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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.<sup>5,8-10</sup>

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.<sup>3,8</sup> 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.<sup>6</sup>

Sweat chloride concentrations >160 mmol/l are not physiologically possible and suggest specimen contamination or analytical error.<sup>10</sup> 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.<sup>5</sup> 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.<sup>9,10</sup>

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.<sup>8,9</sup> More detail with regard to these guidelines may be obtained from [www.acb.org.uk/docs/sweat.pdf](http://www.acb.org.uk/docs/sweat.pdf).<sup>9</sup>

### **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.<sup>10</sup> 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.<sup>7,10</sup>

### **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.<sup>4</sup> 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.<sup>5</sup>

### **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.<sup>5</sup>

### **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](http://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.<sup>1</sup> 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.<sup>2-4</sup>

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

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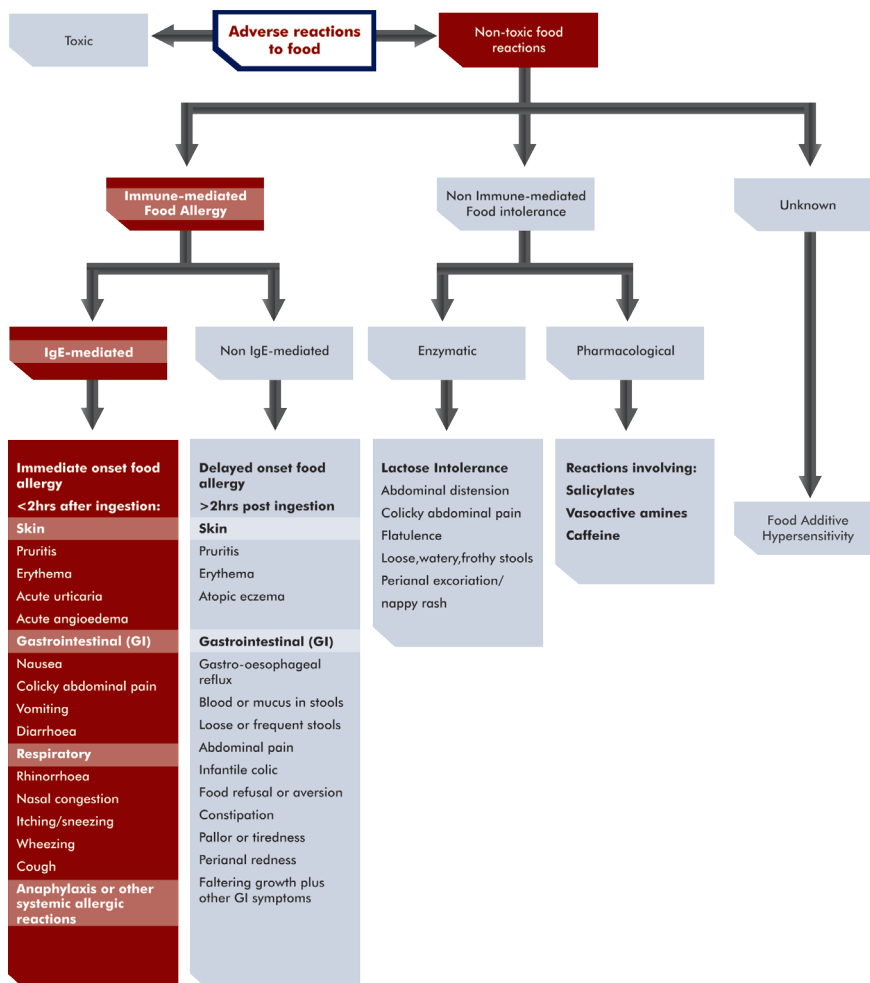


Fig. 1. Adverse reactions to food.

(the presence of sensitisation plus clinical signs and symptoms of allergic reactivity). Skin-prick tests (SPTs) and blood allergen-specific IgE tests measure sensitisation, while oral food challenge tests measure clinical reactivity.

### Skin-prick test

A SPT is an indirect qualitative measurement of IgE sensitisation. A positive SPT is triggered by specific allergen-induced cross-linking of IgE antibodies on sensitised mast cells and the subsequent release of histamine that causes an itch, a flare and a wheal response at the prick site. Although this immune-mediated mechanism is by far the most potent trigger of mast cell degranulation, there are other non-immune mediated triggers that have similar effects. SPTs are sensitive (few false negative results), but they lack specificity (many

false positive results). A SPT only has a 50% positive predictive value (PPV) for the diagnosis of food allergy in the absence of a clear history. Interpreting SPTs without a proper evaluation of the supporting history often leads to the over-diagnosis of food allergy.

Certain constraints need to be considered before ordering a SPT. Medications that inhibit the typical histamine reaction should be stopped (Fig. 2). SPTs cannot be performed in patients with dermatographism, or when there is extensive atopic dermatitis or eczema. SPTs should not be selected as first-line tests if there is a history of severe reactions to specific foods, because of the risk of allergen-induced anaphylaxis during the test. None of the former constraints applies to allergen-specific IgE (sIgE) blood tests.

### Allergen-specific IgE

The acronym RAST (radioallergosorbent test) is still erroneously used to refer to new-generation sIgE tests, which provide a better measurement of allergen-specific IgE antibodies than RASTs. A positive blood test indicates that specific antibodies are/have been made to a specific food antigen. It confirms sensitisation of the individual, but not necessarily allergy, because <50% of sensitised individuals develop signs and symptoms of allergy during their lifetime. A positive diagnosis of IgE-mediated food allergy requires evidence of both sensitisation and clinical reactivity; a positive sIgE alone cannot confirm allergy in the absence of a clear history of allergy to that food.

sIgE is reported in quantitative units. This allows manipulation of cut-off points to improve the diagnostic specificity of sIgE; the higher the chosen diagnostic cut-off level, the more likely the diagnosis of allergy becomes. Diagnostic cut-off points with a 95% PPV for allergy have been determined for a number of the most important food allergens. They are recommended for use when properly validated (Fig. 3).<sup>1</sup> Unfortunately, such cut-off points are not available for other allergens. It should also be borne in mind that >50% of patients will have sIgE values that are <95% PPV cut-off points; their diagnoses will rely on the supportive history and oral food challenge data.

Although sIgE is generally, but not invariably, less sensitive than SPT, current guidelines indicate that negative tests can be used to rule out allergy in most patients. In instances where sIgE and SPT results are both negative and where the history of allergy is highly suggestive, oral food challenge (OFC) tests have to be done to exclude allergy with more certainty.

### Oral food challenge test

The double-blind placebo-controlled food challenge (DBPCFC) is still the gold standard for the diagnosis of food allergy; all allergy guidelines emphasise its importance and encourage its use.<sup>2-4</sup> A DBPCFC is recommended to confirm a diagnosis of food allergy in all instances where there

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| Abstention Period | Drugs that Inhibit Skin Prick Test Reactions   |
|-------------------|--|
| No Abstention     | Low dose inhaled and short term corticosteroids generally don't suppress the wheal and flare reaction, although larger doses may do so |
| 1 Day             | Histamine H2-receptor antagonists e.g. Cimetidine (Tagamet) or ranitidine (Zantac) have a limited inhibitory effect                    |
| 2 Days            | 1st generation antihistamines  |
| 3 -10 Days        | Non-sedative 2nd generation antihistamines   |
| 2 Weeks           | Medication with antihistamine properties eg. Anticholinergic agents, phenothiazine and tricyclic antidepressants                       |

Fig. 2. Medications that inhibit the typical antihistamine reaction.

| Generic Reference Values for sIgE (KU/L) | 95% PPV Cutoff for Specific Foods (KU/L) |
|--|--|
| <0.1 = < detect. limit                   | Egg >7                                   |
| 0.1 - 0.35 = borderline                  | Peanut >14                               |
| 0.35 - 0.70 = very low pos               | Cow's milk >15                           |
| 0.70 - 3.5 = low pos                     | Fish >20                                 |
| 3.6 - 17.5 = <b>positive</b>             | <b>Soy</b> > <b>65</b>                   |
| 17.5 - 50.0 = <b>high pos</b>            | <b>Wheat</b> > <b>80</b>                 |
| 50.0 - 100.0 = <b>very high pos</b>      |  |
| > 100 = <b>extremely high</b>            |  |
|  | <u>Infants (&lt;2 years):</u>            |
|  | <b>Egg</b> > <b>2</b>                    |
|  | <b>Milk</b> > <b>5</b>                   |

Fig. 3. Reference values.

are no reliable 95% PPV diagnostic cut-off points and where the history of allergy is equivocal. A DBPCFC is also indicated if there is a clear mismatch between history and laboratory data. It is costly and time consuming and very few centres in South Africa are equipped to do DBPCFCs routinely.

Single-blind placebo-controlled food challenges (SBPCFCs) and open food challenges are more readily available, but unlike DBPCFCs they do not eliminate clinician and patient bias. Despite this limitation, negative challenges with such tests are considered diagnostic for ruling out food allergy, but the predictive value of positive tests (when allergic symptoms are elicited) is less certain and relies on supportive history and other laboratory test data.

Because of the risk of anaphylaxis in patients with a history of severe allergic

reactions, especially in patients with asthma, OFC tests must be conducted in facilities with on-site medical supervision that are properly equipped to deal with medical emergencies. The same applies to SPTs with food allergens.

### Evaluation of tolerance

Prolonging avoidance diets unnecessarily is harmful and has a negative impact on patients' nutritional health and psychosocial wellbeing.

The majority of allergic children develop tolerance to cow's milk, egg, soy and wheat between the ages of 3 and 16 years. Approximately 20% of peanut-allergic children will develop tolerance, while <10% outgrow allergy to tree nuts. The likelihood of outgrowing allergy to fish and shellfish is slim and adults who develop any type of food allergy are less likely to develop tolerance than children.

A high initial level of sIgE against food is usually associated with a lower rate of resolution of clinical allergy over time, while declining sIgE levels in children (but not always in adults) is an indication that tolerance is developing. Following quantitative sIgE values over time helps to determine when it is safe to do an OFC test to decide whether an avoidance diet can be stopped. Annual sIgE testing is accepted practice for milk, egg, soy, and wheat, while the test interval can be increased up to three years for peanut, tree nuts, fish and shellfish.

SPTs remain positive long after patients have developed tolerance; they are better suited for initial diagnosis rather than follow-up. Inadvertent sensitisation to specific allergens is also a theoretical possibility during a SPT.

### Limitations of traditional diagnostic tests

There are currently no diagnostic tests that can accurately predict anaphylaxis or the severity of future reactions in patients who are allergic to certain foods.

Traditional allergy tests are based on crude natural food extracts that consist of complex mixtures of allergenic and non-allergenic proteins and other molecules.<sup>5</sup> Those based on such extracts are useful screening tests for allergy, but do not discriminate between primary sensitisation to major or minor allergens that have either more or less potential to elicit allergenic reactivity. They also do not discriminate between sensitisation to single or multiple allergens in extract mixtures, and can't establish whether positive reactions are due to cross-reactivity or co-sensitisation to allergenic proteins that are not species specific and that might be very similar to proteins in other foods or pollen to which the patient has been exposed.

Food extracts are very difficult to standardise owing to the complexity of the components in the extracts and the variability of natural products and their endogenous degradation processes. This explains the lack of diagnostic sensitivity and specificity of SPTs and sIgEs that are based on primary food extract mixtures.

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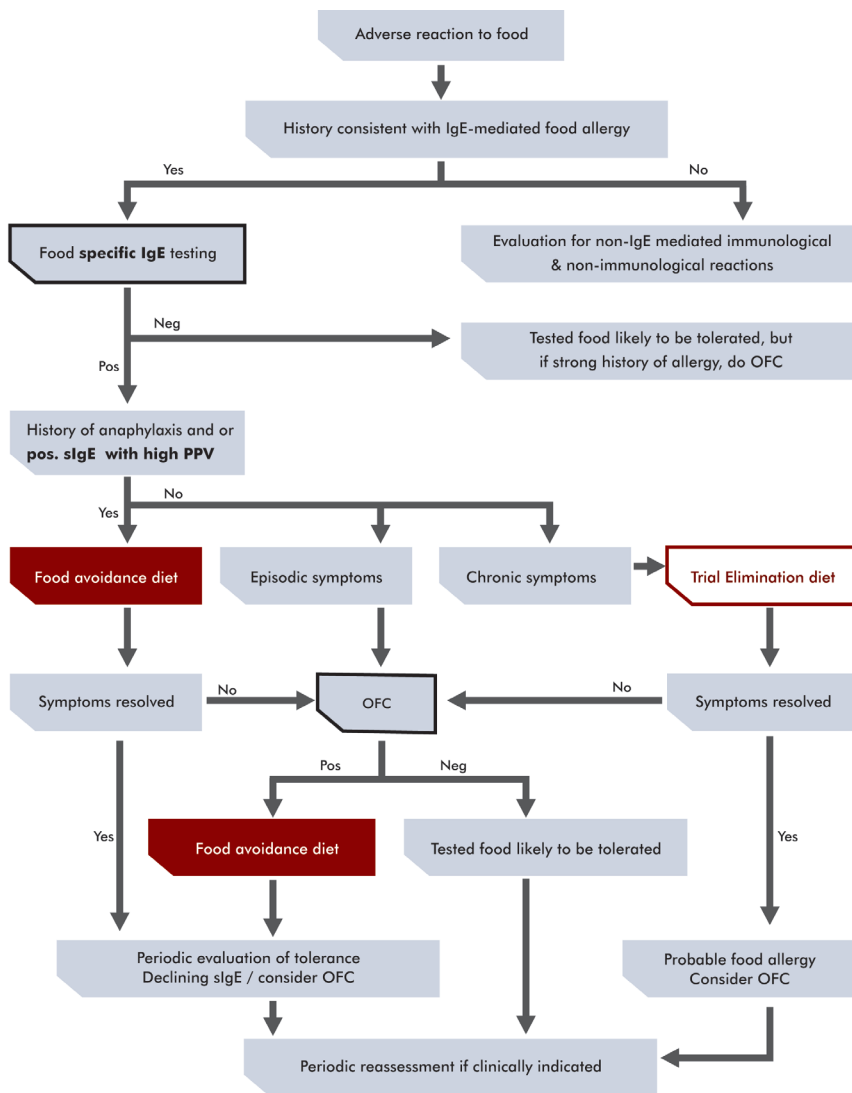


Fig. 4. Investigation of immediate-onset IgE-mediated food allergy.

### Component-resolved diagnostics (CRD) and recombinant allergens

Component-resolved diagnostics (CRDs) and molecular allergology attempt to circumvent the above-mentioned problems. CRDs focus on the use of mono-component sIgE tests that are based on single native allergenic proteins (purified from complex natural food extracts), or on single recombinant antigens (obtained from biogenetically engineered protein fragments that are virtually identical to major IgE-binding epitopes identified on various allergenic food proteins).

Standardisation of recombinant sIgE ( $\mu$ sIgE), either as a single artificial component test or as tests based on mixtures of artificial recombinant allergens, is significantly better than tests based on purified native extracts ( $\mu$ sIgE) and is clearly superior to tests based on crude food extracts. The development of standardised recombinant allergen  $\mu$ sIgE tests has provided clinicians with quantitative tools that can delve more deeply into the precise aetiology of allergy.

Recombinant allergen-based tests have some limitations, however. Individuals are

exposed to allergens from natural sources and not to recombinant proteins. They are capable of developing antibodies to a range of different fragments of natural allergens that will not necessarily be present in recombinant allergen-based tests. The use of CRDs and recombinant allergens is currently not recommended to replace, but rather to complement and refine, the results obtained from traditional diagnostic tests.

### CRD and peanut allergy

Peanut allergy is the most common cause of food-induced anaphylaxis, but not all peanut-allergic patients have the same risk for anaphylaxis and not all require a strict avoidance diet. Each of the 13 peanut allergens currently identified has its own specific risk- and cross-reactivity profile and each peanut-allergic patient may be sensitised to any one or more of those allergens. This variation explains why some peanut-allergic patients have a higher risk for anaphylaxis and are unlikely to become tolerant, and why some of them will tolerate tree nuts while others will not. A positive peanut recombinant Ara h 2  $\mu$ sIgE has for example become an established risk factor to differentiate patients with true peanut allergy from those who are sensitised but clinically tolerant to peanuts.<sup>6</sup>

CRDs have helped to elucidate the varied nature and risk profiles of other important foods, such as tree nuts, fish, milk, egg, soy, wheat, where recombinant allergens play an increasingly important diagnostic role.<sup>5</sup>

### Microarrays and comprehensive allergen profiles

A comprehensive allergen profile is required to fully understand an individual's specific risk and cross-reactivity profile. The development of biochip protein-microarrays in combination with multiplexing technology has enabled the simultaneous analysis of large numbers of different allergens on minute samples in a cost-efficient manner. The ImmunoCap ISAC microarray is available in South Africa and provides semi-quantitative measurement of

112 mono-components from more than 50 allergen sources. Nanotechnology benefits paediatric diagnosis, because it requires very little blood to do a comprehensive allergen profile. It is mainly indicated for patients with multiple food and inhalant allergies. Its main drawbacks are onerous validation procedures and the risk of overdiagnosis; it requires specialist knowledge to prevent misinterpretation of the complex results of these tests.<sup>7</sup>

Ongoing research is required to improve the ability of laboratory tests to assess the presence and severity of food allergy and to predict prognosis and resolution of disease.

### Summary (Fig. 4)

- Commence investigation of food allergy with a detailed allergy-focused medical history and examination.
- Select the appropriate allergens and co-allergens for sIgE or SPTs to confirm or exclude IgE-mediated allergy.
- Confirm equivocal findings with an OFC test.
- Monitor development of tolerance with sIgE (in children) and confirm with an OFC test.
- Allergy tests should only be undertaken by healthcare professionals who are competent to perform and interpret them.
- SPTs and OFC tests should only be undertaken where there are facilities to deal with an anaphylactic reaction.
- CRDs employing recombinant allergens and comprehensive allergen profiles using microarray nanotechnology offer complementary diagnostic tools for the allergy specialist.

Further reading and references available at [www.cmej.org.za](http://www.cmej.org.za)

## Troponins and acute coronary syndrome

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Cardiac muscle injury is defined as the disruption of normal cardiac myocyte membrane integrity resulting in the loss of intracellular constituents such as troponin, creatine kinase, and myoglobin into the extracellular space. The mechanism of injury includes trauma, toxins and viral infections, but ischaemia or infarction – due to an imbalance between the supply and demand of oxygen – is the most common cause.<sup>1</sup>

Acute coronary syndrome (ACS) constitutes a large spectrum of clinical conditions, ranging from unstable angina pectoris to acute myocardial infarction (AMI).

### Diagnosis of acute myocardial infarction

The diagnosis of AMI was traditionally made using the combination of chest pain, electrocardiographic (ECG) manifestations, and elevations in serum or plasma of cardiac biomarkers. The biomarkers traditionally requested are troponins and creatine kinase – MB fraction (CK-MB). Clinical symptoms such as chest pain are frequently atypical or absent, and ECG changes may be nonspecific or absent. This has resulted in the diagnosis of AMI becoming more dependent on the measurement of biomarkers.

Because of their greater sensitivity and specificity,<sup>1</sup> cardiac troponins (cTn) are the biomarkers of choice for the evaluation and management of patients with ACS, and in the diagnosis of AMI. Guidelines set in 2007 by the National Academy of Clinical Biochemistry and the European Society of Cardiology/American College of Cardiology stated that ‘in the presence

of a clinical history suggestive of ACS, the following is considered indicative of myocardial necrosis consistent with myocardial infarction: an elevation in cTn concentrations above the 99<sup>th</sup> percentile of a healthy population, accompanied by an assay imprecision of  $\leq 10\%$  ...<sup>2,3</sup> In addition, a rising and/or falling troponin pattern is an important component of the universal definition of AMI.<sup>3</sup>

### Troponin biochemistry

Cardiac troponins consist of three proteins known as cTnC, cTnI and cTnT based on their function: C for calcium-binding, I for inhibition of actin-myosin interactions, and T for tropomyosin binding to facilitate contraction.<sup>2,3</sup> cTn is released in the setting of irreversible damage to the myocyte and starts rising in blood 4 - 6 hours after cell death, peaks at approximately 18 - 24 hours and remains detectable for up to 14 days. This time frame is observed when using non-high-sensitivity cTn assays.<sup>1</sup>

### ‘Highly sensitive’ cTn

The ever-increasing sensitivity of cTn assays has led to the development of ‘highly sensitive’ cTn (hsTn) assays, capable of measuring cTn levels below the 99<sup>th</sup> percentile in a healthy population. Use of these hsTn assays makes it possible to detect low levels of cTn even in healthy subjects.<sup>4</sup> A rise in cTn can be observed 2 - 3 hours after the onset of an AMI, leading to earlier diagnosis and therapeutic intervention.<sup>1</sup> Serial changes documented by a second measurement will help to differentiate acute cardiac disorders (showing a rise and/or fall) from chronic cardiac disease, which will usually exhibit constant cTn levels.<sup>5</sup> The improved sensitivity involves sacrificing reduced specificity, leading to additional diagnostic challenges for clinicians.<sup>6</sup> With the increased use of hsTn assays and the application of the 99<sup>th</sup> percentile as the decision limit for AMI, a substantial increase in detection of patients with elevated cTn levels will be observed, and a high percentage of patients will be misclassified. It should be emphasised that AMI is not the only cause of myocyte necrosis, and therefore non-ischaemic causes of troponin elevation should be kept in mind.<sup>3</sup> The high