow-up and treatment	
2) BIOMARKERS OF CFTR FUNCTION	
CFTR variants	CFTR genotype as a surrogate marker for CFTR-RD
Sweat test	Sweat chloride concentration as a surrogate marker for CFTR-RD Other sweat tests
Electrophysiology tests	NPD relevance in CFTR-RD ICM relevance in CFTR-RD Relevance in CFTR-RD of <i>in vitro</i> tests in primary cells Variability of results and range of values
3) THE SPECIFIC DISORDERS	
Congenital bilateral Absence of the Vas Deferens (CBAVD)	Definition and diagnosis of CBAVD as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of CBAVD
Bronchiectasis	Definition and diagnosis of Bronchiectasis as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of Bronchiectasis
Recurrent pancreatitis	Definition and diagnosis of Pancreatitis as a CFTR-RD Alternative to CFTR-RD Recommendations for follow-up and treatment of Pancreatitis
Allergic Bronchopulmonary Aspergillosis (ABPA)	Can some cases be considered CFTR-RD? If so: Definition and diagnosis of ABPA as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of ABPA
Upper airways disorders	Can some cases be considered CFTR-RD? If so: Definition and diagnosis of UAD as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of UAD
Primary Sclerosing Cholangitis (PSC)	Can some cases be considered CFTR-RD? If so: Definition and diagnosis of PSC as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of PSC
Aquagenic Wrinkling (AW)	Can some cases be considered CFTR-RD? If so: Definition and diagnosis of AW as a CFTR-RD Alternative diagnosis to CFTR-RD Recommendations for follow-up and treatment of AW
4) OBJECTIVES FOR THE FUTURE	
CFTR-RD registry	Necessity and criteria to establish a CFTR-RD Patient Registry Independent or included in CF registries? Source of data and quality monitoring Towards a homogeneous ICD classification
CFSPID	Similarities and differences between CFSPID and CFTR-RD Is there evidence supporting evolution from CFSPID to CFTR-RD? Information to parents
Barriers/ opportunities to implementation	How different health systems can implement guidelines Stakeholders and their support for implementation Implementation monitoring plan
Dissemination	Strategies to reach and inform stakeholders and non CF physicians Endorsement by scientific societies
Clinical trials and their endpoints	Peculiarities Questions to be answered, endpoints, outcomes Sustainability
Reproductive risk	Does the autosomal recessive paradigm apply? What sort of testing, if any should be proposed? Would cascade testing be appropriate?

1 NPD: Nasal Potential Difference.

2 ICM: Intestinal Current Measurement.

5.2. 1b) Presence of specific phenotypes: multi-system phenotypes

A critical element of the 2011 recommendations was the description of CFTR-RD as a mono-organ condition. The question of whether manifestations of disease in two or more organs with evidence of CFTR dysfunction (but not fulfilling the standard CF diagnostic criteria), should be considered CFTR-RD or an unusual form of CF was left unresolved.

In real-life practice, when organ-specific investigations are performed it is not unusual to find clinical evidence of disease elsewhere, the most evident example being the sweat gland epithelium, as CFTR-RD sweat chloride levels are commonly in the intermediate range between 30 and 59 mmol/L. Radiological evidence of pulmonary hyperinflation and of sinus disease and functional evidence of airway obstruction have been reported in males with CBAVD [118]. Notably, the identification of disease in other organs

Clinical features associated with CF and CFTR-RD and the relationship with CFTR dysfunction

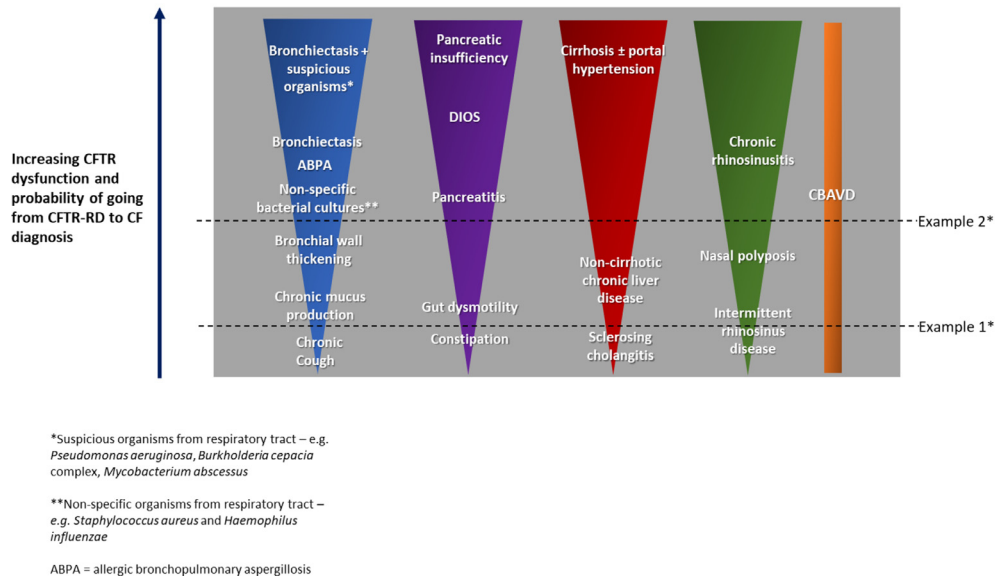


Fig. 1. Hierarchical spectrum of symptoms and conditions present at increasing levels of CFTR dysfunction

The figure shows the potential for poly-organ disease at the CFTR functional levels associated with CFTR-related disorder as different organs can be affected at the same level of CFTR dysfunction (i.e. the same horizontal level on the figure). Not all organs are affected every time. The triangles indicate increasing severity of involvement of that organ (note: CBAVD is not given a triangle as it is not progressive). Aquagenic wrinkling is not included as the relationship with CFTR function is unclear. *Example 1: a male patient with CBAVD, a chronic cough (but no bronchiectasis) and intermittent rhinosinus symptoms. Example 2: a female patient with pancreatitis and nasal polyposis (but no lower respiratory tract involvement).

may depend on how intensively one investigates this, and the complete clinical scenario may be undetected because of subclinical or asymptomatic features, like bronchial wall thickening, sinusitis and absence of the vas deferens.

Diagnostic attitudes differ between clinicians, with some inclined to shift towards a CF designation with increasing organ involvement, whereas others are reluctant to declare a diagnosis of CF with the associated unfavourable prognostic implications and possible repercussions on health insurance. This complex scenario requires a clearer definition of the individual phenotypic range which can be expected in a person with CFTR-RD. Advantages and disadvantages of a mono- versus poly-organ definition are summarized in table 2 and discussed briefly below.

Restricting CFTR-related disorders to single organ conditions would provide a clearer diagnostic pathway for physicians, reducing confusion and the risk of a patient with CF being inappropriately labelled as CFTR-RD, and hence denied access to CF therapies that might be beneficial to prevent progression of the disease. However, caution should be exercised when interpreting clinical features for the final diagnosis as the manifestations can progress over time or may occasionally resolve. Assigning a CF diagnosis to patients with multi-organ disease might also risk labelling a phenotypically 'mild' person with a condition that carries potential significant psychological and social implications. As the vas deferens is exquisitely sensitive to CFTR dysfunction, a higher proportion of CF diagnoses will be in males due to CBAVD, thus impacting on gender distribution of CFTR-RD. Moreover, the level at which a pathology and its associated symptoms move towards CF will need to be carefully defined – e.g. bronchial wall thickening and 'mild' bronchiectasis are often asymptomatic (Fig. 1).

On the other hand, including multi-organ involvement in the diagnosis of CFTR-RD would enable greater disease stratification by CFTR function with the aim of reducing the risk of inappropriately implementing rigid CF standards of care with potential over-

medicalisation. It would also avoid the psychological and social implications of a CF diagnosis as outlined above. A potential negative impact is the lack of access to CF-specific drugs (e.g. CFTR modulators) which improve CFTR function and therefore have a theoretical rationale for use in these patients. Whilst this is a potential problem, it must be recognized that we do not yet know efficacy and safety of modulators in the CFTR-RD population. A key requirement to all of the above is access to appropriate diagnostic tests to ensure the individual has been sufficiently worked up with relevant CFTR functional (e.g. NPD and/or ICM) and genetics tests.

In consideration of the above discussion, the panel agreed on the following:

- Involvement of a single organ in the diagnosis of a CFTR-RD provides a clearer differentiation from CF but this feature is not essential for the diagnosis, particularly as it is recognized that clinical features can evolve over time
- The involvement of more than one organ does not preclude a diagnosis of a CFTR-RD if the diagnostic tests show a level of CFTR function that does not reach thresholds below which CF is diagnosed. This is explained in greater detail in the second document of this series.

5.3. 2) Exclusion of a diagnosis of CF

The diagnostic criteria for CF have been originally defined and subsequently confirmed by several documents [4,10,119], and in most cases a differential diagnosis between CF and CFTR-RD is easily made. It is important to consider that some variants, like 3849+10KbC>T, are CF causing even if associated with sweat chloride levels below 60 mmol/L

As outlined above, clinical phenotypes involving more than one organ may pose harder challenges, as it may be quite difficult to establish where CF ends and CFTR-RD begins in the continuum of CFTR protein dysfunction [120]. These situations deserve a careful

Table 2
Advantages and disadvantages of a mono- versus poly-organ definition of CFTR RD.

	Mono-organ	Poly-organ
Pros	More precise use of the clinical phenotype for definition Enables a clearer differentiation between CFTR-RD and CF Easier to explain and understand Avoids inappropriate medicalisation for CFTR-RD Facilitates appropriate use of variant specific modulator therapy for people with poly-organ disease, who would be designated as CF	Prevents over diagnosis of CF Provides an alternative diagnosis for people with a clinical picture consistent with CFTR dysfunction that does not fulfil the diagnostic criteria for CF. Improved utilisation of information from <i>CFTR</i> gene variant assessment and CFTR biomarkers More precise prognostic information (assuming better survival in people with CFTR-RD) Avoid over-medicalisation of people with poly-organ disease labelled as CF A clear diagnosis has potential psychological benefits Not being inappropriately labelled as CF may improve opportunities for life insurance, etc.
Cons	Represents an inflexible approach to diagnosis of CFTR-RD and its possible evolution Mono disease may represent inadequate clinical assessment rather than the actual situation Gender inequality, as absence of the vas deferens only occurs in males May result in over-medicalisation of people with poly-organ disease labelled as CF	May be a difficult concept to convey to some patients (uncertainty and anxiety) Currently, lack of access to CF-specific therapies (including CFTR modulators) Difficult to reach this diagnosis confidently without access to highly specialised services that can provide CFTR biomarker tests and extensive genetic analysis Weight of clinical evidence not sufficiently considered The diagnosis of CFTR-RD results in a separate clinical pathway with potential for inappropriate follow-up

evaluation of benefits and drawbacks of either one or the other diagnosis. Importantly, given the different function of CFTR according to the organ where it is expressed, it may be that conclusions of the different biomarkers vary. This makes the diagnosis algorithm more complex, particularly because the tests rely on different measurements (e.g. chloride concentration in sweat, measurement of chloride and sodium dependant current in the nasal and intestinal mucosa).

It is important to note that some people with CFTR-RD may over time acquire clinical characteristics or sweat chloride concentrations that justify reconsidering the initial diagnosis and changing it to CF. Typically, these scenarios are associated with sweat chloride levels moving above 59 mmol/L on two separate occasions and a progressive cumulation of clinical manifestations clearly suggestive of CF [4,10,121–122]. The assessment in these situations may sometimes prove challenging, not least because sweat chloride levels may moderately increase with age in healthy individuals as well as in people with CF [123].

A different kind of controversy may emerge at the other end of the CFTR-RD gradient of protein expression. Conditions like ABPA, chronic rhinosinusitis, primary sclerosing cholangitis and aquagenic wrinkling are identified in subjects carrying a single allelic *CFTR* variant. This raises the question of whether heterozygosity *per se*, i.e. 50% reduction of CFTR function, may predispose to / be associated with certain pathologies. We lack studies to better delineate this concept, but it seems reasonable to speculate that in some cases CFTR is part of a multifactorial background, which includes genetic and environmental causes [124–127]. This conceptual framework is detailed further in the second paper in this series.

5.4. 3) Evidence of partially functioning CFTR protein

As for CF, the diagnosis of CFTR-RD requires biological criteria and thresholds defining evidence of CFTR dysfunction and/or the presence of well-characterized *CFTR* variants. Whereas some variants exhibit a high penetrance for CFTR-RD, the interpretation of electrophysiological measurements have so far not been as clearly standardized as they have for CF. Moreover, some epithelia are more susceptible to defective CFTR function, and more specifically to impaired Chloride (lung) or Bicarbonate conductance (pancreas or vas deferens) [128,129].

In recent years *in vivo* and *ex vivo* research has provided important insight into the level of CFTR activity protecting from “classical” CF disease. So far three tests have been standardized to assess transepithelial chloride transport by CFTR, either biochemically by measuring the chloride concentration in sweat, or electrophysiologically by assessing the membrane potential changes due to ion transport in the nasal or intestinal mucosa [7,73,77,120,130–134].

In an individual with clinical manifestations compatible with CFTR-RD and where the diagnosis of CF has been excluded, CFTR dysfunction compatible with CFTR-RD is defined by evidence of *in vivo* or *ex vivo* CFTR dysfunction in the CFTR-RD range in at least 2 different CFTR functional tests (sweat test, NPD, ICM).

Unfortunately, while sweat test can be performed in most CF centers, the other measurements are not so easily accessible. This limits the capacity to diagnose CFTR-RD to the CF Specialist Centers where these tests can be performed. Alternative and convenient ways to assess CFTR dysfunction are necessary, and in this context the *CFTR* genotype can be considered a surrogate marker. Clinical, epidemiological and functional data on several sequence variants have been made available and can be used to estimate the level of CFTR dysfunction associated with variants frequently found in CFTR-RD [66,67,80,134,135]. Reports from the large database CFTR2, data from patients carrying various mutations and *in vitro* functional tests of CFTR, including NPD and ICM, suggest that the functional threshold for CFTR-RD seems to be between 10% and 30% of normal [80,135,136].

The inclusion of sequence variants in the criteria to establish CFTR dysfunction allows alternative combinations of functional tests and *CFTR* genotyping to use for the diagnosis of CFTR-RD. Specifically, in an individual with clinical features consistent with CFTR-RD and where the diagnosis of CF has been excluded, CFTR-RD may be diagnosed in the presence of either one *CFTR* variant known to reduce CFTR function and evidence of *in vivo* or *ex vivo* dysfunction in the CFTR-RD range in at least two functional tests, or two *CFTR* variants shown to reduce CFTR function, with at most one CF-causing variant.

A list of variants frequently associated with CFTR-RD and detailed information on the use of biomarkers of CFTR function to diagnose CFTR-RD is included in the second document of this CFTR-RD series. CFTR-RD diagnostic criteria are summarized in Table 3.

Table 3
CFTR-RD diagnostic criteria.

CRITERIA	SPECIFICS
Distinctive phenotypes isolated or in combination	CBAVD; acute recurrent or chronic pancreatitis; bilateral bronchiectasis Allergic Bronchopulmonary Aspergillosis; Chronic rhinosinusitis; Primary Sclerosing Cholangitis; Aquagenic Wrinkling currently under examination Usually mono-organ, but can be polyorgan (see text)
CFTR dysfunction	<i>In vivo</i> or <i>ex vivo</i> CFTR dysfunction in the CFTR-RD range in at least 2 different CFTR functional tests (sweat test, NPD, ICM) OR one <i>CFTR</i> variant and evidence of <i>in vivo</i> or <i>ex vivo</i> dysfunction in at least two functional tests OR two <i>CFTR</i> variants shown to reduce CFTR function, with at most one CF-causing variant
CF excluded	Criteria to be excluded: CF clinical manifestations and/or positive newborn screening and/or a CF sibling AND sweat chloride > 59 mmol/L/L and/or two CF-causing mutations and/or CF consistent electrophysiology test results [4,119]

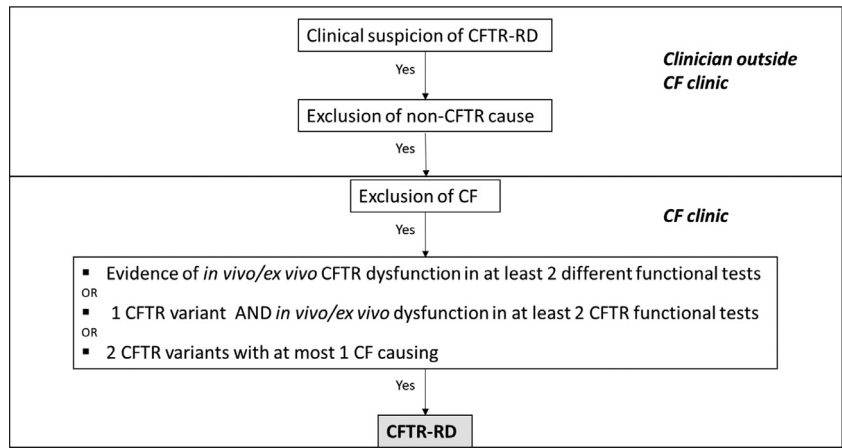


Fig. 2. The path to CFTR-RD diagnosis.

6. The path to diagnosis of CFTR-RD

In most cases the diagnosis of a CFTR-RD will be made in a stepwise way (Fig. 2). Clinical suspicion will be raised more often in clinical services dedicated to the care of conditions like infertility, pancreatic disease or bronchiectasis. Occasionally, assessments such as a sweat test or genetic analysis will be requested at this stage and a tentative CFTR-RD diagnosis formulated. More frequently and appropriately, the patient will be referred to a physician with a special interest in CF or a CF centre for diagnostic evaluation and confirmation. Hence, different professionals may be involved in the diagnostic process, including urologists or andrologists, gastroenterologists, pulmonologists, geneticists and CF doctors. Of these, the latter are usually best equipped for interpreting the results of the tests and assigning the correct diagnosis.

6.1. a. The initial presentation

An individual with a clinical manifestation of CFTR-RD, apart from disseminated bronchiectasis, will probably seek help from doctors other than pulmonologists or CF specialists. These professionals should be able to identify individuals who may have CFTR-RD and refer them for further evaluation to a CF physician or a physician who is familiar with CF.

Hence the importance of creating awareness amongst specialists such as fertility doctors, andrologists, gastroenterologists, ENT doctors, dermatologists and family doctors. They need to have a basic competence in recognizing that some clinical entities in their domain of expertise may be linked to CFTR-dysfunction. They should also keep in mind that while most of these individuals may

not manifest any signs of clinical deterioration, others might develop CF-like disease and need specific CF follow-up and treatment. The documents in this series will be disseminated to a broad group of specialists in order to make them aware of the entity 'CFTR-RD' and of the appropriate diagnostic criteria and references.

It is expected that individuals who have reached this stage will have had confirmatory tests of their presenting (or dominant) condition (e.g. CBAVD) and non CFTR related causes for the disorder excluded. Specific and more comprehensive details relevant to each individual CFTR-RD are presented in later papers of this series.

6.2. b. The CFTR-RD diagnosis

Making a CFTR-RD diagnosis in a CF centre provides greater confidence in the validity of this outcome, in accordance with the criteria described in this document. CF physicians possess knowledge of the diagnostic tests, can interpret them correctly, and have the ability to identify further CFTR morbidities and exclude, or diagnose, CF if appropriate.

In sites where detailed CFTR functional tests (NPD, ICM) are not available, screening for *CFTR* variants known to be associated with CFTR-RD may help to establish the diagnosis. This may preferably be done in a stepwise manner, by first using a *CFTR* variant panel, and if a single pathogenic variant is identified expanding to sequencing. If *CFTR* sequencing identifies a VUS, characterization of CFTR function will help to understand if it is pathogenic or a neutral variant. Detailed knowledge of the subject's *CFTR* genotype is also useful when CFTR functional tests are used to underpin the diagnosis as it may provide prognostic information or be relevant for genetic counselling in the extended family. Sequencing may be also

indicated when there is discordance between the genotype identified by a variant panel and the clinical situation.

As CFTR-RD often presents in later age, the CF specialists managing these patients will most frequently be adult physicians. Paediatric CF teams should be aware and able to recognize patients with CFTR-RD. They should not confuse a CRMS/CFSPID designation with CFTR-RD as these are two different entities with different starting points. Nevertheless, infants with CRMS/CFSPID are at risk of developing CFTR-RD in later life [89–91].

6.3. c. Assessment for possible involvement of other organs

After establishing the presence of CFTR dysfunction in an individual, a comprehensive assessment of their clinical features is required to delineate the extent and severity of CFTR-related organ disease, and determine optimal management. A framework should be followed to ensure a systematic and consistent approach to look for wider evidence of disease due to CFTR dysfunction (Fig. 3). These baseline assessments are similar regardless of the presenting feature but in some situations are age- and sex-specific so will need to be adapted appropriately. Clearly, if the presenting feature is bronchiectasis most of the respiratory assessments outlined below will already have been performed so duplication is not usually necessary. As for the diagnosis, this first assessment should ideally be done by a CF physician and in any case by a physician who is familiar with CF.

History and examination – This must cover family history and all symptoms and signs relevant to organs susceptible to CFTR dysfunction, including pulmonary, rhinosinus, gastrointestinal, pancreatic, hepatobiliary, skin and reproductive. The link between CFTR dysfunction and infertility is obviously most relevant to males, but some evidence exists in females, so the history should be sought. This is an age-relevant question and should be adjusted accordingly, based on the clinician's discretion. Additional important questions – also requiring clinician's discretion – include alcohol (relevant to pancreatitis and liver disease) and smoking history. Although aquagenic wrinkling is a rare association with CFTR dysfunction, questions relating to this should also be asked. The clinical history and examination should cover all the relevant systems and will not be exhaustively listed here, but particularly relevant are chronic cough, especially if productive, clubbing, nasal and/or sinus symptoms, nasal polyps, recurrent abdominal pain suggestive of pancreatitis and nutritional status (weight, body mass index). Palpation of the vas deferens in an adolescent or adult male might be informative but should be performed by a relevant specialist.

Clinical Investigations – A chest radiograph and pulmonary function tests (except if the child is too young to perform them reliably) should be performed in all individuals. If either of these are abnormal, a thoracic CT to evaluate for bronchiectasis and/or bronchial wall thickening is recommended. If the patient reports persistent respiratory symptoms a chest CT is the first choice. Whenever possible a respiratory specimen (spontaneous or induced sputum, or cough/throat swab if not expectorating) should be collected for microbiological analysis. More invasive techniques like bronchoalveolar lavage should be reserved for patients with ongoing symptoms and/or abnormal radiology when it is not possible to collect a reliable sample by easier techniques like sputum induction or cough swab. If rhinosinus involvement is suspected, then a sinus CT and evaluation by an ENT specialist is advised. A low threshold for sinus investigations is recommended as often these symptoms are under reported.

It is recommended that the indication for semen analysis is discussed with all adult and most adolescent males. The lower age limit for this recommendation will vary dependant on the patient's physical, mental and social background, so discretion will be required. Performing an ultrasound scan to evaluate for the presence

and anatomy of the vas deferens can be considered, although usually the trans rectal approach is most sensitive and therefore may not always be available or suitable. There is emerging evidence of the sensitivity of MRI in this area, although this is not yet established in clinical practice and could be difficult for young children [137]. Radiologically, it is easier to be confident that the vas deferens is present rather than absent.

The need for pancreatic, hepatobiliary and gastro-intestinal investigations will vary and should be guided by the patient's clinical features. For example, stool faecal elastase and fat soluble vitamins are only indicated if there is a history suggesting malabsorption and/or nutritional/growth deficiency, which is usually rare in CFTR-RD. A liver ultrasound scan is indicated if clinical features of chronic liver disease are present and/or liver function blood tests are abnormal. Other investigations will depend on the clinical situation and will be discussed in detail in later papers in this series.

7. After diagnosis: the patient's journey

The standards of care on follow-up and treatment of patients with CFTR RD are mostly disease-specific and will be dealt with in detail in a further document of this series. Some general considerations on follow-up and treatment are common to all CFTR-RD and will be introduced here, through a general outline of the patient's journey (Fig. 4).

7.1. A checklist on communication: information to be provided by the caregiver

The complexity, individual variability and evolutionary unpredictability of CFTR-RDs make the communication of the diagnosis particularly challenging, and clinicians need to consider that for some patients this presents a challenging concept. The following questions should be considered:

7.1 a. Does the patient understand the implications of the diagnosis of CFTR-RD?

Does s/he know that the disease spectrum may expand over time and that there is a possibility that the condition may evolve to CF? Although no definite clinical and epidemiological data are at present available, it seems reasonable to speculate that individuals carrying particular *CFTR* variants may be at higher risk to develop CF than others: e.g. a patient with one CF causing variant and a VVCC might have a higher risk than a patient carrying 2 VVCCs. Information regarding genetic counselling in the family and how and when to seek help from the CF centre should be clearly stated. Warning signs that should prompt for help from the CF centre includes the appearance of new events like: chest infection(s), development of a chronic cough, chronic rhinosinusitis, chest tightness, recurrent abdominal pain and any increasing anxiety about the clinical evolution.

Given the uncertainty about the possible evolution, how does the patient (or the parents) cope with this information and is there need for psychological support? In this situation help from the psychologist of the CF team could be offered.

7.1 b. Follow-up

The ideal follow-up of a person with CFTR-RD should be by a clinician with expertise in CFTR-RD and in CF. The CF clinic or specialist CFTR-RD clinic, where the possible involvement of other organs and evolution of the clinical conditions are appropriately monitored, may therefore offer the best service to these patients. The follow-up frequency may be adapted according to the actual or foreseeable clinical impact of the disease. The patient's preference, convenience and the medical facility availability in the region

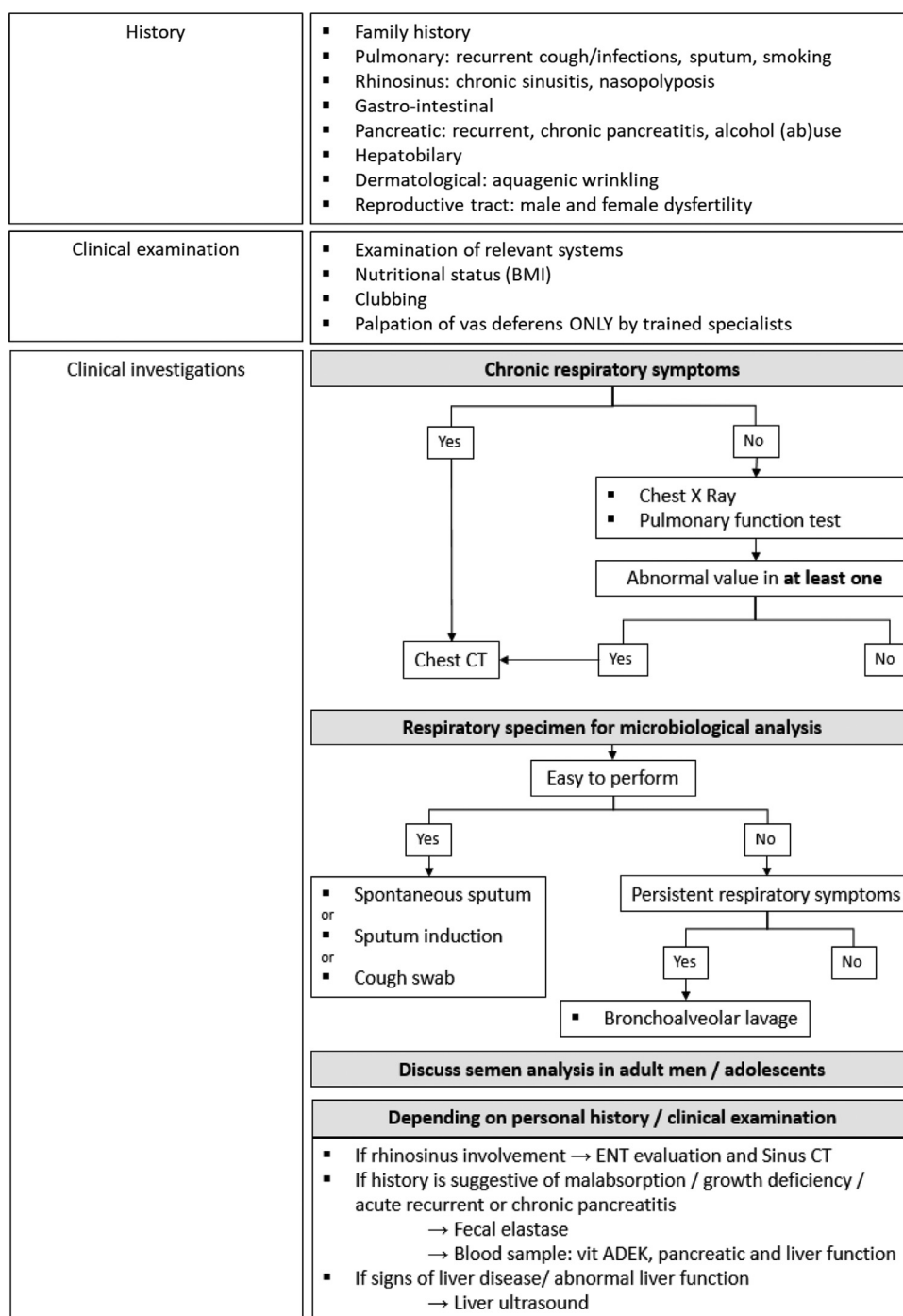


Fig. 3. First assessment after diagnosis of CFTR-RD.

should be taken into account, and a disease specific medical specialist may also be appropriate for people with recurrent pancreatitis, CBAVD, or other forms of CFTR-RD with limited involvement after careful assessment. In this case, the treating physician should work in partnership with the regional CF centre, where the patient will be seen at least once a year. If symptoms arise in another organ system, CFTR functional tests may need to be repeated and possible evolution to CF re-evaluated.

7.1 c. Clinical management

CFTR-RD treatment is largely dependant on the specific disorder, e.g. assisted fertility in the patient with CBAVD, preventing exacerbations and controlling pain in the patient with recurrent

pancreatitis, preventing loss of lung function and pulmonary exacerbations in those with bronchiectasis. It seems reasonable to hypothesize that people with CFTR-RD may benefit from the symptomatic therapies used in CF, but specific clinical trial evidence is missing. A healthy lifestyle, with particular attention to avoiding behaviours potentially more harmful to people with CFTR-RD, like smoking and alcohol abuse, should be recommended.

In the last decade, CFTR modulators have become the new standard of care for patients with CF. These drugs treat the underlying protein defect, improve CFTR function and significantly improve symptoms and disease evolution. Ivacaftor has been available for more than a decade in patients with gating variants and has been

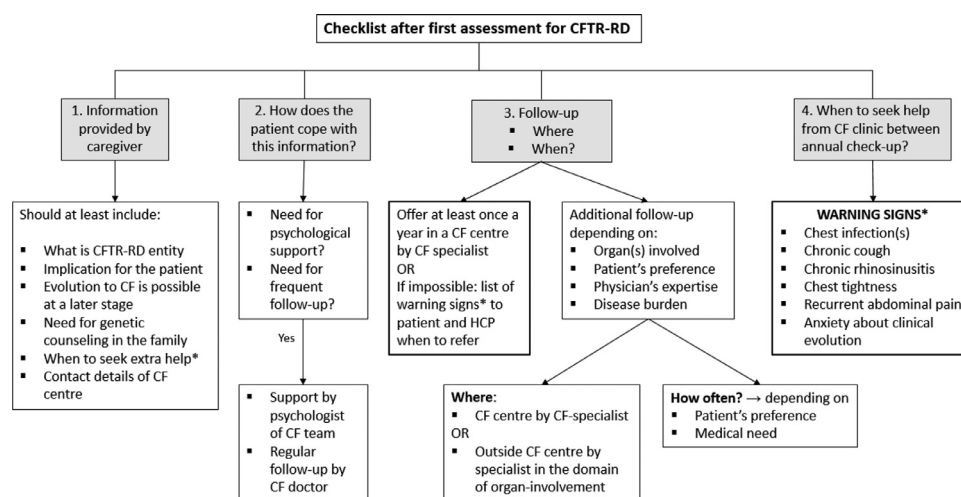


Fig. 4. Checklist after first assessment for CFTR-RD.

well documented in clinical trials to improve many aspects of disease as well as in real life in both the short- and long-term [138–147]. The triple combination of three agents (elexacaftor, tezacaftor, ivacaftor) for patients with at least one F508del CFTR gene variant has demonstrated impressive clinical improvement in both short- and medium-term outcomes [148–150]. Patients with an F508del plus a residual function variant also benefit from triple therapy [151]. In CFTR-RD there is, by definition, a decrease in CFTR function, and it is therefore logical to hypothesize that CFTR modulators might also benefit some of these patients. At present, the evidence for this does not exist, but it should be developed. The high cost of CFTR modulator treatments is a barrier for investigator-initiated trials, but when generic formulations become available this strategy should be pursued. Currently, there is no evidence to support the use of CFTR modulators in CFTR-RD outside of clinical trials. The prospect of potential effective and perhaps preventative treatment further underscores the importance of making the diagnosis of CFTR-RD.

8. Conclusions

It is crucial that people with CFTR-RD are recognized early, accurately diagnosed and appropriately managed. This is the aim underpinning these guidelines and subsequent articles in this series. In this first paper we provide an important new framework in which to reach the diagnosis, but importantly keep the original overarching definition of CFTR-RD, with its requirement for evidence of CFTR dysfunction. This concept is developed further in the second article in this series, with an in-depth review of the use of CFTR biomarkers to diagnose CFTR-RD. The general management of people with CFTR-RD is also discussed here, but it is explored in much greater detail, particularly relating to the specific disorders, in a subsequent article in this series.

It is clear that there are still unmet needs for people with CFTR-RD as gaps in our knowledge remain. Further research is required to better understand key issues, such as the evolution of CFSPID to CFTR-RD (or CF), the effectiveness of ‘CF’ therapies in treating CFTR-RD and genetic counselling/reproductive risk assessment. The role of specific patient registries (separate from CF registries) needs strong consideration as this would facilitate data collection and progress our knowledge of the natural history of CFTR-RD and how it might be modified by treatments. The final article in the series addresses these important issues. By producing these articles, we hope to enhance the ability and confidence of healthcare

professionals in reaching the diagnosis of CFTR-RD and, ultimately, optimise the outcomes for people with this challenging condition.

Declaration of Competing Interest

Carlo Castellani reports payments of honoraria for presentations from Vertex Pharmaceuticals and Chiesi and participation to Advisory Boards of Vertex Pharmaceuticals, Mylan, Actelion and Chiesi. Kris De Boeck reports consulting fees from Vertex Pharmaceuticals, Arcturus, Splisense, Translate Bio and Chiesi and honoraria for lectures from Vertex Pharmaceuticals. Elke De Wachter reports that her Institution received fundings from Vertex for her participation in an Advisory Board and for a presentation. Isabelle Sermet-Gaudelus reports funding for academic research from Vertex Therapeutics and participation to Scientific Boards of Vertex Therapeutics and Elixo; support for attending conferences by Vertex Therapeutics. Nicholas Simmonds reports payment of honoraria for lectures from Vertex, Chiesi, Gilead and Teva; participation to Advisory Boards of Vertex, Chiesi, Gilead and Menarini. Kevin Southern reports no competing interests

CRedit authorship contribution statement

C Castellani: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **K De Boeck:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **E De Wachter:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **I Sermet-Gaudelus:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **NJ Simmonds:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **KW Southern:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing.

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Supplementary materials

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