June 2019 Edition no. 56

AMPATHCHAT

Sarah Walters (MSc (Med) Genetic Counselling) Ampath Genetic Counsellor

Laboratory Diagnosis of Cystic Fibrosis Update

(This article replaces Pathchat edition no 37)

Cystic fibrosis (CF) is one of the most common life-limiting autosomal recessive conditions in South Africa. CF is increasingly being recognised in all of South Africa's diverse population groups. Major advances in CF treatment over the years have increased the median survival age to more than 36 years of age. However, a timely and accurate diagnosis needs to be made to ensure an optimal outcome.

CF is caused by pathogenic sequence variants in a single large gene on chromosome 7 that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein. To date, over 2 000 CFTR variants have been discovered; not all are classified as pathogenic. The CFTR protein functions as a cAMP-regulated chloride channel, which, in turn, may regulate the activity of other chloride and sodium channels at the cell surface. Abnormal transport of chloride and/or other CFTR-affected ions, such as sodium and bicarbonate, leads to thick viscous secretions in the lungs, pancreas, liver, intestine and reproductive tract. It also leads to an increase in the salt content in sweat gland secretions.

In countries with newborn screening programmes in place, patients are identified earlier. Unfortunately, in South Africa, no broad-based population newborn screening programmes are available, thus patients are often only recognised when they are symptomatic. CF newborn screening is available in South Africa as a measurement of blood immunoreactive trypsinogen (IRT) and/or the identification of two CFTR pathogenic sequence variants (because of a clinical suspicion).

Neonates and children present mostly with meconium ileus, respiratory symptoms and failure to thrive. Young adults present mostly due to respiratory symptoms, sinusitis, gastro-intestinal symptoms, recurrent pancreatitis and infertility.

Diagnostic criteria:

(Cystic Fibrosis Foundation consensus report)

- One or more typical phenotypic features of CF, or
- a history of CF in siblings, or
- a positive CF newborn screening test.

PLUS

- An increased sweat chloride concentration on two or more occasions, or
- presence of two disease-causing (pathogenic) sequence variants in the CFTR gene, or
- abnormal ion transport across the nasal epithelium.

Laboratory testing available

Evidence of CFTR dysfunction can be provided by:

- Sweat testing
  - Chloride measurement
  - Conductivity measurement
- Genetic testing
- Immunoreactive trypsinogen (IRT)
- Faecal elastase to demonstrate pancreatic insufficiency

Sweat testing

Figure 1 illustrates the approach in diagnosing CF according to the South African Cystic Fibrosis Association's Medical and Scientific Advisory Committee guidelies (2017). Sweat **chloride** is the only sweat analyte on which a diagnosis of CF should be based. Ampath has recently acquired a chloridometer (Chlorochek®), which is able to measure the sweat chloride at the lower end of the normal range (10 mmol/l) on low specimen volumes, without the need to dilute specimens, as required by international guidelines.

A minimum sweat volume of 15 µl should be collected in the Macroduct® coil. Sweat **conductivity** may only be used for screening and not for the diagnosis of CF. Conductivity results can be obtained either directly by the Nanoduct® analyser (no sweat specimen collected) or by analysing a sweat specimen collected in a Macroduct® coil on the Sweat Chek™ analyser. A conductivity result >49 mmol/l should be confirmed by sweat chloride testing. A Nanoduct® result should be followed by a sweat collection in a Macroduct® coil, while a Sweat Chek™ result can simply be followed by analysis on the Chlorochek® analyser. Sweat conductivity readings may be up to 15 mmol/l higher than sweat

chloride levels, due to the presence of unmeasured anions (e.g. lactate and bicarbonate). Nanoduct® conductivity results are also limited by a high rate of false negative results.

Interpretation: Sweat chloride measurement

Neonates (0–6 months)
- ≤29 mmol/l: Normal (CF very unlikely)
- 30–59 mmol/l : Intermediate (Possible CF)
- ≥60 mmol/l: Abnormal (Diagnosis of CF)

Individuals over the age of six months
- ≤39 mmol/l: Normal (CF very unlikely)
- 40–59 mmol/l : Intermediate (Possible CF)
- ≥ 60 mmol/l: Abnormal (Diagnosis of CF)

Genetic testing

Genetic testing for CF is offered at the Genetics Department of Ampath Laboratories. Genetic testing for CF is conducted by examining the CFTR gene using the Next Generation Sequencing (NGS) technology. Since the genetic status of all affected children and adults with CF has not yet been completed in South Africa, not all pathogenic sequence variants (mutations) are known in all population groups. Therefore, Ampath offers full sequencing of the entire CFTR gene to detect any and all sequence variants (including benign and pathogenic sequence variants). Also, a multiplex ligation-dependent probe amplification (MLPA) or copy number variation (CNV) is conducted to detect the presence or absence of large deletions and duplications of or within the gene. Full CFTR sequencing is useful both for when there is a strong clinical suspicion of CF, and for when there are CF-like features that could be caused by rare milder mutations (atypical CF).

Genetic counselling is always recommended when any genetic testing is being conducted to educate and provide psychosocial support to the patient and the family. Cascade testing for carrier status of other at-risk family members is available and recommended, especially in light of future family-planning options. Testing of pregnancies of two carrier parents of CF is available, as is newer technology in the form of pre-implantation genetic diagnosis (PGD). This is where, through an *in vitro*-type process, fertilised blastocysts are tested for the presence of the two known CF pathogenic variants that have been predetermined in the family.

Identification of two pathogenic variants in the CFTR gene in an affected individual is becoming more important for treatment, as new pharmacotherapeutic agents that have been specifically designed to target certain CF pathogenic variants, known as potentiators and correctors, have become available (currently only available in some overseas countries).

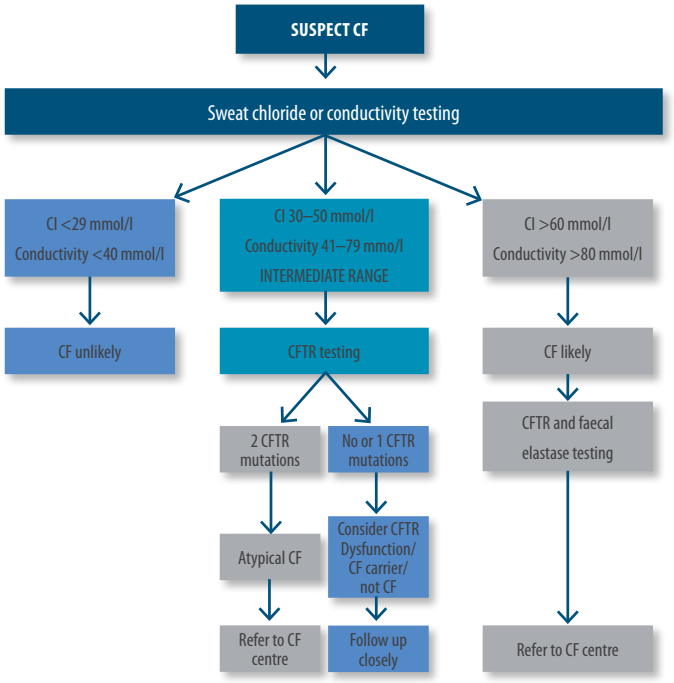
Immunoreactive trypsinogen (IRT)

- IRT is a precursor to trypsin. In neonates with CF and pancreatic dysfunction, release of pancreatic enzymes is impaired, and IRT is not readily removed from the blood stream for conversion to its active form. Most neonates with CF have increased IRT. IRT levels fall rapidly during infancy. After eight weeks, a negative result is not informative.
- Neonates with highly elevated IRT may be considered screen positive. Moderately elevated IRT results are followed with second tier tests for either IRT or CFTR mutation panels. If the screen is positive, follow up with sweat test.
- IRT newborn screening can be done by heel prick blood collection on a Guthrie blood card (dried blood spot). The turnaround-time is three to five days. If a sample is positive, a second sample is recommended for confirmation on day 24.

In a nutshell

- CF is an important common genetic disorder in the South African population, and could present as various clinical scenarios.
- It is important to establish the diagnosis as soon as possible, when any clinical suspicion arises.
- Sweat conductivity (Nanoduct® and Macroduct® collection analysed on Sweat Chek™) is only a screening test and should be confirmed by measuring sweat chloride (Macroduct® collection analysed on Chlorochek®).
- Further confirmatory tests include sequencing of the CFTR gene and faecal elastase.

Figure 1: Approach to testing for CF where sweat testing is available



References

- Zampoli, M, Morrow, B *et al.* (Eds) (2017) *The South African Cystic Fibrosis Consensus Guidelines*, 5th Ed (http://www.sacfa.org.za/wp-content/uploads/2017_09_14_CF_Consensus_Guidelines_2017.pdf , accessed 7/5/2019).
- Ong, T, Marshall, SG, Karczeski, BA, Sternen, DL, Cheng, E, Cutting, GR (2017) Cystic fibrosis and congenital absence of the vas deferens, GeneReviews (www.ncbi.nlm.nih.gov/books/NBK1250).
- Farrell, PM, Rosenstein, BJ, White, TB, Accurso, FJ, Castellani, C, Cutting, GR *et al.* (2008) Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *Journal of Pediatrics* 153: S4–14.