Diagnosing Cystic Fibrosis in Adults

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Abstract

Diagnosing cystic fibrosis (CF) in adulthood is not a rare occurrence for CF centers despite the popular belief that the diagnosis is achieved almost universally in childhood by means of newborn screening or early clinical presentation. The purpose of this review article is to highlight specific considerations of adult diagnosis of CF. Obtaining a diagnosis of CF at any age is exceptionally important to ensure optimal treatment, monitoring, and support. In the new era of more personalized treatment with the advent of transformative therapies targeting the underlying protein defect, accurate diagnosis is of increasing importance. This review highlights the diagnostic algorithm leading to a new diagnosis of CF in adults. The diagnosis is usually confirmed in the presence of a compatible clinical presentation, evidence of cystic fibrosis transmembrane conductance regulator (CFTR) protein dysfunction, and/or identification of variants in the *CFTR* gene believed to alter protein function. Achieving the diagnosis, however, is not always straightforward as CFTR protein function exists on a continuum with different organs displaying varying sensitivity to diminution in function. We highlight the current knowledge regarding the epidemiology of CF diagnosed in adults and outline the various clinical presentations, including pulmonary and extrapulmonary, which are more common in this population. We expand on the stepwise testing procedures that lead to diagnosis, paying particular attention to additional levels of testing which may be required to achieve an accurate diagnosis. There continues to be an important need for both pulmonary and other specialists to be aware of the potential for later presentation of CF, as the improvements in treatment over decades have had large positive impacts on prognosis for people with this condition.

Keywords
- cystic fibrosis
- diagnosis
- late presentation
- CFTR-related disorder
- nasal potential difference
- sweat test

The diagnostic pathway for cystic fibrosis (CF) has evolved over decades as our understanding of the underlying pathology of the disease has increased. Although there is some debate regarding the first description of the condition, the largest case series to describe it focused on autopsy specimens of children with pancreatic fibrosis giving rise to the term "cystic fibrosis."[1] In 1948, a key observation of increased salt losses in sweat during a New York heat wave led to a further understanding of the condition and ultimately resulted in the development of the first test for a CF diagnosis in 1959.[2,3] The sweat test measures chloride concentration in induced sweat and remains a cornerstone of diagnosis to this day. Subsequent research led to the recognition of CF as a monogenic disease, identifying variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene on the long arm of chromosome 7, as being responsible for the condition.[4]

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These breakthroughs resulted in the modern approach to diagnosis, facilitating a complementary pathway of testing for CFTR protein dysfunction by way of the sweat test and the identification of gene variants that may be responsible via genetic analysis.

The purpose of this review is to provide an up-to-date overview of the current diagnostic pathway with a focus on later presentations of CF. Diagnosis in adulthood remains an important topic to be considered across many areas of internal medicine.[5–7] With the advent of CF newborn screening (NBS) in many territories in the world, there may be a belief that the diagnosis is easily delivered in a timely fashion.[8] However, several factors are important to note: (1) NBS is not available worldwide and even in places where it is available false-negative tests are possible,[9] (2) many adults presenting later in life will have been born prior to the advent of NBS programs in their respective countries, and (3) many of those presenting later in life may have a modified clinical phenotype and degree of residual CFTR protein function which may not be identified at NBS. Moreover, an accurate diagnosis is of increasing importance as newer therapies directly targeting CFTR dysfunction are available, which will be indicated for the majority of individuals with CF according to their specific gene variants. These have had a transformative effect on clinical phenotype for many.[10,11] This progress has been built on many successes of multidisciplinary CF care which have led to progressive increases in survival over decades, emphasizing the importance of an accurate and timely diagnosis.

Epidemiology of Late Diagnosis in Cystic Fibrosis

The UK CF Registry provides an annual report on relevant clinical information on people with CF (pwCF) including the number of new diagnoses per year. In their report on 2021 data, the median age at diagnosis was under 2 months (22 days for those diagnosed at < 16 years) which has remained static since 2016, a reflection of the introduction of a NBS program throughout the United Kingdom in 2007.[12] It is notable that consistently there are 30 to 50 new diagnoses per year which were not diagnosed by NBS. A more striking figure is that 14.7% (926 individuals) of adults in the CF registry were diagnosed on or after their 16th birthday, including 20 new diagnoses in that age group in 2021.

Clinical Phenotype of Individuals with a Late Diagnosis of Cystic Fibrosis

The identification of individuals with a possible diagnosis of CF requires that clinicians not specialized in the area are aware of the possibility of a later presentation. It is also necessary to be aware of the multisystem nature of CF disease. Although often believed to be a respiratory disease with recurrent respiratory tract infections and the development of (usually) upper-lobe predominant bronchiectasis, several other presentations can be attributed to CF in adulthood, including infertility in males, severe sinusitis, or pancreatitis. Many may not have been reviewed by respiratory specialists and will present to other services.

The clinical presentation of patients with a late diagnosis of CF has been studied for several decades including in the era prior to NBS. ►**Table 1** summarizes several case series describing the most common presenting features at diagnosis. A 1995 Dutch study described a cohort of 25 patients attending a single adult CF center who were diagnosed after their 16th birthday, comprising 20% of their overall CF clinic attendees.[13] Clinical characteristics of these 25 individuals were compared with 118 individuals with an earlier diagnosis. In the later diagnosis group, 92% presented with recurrent respiratory tract infections. Gastrointestinal symptoms, male infertility, and esophageal varices were the other reasons for presentation. In almost one quarter, the diagnosis was made because of a sibling having CF. Lung function was relatively better preserved in the later diagnosed cohort with a mean forced expiratory volume in 1 second (FEV_1) of 72.5% predicted and these individuals had less than half the rate of annual lung function decline than their earlier diagnosed peers. Infection with *Pseudomonas aeruginosa* was also less common at 24% compared with 70% in the early diagnosed group. Typical extrapulmonary manifestations of CF such as pancreatic insufficiency (12%) and diabetes (8%) were relatively uncommon in the later diagnosed cohort. A more contemporary study from the United States reported similar findings but highlighted that late diagnosis (with an age range from 24 to 72.8 years) defined an important subgroup of pwCF, characterized by better lung function, more pancreatic sufficiency, and, interestingly, more nontuberculous mycobacteria.[6]

Widerman and colleagues also reported distinct differences between patients diagnosed in adulthood and those with an earlier diagnosis by examining data from the 1996 U.S. CF Patient Registry.[14] There were significant differences in the presenting symptoms suggesting diagnosis with respiratory symptoms and nasal polyposis being significantly more common in the later diagnosed group, and gastrointestinal symptoms such as malabsorption, malnutrition, and bowel obstruction being significantly less common.

A single-center study from Oxford in the United Kingdom examined 38 patients who were diagnosed in adulthood.[15] They also describe that by far the most common presenting feature was of recurrent and chronic pulmonary symptoms, with bronchiectasis seen in 94% of those who had a CT at baseline. Spirometry was more impaired in this cohort than has been previously described, with a presenting FEV_1 of 61% predicted. Mean body mass index was 22 kg/m^2, consistent with those of patients diagnosed later in life presenting without nutritional depletion. Data from the Italian CF Registry have been published looking at clinical characteristics of 204 patients diagnosed as adults from 2012 to 2018.[16] They similarly found that patients presented with better nutritional status with only 4.1% of males and 9.4% of females being considered underweight, undoubtedly resulting from a low prevalence of exocrine pancreatic insufficiency of 12.2% in comparison to an estimate of approximately 85% in the overall CF population.

Table 1 Presenting features of CF in patients diagnosed in adulthood from published studies and registry reports (the United Kingdom Cystic Fibrosis Registry Report 2021[12])

	Gan et al[13]	Widerman et al[14]	Farley et al[15]	Padoan et al[16]	The United Kingdom CF Registry
Country	The Netherlands	The United States	The United Kingdom	Italy	United Kingdom
Year	1995	1996	2002–2020	2012–2018	1995–2020
Number of pwCF diagnosed in adulthood	25	786	38	204	926
Lower age at diagnosis (years)	16	18	19	18	16
Mean/Median age at diagnosis (years)	27.7	27	38	36.2	NR
FEV_1 (% pred)	72.5%[a]	59.45%[a]	60.8%	90.8%	NR
Pseudomonas aeruginosa colonization (%)	24%	66.7%[a]	37%	17%	NR
Pancreatic insufficiency (%)	12%	67.8%[a,b]	26%	12.2%	NR
Presenting symptoms					
Recurrent or chronic respiratory tract symptoms	92%	81.6%	100%	NR (70% CF-like symptoms)	53.9%
Abnormal stool/GI presentation	8%	18.5%	21% pancreatitis	NR	5.6%
ENT—nasal polyps or sinusitis	NR	11.1%	32%	NR	7.1%
Male infertility	4%	NR	90% of males were infertile	45.9% of males	5.1%
Genetic testing (FHx or other reason)	NR	3.82%	NR	30%	23.8%

Abbreviations: % pred, percent predicted; CF, cystic fibrosis; ENT, ear–nose–throat; FHx, family history; GI, gastrointestinal; NR, not reported; pwCF, people with cystic fibrosis.
[a]Cross-sectional data and not at the time of presentation.
[b]Pancreatic enzyme use as surrogate for pancreatic insufficiency.

The aforementioned UK CF Registry report also provides information comparing those at an older age of diagnosis to younger presentations who were not identified by NBS.[12] It is interesting to note that the main mode of presentation for older individuals was persistent or acute respiratory infection (53.9%) with an additional 10% presenting with bronchiectasis, highlighting again a key need to further investigate individuals with chronic respiratory symptoms. Nasal polyposis was the presenting feature in 66 people representing 7.1% of the late-diagnosed cohort. Other systemic manifestations of CF such as pancreatitis (1.6%) or fertility issues in men (5.1%) were less common, although this may represent an underestimate as diagnosis secondary to genotype was relatively high at 23.8%, but indication for genetic testing is not clear.

It is apparent therefore that adult-diagnosed patients may present with an altered clinical phenotype in comparison to that seen in the overall CF population.[7] This modified clinical phenotype undoubtedly contributes to the later age at diagnosis. It likely results from a high prevalence of genetic variants which confer some residual protein activity making organs such as the pancreas, which are less sensitive to reductions in CFTR protein activity, less affected early in the course of the disease. It is important to note, however, that the majority of individuals from the studies outlined earlier presented with chronic symptoms which would have seen them present to multiple and diverse medical services. It directly follows therefore that there were likely missed opportunities at achieving a more timely diagnosis. Greater efforts are required among the CF medical community to disseminate this message and ensure that colleagues in pulmonology and across other medical specialties are mindful of CF as a differential diagnosis across a range of multisystem presentations. Work to improve this knowledge and inform the wider medical community is underway by the European CF Society Diagnostic Network Working Group and its CFTR-related disorders (CFTR-RDs) committee.[17]

Does Late Diagnosis Matter?

Accurate diagnosis is crucial to ensure pwCF are given access to the correct treatment and support which CF care centers can provide. An analysis of the adult-diagnosed CF population in the Canadian CF Registry revealed that a more timely diagnosis may also have a prognostic impact.[18] Older age at diagnosis was an independent risk factor for death or

transplantation in a multivariate model. Moreover, a large study using data from the U.S. CF Foundation Patient Registry showed significant improvements in health parameters when adults diagnosed with CF older than 40 years received CF-specific healthcare interventions.[19]

Diagnostic certainty for individuals can be greatly helpful as many will have suffered symptoms for long periods before a definitive diagnosis has been made, as suggested by the high rates of chronic respiratory symptoms at diagnosis. CF care models are based on multidisciplinary care teams with expertise in different aspects of CF care. A diagnosis of CF provides individuals with access to this expertise and support from medical, nursing, and psychological services, which can be profoundly helpful at these times.

A formal diagnosis of CF can also enable better access to therapies. More treatments and in particular nebulized antibiotics are licensed for the treatment of CF-related bronchiectasis than non-CF bronchiectasis.[20–22] The recent development of CFTR modulator therapies has increased the importance of a correct diagnosis.[10,11] Through three generations of compounds, CFTR modulators are now indicated for more than 90% of pwCF. Clinical trial results suggest that in many they can have transformative effects in terms of pulmonary function, risk of infection, and quality of life. This appears to be the case even for those with a milder phenotype with clinical trials suggesting significant benefit with dual CFTR modulator therapy for those individuals heterozygote for the most common variant *Phe508del* and a variant associated with residual function (commonly termed "residual function variants").[23] More recently, the combination of three therapies, elexacaftor, tezacaftor, and ivacaftor (ELX/TEZ/IVA), has confirmed additional benefit over and above dual therapy.[24] CFTR modulator use has also been shown to reduce admissions with acute pancreatitis for pwCF, suggesting a possible further benefit for patients diagnosed later in life, although the evidence for this is still conflicted, as some patients may also have a worsening of symptoms.[25–27]

Current Diagnostic Pathways

The diagnosis of CF can be firmly established when a reliable biomarker of CFTR protein activity shows aberrant protein function and is associated with compatible gene variants situated *in trans* in each of the individual's *CFTR* alleles. With greater elucidation of the functional consequences of individual *CFTR* gene variants, there is a greater appreciation that although for most the diagnosis is clear and can be established at an earlier age, for others it can be more challenging.[28] The CF community has made great efforts to characterize individual genetic variants and thereby determine the potential disease liability afforded to each.[29] Currently, there are more than 2,000 reported *CFTR* variants, some of which are classified as not CF disease causing, as CFTR function is not diminished enough to cause CF. CFTR protein dysfunction exists on a continuum and for some with variants which alter protein activity, the reduction in activity may not be sufficient to lead to multiorgan dysfunction; for others whose variants confer no residual protein activity, the consequences lead to the systemic consequences of a "full" CF diagnosis. A further complexity occurs when a variant of varying clinical consequence (VCC) is identified. In this situation, reduction in protein function can be variable and disease liability will be affected by penetrance and expressivity, in addition to the functional consequences of the gene variant carried on the second allele. The current CF diagnostic guidelines were revised in 2017 and embrace some of these nuances.[30]

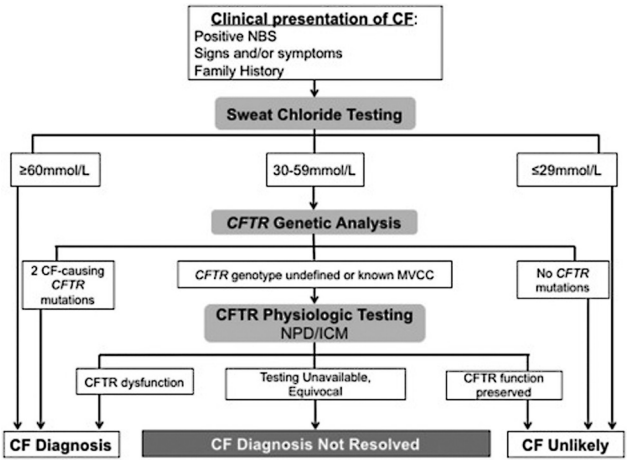

Fig. 1 Current diagnostic algorithm for cystic fibrosis (CF). CFTR, cystic fibrosis transmembrane conductance regulator; ICM, intestinal current measurement; NBS, newborn screening; NPD, nasal potential difference. (Reprinted from Farrell et al,[30] with permission from Elsevier.)

These guidelines provide a robust diagnostic algorithm for the diagnosis of CF (see ►**Fig. 1**). The algorithm begins with a statement that an individual has to have a clinical presentation of the disease and evidence of CFTR dysfunction. Clinical presentation of the disease is defined as signs/symptoms, a positive NBS, or a family history. The first level of CFTR protein testing is the sweat test indicating how well this test has stood the test of time with levels of $\geq$60 mmol/L consistent with a diagnosis of CF, 30 to 59 mmol/L being designated borderline and $<$30 mmol/L deemed normal, whereby a CF diagnosis is unlikely (although in rare cases it is still possible as some variants are associated with borderline or even normal sweat chloride values).[31] Further strata of testing are then suggested with CFTR genetic analysis and then additional functional CFTR testing using nasal potential difference (NPD) or intestinal current measurements (ICMs) to reach a more conclusive diagnosis, if indicated.

This approach remains the standard of care for diagnosing adults with CF. The subtleties of diagnosis are alluded to at the end of the algorithm, with the final outcomes categorized as CF diagnosis, CF unlikely, and finally CF diagnosis not resolved. In some cases, individuals under investigation will be determined to fit better into the diagnostic category of CFTR-RD, a diagnosis more common in adults than in children. The term CF screen positive inconclusive diagnosis (CFSPID) is exclusively used for asymptomatic babies who have a positive NBS but in whom the diagnosis of CF is not confirmed; so, it is not relevant to adult diagnosis. CFTR-RDs are clinical conditions

associated with CFTR dysfunction but do not meet the full diagnostic criteria for CF.[32] Classical examples of these presentations include congenital bilateral absence of the vas deferens (CBAVD), recurrent acute or chronic pancreatitis, and diffuse bronchiectasis. While these are still the most common presentations of CFTR-RD, recent updated published guidelines highlight that a more diverse clinical spectrum is feasible, including the possibility of polyorgan (and symptomatic) involvement.[17] Accurate separation between CFTR-RD and CF can be particularly challenging and should be conducted in specialist centers, as the diagnosis of CF may carry with it important psychological and financial consequences potentially influencing an individual's ability to secure life insurance or indeed a mortgage.

Diagnostic Tools in Cystic Fibrosis

Sweat Test

The sweat test is the oldest and most widely applied and available test of CFTR protein function. The sweat test relies on the role of CFTR protein in reabsorbing chloride ions from sweat into the cells of the sweat duct. Dysfunction of CFTR protein leads to elevated levels of chloride in sweat with agreed threshold values as outlined earlier. It is recommended that sweat testing is performed on more than one occasion to achieve two measurements before a diagnosis can be confirmed in the correct context. Borderline sweat chloride values can also lead to a diagnosis of CF in the right clinical context if accompanied by two variants in the *CFTR* gene known to be CF causing. As outlined earlier, as certain *CFTR* variants associated with disease can be associated with normal or borderline sweat chloride values (such as D1152H and 3849 + 10 kb C > T),[31,33] it is advisable to pursue further testing and not resolve a diagnostic quandary based on sweat chloride values alone.

Genetic Testing

The next step in the diagnostic pathway involves genetic testing to identify variants within the *CFTR* gene which may lead to a disruption or loss of function of the CFTR protein. Genetic testing for variants in *CFTR* is usually a multilevel process with the initial screen using a polymerase chain reaction (PCR) analysis to identify the most common pathogenic variants identified in a specific population. As variant frequency varies according to geographical region, it is essential that the ethnicity of the person being tested is known to permit targeted variant analysis for the most relevant variants for them. In the United Kingdom, the most common panels cover 36 to 50 variants for initial testing, which is approximately 90 to 95% of alleles in British Caucasians.

Further testing, however, may be required in isolated cases and this may be more common in those with a later diagnosis. It is a relatively common misconception that a negative initial screen by itself is sufficient to rule out a diagnosis of CF. In the context of adult diagnosis, for patients presenting to secondary respiratory services or other medical specialties, the sole use of an initial PCR test can lead to false reassurance, particularly due to the limited availability of specific functional CFTR testing, including sweat chloride, outside of a pediatric setting. In the correct clinical context, functional CFTR testing should always be performed and if clinical suspicion is high, a more thorough evaluation of the *CFTR* gene is indicated. Usually, this includes exon sequencing and multiple ligation probe amplification to look for large deletions and duplications in the 27 coding exons of the *CFTR* gene. However, whole gene sequencing may be more appropriate in select cases, as demonstrated by a recent publication by Morris-Rosendahl and colleagues from a large adult center in the United Kingdom, as they identified a disease-causing deep intronic variant by next-generation sequencing, which would not be identified by the aforementioned methods.[34] In cases of diagnostic uncertainty, the surveillance of noncoding regions of the *CFTR* gene may be necessary to facilitate an accurate diagnosis.

Interpretation of Genetic Variants

The detection of rarer variants is accompanied by distinct challenges of its own. Little may be known or published regarding the functional consequences of individual variants and accurate genotype–phenotype correlations may be challenging. The online resource CFTR2.org provides invaluable assistance in this context by examining the disease liability of variants in *CFTR*.[35] By utilizing clinical, laboratory, and epidemiological data provided by CF registries worldwide they have adjudicated on the likely pathogenicity of more than 480 variants. At the time of writing this article, 82.7% of these variants have been determined to be CF-causing, 4.9% thought to be non–CF-causing, 10.1% are variants of VCC, and 2.3% are of unknown significance. In individuals with two variants identified, utilization of this resource can be invaluable in determining accurate disease classification.

Providing a diagnosis of CF is clear in those with two known CF-causing variants and a compatible clinical phenotype. As CF is biallelic, the disease-causing propensity of each individual variant an individual harbors has to be evaluated. In those with milder phenotypes, interpretation of results remains a challenge in some instances. In the vast majority of cases, individuals with two variants of VCC, residual function of the mutated CFTR protein is unlikely to lead to the multisystem consequences of classic CF. This is not universal; so, clinical phenotype and functional CFTR testing are essential for accurate diagnosis. Similarly, in those harboring one CF-causing variant and a second variant of VCC, a combined approach is necessary.

Genetics of Patients with an Adult Diagnosis of Cystic Fibrosis

The specific genotype of those with a late diagnosis will vary according to geographical location. In Western countries where homozygosity for *Phe508del* is the most common genotype among CF patients diagnosed at any age (47.7% population of the United Kingdom),[12] it is markedly less common (proportionately) in later diagnosed patients due to

the severity of this genotype and likely early clinical manifestations. Later diagnosed patients are more likely to harbor residual function variants, including variants of VCC, in combination with a CF-causing variant. An Italian study looking at the characteristics of 204 adult-diagnosed patients in the period 2012 to 2018 revealed *Phe508del* to be the most common genetic variant, but only 3 (1.5%) of the patients were homozygous.[16] In 35% of cases, the genotype combination was CF-causing/CF-causing, but slightly more common was the genotype CF-causing/variant of VCC (36%). In almost 18% of causes, the pathogenicity of the second allele was unknown. These data again highlight the difficulty in diagnosing some adults and the need for additional testing in many cases.

The ability of individuals to benefit from variant-specific treatment such as CFTR modulators is a key consideration given the benefits reported in clinical trials. Farley and colleagues described that 84% of their 38 adult-diagnosed patients would qualify for CFTR modulator therapy under a European license with the figure increasing to 89% if U.S. licensing was used.[15] Most published case series represent data from Europe or North America where the majority of adult-diagnosed patients are heterozygous for *Phe508del*, which is the licensed indication for the combination therapy ELX/TEZ/IVA. This may not be the case in other jurisdictions where *Phe508del* is a less common variant.

Other Tests of Cystic Fibrosis Transmembrane Conductance Regulator Function

As highlighted earlier, in select cases, the diagnosis of an adult with CF can be challenging. Initial sweat testing may be in the borderline (or even low) range and the disease-causing potential of identified variants may be unknown or variable. Therefore, if the diagnosis remains unresolved, further evaluation of CFTR function should be undertaken. As outlined earlier, the two principal tests with clinical utility in this context are NPD and ICM.

Nasal Potential Difference

The role of NPD in assessing a CF diagnosis has been elevated over recent iterations of diagnostic guidelines.[30] In cases where there continues to be ambiguity, further electrophysiological measurements are recommended. Testing of NPD and ICM is the best evidenced next step. A full review of NPD is beyond the scope of this article and it is covered in depth in other review articles.[36,37] Briefly, NPD assesses CFTR and the epithelial sodium channel (ENaC) function by measuring the change in voltage across the nasal epithelium in the presence of solutions that will modify ion channel activity. This is done by measuring the potential difference between the subcutaneous compartment and the nasal epithelium (under the inferior turbinate where epithelium becomes ciliated pseudocolumnar epithelium). As CFTR is an ion channel, its function can be assessed by the sequential perfusion of solutions that inhibit ENaC (amiloride) and induce CFTR activity (chloride-free solution and isoprenaline) giving rise to characteristic traces separating CF patients from healthy controls (►**Fig. 2**). Due to the technical nature of the procedure, the need for precise conditions, and the relatively low proportion of patients requiring the test, it is recommended that these procedures are conducted only in expert centers, with a critical mass of expertise and throughput. In the correct setting, NPD measurements have been shown to be reproducible and effective in discriminating between CF and non-CF.[38–41] Furthermore, NPD measurements may provide useful information on patients who are at higher risk of CF complications and potentially select individuals who may need more intensive follow-up.[42,43]

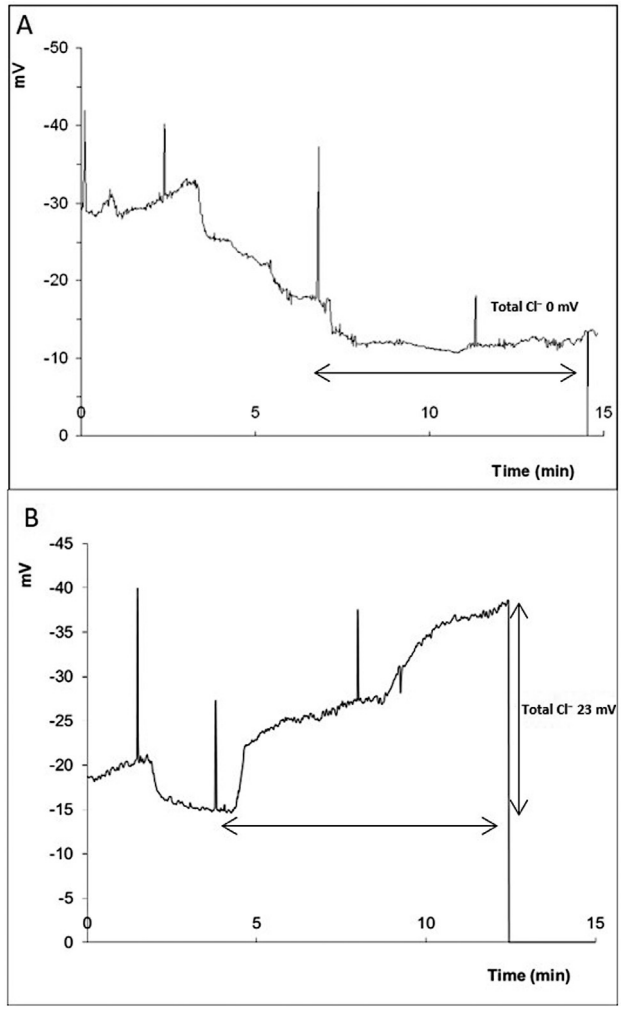

Fig. 2 (**A**) An abnormal nasal potential difference tracing from a patient with the *Phe508del/Asp1152His* genotype. Although basal sodium secretion is normal, there is an absence of chloride secretion. The abnormal NPD trace is contrasted with a healthy control (**B**), showing a normal basal reading and excellent chloride secretion; well above the diagnostic threshold of ≥5 mV). NPD, nasal potential difference.

Intestinal Current Measurements

ICM is an ex vivo method of examining CFTR protein function on rectal biopsies from patients suspected as having CF. Freshly obtained rectal biopsies are tested in a Ussing chamber for electrical responses to a series of secretagogues. By examining differential responses determined by the level

of CFTR protein function, discrimination can be made between healthy controls, CFTR-RDs, and CF. ICM shares many of the challenges of NPD measurements (such as specialist equipment and highly skilled operators) but requires the additional step of obtaining rectal biopsies from patients. This procedure is conducted in a limited number of CF centers worldwide and availability of testing can represent a challenge in certain jurisdictions. Evidence to date suggests that it is at least as effective as NPD in accurately diagnosing patients and has the added advantage of not being affected by epithelial inflammation, which can reduce the accuracy of NPD, particularly when severe nasal polys and/or rhinitis is present.[44–46]

Other diagnostic tools are under development, including the β-adrenergic sweat stimulation test and, more recently, the use of patient-derived rectal organoids.[47,48] Organoids are three-dimensional structures derived from stem cells commonly generated from an intestinal tissue sample. Work has been performed assessing the response of rectal organoids to CFTR modulator drugs and an ongoing EU-funded project is examining their utility as individual responsiveness biomarkers to assess novel medications aimed at improving CFTR function.[49] However, more recently, their diagnostic capabilities have been investigated; in a recent publication by the Belgian Organoid Project, morphology analysis of rectal organoids was performed to investigate the potential utility of this technology as a diagnostic tool.[50] By examining the presence or absence of a central lumen and the roundness of the organoid structures, this analysis was able to discriminate between patients with CF (including those with milder phenotypes) and healthy controls. This technology, however, is early in development and further validation will be required before it can be introduced into clinical practice.

The Diagnosis: Where Are Individuals with a Potential Diagnosis of Cystic Fibrosis Identified?

There are many sources of adult referrals to CF centers for the purpose of further diagnostic assessment. Male subjects may be referred as a result of infertility investigations which have identified azoospermia and CBAVD. However, one of the most common sources of referral is still via the respiratory specialist as recurrent respiratory tract infection and the identification of bronchiectasis should be potential triggers for further investigation. Ear, nose, and throat specialists may be alerted to the possibility of a diagnosis of CF due to the degree of nasal polyposis present. Other presentations yielding further investigation include recurrent pancreatitis and some individuals will present due to known or a recently discovered family history. In rare situations, dermatologists may consider the diagnosis as aquagenic wrinkling of the hands is a known rare association of *CFTR* gene variants.[51]

Illustrative Case

A 63-year-old white female presented with a history of bronchiectasis since early adulthood. Test results at that time were "inconclusive" for CF; so, she stayed in a general respiratory service. Her airways became chronically infected with *P. aeruginosa* and her FEV_1 deteriorated to 38% predicted; so, she was referred for further CF investigations. She was found to have a normal/borderline sweat (chloride) test of 24 and 30 mmol/L. Genetic analysis identified *Phe508-del* and *Asp1152His*, the latter being a variant of VCC. She underwent NPD measurements (►**Fig. 2**)—despite showing a normal basal (sodium) value, there was no chloride secretion, thus confirming the diagnosis of CF. Confirming CF brought significant improvements to her health with CF multidisciplinary team input and access to effective CF therapies such as dornase alfa and inhaled antibiotics. Importantly, the CFTR modulator ELX/TEZ/IVA is indicated for this genotype.

Key Factors that May Suggest Cystic Fibrosis as a Possible Diagnosis

As CF is a multisystem disease, the recognition of a combination of symptoms, signs, or past illnesses is crucial to alerting the treating clinician. A history of recurrent or chronic pancreatitis or recurrent respiratory symptoms, or in males a history of infertility, should initiate the consideration of a systemic problem. Adult diagnosis of CF can result from a failure to identify these patterns earlier in life and thus can lead to excess patient morbidity, frustration, and a lack of faith in medical professionals.

Pasteur and colleagues published a case series of 150 patients with bronchiectasis who were further investigated in a large center in the United Kingdom.[52] A total of 142 of the referrals were from respiratory physicians, 4 from a lung transplantation unit, and 4 from family doctors. In 11 of these subjects, using a limited genetic panel covering only 86% of local mutant *CFTR* alleles, at least one CFTR variant was found. Although the authors state that CF was ultimately diagnosed in only 4 patients, 8 of the 9 patients who had sweat testing performed had a sweat chloride level of $\geq$60 mmol/L consistent with a CF diagnosis. It is notable that these investigations were not pursued prior to tertiary referral even among pulmonary specialists, highlighting that awareness of a late presentation is a significant issue. The development of more effective treatments since this publication clearly highlights that failure of diagnosis may lead to unnecessary morbidity.

Key features that should alert pulmonary physicians to the possibility of CF are recurrent infections without an underlying cause. Patients may have been mislabeled as asthmatics for many years prior to diagnosis and a thorough assessment for those with recurrent infections is indicated. The isolation of certain pathogens may also alert the treating physician.[53] Certain pathogens such as *Staphylococcus aureus* and *P. aeruginosa* are commonly seen in other respiratory conditions. However, the identification of other pathogens such as *Stenotrophomonas maltophilia* and *Achromobacter* species, in addition to atypical species such as *Pandoraea*, *Ralstonia*, *Serratia*, and *Burkholderia*, would be considered unusual and merit further exploration in the correct clinical

context. Similarly, the identification of predominantly upper lobe bronchiectasis, allergic bronchopulmonary aspergillosis (in the absence of asthma), hemoptysis, and clubbing may provide clues that a systemic pathology is the underlying cause.

Communicating the Diagnosis

Making a diagnosis of CF is a significant life event for each patient. Many have had a long history of symptoms which have not been fully addressed; so, an accurate diagnosis may come as a relief initially as they envisage a clear treatment pathway. The diagnosis can also be unexpected and result from potentially minimally symptomatic disease. In a growing age of medical information being widely available on the internet, the diagnosis of CF can be worrying for them as most sources will describe it as a life-limiting or lethal condition. In spite of best advice, most people newly diagnosed will carry out their own additional research. Although accurate prognostication can be very challenging, it is likely that their disease trajectory will differ from that reported for the majority of CF patients, with better survival[54,55]; so, these nuances should be carefully explained to them.

The diagnosis of a genetic condition can lead to significant worry about the patient's own family and offspring. The realization that they now are diagnosed with an incurable chronic condition can be exceptionally harrowing and confusing. Adjustment to living with a lifelong condition that may lead to premature death takes time and the support of the CF medical and nursing teams is crucial. Many benefit from the input of CF psychology services during this period of adjustment. The diagnosis may also have financial implications with many employers unaware of precautions that may need to be taken to accommodate pwCF and the need for recurrent hospital appointments. Patients may find it difficult to secure life insurance or mortgages which adds additional burden. Sensitivity and empathy from the CF care team can be crucial in helping newly diagnosed patients adjust to a changing future.

Cystic Fibrosis Transmembrane Conductance Regulator–Related Disorders and Unresolved Cases

After thorough evaluation, the diagnosis of CFTR-RD—not CF—may be more appropriate for some individuals. Patients with this diagnosis should be followed up longitudinally to manage their symptoms appropriately and to monitor for the development of more CFTR-related complications in the future as their diagnostic label could change to a more formal diagnosis of CF. Long-term management and follow-up is discussed in recent published guidelines, but essentially CF center (or centers with a specialist interest in CF/CFTR-RD) follow-up is advised, but usually on a less intensive basis than CF standards of care.[17]

When no evidence of CFTR dysfunction or CFTR-associated disease is identified, the individual can be discharged back to their referring team. In a minority of cases, the situation may remain unresolved; there may be suspicion of another channel defect (e.g., ENaC),[56] but the evidence remains unclear. Follow-up in this situation will depend on the level of disease and at the discretion of the clinician and patient.

Ongoing Challenges for Adults Diagnosed with Cystic Fibrosis

There are several ongoing challenges in this field. The greatest initial challenge is increasing the awareness of the spectrum of CF and late diagnosis among the medical community; experience in CF centers should be an essential requirement for all pulmonology trainees. Increasing education across medical specialties is also necessary as presentations may be subtle and easily missed, particularly if each pathology is considered in isolation.

The diagnostic entities of CF and CFTR-RD are not always easy to separate and are somewhat fluid. There is also a gender discrepancy among CF/CFTR-RD as males have the additional diagnostic entity of CBAVD. For example, should a female and a male both present with chronic pancreatitis in their 30s with identical genetic variants and physiological testing, the male would be considered to have more systemic disease and likely diagnosed with CF, whereas the female may receive the diagnosis of CFTR-RD.

In equivocal cases, it can be a matter of interpretation whether one attributes symptoms such as nasal polyposis, sinusitis, or a cough in the absence of bronchiectasis to CFTR dysfunction and these subtleties may lead to inconsistent diagnoses between physicians. For the identification of bronchiectasis, high-resolution CT thorax remains the gold standard and is often utilized to rule out bronchiectasis. However, we know from infant studies that inflammatory changes and ventilation abnormalities can occur in CF that precede the development of bronchiectasis.[57] In adults, questions remain unanswered in this area: Should we consider the use of more sensitive techniques, such as lung clearance index, to identify pulmonary involvement in someone without bronchiectasis, when the diagnosis of CF or CFTR-RD has been confirmed? The CF community has recently published guidelines to try to improve this situation and increase consistency, producing updated recommendations on the use of diagnostic biomarkers, diagnosis and management of CFTR-RD, to help clinical teams and improve information for patients.[17,58]

Conclusion

Diagnosing CF in adults is not a rare occurrence for CF centers—delays in the diagnosis have a meaningful impact on an individuals' well-being, something that will extend further in the new era of CFTR modulation therapy. Patients diagnosed later in life largely represent a cohort of individuals with variable clinical presentations, but one that is progressive and requires long-term care in a CF center. Improving the awareness of this issue is essential to ensure a prompt and accurate diagnosis, so that outcomes for all people affected can be optimized for the long term.

Conflict of Interest
None declared.

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