that can influence laboratory assays as well as the pitfalls of the assays to aid with interpretation of results.

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Laboratory tests in the diagnosis of cystic fibrosis

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Cystic fibrosis (CF) is the most common autosomal recessive disorder among white individuals, and occurs in all South African population groups. Recent evidence suggests a prevalence of 1 in 2 000 among white South Africans and 1 in 12 000 in the coloured population. In black South Africans carrier frequency estimates have been used to project the incidence of 1 in 4 624 live births.1

Generally, South Africa offers diagnostic services and expertise similar to those available worldwide for CF patients.2

The genetic defect

CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) protein, a product of the CFTR gene located on chromosome 7. This gene encodes a cAMP-regulated chloride channel that regulates chloride transport at the apical membrane of epithelial surfaces, such as the airways, pancreatic ducts, biliary tree and sweat ducts.3 Abnormal transport of chloride and/or other CFTR-affected ions leads to thick, viscous secretions in these organs. Consequently, the CF patient typically presents with multisystem disease, e.g. suppurrative lung disease, pancreatic insufficiency, multifocal biliary cirrhosis, male infertility and high electrolyte loss in sweat.4,5 The last mentioned forms the basis of the sweat test for the diagnosis of CF.3

Diagnosing CF

Current consensus is that the diagnosis of CF should be based on the presence of one or more characteristic clinical features, a history of CF in a sibling, or a positive newborn screening test plus laboratory evidence of an abnormality in the CFTR gene or protein. This can include biological evidence of channel dysfunction (abnormal sweat chloride or nasal potential difference) or identification of a CF disease-causing mutation in each copy of the CFTR gene. The vast majority of persons with CF are diagnosed based on classic signs and symptoms and corroborative laboratory results; however, in approximately 5 - 10% of patients the diagnosis is not clear-cut.6

Role of the laboratory in the diagnosis of CF

The laboratory plays a key role in the screening, diagnosis and follow-up of patients with CF. Screening tests include sweat conductivity measurement and newborn testing for immunoreactive trypsinogen (universal screening is not currently done in South Africa). Diagnostic tests include mutation analysis, and quantitative measurement of sweat chloride (the sweat test) – the gold standard. Other ancillary tests may also support the diagnosis of CF, e.g. faecal elastase and semen analysis. In addition, certain features such as chronic metabolic alkalosis and acute salt depletion may suggest the diagnosis.6

Genetic testing

Since the discovery of the CFTR gene in 1989, it has been possible to use gene mutation analysis as an adjunct to sweat testing for the diagnosis of CF.3 The most common mutation in the white South African population is the ΔF508 (delta F508) mutation and the most common in the black population is the 3120+G→A mutation. Both are present in the coloured population.7,8

More than 1 200 mutations and polymorphisms have been identified, and as routine screening tests are not able to detect all CFTR gene mutations a negative genetic test does not ensure a normal CFTR genotype.5 Testing profiles may vary, from testing for the two most common above-mentioned mutations, to extended panels, including up to 50 of the most frequently observed CFTR mutations within populations of European origin. Using the ΔF508 only, about 80% of white carriers and 67.4% of coloured carriers will be identified. About 46% of black CF carriers will be detected using the 3120+G→A test.2

Requesting molecular analysis instead of performing a sweat test may confirm a diagnosis of CF, but cannot exclude it.1

Sweat testing is a measure of CFTR function and therefore remains an essential test for the diagnosis of CF, even in the genomic era.3

The sweat test

The measurement of sweat chloride concentration remains a pivotal test for the diagnosis of CF and needs to be performed to established guidelines to prevent pitfalls as well as false positive and false negative results. Sweat collection is generally performed in one of two ways: The Gibson Cooke method uses pilocarpine iontophoresis to stimulate sweat production, with subsequent collection of sweat onto gauze or filter paper for analysis, but more recently many laboratories have changed to using the Wescor® apparatus. This also employs pilocarpine iontophoresis, but the sweat is collected into microbore tubing.7

Both collection methods are followed up by quantitative sweat chloride and sodium analysis.7

Generally, sweat tests are not performed until the subject is more than two weeks of age and weighs more than 3 kg. It can be attempted in term infants after 7 days of age if clinically important, but insufficient sweat collection is often a problem.3 Sweat tests should never be performed on babies under 48 hours of age, as falsely high values may be obtained.12

Testing should be postponed in acutely ill patients. If the patient is malnourished or dehydrated, has eczema, untreated Addison’s disease, ectodermal dysplasia, certain types
of glycogen storage disease or untreated hypothyroidism, false positive results may be obtained. Sweat electrolytes are decreased in patients with oedema and during the administration of mineralocorticoids, resulting in a false negative sweat test. Under these circumstances the test should be delayed or avoided.5-10

Currently the universally accepted reference intervals for sweat chloride concentrations are: >60 mmol/l is considered diagnostic of CF; 40 - 60 mmol/l – borderline; and <40 mmol/l – normal.6,7 Some guidelines propose additional reference ranges for infants up to age 6 months: ≤29 mmol/l – CF unlikely; 30 - 59 mmol/l – intermediate; ≥60 mmol/l – indicative of CF.5

Sweat chloride concentrations >160 mmol/l are not physiologically possible and suggest specimen contamination or analytical error.6,9 Sweat sodium concentration may also be measured. If the difference between the sweat sodium and chloride concentrations is >20 mmol/l, the test should be repeated. Sodium concentrations must not be measured in isolation.6 In addition, patients with CF usually have a chloride/sodium ratio >1. While a ratio >1 supports the diagnosis of CF, a ratio of <1 does not exclude CF and is not recommended for interpretation.5,10

Laboratories are required to follow strict guidelines with regard to iontophoresis time and current, medium of collection, collection time, quantitative sweat electrolyte analysis and competency testing.7,8 More detail with regard to these guidelines may be obtained from www.acb.org.uk/docs/sweat.pdf.9

**Sweat conductivity**

Examples of qualitative screening sweat tests currently in use are the Wescor Sweat-Chek® and the Nanoduct® conductivity analysers. When evaluating sweat conductivity results, it should be noted that values are approximately 15 mmol/l higher than sweat chloride concentration. It is most likely that the difference is caused by the presence of unmeasured anions such as lactate and bicarbonate.5,10 Sweat conductivity must be regarded as a screening test only, with conductivity values >80 mmol/l (expressed as sodium chloride equivalent) very likely due to CF. All values >50 mmol/l must be followed up with quantitative measurement of sweat chloride.7,10

**Immunoreactive trypsinogen**

In South Africa, population screening for CF is not currently performed. It is, however, possible to identify most CF infants during the first days of life by measurement of blood immunoreactive trypsinogen (IRT) (pancreatic pro-enzyme precursor of trypsin) and identification of CFTR mutations.4 In countries where newborn screening is available, heel-prick blood specimens collected on days 2 - 4 after birth to measure IRT is a primary screen. Babies with increased IRT levels are selected for sweat testing and mutational analysis. It is thought that pancreatic acini in infants with CF are capable of producing trypsinogen, but ductules are blocked, preventing trypsinogen from reaching the small intestine to be converted to trypsin, hence leading to ‘spillage’ into the circulation. The IRT levels decrease after 1 - 2 months, indicating that pancreatic acini are no longer functioning well enough and therefore the IRT test becomes unreliable after this period.5

**Ancillary tests**

Faecal elastase can be measured to assess pancreatic exocrine function. This pancreas-specific protease present in the pancreatic juice is not degraded during passage through the gut. Low faecal elastase (<200 μg/g) after 4 weeks of age is indicative of pancreatic insufficiency and provides supporting evidence for a diagnosis of CF. Respiratory tract microbiology (sputum or bronchoalveolar lavage) and urogenital evaluation (semen analysis) can also be useful in the diagnosis of CF.5

**Conclusion**

CF is a common genetic disease with a diverse clinical presentation, and should be considered in the differential diagnosis in all South African population groups with suggestive symptoms. The laboratory plays a central role in the diagnosis and follow-up of these patients, but clinicians must be aware of the limitations, pitfalls and systematic use of these tests when interpreting results.

References available at www.cmej.org.za

**Investigation of immediate-onset IgE-mediated food allergy**

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It is important to differentiate between food allergy and other causes of adverse reactions to food because patients with severe immediate-onset IgE-mediated food allergy are at risk of developing anaphylaxis that may cause death (Fig. 1).

Up to 35% of the population in Western countries self-report ‘food allergies’, but the true prevalence is probably between 3% and 6% in children and 1% and 4% in adults.1 Approximately 90% of documented cases of food allergy in the USA are caused by a relatively small number of foods that comprise cow’s milk, egg, soy, wheat, peanut, tree nuts, fish and shellfish. Food-induced anaphylaxis is caused mainly by peanut, followed by tree nuts, fish, cow’s milk and egg.

**Laboratory investigations**

Current food allergy guidelines emphasise that a true diagnosis of immediate-onset IgE-mediated food allergy requires a positive history of clinical allergy to a specific food as well as a positive allergy test that matches that history.2,4

Any investigation into food allergy has to commence with a detailed allergy-focused history and examination, followed by a selection of appropriate tests to confirm or exclude allergy. It is important to distinguish between sensitisation (the presence of allergen-specific IgE antibodies) and allergy...