The diagnosis of cystic fibrosis

Kris De Boeck¹, Francois Vermeulen¹, Lieven Dupont²

Available online: 31 May 2017

1. University of Leuven, Department of Pulmonology, 3000 Leuven, Belgium
2. University of Leuven, Department of Pulmonology, Leuven, Belgium

Correspondence:
Kris De Boeck, University of Leuven, Department of Pediatrics, Pediatric Pulmonology, 3000 Leuven, Belgium.
christiane.deboeck@uzleuven.be

Summary

Establishing the diagnosis of cystic fibrosis (CF) is straightforward in the majority of patients: they present with a clear clinical picture (most frequently chronic respiratory symptoms plus malabsorption), the sweat chloride value is > 60 mmol/L and two known disease causing CFTR mutations are identified. In less than 5% of subjects, mainly those with a milder or limited phenotype, the diagnostic process is more complex, because initial diagnostic test results are inconclusive: sweat chloride concentration in the intermediate range, less than 2 CF causing mutations identified or both. These patients should be referred to expert centers where bioassays of CFTR function like nasal potential difference measurement or intestinal current measurement can be done. Still, in some patients, despite symptoms compatible with CF and some indication of CFTR dysfunction (e.g. only intermediate sweat chloride value), diagnostic criteria are not met (e.g. only 1 CFTR mutation identified). For these subjects, the term CFTR related disorder (CFTR-RD) is used. Patients with disseminated bronchiectasis, congenital bilateral absence of the vas deferens and acute or recurrent pancreatitis may fall in this category. CF has a very wide disease spectrum and increasingly the diagnosis is being made during adult life, mainly in subjects with milder phenotypes. In many countries, nationwide CF newborn screening (NBS) has been introduced. In screen positive babies, the diagnosis of CF must be confirmed by a sweat test demonstrating a sweat chloride concentration above 60 mmol/L. To achieve the benefit of NBS, every baby in whom the diagnosis of CF is confirmed must receive immediate follow-up and treatment in a CF reference center. CF NBS is not full proof: some diagnoses will be missed and in some babies the diagnosis cannot be confirmed nor ruled out with certainty. Screening algorithms that include gene sequencing will detect a high number of such babies that are screen positive with an inconclusive diagnosis (CFSPID). Even in 2016, the most reliable and widely available diagnostic test for CF is the measurement of chloride concentration in sweat. The method of choice is sweat induction by pilocarpine iontophoresis, followed by sweat collection on a gauze or filter paper or in a Macrodust coil. Since mutation specific therapies have become available, it is important to identify the mutations responsible for CF in each individual patient.
The need for a consensus on the diagnosis of cystic fibrosis (CF)

CF is in essence a clinical diagnosis supplemented with proof of cystic fibrosis transmembrane conductance regulator (CFTR) channel dysfunction. But the clinical picture spanning a broad range of symptoms and the CFTR dysfunction varying from slight decrease to no function at all, call for more precision in diagnostic criteria. In addition, most infants identified via a positive newborn screening (NBS) test are asymptomatic at time of diagnosis. Clinical observations compatible with the disease CF date back to the 16th century, either via poetry about children with a salty taste, or reports about severe steatorrhea or malabsorption by astute clinicians. For those interested, we refer to www.cfmmedicine.com, the well-illustrated website developed by Doctor Jim Littlewood, documenting the history of CF. Despite these earlier descriptions, the report on “fibrocytic disease of the pancreas” in pathology samples by Dorothy Anderson from Columbia University in New York is usually considered as the “official” discovery of the disorder. Another break-through was the recognition of the increased salt content in the sweat of people with CF by Paul di Sant’Agnese in 1953. This discovery led to the first diagnostic test for CF. In patients with chronic lung disease and/or malabsorption, the diagnosis CF could be confirmed by measuring a sweat chloride concentration above 60 mmol/L. It was however mainly from 1985 onward, following discovery of the CFTR gene, that the extreme variability in phenotype associated with mutations in this CFTR gene became apparent. That brought about the need for a diagnostic consensus on CF.

Development of diagnostic consensus and diagnostic algorithms

The first consensus report on the diagnostic criteria for CF dates from 1998 [1]. The panel, convened by the North-American Cystic Fibrosis Foundation, concluded that the diagnosis of CF requires the presence of CF specific symptoms or a positive newborn screening test result or a history of CF in a sibling plus two elevated sweat chloride values (> 60 mmol/L) on separate days or identification of 2 CF mutations (of a panel of 23 CF mutations) or (rarely, in the absence of the two previous proofs of CFTR dysfunction) abnormal nasal potential difference (NPD) test results on 2 separate days. The NPD test measures the nasal transepithelial potential difference in vivo on the nasal mucosa and how this potential difference changes during perfusion of solutions to block the epithelial sodium channel (ENaC) or to activate the CFTR channel [2-4]. In the consensus document, the panel of mutations only listed 23 CF mutations. However, an increasing number of CFTR mutations were being described and collected in the CFTR1 database (http://www.genet.sickkids.on.ca/app); their total number already exceeded 1400 in the early years 2000. In addition, the vast phenotypic spectrum of CF that became apparent and terms like typical CF, atypical CF and non-classic CF surfaced.

In 2006, the European CF Diagnostic Network Working Group agreed on the terminology to use (Table 1) and proposed algorithms for the CF diagnostic process [5]. Patients diagnosed with classic or typical CF have at least one phenotypic CF characteristic plus a sweat chloride above 60 mmol/L. In contrast, patients with non-classic or atypical CF have phenotypic features in at least one organ and a sweat chloride value in the intermediate range (30–60 mmol/L) but proof of CFTR dysfunction via identification of 2 disease causing CFTR mutations and/or an abnormal nasal potential difference measurement (NPD). The distinction between classic or typical and non-classic or atypical CF was further motivated by an on average less severe phenotype in the latter group [6].

A renewed American consensus was reported in 2008 [7] that differed slightly from the European consensus: the distinction between typical and atypical CF was not accepted; the lower limit of intermediate sweat chloride was 40 mmol/L from the age of 6 months on; NPD was not accepted as a diagnostic test. A comparison of both algorithms for 208 consecutive patients with single organ disease entering the diagnostic process found 85% concordance between the European and American consensus [8]. The higher sweat limit in the American consensus was responsible for a good part of the discordance. Adding NPD, data allowed an extra 17% of subjects to enter a diagnostic category, whereas extended genotyping did not. This was later confirmed in a second report by the same authors [9].

With the increasing impact of CF NBS on the CF diagnostic process, and with the increasing recognition that some screen positive infants do not meet CF diagnostic criteria, new American guidelines for diagnosis of CF will be published in 2017. The concordance with the European diagnostic consensus increases; the lower limit for an intermediate sweat chloride value will be 30 mmol/L; the American and European definition of screen positive newborns who do not meet CF diagnostic criteria will be similar; but the American consensus still does not accept the term atypical CF (see further sections under CF NBS and splitters versus lumpers).

---

**Glossary**

- **CF**: Cystic fibrosis
- **CFSPID**: Cystic fibrosis screen positive inconclusive diagnosis
- **CFTR**: Cystic fibrosis transmembrane regulator
- **CFTR-RD**: Cystic fibrosis transmembrane regulator related disorder
- **ECFSPR**: European cystic fibrosis society patient registry
- **ENaC**: Epithelial sodium channel
- **FIS**: Forskolin induced swelling
- **ICM**: Intestinal current measurement
- **IRT**: Immunoreactive trypsinogen
- **NBS**: Newborn screening
- **NPD**: Nasal potential difference
Despite minor differences between American and European consensus, we should highlight that establishing the diagnosis of CF is straightforward in the vast majority of patients who present with a clear clinical picture and elevated sweat chloride values (> 60 mmol/L). Only in less than 5% of subjects, mainly those with a milder or limited phenotype, the diagnostic process is more complex, because initial diagnostic test results are inconclusive: sweat chloride concentration in the intermediate range, less than 2 CF causing mutations identified or both. If at all possible, these patients should be referred to expert centers where further diagnostic testing by NPD or intestinal current measurements (ICM) can be done. There is now ample proof that these tests do assist in the CF diagnostic process [8–12]. Still, in some patients, despite symptoms compatible with CF and some indication of CFTR dysfunction (e.g. only intermediate sweat chloride value), diagnostic criteria are not met (e.g. only 1 CFTR mutation identified). For these subjects, the term CFTR-related disorder (CFTR-RD) is used [13]. Patients with disseminated bronchiectasis, congenital bilateral absence of the vas deferens and acute or recurrent pancreatitis may fall in this category. Of course, alternative diagnoses must have been explored before concluding to CFTR-RD; e.g. in patients with bronchiectasis, diseases like primary ciliary dyskinesia, immunodeficiency, lung malformations, gastro-esophageal reflux disease etc. must be excluded.

**Symptoms and age at diagnosis**

In countries where national CF NBS has been implemented, the number of patients in whom the diagnosis is made on clinical grounds is slowly decreasing. However, every NBS program will have screen failures. Physicians must remain alert to the diagnosis CF in children or adults who present with chronic or recurrent respiratory tract infection, especially when associated with signs of malabsorption. These symptoms, isolated or combined, remain the most common presentation in countries where NBS has not been implemented. The list of symptoms leading to the diagnosis of CF is however much longer and also includes: salt loss syndromes (dehydration and electrolyte imbalance acutely on hot days or progressively due to low salt content in breast milk and infant formula); male infertility due to azoospermia and absence of the vas deferens; intestinal obstruction; liver disease; pancreatitis; consequences of malabsorption of protein, fat soluble vitamins or minerals; nasal polyps… As an illustration, see table II with the presenting symptoms of patients with CF in Belgium, a country where national NBS has not yet been implemented. All physicians, including adult lung, gastro-intestinal, liver or infertility specialists, must remain familiar with the very wide spectrum of the initial manifestations of the disease CF. Nonetheless CF NBS, CF must remain part of the differential diagnosis in many children and adults with chronic or recurrent symptoms. CF spans a vast disease severity spectrum and at least 10% of patients are only diagnosed during adult life (figure 1).

In countries without national NBS program, the median age at diagnosis varies from 4 months to almost 2 years [European CF Society Patient Registry (ECFSPR) annual reports 2013 and 2014 at https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports/]. To obtain maximal benefit from NBS, the age at diagnosis must be as young as possible and certainly below 2 months [14]. This goal is being achieved in most European countries with CF NBS (ECFSPR annual reports). But even at the age of 1 to 2 months, some babies are already symptomatic [15,16].

**CF diagnostic tests**

**Sweat test**

The most reliable and widely available diagnostic test for CF is the measurement of chloride concentration in sweat. Also, in infants with a positive NBS test, the diagnosis CF must be confirmed by a sweat test [17]. Isotonic sweat is produced in the secretory coil, but in the sweat duct most of the chloride is reabsorbed via the CFTR channel. Sweat of healthy people is thus
**Table I**

Symptoms or facts leading to diagnosis of cystic fibrosis

<table>
<thead>
<tr>
<th>Category</th>
<th>All subjects In registry in 2013</th>
<th>New diagnosis of CF In 2012</th>
<th>In 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Acute or recurrent respiratory problems</td>
<td>493</td>
<td>43.6</td>
<td>11</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>282</td>
<td>24.9</td>
<td>7</td>
</tr>
<tr>
<td>Chronic diarrhea/steatorrhea/malabsorption</td>
<td>231</td>
<td>20.4</td>
<td>4</td>
</tr>
<tr>
<td>Neonatal screening test positive</td>
<td>191</td>
<td>16.9</td>
<td>4</td>
</tr>
<tr>
<td>Meconium ileus (MI)</td>
<td>161</td>
<td>14.2</td>
<td>4</td>
</tr>
<tr>
<td>Family history of CF</td>
<td>115</td>
<td>10.2</td>
<td>4</td>
</tr>
<tr>
<td>Nasal polyps/chronic sinusitis</td>
<td>50</td>
<td>4.4</td>
<td>1</td>
</tr>
<tr>
<td>Rectum prolapse</td>
<td>30</td>
<td>2.7</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal obstruction past neonatal period</td>
<td>25</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>Prenatal diagnosis</td>
<td>34</td>
<td>3.0</td>
<td>2</td>
</tr>
<tr>
<td>Dehydration/electrolyte imbalance</td>
<td>19</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>Neonatal jaundice</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Infertility</td>
<td>11</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>87</td>
<td>7.7</td>
<td>0</td>
</tr>
<tr>
<td>No information(^1)</td>
<td>54</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Data from the Belgian CF Registry (https://www.wiv-isp.be/epidemie/epien/prog20.htm).

\(^1\)The overall percentages are based on 1132 patients; since in 54 patients the information was missing. There were 28 new diagnosis of CF in 2013 but the percentages are based on 26 patients. More than 1 symptom or fact may be listed in some patients and were not mutually exclusive.

hypotonic. When CFTR is dysfunctional, reabsorption of chloride will not occur and this explains the high chloride content in the sweat of people with CF. The method of choice is sweat induction by pilocarpine iontophoresis, followed by sweat collection on a gauze, filter paper (both requiring about 75 mg of sweat) or macroduct coil (requiring 50 μL) (figure 2). A minimum sweat rate of 1 g/m² body surface area per minute is required so that the collection time typically takes around 30 minutes. A sweat test can be performed from the age of 2 weeks on in infants who are normally hydrated and not acutely ill. There are extensive guidelines for correct sweat test performance (http://www.rcpch.ac.uk/system/files/protected/page/Sweat%20Guideline%20v3%20reformat_2.pdf) [18]. Unfortunately, these guidelines are not always adhered to. Most patients with CF will have a sweat chloride concentration above 60 mmol/L. Elevated sweat chloride values not due to CF are uncommon and include endocrine diseases and some rare disorders that can usually be easily discriminated from CF [19]. Measuring the sweat chloride concentration is considered more accurate than measuring sweat conductivity (reflecting the total ion content) using the nanoduct, that only needs 3 μL. Obtaining sufficient sweat (even using the macroduct collection system) is not always possible in newborns. Recent comparisons of sweat conductivity in samples obtained by nanoduct and sweat chloride concentration in samples from the macroduct yielded highly concordant results [20,21]. In addition, the nanoduct had a much higher success rate.

In recent years, the intra- as well as intersubject variability in sweat chloride value in patients with CF has been better studied. The majority of the variability in sweat chloride (56%) is determined by the specific CFTR genotype. Other causes of variability are: variation over time (14%), environmental factors (13%), residual factors such as test variability (10%) and unique individual factors (7%) [22]. Real life sweat test variability has also been calculated from repeated measurements during the placebo arm in clinical trials. The intra-patient standard deviation in patients with G551D amounts to 8.1 mmol/L [23]. As such, especially in subjects with an intermediate sweat chloride value,
repeating the sweat chloride measurement can at times even change the diagnostic conclusion [24], hence pointing out the importance of other measures of CFTR function.

**CFTR gene mutations**

Since mutation specific therapies have become available, it is very important to identify the mutations responsible for CF in the individual patient. This is done by first running a standard CFTR mutation panel containing the most common disease-causing mutations in the studied population (covering around 80–85% of all mutations in the population). When 2 CFTR mutations are not identified and the diagnosis CF is almost certain (sweat chloride concentration above 60 mmol/L) or highly probable (e.g. suggestive clinical picture), the second step is extensive sequencing of the entire CFTR gene plus assessment of large deletions or insertions. Certified laboratories are mandatory for the latter more complex analyses. Even then, CFTR mutation analysis is not the answer to every dilemma, since the pathogenic potential of many CFTR mutations is unclear.

The CFTR gene was discovered more than 30 years ago. Only one mutation, F508del, is frequent and occurs in about 70% of CF alleles; by comparison all other mutations are rare. In most countries, only 6–8 mutations have a frequency above 1% (ECFSPR annual report 2014). Hence, in total, many patients have mutations that are rare or ultra-rare i.e. only occurring in a few or even a single individual. At present, more than 2000 different mutations in the CFTR gene have been reported in patients with CF or CF-like symptoms. The CFTR 1 database (http://www.genet.sickkids.on.ca/app) lists these mutations plus the clinical information reported about subjects with these mutations. This is however an unfiltered dataset and the detail in the information provided varies. The CFTR2 database (http://www.cfr2.org) provides much more robust information about the disease liability of selected CFTR mutations via analysis of data compiled from the European, American and Australian CF registries. Currently, information is available for about 300 CFTR mutations [25]. Mutations are labeled as disease causing, not disease causing, of variable clinical significance or of unknown clinical significance. A mutation is considered disease causing by

---

**Figure 1**

Age at diagnosis in patients with cystic fibrosis in Belgium, a country without a national newborn screening program. The median age at diagnosis is 6 months. In more than 10% of subjects, the diagnosis is made during adolescence and adult life. Data source: https://www.wiv-isp.be/epidemio/epinl/index20.htm
K. De Boeck, F. Vermeulen, L. Dupont

Figure 2
Sweating is induced by pilocarpine iontophoresis during 10 minutes. Sweat is collected on a gauze or—as seen in this figure—using a Macroduct® coil, that allows easy visualization of the progression of the sweat collection by the blue dye.

Clinical criteria if the mean sweat chloride concentration derived from at least 3 patients carrying the variant is \( \geq 60 \) mmol/L [25]. The clinical features associated with a CFTR mutation have been derived from patients carrying the mutation of interest in trans with a CF causing mutation i.e. previously shown to have minimum residual function. Mutations have been evaluated for clinical severity and for functional consequence. If needed, disease penetrance is assessed in fathers of CF patients, since infertility is a clinical characteristic with a very high penetrance. The CFTR2 website lists the number of patients with that particular mutation, their mean age, their lung function according to age group, the proportion of subjects with pancreatic insufficiency and Pseudomonas lung infection. For every mutation of interest, data can be displayed according to the type of mutation present on the second allele (all mutations, the 23 CF mutations listed by the American College of Medical Genetics, F508del mutation or all patients with pancreatic insufficiency). The functional characteristics of the mutations are reported. Selected papers about the mutation of interest complete the information provided. As such, this website is an extremely useful resource for clinicians and researchers.

Nasal potential difference (NPD) measurement and intestinal current measurement (ICM)
Like the sweat test, NPD and ICM are bioassays of CFTR function. Both NPD and ICM require great expertise and are not widely available. Both tests have by now been standardized and have proven their usefulness to rule in or rule out CFTR dysfunction [3,8-12].
NPD is an in vivo test that measures the transmucosal voltage potential. In CF compared to healthy, the nasal mucosa has an increased sodium absorption and a defective chloride secretion. In CF compared to normal, this is reflected by a more negative basal nasal potential, a larger change in potential when the sodium channel is blocked and little or no change in potential when the CFTR channel is stimulated by zero chloride solution and isoproterenol (figure 3). The first 2 values of the NPD measurement reflect ENaC function, the latter the CFTR channel function. There is a gradient of abnormality in these NPD values from patients with CF and pancreatic insufficiency, over CF with pancreatic sufficiency, to CFTR-RD, heterozygotes and healthy [2]. Using NPD results, the best discrimination between patients and healthy comes from the zero chloride plus isoproterenol value or ratio's reflecting both the ENaC and the CFTR channel function [3].
The major weakness of NPD is the relatively large test-to-test intra-subject variability [26,27]. This can be diminished by averaging values of measurement in both nostrils [28] or by increasing the sampling surface by modification of the nasal catheter [29]. Also, the fact that universal reference values have not been accepted is a draw back; at present every lab uses his own cut-off values to distinguish CF, non-CF and atypical CF. Despite these shortcomings, there is ample evidence that NPD can help to rule in or rule out the diagnosis of CF [8-12,30]. Although the test can be done in babies using an abbreviated protocol [31], performing an NPD measurement becomes much more feasible from the age of 6 years on.
ICM is an ex vivo test of CFTR function. To measure ICM, a small tissue sample obtained via rectal biopsy is mounted in an Ussing chamber. The set-up can be open circuit so that transmucosal potential is measured or open circuit so that the electric current as a consequence of the ion fluxes is measured [26,32]. In patients with CF, intestinal chloride secretion is impaired, while absorptive processes remain normal or are increased. There is thus a clear difference in ICM between CF and healthy [32,33]. The response to carbachol and histamine in intestinal tissue contains two components: a lumen positive current by apical potassium efflux and a lumen negative current by CFTR mediated chloride secretion. In ICM of healthy persons, the potassium efflux is masked by the much larger chloride efflux. In people...
with CF, the current is reversed because only the potassium efflux is present.

Like NPD, ICM is especially useful to diagnose or rule out CF in patients with an intermediate sweat chloride, with less than 2 CFTR mutations identified or with CFTR mutations of unknown clinical significance [11,12,33,34]. This can be particularly helpful in regions with a low prevalence of the F508del mutation and a great diversity of CFTR mutations such as e.g. Brazil or Israel. Taking a rectum suction biopsy is painless, well accepted by patients [11] and can be done in people of all ages.

**CF newborn screening**

As the term states, CF NBS is a screening test, not a diagnostic test. In screen positive babies, the diagnosis of CF must still be confirmed. In addition, to achieve the required benefit, every baby in whom the diagnosis of CF is confirmed must receive immediate follow-up and treatment in a CF reference center.

Different CF screening algorithms are in use. In all screening programs, measurement of immunoreactive trypsinogen (IRT), a marker of pancreatic injury, in the heel prick blood sample is the first tier screen [35]. A second tier test is needed to improve test specificity because the initial IRT can be increased due to perinatal stress, critical illness, CFTR mutation carrier status and other causes. The second tier test differs between programs and can be CFTR mutation analysis, measurement of pancreas-associated protein or repeat IRT. The latter 2 methods avoid detection of CFTR mutation carriers. For repeat, IRT as second tier screen, patient recall is needed at the age of 2 to 4 weeks, with an increased risk of loss to follow up. Meconium ileus at birth is a cause of a falsely low IRT and should always prompt investigations for CF. For a full discussion of pro and con of specific CN NBS programs, we refer to recent reviews [35,36]. CF NBS is not full proof. Some diagnoses will be missed and the diagnosis of CF will not be confirmed in all screen positive babies. In some, it will be impossible to conclude to presence or absence of CF. These babies are named CF screen positive inconclusive diagnosis (CFSPID) on the European continent [37] and CF related metabolic syndrome (CRMS) in the North-American nomenclature [38]. Depending on the algorithm used and especially on the number of CFTR mutations included in the second tier, the ratio of babies with an inconclusive diagnosis versus patients with CF will increase [35,39]. In programs using gene sequencing as second or third tier, more babies with CFSPID than patients with CF are detected [40]. This is of course worrisome since these families are at risk of increased anxiety and the babies may be exposed to unnecessary tests and be “medicalized” without need. Only a minority of subjects with CFSPID will eventually meet CF diagnostic criteria [41-43]. Hence, every CF NBS program should be under continuous re-evaluation so that the algorithm used can be adapted [35,39]. Some suggest a continuum between CFSPID and CFTR-RD [35].

At present, we have insufficient evidence for this assumption. An enormous difference between both conditions is the context: CFTR-RD is a label-often during adult life- for a symptomatic subject; CFSPID is just an abnormal screening test result in the newborn period in a baby who is asymptomatic.

The most important fact however remains that CFNBS is not a diagnostic test. The diagnosis must be confirmed by a positive sweat test, detecting 2 CFTR disease-causing mutations or rarely by NPD or ICM measurement [5]. CF NBS is however very
important since it paves the way for an early diagnosis, avoiding a diagnostic odyssey, improving outcome [44,45] and allowing informed decision making for further pregnancies. In regions such as e.g. Australia, Wisconsin, Brittany and Veneto, CF NBS has been implemented since decades. Where longstanding CNBNS programs have been coupled to cascade carrier screening, the incidence of CF has decreased [46,47].

**Controversies**
The pro and con of detection and follow up of subjects with the R117H mutation

Soon after starting CF NBS, the French alerted to the surprisingly high number of subjects with an F508del/R117H genotype compared to the low frequency (1%) of subjects with this genotype in the existing French CF registry [48]. More importantly, the majority of these subjects remained asymptomatic during a decade of follow-up. All CF NBS programs have since confirmed the high detection of this genotype. Although the R117H mutation will lead to a CFTR channel with some decrease in conductance as well as gating properties [49], the disease penetrance (likelihood of symptoms in these subjects) is very low and depends on other polymorphisms on the same allele. Indeed, the length of the poly T-tract (5T, 7T or 9T) in intron 8 will influence the inclusion of exon 9 in the CFTR protein [50]. Disease penetrance is higher in subjects with 5T-R117H, but very low in subjects with 7T-R117H and 9T-R117H. Most subjects detected in France have the 7T phenotype. This may differ between countries: initial data in the ECFS registry point towards a higher percentage of 5T-R117H on the British Isles but we lack reliable data.

Supporters of detecting R117H at young age point out occasional adults with severe bronchiectasis only diagnosed during adulthood [51]. Opponents of detecting R117H at young age stress the large number of subjects picked up in whom disease will never develop. Indeed, in France it was calculated that there are at least 3650 subjects with this genotype [52]. This is of course a very high number, especially when compared to the 6300 subjects currently in the French CF registry (http://www.vaincrelamuco.org/sites/default/files registre-2013.pdf). Recent data from the CF NBS program in Norway [53] report an even higher prevalence of the R117H allele than the F508del allele. For this reason, the R117H mutation has been removed from the French NBS panel.

**Splitters versus lumpers: typical and atypical disease or just CF and no CF**

Although on average, disease severity differs between subjects with a sweat chloride value above 60 mmol/L and subjects with a sweat chloride in the intermediate range [6], the American consensus does not accept the terminology of typical and atypical CF. Indeed, an intermediate sweat chloride value does not equal milder disease, just as the disease course is not always severe in subjects with a high sweat chloride value. There is overlap in disease course between these groups. Still, several genotypes such as those of subjects heterozygous for a CFTR mutation associated with residual function (mutations of class IV or V or mutations usually associated with pancreatic sufficiency) are on average associated with a milder disease course, a lower treatment burden and a higher age at diagnosis [6,54-56]. The fact that subjects are only diagnosed during adult life, sometimes even during the fifth or sixth decade [51] implies that they have a better prognosis, since they long surpassed the median age of survival. Similarly, in young subjects with an expected milder disease course, a different terminology than typical CF can decrease the anxiety in families without interfering with appropriate follow up and treatment in the individual patient. Hence, from a medical and psychosocial point of view, stratifying for expected disease severity makes sense. It is also customary in other chronic genetic diseases e.g. spinal muscular atrophy where having more than 1 pseudogene is usually –but not always- associated with a slower disease progression [57]. Also, in other respiratory diseases, like asthma, stratification is done according to severity [58]. On the other hand, giving the disease “mild or severe” only one name “CF” may in the long run de-dramatize the sound of the diagnosis CF.

**Diagnostic tests on the horizon**

The beta adrenergic sweat test

Sweat secretion has a CFTR-independent and a CFTR-dependent component that can be stimulated and measured individually by the sequential intradermal injection of metacholin (CFTR-independent secretion) followed by a β-adrenergic cocktail (CFTR-dependent secretion). The ratio of CFTR dependent and CFTR independent sweat is then measured optically on a gland-b by gland basis or by evaporometry [59-61]. Beta-adrenergically-stimulated sweat rates appear to have a near-linear readout across the entire range of CFTR function and differentiate CF, CF carriers and controls. Optical measurement seems to be more sensitive that evaporimetry [61]. This test may have potential as a diagnostic test and possibly even more as a biomarker of CFTR function in clinical trials.

**Organoids**

Using very specific culture conditions, stem cells contained in intestinal tissue from a rectum suction biopsy can multiply, differentiate and spontaneously assemble into hollow 3-dimen sional structures called “organoids” [62]. This requires a delicate balance of several growth factors like Wnt, R-spondin and Noggin plus a specific basement membrane matrix. These “organoids” or "mini guts" contain all intestinal cell types. They are genetically and phenotypically extremely stable. They can be expanded over long time periods by mechanical disruption and re-plating of the stem cells still contained in the organoids.
Organoids can be biobanked, thawed, re-expanded and retested if needed. When grown in extracellular matrix, the cells orient with their basal membrane to the outside. In the context of CF, organoids can be used to test an individual’s CFTR function [62,63]. Indeed, intestinal organoids have a high expression of the CFTR protein. When intestinal organoids derived from healthy controls are stimulated with forskolin to increase intracellular cAMP and activate the CFTR channels, chloride and water secretion into the lumen of the organoid will occur. During this process, the organoids will swell and this swelling is quantified using microscopy, hence the assay’s name: forskolin induced swelling assay or FIS assay. The swelling can be expressed as the maximum surface area increase compared to baseline after 60 min under one particular stimulation condition or as area under the curve, taking into account stimulation with increasing forskolin concentrations (figure 4). This assay is CFTR specific since swelling is not seen when a CFTR inhibitor is added to the assay. In contrast, depending on the CFTR mutations present and the forskolin concentrations used, organoids derived from subjects with CF will either not swell (e.g. G542X homozygous subject), swell only minimally (F508del homozygous subject), or have some residual degree of swelling (R117H/F508del). As such the FIS assay has the potential to establish the diagnosis of CF by measuring CFTR function in subjects with rare CFTR mutations of unknown clinical significance.

Making the diagnosis of CF in adults
Just as we have become aware that CF is not only a disease of Caucasians but occurs in all populations, we now have full evidence that CF is not only a disease of children. In several western European countries (https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports) in the United States (https://www.cff.org/Our-Research/CF-Patient-Registry/Highlights-of-the-2014-Patient-Registry-Data), in Canada (http://www.cysticfibrosis.ca/our-programs/cf-registry) and in Australia (https://www.cysticfibrosis.org.au/media/wysiwyg/CF-Australia/medical-documents/CF_ADataRegistryReport_2014_Final.pdf), there are more adults with CF than children with CF. This is not only the consequence of a better survival of people with a childhood diagnosis of CF [64] or with very low lung function [65]. Increasingly, CF is being diagnosed in adults with chronic respiratory symptoms, male infertility or even multisystem disease. The age at diagnosis of this genetic disease extends to the 6th and even the 7th decade of life [51] (https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports). The cohort of subjects with a diagnosis during adult life differs from that with an early diagnosis [66,67]. More often, these patients are pancreatic sufficient and have mutations other than F508del. These people often have had a protracted course of chronic cough, sputum production and bronchiectasis, without the diagnosis of CF ever being considered. Their disease is usually less advanced compared to subjects of the same age who are F508del homozygous. However, their disease progression, although with a later start, is equally fast compared to F508del homozygous [68]. Finally, arriving at a correct diagnosis, will often give these patients a feeling of a relief, the end of a long quest. And when appropriate CF treatment and follow up are started, patients experience improvement in lung function, decreased symptoms and better stabilization of lung function [68].

Communicating the diagnosis of CF
Parents will remember their entire lifetime when, how and by whom the diagnosis of CF was communicated [69]. As can be expected, hearing the diagnosis of CF can evoke strong emotions in parents. Announcing the diagnosis is thus a very
important landmark and can influence future coping. The diagnosis must be communicated in a quiet environment, unhurried, compassionate and by a person, preferably the doctor, who is an expert in the disease and can accurately answer the parents’ questions. The major facts about the disease must be communicated: life shortening, need for life long complex treatment, risk of a high disease burden and several complications, risk of recurrence of CF in future pregnancies, male infertility. The communication style must be adapted to the parents’ needs: some are so shocked that any further information will not sink in; some will want as much information as possible. Repeated encounters over the course of a few days will usually work better than only one long encounter. Written information will enforce and consolidate the oral information given. Coping by the parents can be difficult and will require time, especially if their children are severely ill at the time of diagnosis. But also, parents of an asymptomatic newborn who get the diagnosis after NBS will need time to come to terms with this entirely new fact. Some may go into denial and find it difficult to believe their “healthy looking child” has a serious disease and needs this complex treatment. At times, there is the feeling of guilt, especially when a second child with CF is born and prenatal testing was not done.

As much as announcing the diagnosis CF is bad news, also hope must be given. The life expectancy of children with CF born today is estimated at 50 years [70]. A highly effective mutation specific therapy is available for the 3% of patients with a class 3 mutation and a treatment with modest efficacy for patients homozygous for F508del. The therapeutic pipeline is very busy for nearly all mutation types [71]. The intensive research to find a treatment for the basic defect of the most common life shortening genetic disease will almost certainly lead to further improved outcome.

Announcing the diagnosis CF in adults is different [72]. Of course, the information goes directly to the patient, not via his parents. For many patients and families, announcing the diagnosis will raise a dust cloud: why was CF not considered earlier; what with other family members; what will my life expectancy be; I have no time for such a complex treatment; I do not want to share that info with my environment. The very high risk of male infertility may seriously trouble the young men. When the diagnosis is announced past the third or fourth decade, patients may be very surprised, but they may more easily see the advantages: understanding the reason of their previous complaints; finally starting a specific and more effective therapy. But the bottom line is that patients and parents differ, there is no uniform nor best way of announcing the diagnosis of CF [69,72].

Acknowledgements: we thank the Belgian CF registry for use of figure 1 and table 1; we thank Dr Anabela Ramalho for generating figure 4.

Funding statements: none.

Disclosure of interest: the authors declare that they have no competing interest.

References

The diagnosis of cystic fibrosis


