Annotations

Diagnosing cystic fibrosis: blood, sweat, and tears

Making a diagnosis of cystic fibrosis has lifelong implications and repercussions for the affected individual, their family, and the many people they will encounter throughout their lives. Clearly, the decision needs to be made accurately and as early as possible. A late diagnosis is often preceded by a catalogue of hospital visits, family anguish, anger, and guilt and a delay in the initiation of early treatment may have an impact on long term outcome. Equally disturbing is a small but increasing experience in cystic fibrosis clinics of the child, diagnosed as having cystic fibrosis, whom on review—often years later—is found to be normal.

In most instances the decision is easy: a child has suggestive clinical symptoms or a family history and positive sweat tests confirm your suspicions of cystic fibrosis. The diagnostic criteria, developed in the 1960s and based on phenotypic considerations, have been useful in guiding clinical practice for nearly 30 years. Although in the past false positive and negative diagnoses have been documented, most errors have been attributed to the vagaries of sweat test technique. The concept of a borderline sweat test or even negative sweat test in the face of convincing clinical evidence for cystic fibrosis ('sweat test negative cystic fibrosis') has previously been considered. Case reports have questioned a broader phenotypic range for cystic fibrosis but such cases, if indeed they existed, were considered exceptionally rare.

With the discovery of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) and readily available laboratory techniques to characterise the common mutations, it was anticipated that diagnostic accuracy would improve and the status of these borderline decisions would be clarified. Misdiagnosis, both false positive and false negative, need no longer occur and the cystic fibrosis phenotype could be correlated with the genotype providing useful prognostic information. This has not necessarily been the case. Since genotype analysis has become a major player in the diagnostic armoury for cystic fibrosis, the situation can remain as confusing and clinics are increasingly facing the question: does this patient have cystic fibrosis?

This annotation examines the diagnostic tools available for cystic fibrosis and reviews an expanding phenotypic spectrum for this condition.

Sweat test

First described in 1959, the sweat test remains the gold standard for the diagnosis of cystic fibrosis. A positive sweat test is diagnostic in the absence of known entities associated with a false positive test (generally rare and clinically distinct conditions) as long as it is performed by staff experienced in the technique and on an adequate weight of sweat (>100 mg). In cystic fibrosis the sweat sodium or chloride concentrations are above 60 mmol/l (>70 mmol/l in adolescents and adults) with a mean around 100 mmol/l. In normal individuals and carriers of the cystic fibrosis gene the mean sweat sodium or chloride concentrations are around 30 mmol/l. Interpretation must be corrected for age.

The standard sweat test or quantitative pilocarpine iontophoresis test is laborious both in name and execution. Reliability increases with the increased frequency that an individual centre undertakes the procedure but the search for a cheaper, easier sweat test continues. Recently the macroduct collection system and the use of a conductivity analyser has received favourable attention, but many pediatricians would still confirm this technique with the traditional method. It has become common practice to repeat the sweat test up to three times before the diagnostic label is enshrined as a truth to ensure that technical aberrations do not lead to misdiagnosis. The commonest cause of both false positive and negative values follows suboptimal technique. Difficulties still arise in the use of the sweat test in infancy. Although expertise in certain cystic fibrosis centres can provide results in this age group, testing is often delayed until sufficient sweat volumes are obtained for reliable interpretation.

It is recognised that older children and adolescents present specific difficulties in the interpretation of sweat testing; 10% of normal adolescents will have sweat salt concentrations greater than 60 mmol/l. The fludrocortisone suppression test may clarify the situation in these cases. The administration of oral 9-alpha fludrocortisone (3 mg/m²/day for two days) increases sodium reabsorption in the normal sweat duct and sweat electrolytes fall towards normal in unaffected individuals but not in cystic fibrosis.

The sweat test is not a reliable method for screening, is not always useful in infancy, even in skilled hands, and may be normal or borderline in some children who have subsequently been found to have cystic fibrosis. For this reason
the discovery of the cystic fibrosis gene was met with considerable enthusiasm for its potential as a diagnostic tool and screening device.

Genotyping of the CFTR mutation

Cystic fibrosis is caused by mutations in the gene for the CFTR, which encodes a protein of 1480 amino acid residues and functions in part as a CAMP chloride channel. Approximately 70% of cystic fibrosis chromosomes carry at least one mutation predicted to alter the predicted cystic fibrosis polypeptide.\(^{14}\) The final protein product can also be affected by a large number of additional mutations. The total now exceeds 600 and the prediction of only one cystic fibrosis mutation—does she have cystic fibrosis? Some mutations such as the 5T allele (the 5 thymidine residues disrupt the intron 8 acceptor splice site) are associated with a widely variable clinical presentation from entirely healthy fertile individuals to typical cystic fibrosis patients.\(^{24}\)

Homozygosity for the mutation 3849+10kbC→T predisposes to severe bronchiectasis but normal sweat chloride concentration and male fertility.\(^{21,25}\)

Unusual presentations warrant further evaluation but how far should one go?

Further evaluation of atypical cases

A positive sweat test and two cystic fibrosis mutations on genotyping will provide clear evidence of a cystic fibrosis diagnosis in the majority of cases. Increasingly, however, paediatricians and cystic fibrosis clinics are having to provide further evaluation of the patient with an unusual phenotype or borderline sweat electrolyte concentrations in whom genotyping has failed to demonstrate homozygosity for cystic fibrosis.

A systematic approach would include the following elements:

1. The ‘golden rule’: always repeat the sweat test in a centre that undertakes the test regularly and measure both the sodium and chloride.

2. Thoroughly evaluate the respiratory system: depending on the age and the clinical indications consider:
   - Microbiology of the sputum including bronchoalveolar lavage looking specifically for pseudomonal or staphylococcal colonisation
   - Spirometry, including tests of small airway function
   - Radiological imaging, including computed tomography, of the chest and sinuses.

3. Exclude alternative aetiologies of chronic lung disease:
   - Host defence defects: IgE, immunodeficiencies, α₁-antitrypsin disease
   - Recurrent aspiration
   - Ciliary dyskinesia.

4. Evaluate pancreatic, gut, and liver function if indicated (see below).

5. Urogenital assessment where appropriate:
   - Semen analysis
   - Rectal ultrasound of the urogenital tract.

6. Initiate an extended search for rarer CFTR mutations. Frequently, only four or five mutations will have been excluded and further evaluation should be tailored to the individuals ethnic or geographical origins, their clinical presentation, and course.

How far you search for elusive mutations depends on the clinical need and the resources available. Improved molecular techniques continue to be developed especially to detect the missense or splice site mutations that are associated with partial CFTR function or very low levels of normal CFTR.\(^{27}\) The capabilities of highly sensitive mutation scanning techniques such as denaturing gradient gel electrophoresis may alleviate the current problems with precise mutation detection.

Ancillary testing for cystic fibrosis

Attempts to mount additional evidence for or against the diagnosis of cystic fibrosis could include the following ancillary tests.
NASAL POTENTIAL DIFFERENCE
Patients with cystic fibrosis demonstrate a more negative potential difference across respiratory epithelium than normal or diseased controls. Techniques to measure the transepithelial voltage in the mucosa of the inferior turbinates have been described and in selected cases may provide useful collaborative evidence in the process of accumulating evidence to support a diagnosis of cystic fibrosis. Studies looking at nasal transepithelial potential difference in patients with various defined CFTR mutations showed a high sensitivity for this measurement of CFTR conductance property, however, the measurements are difficult to perform and in children it is frequently more troublesome to achieve meaningful results using this method than the sweat test. Measurements are best undertaken by laboratories experienced in the technique.

Results are influenced by recent viral infections, the precise anatomic location in the nose, previous nasal surgery, allergic rhinitis or polyps, and genotype. IMMUNOREACTIVE TRYPsin
Immunoreactive trypsin can be measured in a blood spot from the Guthrie card and is 2–5 times higher in neonates with cystic fibrosis. The level decreases after 1–2 months and thereafter becomes unreliable. The test is bedevilled by high false positive and false negative rates and has no part to play in the definitive diagnosis of cystic fibrosis. It does have some potential as a useful screening device in a two stage approach incorporating CFTR mutation analysis.

TESTS OF MALABSORPTION
The malabsorption of cystic fibrosis is highly variable and the diagnostically challenging patients are often those with mild clinical symptoms of pancreatic insufficiency. For this reason, tests for pancreatic function have to date been undertaken to assess the need and adequacy of treatment rather than prove the diagnosis.

Pancreatic function tests can be relatively simple to perform such as those designed to show excess faecal fat in stool or deficiency in chymotrypsin while others are technically complex, difficult to interpret, and rarely undertaken even in specialist centres. The latter group includes the measurement of pancreatic enzyme concentrations, duodenal fluid volumes, pH and electrolyte concentrations, and requires duodenal intubation and sequential stimulation of the pancreas with intravenous pancreozym and secretin. It has been noted that significant reductions in the electrolyte and bicarbonate concentrations of duodenal fluid can occur, even in those patients considered pancreatic sufficient on clinical grounds or simple testing. The role of these complex tests in the diagnosis of difficult cases or unusual cystic fibrosis genotypes has yet to be determined.

Microscopic evidence of fat malabsorption on a single stool sample is insufficiently specific or sensitive to be a useful diagnostic tool. The three day stool collection (hated by patients, parents, and laboratories alike) for estimation of faecal fat and coefficient of fat absorption may provide further evidence for or against a cystic fibrosis diagnosis. Additional tests proposed include faecal chymotrypsin, faecal pancreatic elastase 1, and the steatoctit test. The strong desire of workers in this field is to avoid any form of faecal collection or stool gazing. This has led to the avid exploration of alternative means for assessing pancreatic insufficiency and fat malabsorption including consideration of urinary markers (NBT-PABA, fluorescein diluate), serum enzymes (immunoreactive trypsin, lipase, amylase), and the breath (13CO2 in the breath after injection of 13C labelled isotopes). Few show promise at this stage of sufficient sensitivity.

Conclusion
The diagnosis of cystic fibrosis needs to be made early and confidently. No racial group is exempt and children of ethnic minorities or mixed heritage are at greatest risk of delayed or missed diagnosis. Although raised sweat electrolytes confirm most cases of cystic fibrosis, normal values need not reject this diagnosis. Sweat testing remains the standard, (blood or mouth wash) for genotyping can be confirmatory, but the tears of the missed or mistaken diagnosis must be avoided.

A small group of patients is emerging who do not have the classical cystic fibrosis phenotype of severe chest disease, malabsorption, and a reduced life span but have one or more features of ‘milder’ CFTR malfunction (for example decreased nasal potential difference, infertility, positive sweat test ). Perhaps best considered as ‘CFTR associated disease’, they do not necessarily deserve the same rigours of treatment and follow up designed for classical cystic fibrosis. A rational approach would be to identify those patients who are homozygous for mutations in the CFTR gene, recognise potential end organ vulnerability from prior genotype-phenotype correlation studies where possible, and tailor treatment regimens according to individual patient needs.

The call for national screening for cystic fibrosis is gaining impetus and increasingly regions in the UK will have access to this facility. Screening protocols are designed to detect positive cases early and reliably but even with a two tier approach, approximately 10% of cases will still be missed, especially those children of mixed heritage or ethnic minorities with rare mutations. The possibility of false positive labelling could also increase. Clinicians must welcome screening for cystic fibrosis but cannot be lulled into complacency. Vigilance will be required to counter the shortcomings of a screening programme by identifying missed cases, acting on suspicions of a false positive diagnosis, and remaining alert to the unusual clinical presentations and full phenotypic range that encompasses this entity called cystic fibrosis.

Colin Wallis
Respiratory Unit,
Great Ormond Street Hospital for Children NHS Trust,
Great Ormond Street,
London WC1N 3JH

Improving care for central nervous system tumours: a mood for change

Central nervous system (CNS) tumours are both numerically and clinically important. They share a similar incidence with acute leukaemia, making them the commonest group of solid tumours. However, such a statement masks the histological and clinical diversity of this group of tumours. Included within the category are both classically embryonal tumours and between 60–90% of paediatric extracranial solid malignancies are managed in accredited UK (UKCCSG) centres by a paediatric oncologist. The treatment of these tumours is therefore subject to a coordinated national approach and subsequently the setting up of national and international clinical studies or trials. Childhood acute lymphoblastic leukaemia and Wilms’ tumour were the first diseases to be tackled by a coordinated national approach and this model has been extended to all of the major childhood tumours with the notable exception of many CNS. The result is that 87% of haematological malignancies and between 60–92% of paediatric extracranial solid malignancies are managed in accredited UK (UKCCSG) centres by a paediatric oncologist. The conduct of each trial has been associated with sequential improvement in survival and there is a significant survival benefit linked to treatment in a specialist centre. Through a multidisciplinary approach and feedback, the survival rates have increased, and the influence of treatment on normal organ development. It is a sign of the maturity of these studies that current clinical trials are able to focus on improving care rather than concentrating on the diagnosis and staging of these tumours.
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COLIN WALLIS

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