5. Making the diagnosis

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Since October 2007, newborn screening for CF has been in place throughout the whole of the UK (1st July 2007 for those born in our region). At our centre, the majority of new diagnoses are now through this route. Conventional methods of diagnosis are still used to confirm the screening results and will be needed for the small proportion of CF children (estimated at 3 per year for London and South East England) in whom the diagnosis was missed by screening, or who have come from abroad where screening might not take place. About 5% of babies & infants diagnosed with CF were missed on screening in the 4-year audit. These will often have a mild phenotype. We no longer use the term 'atypical' CF.

5.1 Newborn screening

Immunoreactive trypsinogen (IRT) is measured on a dried blood spot obtained on the Guthrie card at day 6 of life. Samples with abnormally raised IRT levels will undergo CFTR mutation screening as per the flow chart (see below). Some children require a second heel prick.

Positive screen results are conveyed directly by the screening laboratory to the specialist centre and the screening pathway initiated.

The CF Nurse Specialist liaises with the baby's Health Visitor to discuss the result and arrange a joint visit to the family. This takes place within 5 working days on a Monday or Wednesday afternoon, enabling the sweat test to be performed the following day on a Tuesday or Thursday morning. The Health Visitor is requested by RBH not to contact the family until 9 am on the day of the visit, to arrange the appointment with them, so we do not prolong the waiting time and anxiety. The HV will be briefed by the nurse specialist to explain to the family that a nurse from the hospital will accompany them regarding part of the newborn screening results and that they suggest both parents may wish to be present at the visit.

In the home it is explained that CF is likely, but that a sweat test is required, and an appointment has been arranged at the Royal Brompton the following day. The family will be seen by one of the CF nurse specialists, and if possible, briefly by the consultant, to introduce themselves. The sweat test is performed, which is mandatory (even if two genes have been identified), to rule out any possibility that the screening sample has been misidentified. Results are available within an hour, allowing, in the majority of cases, the diagnosis to be confirmed to the family by the Consultant; in rarer cases where the diagnosis is unclear, we follow a different pathway - see below. The Consultant will then take a full history, carry out

an examination and answer the parents' questions. The basics of CF may be discussed but at this time of great stress, we attempt to limit the amount of information conveyed to parents, most of which will be discussed at the Education Admission. Similarly, screened babies are usually well.

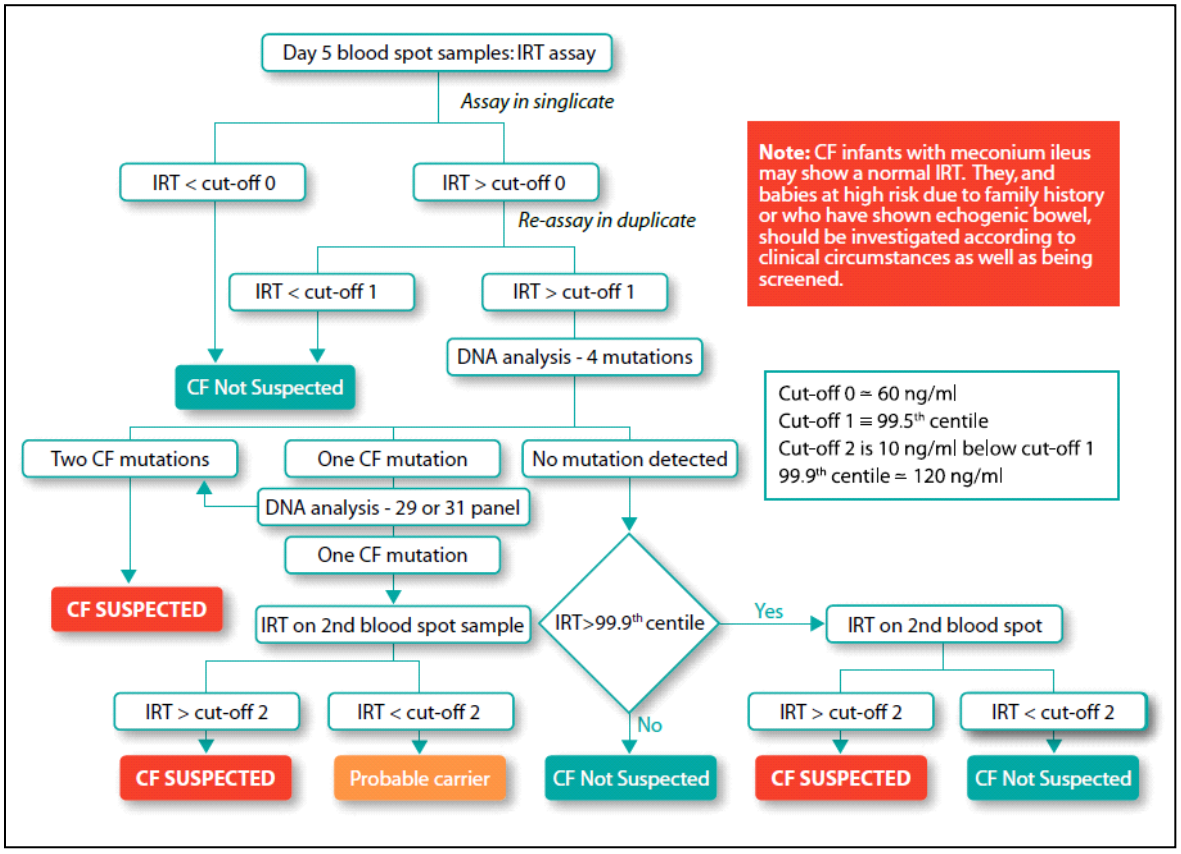
Treatment will usually not be initiated at this time with the exception of pancreatic enzyme supplements if symptoms are indicative of pancreatic insufficiency (abnormal stools, very hungry baby, concerns over weight); if there is doubt the dietitian will see the baby that day. A sample will be collected for stool elastase or parents are given a pot to send back. Just occasionally oral antibiotics are needed as the baby has chest symptoms.

The child's GP will be informed by the consultant or nurse specialist once the diagnosis of CF is confirmed, and in some cases a shared care consultant is also contacted.

A two day educational admission will be arranged for the week after diagnosis. Families are usually admitted to a cubicle in the Sleep Unit and may go home overnight if they wish. A timetable is pre-arranged to ensure that each member of the MDT has an allocated slot in which to teach the family about their role within CF. They will meet with the consultant, nurse specialist, home care nurse, dietician, physiotherapist, clinical psychologist, pharmacist and family liaison officer. The consultant, dietician, nurse specialist and physiotherapist meet with the family on both days to answer any questions that may have arisen. **Education visit is also done for older patients being transferred into our unit from abroad.**

Medication and physiotherapy are started during the admission.

After the two day admission the home care nurse visits the family the next week to offer support and go over what was taught during the admission. They review medications, physiotherapy and any problems that have arisen. The child is then seen in clinic the following week. These appointments are made during the two day admission.



Older siblings of babies diagnosed by screening will have a sweat test; usually the parents are keen for this to be done soon to allay their worries. However, it is not advisable to do this during the education visit as we have had a case of an asymptomatic older sibling being diagnosed at that difficult time; offer to do this before the visit or arrange for the local hospital to do it.

5.2 Clinical presentation

This is rare now that newborn screening is well established. However, it is **essential** that the possibility of a CF diagnosis is not ignored or 'ruled out' if a baby has been born since screening began as screen failures do occur. Additionally, children born before screening may present late with clinical features, as may babies born abroad. Lack of experience of clinical staff may actually lead to further delays in diagnosis in such groups of children. The history and/or examination will usually raise suspicions of the CF diagnosis. Common features are recurrent respiratory infections and faltering growth with steatorrhoea (but do not be fooled by the thriving child). Other features in a baby that mean CF must be excluded include meconium ileus, rectal prolapse, salty tasting skin, prolonged obstructive jaundice, electrolyte disturbance suggestive of Pseudo-Bartter's syndrome and unexplained haemolytic anaemia, hypoalbuminaemia and oedema. Finger clubbing and nasal polyps in an older child are also important, as is isolation of *S. aureus* or *P. aeruginosa* from the respiratory tract. Confirmatory investigations are outlined below. If in any doubt, we do a sweat test, and if anyone at all (including parents) is worried about CF, we do a sweat test.

5.3 Sweat testing

Sweat testing will reliably make the diagnosis in 98% of patients. Despite the availability of genotyping (and because of its limitations) the majority of children in whom CF needs to be excluded will undergo sweat testing. This group will include the following:

- Baby with a positive newborn screen.
- Child with suggestive history / symptoms/ examination.
- Sibling of a known case (even if asymptomatic). We do this routinely for under 5 year olds, and older children if there is clinical suspicion or if the parents wish due to their need for reassurance.
- More distant relative of known case if clinical suspicion.

We perform the sweat test using the macroduct system, and analysis can be reliably performed on small quantities. Minimum of 20 minutes and maximum of 30 minutes is the recommended testing time. Sweat testing can be performed once a baby is > 48 hours old although often inadequate samples are obtained in the first few weeks.

As with any of these techniques, it is extremely important that they are performed by personnel who are experienced. Only the CF nurse specialist, Day-case nurse or trained out-

patients nurses carry out our sweat tests. The sweat is analysed by the Biochemistry lab and results include sweat volume and Cl^- levels. National guidelines for sweat testing have been updated in 2014 - http://www.acb.org.uk/docs/default-source/committees/scientific/guidelines/acb/sweat-guideline-v2-1.pdf

Results must be interpreted in the clinical context

Normal range Cl^- <30 mmol/l;
Borderline Cl^- 30 to 60 mmol/l (although in infants, this may still be CF).
CF confirmed Cl^- >60 mmol/l.

Chloride is the primary ion measured; sodium should not be measured alone. We do not measure conductivity and the available evidence does not in our opinion advocate its use. The diagnosis of CF should be made on the basis of 2 sweat test results not one, we take 2 samples at the same time from different limbs. If there is any doubt over a result, repeat the test or discuss it with a consultant. Flucloxacillin, commonly listed in old texts as a cause of false positive sweat tests, has no effect on a sweat test result.

False negative results. Cases are increasingly recognised where the clinical picture of CF is supported by genotyping, but in the presence of a normal sweat test (<1% CF patients). Beware therefore of excluding the diagnosis (in highly suggestive cases) on the basis of a normal sweat test alone. Genetic testing would be the appropriate next step (see below). Discuss the case and the possibility of nasal potential difference testing with Prof Jane Davies (see later).

False positive results. Many theoretical causes as listed in textbooks, most of which do not appear to cause problems in routine clinical practice. Those which may be encountered include malnutrition or skin disorders such as severe dermatitis/eczema. Transient increases in sweat electrolytes have also been reported in young patients with immunodeficiency states.

5.4 Genetic analysis

There are currently at least 2000 identified variants in the *CFTR* gene, although not all of them are definitely associated with the clinical picture of CF. Mutations fall into different classes (I-VII), with commonest in the Caucasian population being a class II mutation, F508del (formerly) ΔF508. Nomenclature has changed (see appendix 11).

The CFTR2 website is a growing resource which provides excellent data on gene mutations and their expected effects. See www.cftr2.org.

In all children with a diagnosis of CF, we will now seek to fully identify two gene mutations including full gene sequencing if required because:

- In a child diagnosed with CF:
  - it facilitates screening for other family members.
  - and allows prenatal diagnosis of future pregnancies.
- Is an eligibility criterion for mutation-specific therapy (*e.g.* ivacaftor) and may allow enrolment into clinical trials of other agents.

- For pregnant mothers of affected children, cord blood testing should be planned for the newborn sibling at the time of birth (arrange with mother in clinic, give form and blood bottle).
- Generally older siblings will have a sweat test for diagnosis rather than genetic analysis. The latter would detect carriers, which is something that should be postponed until the sibling is old enough to decide whether they wish to know their carrier status (usually mid teens and older).
- We will also use extensive genotyping in cases of borderline diagnosis.

Based on current knowledge, genotype analysis should *not* be used to guide prognosis in an individual child, except rarely (and very cautiously) in the case of mutations usually associated with pancreatic sufficiency (*e.g.* R117H). Pancreatic status should be confirmed with a faecal elastase in all cases; PS may evolve into PI over time, so repeat measurements should be considered, and attention paid to symptoms and nutritional progress. Although studies have shown a milder lung phenotype in certain groups such as these, patients with typical, severe lung disease have also been described, hence it is best not to prognosticate in individual cases. There can also be problems occasionally with a genetic diagnosis of CF in a patient who is asymptomatic with no apparent CF phenotype. These must be discussed with the consultant.

Limitations of mutation analysis

The term 'genetic variant' is now preferred to 'mutation'. Due to the large number of identified genetic variants (>2,000 although not all confirmed as truly disease-causing), and the extreme rarity of many of these, it is only practical to screen for a few on a routine basis. The first-line screen currently includes the commonest 50. Clearly therefore failure to detect variants does not exclude the diagnosis. This is particularly true in a child of non-Caucasian origin. There is now a specific panel of gene variants, which are common in the Asian community. It is therefore CRITICALLY IMPORTANT that in every case the child's ethnic origin is included on the request form so that the most likely variants can be looked for. Full gene sequencing can be performed if specifically requested but is expensive (in the order of £500) and time-consuming; whilst previously we would not have performed this routinely in patients with a clear-cut biochemical diagnosis, all children should now have a genetic cause actively pursued as they may be eligible (now or in the future) for small molecule modulator therapies or trials of these drugs. Samples for both first-line screen or full sequencing should be sent to the Clinical Genetics Lab in house at the Royal Brompton using the genetic testing form available on the intranet. Note - these forms require parental signature to indicate consent. The lab will also perform non-CFTR ion channelopathy testing in complex cases; please contact Prof Jane Davies to discuss any such cases.

5.5 CF Screen-Positive, Inconclusive Diagnosis (CFSPID)

There are two scenarios in which making a diagnosis after a positive NBS is less easy:

1) Borderline sweat test (30-60 mmol/L) in the absence of two *disease causing* gene variants. *
2) Normal sweat test in the presence of 2 CFTR variants, at least one of which is of uncertain significance.

In both these cases, the 'significance' of gene variants can be looked up on the CFTR2 website on ww.cftr2.org, which categorises them into 'disease-causing', 'variable clinical consequence (VCC; see below)' and 'non-disease causing'. The website currently covers the commonest mutations, although the database is growing with time.

* This is a change from our older guideline based on a Delphi consensus process (*J Cyst Fibros 2015;14:706-13*) in which a borderline sweat test (30-60 mmol/L) with two CFTR variants would not be classified as CFSPID, even if one or both of these were variable clinical consequence. The more recent consensus diagnostic guidelines (Farrell PM et al. J *Pediatr*. 2017;181S:S33-S44) now state that in the absence of two *disease-causing* variants, a positive (≥60 mmol/L) sweat chloride is required for a CF diagnosis. The main group affected by this change is babies with F508del/ R117H-7T (or another VCC) with intermediate sweat Cl-, who were previously regarded as CF but should now be labelled as CFSPID. Babies with <2 CFTR gene variants identified should have full *CFTR* sequencing *without delay.*

It is essential that the diagnostic uncertainty is shared with the parents and we avoid any temptation to 'label' a baby prematurely; undoing a CF diagnosis poses its own problems for families.

Genotypes of varying clinical consequence

- There are a number of mutations in this category. Most common one leading to this scenario is R117H-7T (if R117H is reported, it is *essential* the 7T/5T variant is included, otherwise check with lab).
    - R117H-**5T** leads to low levels of CFTR function and is considered a CF disease-causing mutation;
    - R117H-**7T** leads to variable amounts of CFTR function and is so commonly found in non-CF populations in combination with F508del, that this is not considered diagnostic. Some patients with these mutations will have CF, usually pancreatic sufficient and often presenting with symptoms much later in life, and others will not.
    - **9T** is very rarely seen associated with R117H, so if lab report says F508del/ R117H and 9T/5T, the R117H and the 5T are together, (sometimes termed *in cis),* and the 9T can be ignored. The child therefore has the disease-causing R117H-5T mutation.
- Other mutations in this category (this not comprehensive, so if in doubt, check CFTR2): D1152H, L997F, 5T.

Follow up

All CFSPID babies should be referred to Prof Jane Davies' general respiratory clinic, where they will receive further information, clinical surveillance and further CFTR functional testing (repeated sweat testing, stool elastase) and low level clinical monitoring until the clinical picture becomes clearer.

The possibility of male infertility related to CBAVD (congenital bilateral absence of vas deferens) is always discussed; the vas deferens is the most sensitive organ in the body to loss of CFTR function and CBAVD will be present in a proportion of cases.

The main aim is to avoid over-medicalisation, whilst maintaining sufficient observation of the baby to detect any concerns. CFSPID is not a diagnosis, nor was it intended for long-term

use; it should rather be regarded as a holding label. We seek clarification over time of the clinical status:

(1) Evolve into CF:

- increasing sweat Cl- into diagnostic range
- 2 disease-causing CFTR variants recognised on subsequent genetic testing of a child with a previously incomplete genotype
- evolution of clinical symptoms –
    - development of pancreatic insufficiency would be enough in itself, but is very rare in this group
    - respiratory symptoms can be more difficult, as all young children get coughs. Severe or persistent symptoms would be of concern, as might those accompanied by positive cultures such as *P. aeruginosa.*
- CFTR dysfunction confirmed on nasal PD (although this is very difficult in young children and will only be undertaken in cases of high clinical suspicion)

After MDT discussion, children in any of the above groups may be transitioned into the CF clinic and will then be entered onto the national CF registry; they may continue to have a milder clinical course than their conventionally diagnosed counterparts. The parents will be invited to come for our standard 2 day education package for newly diagnosed children.

(2) Remain well with normal or borderline tests:

- We review babies every 6-12 months in a general respiratory clinic
- There is a lack of consensus throughout Europe on whether to routinely culture airway secretions in this group; in our clinic, we are undertaking symptom-guided rather than routine cough swabs
- We are considering how best to undertake long term care of these children. It is likely that we may see them with decreasing frequency and/ or transition to telephone follow up during childhood.
    - Consider sensitive pulmonary tests at this time *e.g.* LCI as a benchmark
    - Ask the family to alert us early of any clinical concerns, particularly chest problems
    - Review in person when the child reaches adolescence:
        - Consider lung function testing and LCI
        - Discuss the risk that some of these patients may acquire chest disease (sometimes significant bronchiectasis) in adulthood, importance of smoking avoidance etc. will be discussed.
        - Revisit the issue of infertility in a male CFSPID case
- Jackie Francis is the CFSPID nursing link, through whom any enquires can be passed.
- Michele Puckey, Consultant Paediatric Psychologist, has an interest in this field and can be consulted for help; these parents often find the uncertainty extremely difficult to deal with.
- At the current time, these babies should not be entered onto the national CF registry, although work is in place to adapt the registry to include a specific CFSPID section. Should they acquire a diagnosis of CF later, they will be added then.

5.6 Antenatal testing

Carrier parents contemplating another pregnancy should be referred for genetic counselling in order to decide whether they would like antenatal screening (CVS, which can be performed around 10-12 weeks gestation or amniocentesis which is usually slightly later).

On the basis of the limited number of mutations screened for, some CF children will be, for example, F508del/-, meaning one detected and one undetected allele. Failure to detect both mutations in the proband does not rule out the possibility of antenatal or sibling diagnosis, as linkage analysis may be possible. Parental blood samples would be required.

When the mother of a child with CF has a subsequent pregnancy, it is important that when they are in clinic with their CF child, we discuss the possible outcomes of the pregnancy. Specifically, the baby is at risk of meconium ileus (particularly if we know the first child is F508del homozygous should it turn out to have CF. Our advice is that the child is not taken home until it has established feeding and had a normal bowel motion. In addition, we recommend that a cord blood sample is taken for DNA analysis, and we give the mothers a form for CF genetics with the relevant blood bottle (EDTA red bottle) to hand to their midwife. The cord blood result is usually ready before the Guthrie card CF screening result is available. We expect that the mother will have informed their obstetrician that they already have a child with CF.

5.7 Pre-implantation diagnosis

For parents wishing to consider pre-implantation diagnosis, to ensure an unaffected fetus, we usually ask their GP to refer them to Mr Yacoub Khalaf at Guy's and St Thomas' Hospital Centre for Preimplantation Genetic Diagnosis. https://www.guysandstthomas.nhs.uk/our-services/pgd/about-us/welcome.aspx

Their website states the criteria for starting PGD treatment -

- You are under the age of 39 for women;
- You complete and return our questionnaires;
- You are living together in a stable relationship;
- (For women) your hormone levels are within a range that suggests that your ovaries will respond to treatment;
- an accurate test is available and there is a license from the HFEA;
- the PGD team agrees that you are suitable for treatment;
- there are no concerns about the welfare of any child conceived using our treatment; and
- Funding is available– either from the NHS or yourselves if you choose to pay for your own treatment. Private costs are £8000 per cycle plus drug costs (£1000-2000).

There may be an issue with CCGs agreeing to pay for the procedure. Referral forms are downloaded from their web address above and sent to -

Centre for Preimplantation Genetic Diagnosis
11th Floor, Tower Wing
Guy's Hospital
Great Maze Pond
London SE1 9RT

Tel: 0207 188 1364
Email: pgd@kcl.ac.uk

5.8 Other tests

These may be supportive of the diagnosis:

- **Stool elastase**: low in CF with pancreatic insufficiency (usually <15 mcg/g). Normal levels (are expected by day 3 in term infants and by 2 weeks of age in those born less than 28 weeks gestation, so tests should not be performed before this time.

Normal	> 200 mcg/g stool
Mild/moderate pancreatic insufficiency	100-200 mcg/g stool
Severe pancreatic insufficiency	< 100 mcg/g stool

  These are sent by our biochemistry lab to Biochemistry Department of Sandwell and West Birmingham City Hospital. For newborn screened babies, the lab will prioritise samples to try to get the result back in 4 days, so that it will be ready for when the parents come in for their Education Visit.

- **Nasal potential difference (PD)**: difficult in small children as requires co-operation but may be useful in older indeterminate cases (over 8-10 years). Can be done easily on young children whilst under general anaesthetic, *e.g.* for bronchoscopy. We rarely obtain useful readings in the presence of nasal polyps or if there has been previous nasal surgery, and it should definitely be postponed if the child has had a cold within the last 2 weeks. It is a difficult and time-consuming investigation and will therefore usually only be done once all other CF investigations are complete. We are not currently undertaking this in the CFSPID population but will consider it when a clinical concern arises. Please refer to Prof Jane Davies (via PA, Gina Rivellini, g.rivellini@imperial.ac.uk, 0207 594 7986), who runs a specialised nasal PD clinic approximately monthly.

5.9 Routine investigations for newly diagnosed patients

For many years we have carried out routine surveillance bronchoscopy and pH study on all newborn screened infants at 3 months of age. Our latest audit of data no longer supports use of routine bronchoscopy over surveillance swabs, which has been aided by the introduction of induced sputum cultures. We are also aware that it is quite difficult for the families to have their baby undergo this general anaesthetic procedure. We will certainly have a low threshold though for diagnostic bronchoscopy and BAL in young children if there is any clinical concern, and induced sputum has not been helpful.

Our data has revealed 50% infants have gastro-oesophageal reflux. We will continue to have a low threshold for starting reflux therapy when babies have symptoms. Furthermore, if infants have recurrent growths of coliforms (*e.g. Enterobacter, Escherichia, Klebsiella, Citrobacter*), we will assume the child has reflux, treat accordingly and consider a pH study.

Blood is taken if the CF genotype is not known from the heelprick screening sample, although the laboratory may have stored DNA if extended genotyping is required so check first. Otherwise blood is not taken until 1st annual review.