Getting to the Heart of the Matter:

Evolving Troponin Assays for Earlier Diagnosis of Acute Myocardial Infarction

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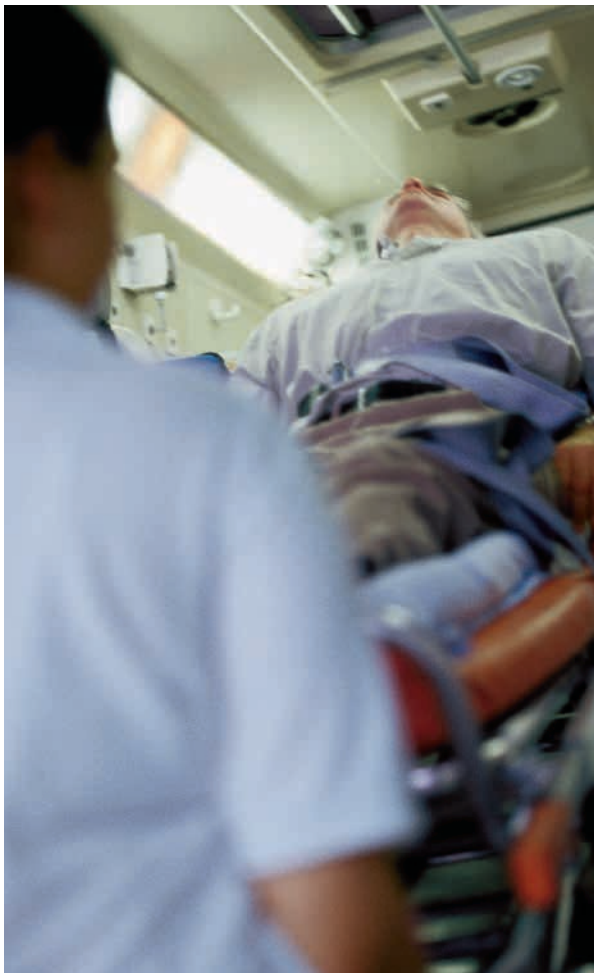

Imagine the following scenario:

- A 45-year-old man arrives in the emergency department (ED) complaining of non-radiating chest pain and dyspnea from which he has suffered for over 6 hours.
- He denies having engaged in strenuous physical activity that might have resulted in injury or muscle pain, and in fact the pain is not exacerbated with deep breathing.
- The patient reports previous treatment for hypertension, but has not continued anti-hypertensive therapy for several years and reports no history of other cardiac or general medical issues.
- A 12-lead ECG indicates no ST elevation, abnormal Q waves, or Left Branch Bundle Block (LBBB).
- Cardiac troponin (cTn) was detectable neither at admission nor 6 hours later according to values obtained using a conventional cTn assay.
- How should the ED physician continue evaluating this person?
- Should more tests be conducted, such as an echocardiogram or stress test?
- Should more invasive testing such as coronary angiography be carried out?
- Should the patient be discharged with the advice to seek outpatient care?

Although radiating pain is considered a hallmark of acute myocardial infarction (AMI), not all individuals exhibit typical symptoms. Therefore, when evaluating chest pain, AMI should not be ruled out on the basis of symptoms alone. Because the majority of patients experiencing AMI have no ECG abnormalities, ECG alone is not sufficient for rule-out in approximately 90% of chest-pain patients arriving in the ED. For these reasons, in accordance with the universal definition of MI first adopted by the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) in 2000, and redefined in 2007 and 2012 by the joint ESC / ACCF / AHA / WHF, several criteria need to be considered before AMI can be either diagnosed or ruled out. The current definition is summarized in Figure 1.

The element central to the current algorithm is the observed change of a cardiac biomarker, preferably either cardiac troponin I (cTnI) or T (cTnT). If the cardiac biomarker requirement is not met, none of the other conditions alone or in combination are sufficient to classify or treat the event as an MI. Because its presence in serum or plasma is specific for cardiac myocyte damage and necrosis, cardiac troponin is preferred as a biomarker over CK-MB.

However, cTn assays have evolved considerably over the last decade and differences in detection capabilities have affected how results might be interpreted within the framework of the universal definition/redefinition. Earlier assays (referred to in this article as "conventional assays") were unable to detect very low levels of cTn with the accuracy achievable by many newer assays, and this typically affected whether or not cTn detection could be ascribed to cardiac injury. The universal definition/redefinition approach originally improved diagnostic accuracy over symptom-only algorithms using conventional assays because – for the most part – such assays detect cTn in no more than 1% of individuals presumed healthy. Thus any patient with at least one cTn measurement above the diagnostic cutoff using a conventional assay has a very high probability of having suffered recent cardiac injury. Although such an injury may be associated with trauma and chronic cardiovascular or kidney disease, in concert with ischemic symptoms, conventional cTn assays are highly specific and sensitive for AMI.

In the past few years, assays have been developed which are more sensitive: they can detect cTn with greater accuracy and at much lower levels, and in most such assays, cTnI may be observed in approximately 50% or more of individuals who are presumed healthy.[2] Assays with varying capabilities of detecting cTn in large segments of the presumed healthy population are referred to in the literature as "sensitive" or "high sensitivity". In fact, at least one such high-sensitivity cTn assay (hs cTn) has been reported to detect cTnI in 100% of healthy individuals, indicating that Tn normally circulates at low levels that are simply below the analytical sensitivity (i.e., limit of detection [LoD]) of the older assays.[3] This means that it may now be more difficult to determine whether, in the absence of trauma, a positive result actually reflects an ischemia-related rise, whether it is indicative of some other acute or chronic cardiac, renal, or other disease, or whether it is simply normal for that individual.

Detection of a rise and/or fall of cardiac biomarker values (preferably cardiac troponin) with at least one value above the 99th-percentile upper reference limit

and with at least one of the following:

- Symptoms of ischemia
- New or presumed new significant ST-segment T-wave (ST-T) changes or new left bundle branch block (LBBB)
- Development of pathological Q waves in ECG
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- Identification of an intracoronary thrombus by angiography or autopsy

Figure 1: Key aspects of the 2012 Third Universal Definition of Myocardial Infarction.[1]

Needless to say, this has created confusion as to how to interpret sensitive and hs cTn assay results in a clinically relevant way. In which cases should sensitive and hs cTn assay results actually be considered to be of diagnostic relevance for AMI? What criteria should be applied to these tests to rule out AMI? Christian Mueller, MD, FESC, Professor of Medicine and Head of Clinical Research and Acute Cardiac Care at the University Hospital in Basel, Switzerland, addressed these issues in a 2013 webinar which he delivered courtesy of Siemens Healthcare. Before discussing Dr. Mueller's recommendations on how best to use sensitive and high-sensitivity cTn assays, it is helpful to understand what they measure, how assay precision is defined, and what the difference is between analytical sensitivity (i.e., the detection limit of the assay) and clinical sensitivity (i.e., the cutoff for diagnosing AMI).

Biochemistry and precision

Cardiac troponin assays use antibodies directed at either the I or T subunit of the troponin molecule. The universal definition of MI specifies that cTn assays should be able to detect cTn (either I or T) at ng/L (pg/mL) levels. The coefficient of variation (CV) is an estimate of assay imprecision achieved by measuring multiple replicates of a single sample. According to the universal definition, %CV should be ≤ 10% at the level defining the 99th-percentile in a normal reference population (also referred to as the upper reference limit, or URL). Very few of the earlier-generation cTn assays actually achieved the 10% CV criteria at the URL. In many of these assays, CV at the URL ranged between 20% and > 50%, and a 10% CV could only be attained at a level 2 to 3 times greater than the URL. For example, in 2005, five different assays claimed detection of the 99th percentile at 0.1 µg/L (100 ng/L), but the 10% CV (and hence the diagnostic cut point) for these assays ranged from 0.33 to 0.44 µg/L (330 to 440 ng/L). This low precision meant that a value measured at or near the URL might be pathological, but it could just as easily reflect an imprecise measurement of a non-pathological level (false positive). Because measurement was not reliably

accurate within 10% of the measured value until it was 3 to 4 times the URL,[4] and because it can take several hours longer for cTn to reach the trustable level than the actual 99th percentile, diagnosis could be delayed, costing the patient valuable treatment time and requiring more ED resources than if a more rapid diagnosis were possible. Additionally, imprecision near the URL can mask small changes in level over time. In this case, the absence of a discernible change over an extended observation period – even 12–24 hours or more into an acute coronary event – might lead to the patient being discharged without appropriate treatment.

What qualifies an assay as "sensitive" or "high-sensitivity"?

In the last decade, many manufacturers have developed new generations of cTn assays with improved analytical sensitivity. Although a classification standard has yet to be fully agreed upon, Dr. Mueller uses analytical precision to differentiate conventional assays from sensitive and hs assays, noting that sensitive and hs assays can detect cTn at the 99th-percentile with imprecision (CV) ≤ 10%, whereas as mentioned above, imprecision at the 99th-percentile upper limit of normal (ULN) is > 10% CV for conventional assays. Thygesen et al., further specifies that hs assays should have an LoD between 0 and 9 ng/L. In comparison, the LoD of most conventional assays detected cTn around 0.04–0.20 µg/L (note that the official units for conventional assays are reported in µg/L, whereas hs assays are reported in ng/L or pg/mL).[5] Assays with a lower LoD increase analytical sensitivity by an order of magnitude or more. Many experts in the field (such as the members of the International Federation of Clinical Chemistry [IFCC] task force on cardiac biomarkers) also maintain that assays should only be labeled hs if they can detect cTn in more than 50% of the normal reference population. Many hs assays have been reported to detect cTn in ≥ 95% of presumed healthy individuals, and, as mentioned earlier, at least one has been shown to detect cTn in 100% of the healthy reference population.[3, 5] In fact, high detection rates in the reference population make calculation of the 99th percentile more precise. This, along with other modifications to assay design, has improved assay precision such that the CV for sensitive and hs cTn assays is ≤ 10% at the true 99th percentile.[1] Thus, in contrast to conventional assays, sensitive and hs assays should indeed meet the requirements outlined in the universal definition consensus statement (Figure 2).

Diagnostic speed and accuracy

Because the analytical sensitivity is so much greater and imprecision at the cut point is considerably improved in hs assays and some sensitive assays, rises in cTn can be seen at much lower levels. This makes little difference in the treatment of the 5% of patients with clear ECG-identified STEMI as they can be diagnosed based on ECG and symptoms and treated within minutes. However, this increased sensitivity provides a tremendous advantage in diagnosing and treating the much larger population of patients who are experiencing either NSTEMI or unstable angina, and for ruling out AMI in the largest population of patients in whom chest pain is not associated with an ischemic event.

Previously, guidelines for diagnosing NSTEMI using conventional assays required monitoring for cTn elevation above the decision cut point over a period of at least 6 hours, and sometimes for as long as 24 hours. However, in early AMI, cTn concentrations may not be sufficiently elevated to be detectable in the peripheral blood for several hours; in fact, conventional assays are "detection blind" for approximately 4–6 hours after MI onset (Figure 3). Because of this limitation, a patient evaluated in the ED within 2–4 hours of symptom onset might not be diagnosable on the basis of cTn levels upon arrival and would have to remain in the ED with ECG monitoring for several more hours before MI could

[1] The URL and CV for most sensitive and hs should be assays vary depending on the population, study, and testing site.

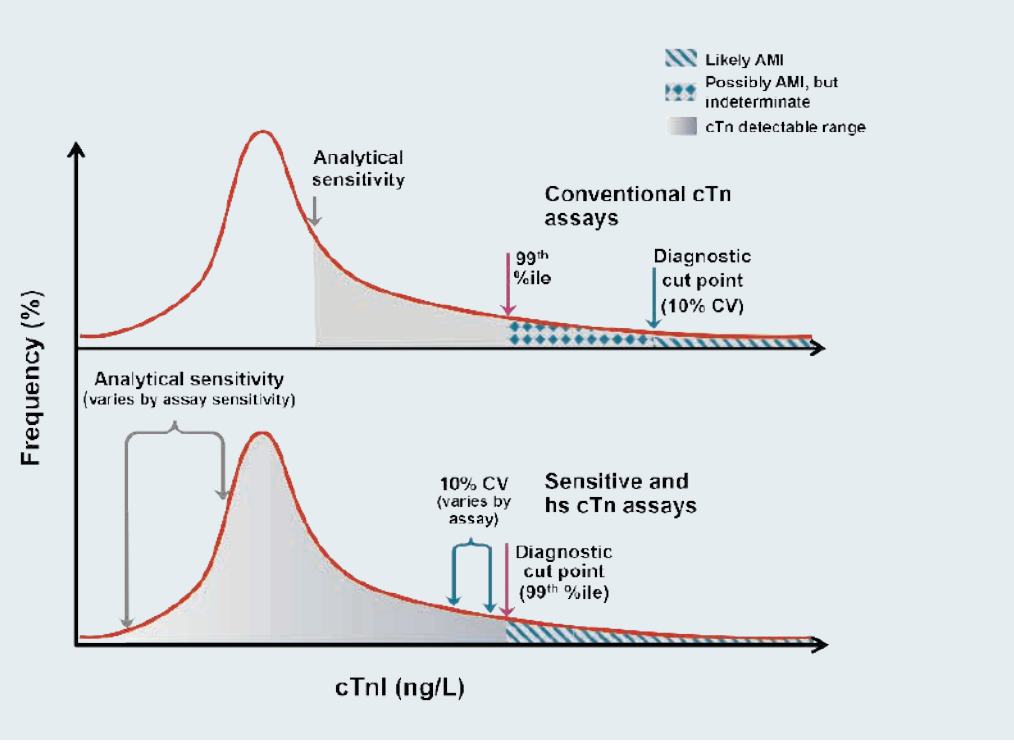

Figure 2: Detection by conventional vs. sensitive and high-sensitivity cTn assays.

be ruled in or ruled out – a situation that contributes to increased resource expenditure, hospital costs, and ED overcrowding. Thus, using conventional assays, unless cTn levels are indisputably elevated at presentation, the shortest time to either rule in or rule out is typically around 7 hours after ED admission (depending on whether or not a second measurement indicates that the cTn level has changed).

Improved rule-in and rule-out of NSTEMI by sensitive and hs cTn assays

In 2011, the ESC recognized that more sensitive assays could begin to detect a change in elevation in blood drawn within 3 hours after symptom onset and recommended that more sensitive cTn assays be used to both rule in and rule out NSTEMI. The ability to detect myocardial damage earlier can be understood by comparing where the decision cut points fall in relation to multiples of the URL for conventional versus sensitive and hs cTn assays (Figure 3). Currently, using a sensitive or hs cTn assay can reduce diagnostic time to between 3 and 4 hours, and Dr. Mueller and his team project that assay improvements and other considerations (to be discussed below) will eventually lead to a 1-hour diagnostic window. Earlier treatment and reduction of cardiac damage will clearly benefit patients in both the short and long term. At the same time, hospitals benefit by reducing ED congestion, costs, and impact on resources.

In addition to shorter time to diagnosis, diagnostic accuracy of NSTEMI improves with sensitive and hs cTn assay use. With conventional assays, the area between the URL and the 10% CV diagnostic cut point constitutes a gray area or "indecision zone" where levels are likely pathologic but somewhat indeterminate. In his presentation, Dr. Mueller explained that patients with a normal ECG and cTn elevation in this indecision zone would previously have been diagnosed with unstable angina. However, because the CV at the URL is ≤ 10%, sensitive and hs cTn assays can reliably detect elevation at their analytical cutoffs, eliminating the indecision zone. As a result, many of these patients are now correctly diagnosed with NSTEMI and treated accordingly. In general, Dr. Mueller's team showed that the diagnostic accuracy of a number of sensitive and hs cTn assays is much higher for early diagnosis than conventional assays (Figure 4).[6]

The importance of serial measurements and change

Similar to conventional cTn assays, however, it appears that the greatest strength in sensitive and hs cTn assay use and interpretation lies in serial measurements for both rule-out and rule-in of AMI. Observation of a serial change is especially important for ruling in AMI in cases where cTn may be chronically elevated, such as in patients with structural heart disease, kidney disease, or other conditions in which chronic elevation is common. In 2011 Keller et al. demonstrated that, in combination with using the 99th-percentile as the admission cut point, a relative increase in cTn between admission and 3 hours later

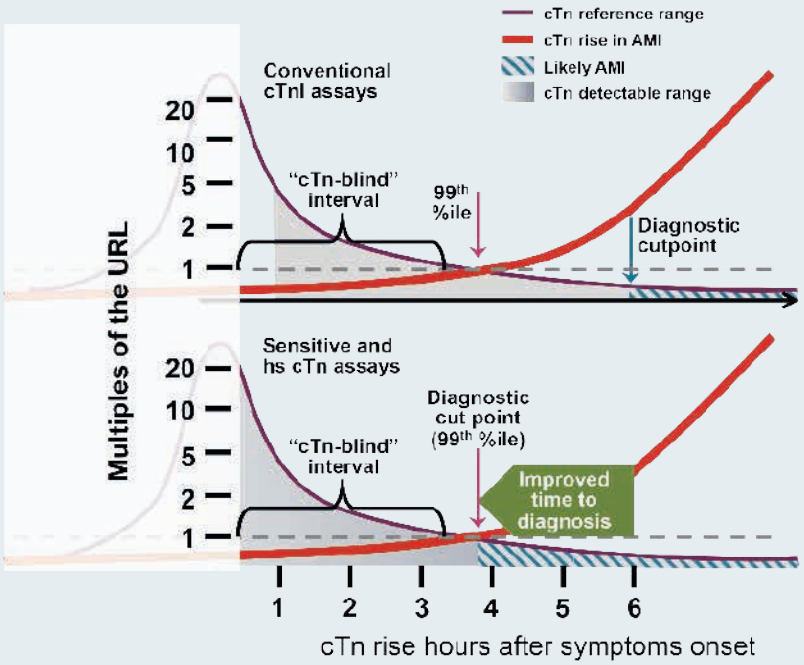

Figure 3: Relation between increasing cTn and the diagnostic cut point.

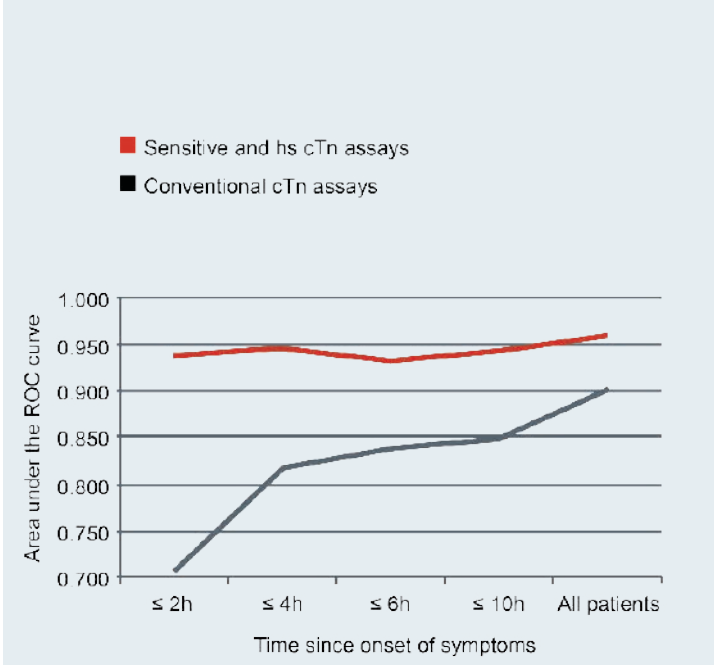

Figure 4: Diagnostic accuracy of conventional vs. sensitive and hs cTn assays.

had a greater diagnostic value than a single elevation at admission.[7] Reichlin et al. made a similar observation for absolute and relative changes at 1 and 2 hours after admission, but also noted that the negative predictive value for ruling out AMI was 98% at 1 hour and 99% at 2 hours after admission if there was no significant change in the cTn level using a sensitive or hs cTnI assay (Negative Predictive Value [NPV] was slightly lower when the relative change was evaluated).[8] Earlier rule-out of non-AMI-associated chest pain, which accounts for ~50% of the ED chest-pain population, would help to substantially reduce ED costs and patient wait times.

These observations and others have led the IFCC to propose guidelines for use of either absolute or relative changes to support rule-in and rule-out of AMI. In this guidance, the IFCC notes that large changes in levels indicate high specificity for AMI at the expense of sensitivity (i.e., some patients with AMI will be missed if the practitioner decides the serial change is too small to justify an AMI diagnosis). Conversely, if a practitioner prefers to diagnose AMI when the serial change is small for increased sensitivity, specificity will be lower, and more patients might be incorrectly diagnosed with an AMI when the underlying cause of chest pain is not an acute event. Regardless, the serial change value will depend on the assay and timing interval used and will have to be empirically determined. They will still require, however, that at least one measurement is elevated above the URL and that the magnitude of the change observed in a serial reading can be accurately measured according to the CV at the level of change for the assay used. Use of serialchanges in cTn levels for diagnosis was the thrust of major discussion at the UK NEQAS Cardiac Markers Dialog meeting held in June 2014.

Quantitative aspects of sensitive and hs cTn assays

Finally, Dr. Mueller's research suggests that positive and negative predictive values may be assignable using specific quantitative results. Very high levels above the 99th percentile are more likely indicative of a large AMI, while lower-magnitude elevations may be associated with smaller or micro AMIs. More studies are needed, however, to fully develop and substantiate predictive values.

Case study: how using sensitive and hs cTn assays can improve diagnosis

Let us return to the patient presentation at the opening of this article. This hypothetical patient was actually a case study presented by Dr. Mueller. In this case, the man was discharged from the ED with instructions to follow up with his own physician. However, this patient returned to the ED 4 days later with acute chest pain that was more severe and radiated to his left arm and back. A 12-lead ECG was very clearly diagnostic for an acute STEMI, and the patient was immediately revascularized in the cath lab. However, asked Dr. Mueller, might this man have been treated differently 4 days earlier at the initial presentation if a sensitive or hs cTn assay had been used? The answer is likely yes. Dr. Mueller reanalyzed this patient's stored samples using a sensitive cTn assay and two hs cTn assays (Table 1). In this case, unlike the con-

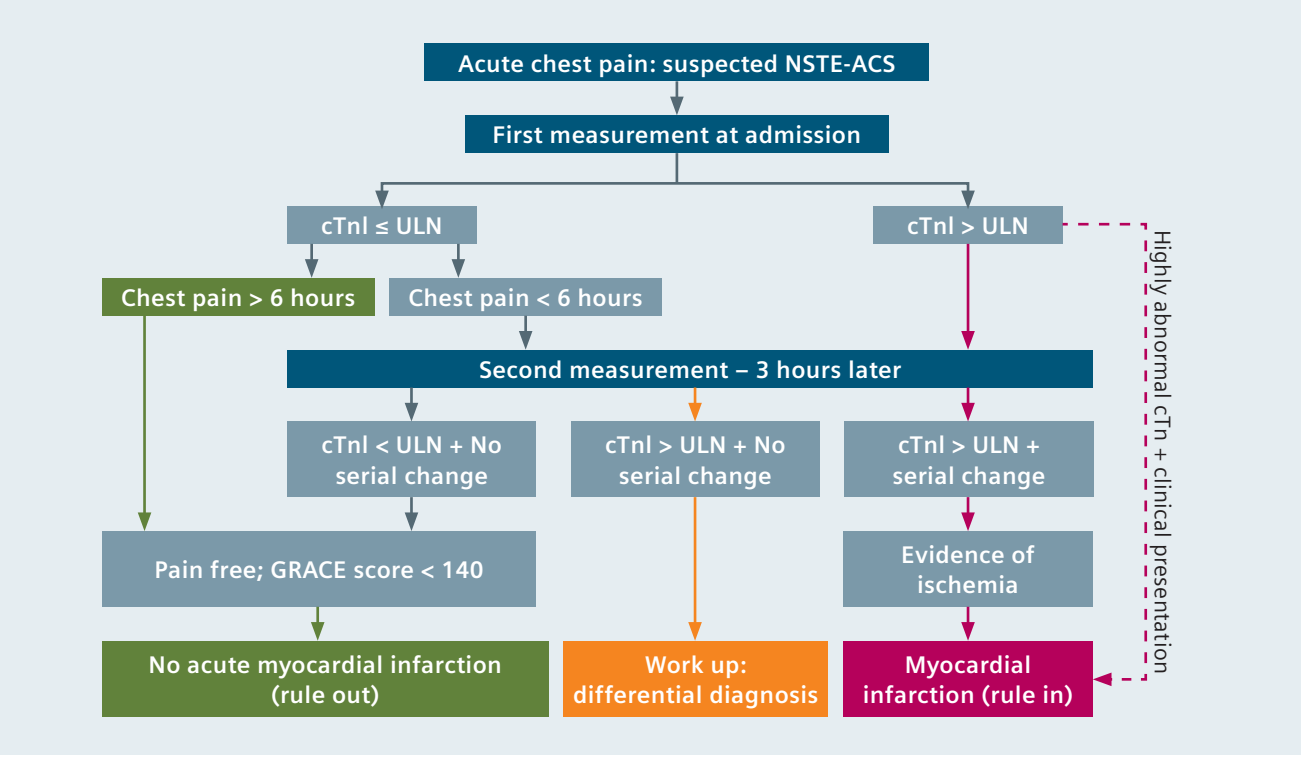

Figure 5: Algorithm for evaluating suspected NSTEMI (adapted from the 2011 ESC guidelines).[10]

		cTn Concentration				
Assay	**Diagnostic Cutoff**	**0 h**	**1 h**	**2 h**	**3 h**	**6 h**
Conventional cTnT (Roche, fourth-generation)	0.35 µg/L	<0.01 µg/L	<0.01 µg/L	<0.01 µg/L	<0.01 µg/L	<0.01 µg/L
Siemens ADVIA Centaur® TnI-Ultra assay	40 ng/L	16 ng/L	39 ng/L	88 ng/L	102 ng/L	
Roche hs cTnT[9]	13.5 ng/L	11.2 ng/L	22.4 ng/L	31.0 ng/L	32.0 ng/L	
hs cTnI in development by Siemens	9 ng/L	18.2 ng/L	44.8 ng/L	66.7 ng/L	100.0 ng/L	

Table 1: Serial measurements of patient samples taken at initial presentation (4 days before STEMI diagnosis) using conventional, sensitive, and hs cTn assays.

ventional assay used at the original presentation, all three sensitive and hs cTn assays detected a serial rise in cTn with at least one measurement above the 99th percent cutoff within 0–2 hours after presentation. In all likelihood, had a more sensitive assay been used at the initial presentation, this patient would have received earlier treatment, resulting in less myocardial damage and scarring, at a lower overall cost of care (considering two ED visits requiring additional monitoring and more resources than a single visit would have required).

Key parameters for using sensitive and hs cTn assays: summing it all up

Clearly, there is much to consider and understand if your hospital laboratory has migrated to a sensitive or hs cTn assay, or is considering doing so. Despite the potential confusion over the interpretation of such assays, Dr. Mueller emphasizes their value for both ruling in and ruling out AMI much more rapidly than conventional assays by summarizing tips for an early diagnosis:

1. Use an assay for which CV is ≤10% at or below the 99th-percentile.
2. The 99th-percentile is assay-specific, and either the value supplied by the manufacturer or a value garnered from studies must be used consistently.
3. Quantitative use can increase the predictive value of the test.
4. Absolute changes in serial measurements can help to differentiate chronic from acute cardiac disorders (Figure 5).[10]

Following these recommendations, diagnostic time can be shortened from 6 hours or more to 3–4 hours. As more data is being collected, Dr. Mueller foresees a day when both rule-out and rule-in might be achieved in as little as 1 hour, which would undeniably be a boon to both patients and hospitals.

Clinical implications of a sensitive or hs cTn assay

- Lower levels of troponin can be detected earlier
- Changes in serial samples support AMI diagnosis
- Serial sampling can reflect a change ≤ 3 hours after first presentation
- Earlier detection and serial samples translate to faster diagnosis, improved care, and reduced costs

Further Information

www.siemens.com/healthcare

Dr. Mueller's complete presentation (registration on the site is free and required for viewing)

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The outcomes achieved by the Siemens customers described herein were achieved in the customer's unique setting. Since there is no "typical" hospital and many variables exist (e.g., hospital size, case mix, level of IT adoption), there can be no guarantee that others will achieve the same results.