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The role of troponin testing in the diagnosis of acute coronary syndrome: when and how?

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ABSTRACT

Background and aim: The large number of publications on the role of troponin testing in the setting of patients with suspected acute coronary syndrome, in patients with stable angina, in patients with comorbidities, and in elderly residents makes it difficult to determine what to do (which troponin test), in what patients (acute or chronic coronary disease), and in which setting (testing in the ambulance, emergency department or Intensive Care Unit). The development of point-of-care troponin I and T tests has opened the door for early testing, but testing too early will conflict with the "troponin blind interval". The recent improvements of analytical sensitivity of point-of-care troponin tests have created a vast number of new situations in which an early generated troponin result may lead to rapid diagnosis (in conjunction with ECG, anginal complaints, and physical examination) and rapid "rule in" or "rule out" decisions, which will lead to less admissions to the emergency department, less diagnostic activities like imaging procedures, and less costs of clinical care.

Methods: This review addresses developments of this field in the past 10 years since we asked the question "Will future troponin measurement overrule the ECG as the primary diagnostic tool in patients with acute coronary syndrome?"[1]

Findings: The sensitivity and reliability of the cardiac troponin I or T tests, including point-of-care cardiac troponin I or T tests, have been improved to a level that is indicated by the indication "high-sensitivity" (hs). These tests make it possible to determine cardiac troponin I or T values (i) in ≥50% of blood samples of healthy individuals, (ii) in blood samples of patients with acute myocardial infarction of very recent origin, (iii) in blood samples that show a rise of cardiac troponin I or T levels, and (iiii) in blood samples that show a fall of cardiac troponin I or T levels. An interesting development is the use of a second biomarker, of which copeptin is a promising adjunct. Instead of a second biomarker, clinicians are assisted by employing acute coronary syndrome scoring systems (such as the HEART score), for instance, to identify patients with low risk. The issue of specificity of elevated cardiac troponin I or T levels for acute myocardial infarction is scrutinized.

Conclusion: We conclude this review by stating that for patients with suspected acute coronary syndrome the protocols are refined for how and when to use cardiac troponin I or T tests for (i) prehospital triage to rule out low risk patients, and (ii) in hospital risk assessment of (very) high risk patients, including anamnesis, physical examination, and ECG. The continuing development of cardiac troponin tests improves the quality of diagnosis and shortens the interval to diagnosis and subsequent discharge from hospital, leading to less further clinical assessments, and less health care costs. In general, any new biomarker test should comply with filling in existing gaps in clinical care.

Keywords: hs-cardiac troponin, non-hs-cardiac troponin, acute coronary syndrome, acute myocardial infarction, point-of-care test

1. Introduction

Individuals with suspected acute coronary syndrome (ACS) receive their first diagnosis usually outside a hospital. On the basis of risk assessment these patients are either ruled-out, or sent to a hospital. In both situations ECG and troponin (Tn) tests, in combination with anamnesis and physical examination, are the pillars of a definitive diagnosis of acute myocardial infarction (MI), unstable angina pectoris, and chest disorders, such as tachyarrhythmias, heart failure, left ventricular hypertrophy, hypertensive crises, cardiomyopathies, valvular heart disease, myocarditis, and stable coronary artery disease[2]. In the past decade, it has been studied whether pre-clinical cardiac troponin I/T (cTnI/T) tests may reduce the burden of patients with suspected ACS sent to the hospital. The rapid development of point-of-care cTnI/T tests stimulated these studies. As we stipulated some years ago[1], the point-of-care cTnI/T tests at that time had insufficient analytical sensitivity of diagnosing MI, unstable angina pectoris, and myocardial injury. Secondly, we wondered whether in patients with non-ST-segment elevation ACS the hs-cTnI/T tests should be combined with other tests, such as tests of B-type natriuretic peptide (BNP) or NT-proBNP. In the past 10 years a number of issues concerning the improvement of the clinical performance of hs-cTnI/T tests have been reported, such as the 99th percentile of hs-cTnI/T concentration of a healthy population, the gender-specific 99th percentiles of normal hs-cTnI/T concentration[3], concomitant cardiac and non-cardiac abnormalities influencing the level of hs-cTnI/T, the 1 h difference (Δ) and the 3 h Δ protocols for diagnosticly accurate "rule-out" and "rule -in" decisions, the absolute or relative Δ, interference of the Tn test by biochemical issues such as auto-antibodies and anti-troponin I antibodies[4], and high interindividual biological variability of cTn[5]. Moreover, in 2019 the Fourth Universal Definition of Myocardial Infarction has subclassified MI into five phenotypes of MI that may or may not influence the diagnostic value of hs-cTnI/T levels[6]. Besides the classification by the Fourth Universal Definition of Myocardial Infarction, MI that may occur after intervention procedures have also be classified according to the Academic Research Consortium-2 definition[7] and the Society for Cardiovascular Angiography and Interventions definition[8]. The Fourth Universal Definition of Myocardial Infarction together with the 2012 European Society of Cardiology guidelines for the management of acute MI in patients presenting with ST-segment elevation[9], and the 2015 European Society of Cardiology guidelines for the management of ACS in patients presenting without persistent ST-segment elevation[10] give a detailed outline of (i) diagnosis of a patient with suspected ACS, (ii) acute treatment of a patient after following the "rule-in" or "rule-out" protocol, (iii) therapy in the hospital, and (iiii) further therapy after discharge. The performance of the hs-cTnI/T tests necessary to guide these clinical steps has reached a higher analytical and clinical performance for the new and refined intended uses. In this paper the quality improvements of hs-cTnT/I tests in the past 10 years, including their sensitivity and specificity for diagnosis of acute MI, are reviewed.

2. Point-of-care high-sensitivity cardiac troponin I or T tests

Assessment of chest pain, ECG findings and cardiac troponin (cTn) values form the diagnostic cornerstones of diagnosis and clinical work-up of any patient with suspected ACS. The ECG may present ST-segment elevation, new Q-waves, or new left bundle branch block in patients with ST-segment elevation myocardial infarction (STEMI), ST-segment depression and/or repolarization disturbances in patients with non-ST-segment elevation myocardial infarction (NSTEMI), and transient ST-segment changes and T-wave changes in patients with unstable angina pectoris. An effective pre-hospital triage strategy that could reliably identify chest pain patients with a low probability of having an acute MI could lead to a reduction of unnecessary (pre)clinical treatments, overcrowding at the intensive care unit, health care costs, and unnecessary patient anxiety. A preclinical cTn test result by a point-of-care test would increase the speed of the diagnostic procedure. Implementation

of a point-of-care test with high sensitivity for cTnI/T may potentially enable faster and more accurate results provided that these tests are undertaken as part of a standardized hospital procedure under the supervision of a central laboratory, connected to the laboratory information system, and used by adequately trained staff[11].

Around 2013 the point-of-care cTnI/T tests had insufficient analytical sensitivity of diagnosing MI, unstable angina, and myocardial injury. The International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB) proposed that for an assay to be defined as high-sensitivity, two analytical criteria need to be met. First, the %CV of the test at the 99th percentile of normal should be ≤10%. Second, measurable concentrations should be attainable at a concentration at or above the assay's limit of detection for ≥50% of healthy individuals[12].

In the past 10 years enormous advances in sensitivity and precision of point-of-care hs-cTn tests have been achieved. In 2018 Wu and coworkers, on behalf of the Expert Opinion from the Academy of the American Association for Clinical Chemistry and the IFCC TF-CB, published clinical laboratory practice recommendations for the use of cTn in patients with ACS[13]. Using these recommendations the developers of novel hs-cTnI/T tests now know exactly the conditions and properties their tests have to comply with. A novel point-of-care cTnI assay was described by Pickering and coworkers in a pilot study of 354 patients. While not formally designated as a high-sensitivity assay, the Abbott TnI-Nx is capable of reporting concentrations from 1 to 1500 ng/L. They found this assay could rule out acute MI on the basis of a single cTn result, with negative predictive value and sensitivity comparable with the established ARCHITECTSTAT hs-cTnI laboratory platform[14].

The International Federation of Clinical Chemistry and Laboratory Medicine has published a list of currently available cTn tests, including point-of-care cTn tests, with analytical performance that enables their intended use[15].

Sörensen and coworkers evaluated a point-of-care cTnI assay (PATHFAST hs-cTnI, LSI Medience) in a cohort of patients with suspected MI presenting to the emergency department. A diagnostic algorithm for a hs-cTnI point-of-care assay was developed. The test showed a negative predictive value of 99.7% (95% CI, 98.1%–100.0%) and 48.0% of patients were ruled out, whereas 14.6% were ruled in with a positive predictive value of 86.5% (95% CI, 77.6%–92.8%). The diagnostic performance of the point-of-care hs-cTnI assay was highly comparable to guideline-recommended use of a laboratory-based hs-cTnI assay. Specifications of the hs-cTnI point-of-care cTnI assay: limit of detection=2.9 ng/L, 10% CV=11.0 ng/L, 99th percentile of normal= 24.2 ng/L in women and 27.0 ng/L in men. By combining the benefits of a point-of-care assay, easy access and short turn-around time with the high sensitivity and accuracy of modern hs-Tn assays, the management of patients admitted with suspected ACS to the intensive care unit are expected to be improved[16].

Ter Avest and coworkers compared the analytical and diagnostic performance of a point-of-care test for cTn (AQT90-Flex point-of-care cTnT assay, Radiometer), characterized by a low 99th percentile cut-off, with hs-cTn assays routinely used in the laboratory, and performed on a single blood sample collected at presentation, from patients with chest pain and suspected ACS. The sensitivity of the point-of-care Tn test was much lower (68%) than that shown for laboratory hs-cTn assays (91%) in the prediction of MI. However, the negative predictive value for MI for both technologies was quite similar (95% versus 98%, respectively)[17].

Earlier data showed a good diagnostic accuracy for hs-cTnI using the Alere Triage point-of-care cTnI platform (Abbott), to diagnose MI at 3 h in early presenters with chest pain[18].

The ARTICA trial showed that pre-hospital rule-out of NSTE-ACS in low-risk patients by a single point-

of-care TnT measurement (Cobas h232 point-of-care cTnT assay, Roche Diagnostics) results in a significant reduction of healthcare costs in the first 30 days and low incidence of major adverse cardiac events (MACE)[19,20]. Pre-hospital rule-out of ACS has recently been shown to reduce healthcare costs in a trial in Norway[21]. Implementation of routine pre-hospital point-of-care Tn measurement has been shown to effectively identify high-risk patients in Denmark, whereas pre-hospital HEART score assessment including a point-of-care Tn measurement has been shown to adequately identify low-risk patients in the Netherlands and the United States[22-24]. At present the most sensitive hs/cTnI point-of-care device is the ATELLICA VTLi hs-cTnI test (Siemens). This device offers acceptable performance for use within the intended medical settings[25].

In a recent study from the Artificial Intelligence in Suspected Myocardial Infarction Study (ARTEMIS) this assay (Siemens Atellica VTLi point-of-care hs-cTnI) was used to determine the safety and direct rule-out of acute MI by a personalized algorithm that can predict the individual probability of acute MI with a single point-of-care hs-cTnI measurement in patients with suspected MI, STEMI excluded. This procedure allows for very rapid, safe, and more efficient direct rule-out of acute MI than guideline-recommended pathways, and accelerates the safe discharge of low-risk patients from the intensive care unit. This algorithm might open new opportunities for the triage of patients with suspected acute MI even in ambulatory, preclinical, or geographically isolated care settings[26].

3. The "troponin blind interval"

The very early Tn test may suffer from the "troponin blind interval", which is the time delay between the onset of an acute MI (*e.g.*, a coronary occlusion) and the first substantial rise of cTn in blood. If home physicians and ambulances are working quickly, the first blood draw may not manifest a cTn rise. Recent studies have examined how the use of hs-Tn testing lead to a more rapid diagnosis of acute MI, suggesting that acute MI may be diagnosed as quickly as 2–3 h[27].

Using hs-TnT, for example, the optimal threshold for rapidly excluding ACS is likely to be its limit of detection of 5 ng/L, while the 99th percentile cut-off of 14 ng/L is considered the diagnostic threshold, and it may take time to rise across the 99th percentile[27].

4. The 99th percentile cut-off of high-sensitivity cardiac troponin I or T levels of a healthy population

The selection of a normal reference population for the 99th percentile cut-off determination remains a critical but also controversial issue as the characteristics of the reference population have a profound effect on the 99th percentile cut-off value. A working group of the ESC recommends, by consensus, an increase of >50% of the 99th percentile value if the baseline value lies below the 99th percentile and the second value exceeds the 99th percentile. In individuals who have an initial cTn value already above the 99th percentile value, an increase of only 20% in the second sample is necessary to diagnose NSTEMI[28]. Multiple factors may influence the determination of this critically important decision limit (*i.e.*, selection criteria including biomarkers, statistical approach, gender, smoking status, diabetes, and age of reference individuals). Even atherogenic lipid markers, particularly apoB or apoB:apoAI ratio, influence the 99th percentile cut-off for hs-cTnI[29]. In univariate analysis, hs-TnT was found to be associated with age, sex, total cholesterol, LDL-cholesterol, HDL-cholesterol, but not with triglyceride. In multivariate linear regression analysis, age (positive correlation; $p<0.001$) and HDL-cholesterol (negative correlation, $p<0.032$) remained significantly associated with hs-TnT[30].

Marjot and colleagues examined how much myocardium may be kept responsible for a cTn concentration of 99th percentile of normal. They calculated that the 99th percentile level of cTn in blood may be exceeded by necrosis of >40 mg of myocardium. This volume is too small to be detected by noninvasive imaging[31].

Bjurman and colleagues found that several patients with a clinical diagnosis of NSTEMI had hs-cTnT levels within the normal range, most probably because of long ischemic time. These findings question the use of in-hospital hs-cTnT values below the 99th percentile cut-off as a way to exclude NSTEMI[32].

Apple and coworkers suggested that the screening and enrollment of presumably healthy individuals into a study to determine the 99th percentile of normal for hs-cTn assay should minimally address the following: clinical history for known cardiovascular disease as well as history of diabetes or renal disease, possible inclusion of an imaging modality, and a description of specimen type used[33]. The healthy reference group should ideally comprise of at least 300 individuals who are matched for age and gender[12,34]. A high interindividual biological variability of cTn has been described which limits the clinical application of any fixed cut-off value. Moreover, the wide disagreement between hs-cTn methods to identify patients with hs-cTn ≥99th percentile cut-off prevents the use of a fixed cut-off value[5,35].

5. Should we use gender-specific 99th percentiles of high-sensitivity cardiac troponin I or T levels?

The implementation of sex-specific thresholds for hs-cTnI had several important implications for diagnosis, management, and outcomes of women and men with suspected ACS. First, it reclassified five times more additional women than men with myocardial injury. As such, the same proportion of women and men are now identified having myocardial injury. Second, implementation was associated with increased rates of coronary angiography and revascularization. Third, despite the identification of more women with myocardial injury following the adoption of sex-specific thresholds, women remained less likely to be investigated and treated for coronary artery disease than men. Finally, the rates of subsequent MI or cardiovascular death were unchanged in both women and men following implementation of hs-cTnI testing[3]. However, other studies denied the necessity of gender-specific 99th percentile limits. Rubini-Gimenez and coworkers reported very high and well-balanced sensitivity and specificity data of the uniform cut-off level for hs-cTnT at presentation in women and men with suspected acute MI[36]. With this finding, a very low number of women reclassified, and hs-cTn measurements using uniform 99th percentile cut-off, in combination with clinical assessment, remain the optimal approach[36]. However, reference interval studies using both hs-cTnI and hs-cTnT assays have demonstrated that the 99th percentile of normal for men is substantially higher than that for women[13].

The application of gender-specific values for the 99th percentile of normal for hs-cTn did not lead to an increase in MI diagnoses in females, or in the number of women undergoing angiography[37]. Similarly, in a study by Eggers and coworkers the use of gender-specific cutoffs did not improve prognostication[38]. Studies regarding gender-specific lower thresholds for women for the diagnosis of acute MI have not shown consistent results[36,39]. Significantly lower values for the 99th percentile of normal for hs-cTn are observed among women compared with men, and therefore sex-specific 99th percentile cut-offs are recommended for hs-cTn assays[33,34,40-42].

6. Specificity (or lack of specificity) of elevated high-sensitivity cardiac troponin I or T levels for diagnosis of acute myocardial infarction

Hs-cTnI/T values may be influenced by concomitant cardiac and non-cardiac abnormalities. Employment of a very sensitive hs-cTnI/T test results in the early identification of MI ("rule in") or "rule out" of MI. The high sensitivity also results in the frequent identification of patients with hs-cTn elevation due to other reasons than MI. Due to cardiomyocyte damage, an elevated level of hs-cTn is not unique to STEMI and NSTEMI, but is also associated with other cardiac disorders, including heart failure, tachyarrhythmias, left ventricular hypertrophy,

hypertensive crises, cardiomyopathies, valvular heart disease, myocarditis, and even stable coronary artery disease. Moreover, hs-cTn has allowed the detection of cardiomyocyte damage as a probable consequence of severe (primarily) noncardiac disease, such as chronic kidney disease (CKD), severe sepsis, septic shock, stroke, and pulmonary embolism[11,43]. Patients admitted to the intensive care unit with aspecific symptoms of angina may have low probability for ACS, but should be tested by complaints of chest pain, physical examination, ECG and hs-cTnI/T to rule out an acute MI. Even in patients with low ACS probability the specificity of hs-cTnT/I for acute MI remained high at ≈90% of the patients when using the 99th percentile cut-off, and further increased when using higher cut-off values. Thus, the higher the hs-cTnT/I blood concentrations, the higher is the likelihood for acute MI even in patients with low ACS probability[43]. In a large, biracial, community-based cohort without known prior cardiovascular disease or heart failure is was found that cTnI measured by a high-sensitivity assay was positively and strongly associated with incident coronary artery disease, ischemic stroke, atherosclerotic cardiovascular disease, heart failure hospitalization, and all-cause mortality[44].

A cTn elevation reflects acute or chronic myocardial damage but is not exclusive for ACS, leading to ambiguous interpretation of results. Sometimes the term false-positive is being used to describe a patient with suspected ACS and elevated cTn but subsequently absence of significant coronary artery disease on coronary angiogram[45]. Even when a cTn rise is consistent with a diagnosis of acute MI, other cardiac diseases such as myocarditis, Tako-tsubo cardiomyopathy or shock may produce significant changes of cTnI/T as well. Interpretation of the results is heavily dependent on the clinical context in which the cTn test is requested[46,47].

In patients with COVID-19 infection myocardial injury is quite common, leading to elevations of cTnI/T ≥99th percentile of normal, but its frequency varies among the studies. The cTn elevations are usually modest and the criteria for acute MI are infrequently met. Nevertheless the magnitude of cTnI/T elevations has prognostic significance[48].

Stress may produce transient myocardial ischemia without any permanent damage. But in a study of stress induced by atrial pacing, cTnT was found to be increased in coronary sinus blood and peripheral blood of subjects without angiographic correlates of myocardial ischemia[49]. Likewise, Mousavi and coworkers demonstrated that stress to the heart induced by marathon running is sufficient to release cTn without the presence of permanent cardiac injury[50].

The basal cTn concentration is determined by gender (males higher than females), age (the older the higher the cTn) and renal function (the more renal dysfunction the higher the cTn). Thus, the practical cut-off level is higher than the so-called 99th percentile of normal in cases of estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 and in patients older than 70 y[51].

Exercise-induced cTnI elevations of ≥99th percentile cut-off after 30 to 55 km of walking independently predicted higher mortality and cardiovascular events in a cohort of older long-distance walkers. Exercise-induced increases in cTnI is considered to be a pathophysiological response to exercise, and an early marker of future mortality and cardiovascular events[52].

Kubo and coworkers found abnormally high hs-cTnT values in 54% of patients with hypertrophic cardiomyopathy. In these patients an elevated hs-cTnT value remained an independent predictor of cardiovascular events after multivariate analysis (HR: 3.23, p=0.012), such as cardiovascular deaths, unplanned heart failure admissions, sustained ventricular tachycardia, embolic events, and progression to New York Heart Association functional class III or IV status[53].

In adult noncardiac surgical patients preoperative cTn was clearly predictive of short-term adverse outcome defined as major adverse coronary events (MACE) and/or all-cause mortality[54]. Preoperatively

elevated hs-cTnT concentrations have a significant association with long-term mortality after noncardiac surgery, one-third of which may by accounted for by myocardial injury[55].

Using one hs-cTnI measurement in patients with suspected stable angina the pretest-probability of obstructive coronary artery disease fell considerably, thereby reducing the need for further diagnosis, *e.g.*, by stress imaging[56].

The specificity of cTnI/T for acute MI has steadily declined, with abnormal concentrations seen in a variety of conditions associated with cardiac injury, such as myocardial damage after chemotherapy such as with trastuzumab and doxorubicin[57]. In oncological patients treated with immune-checkpoint inhibitors (ICI) immune-related toxicities may occur, one of which is ICI-induced myocarditis (shortly ICI-myocarditis). This is a life-threatening side-effect of ICIs that is associated with cardiomyocyte death and infiltration of macrophages and T-cells in muscles. Lehmann and coworkers have reported elevated cTnT levels within 3 days of an ICI-myocarditis diagnosis: if patients had cTnT levels higher than 32 times the 99th percentile of normal, the risk of MACE was high[58]. In general, the elevated cTnT remained high for more than 6 months. Surprisingly, the levels of cTnI rose less high than those of cTnT, and returned to normal within 1-2 months. The differences between the cTnT and cTnI curves have been reported before for patients with a variety of skeletal muscle diseases, as under certain -as yet unspecified- conditions diseased skeletal muscle may produce cTnT, leading to increased cTnT concentrations in blood[59-61] .

7. Chronic (stable) high-sensitivity cardiac troponin I or T elevation

To maintain a high diagnostic specificity, it is important to distinguish acute from chronic hs-cTn elevation. Acute causes of hs-cTn elevation are associated with a corresponding rise or fall of hs-cTn, indicative of acute MI. Myocardial injury was defined as elevated cTn concentration without signs or symptoms of myocardial ischemia. Although elevated cTn concentrations are specific for indicating damage to the myocardium, they are not specific for the etiology of injury. Thus, elevated cardiac cTnI/T levels may be associated with acute as well as chronic pathological conditions[62-64]. Acute cardiomyocyte injury causes a steep release of cTnI/T, such as in acute MI, shock, myocarditis, pulmonary embolus, and Tako-tsubo (stress-induced) cardiomyopathy. Chronic, stable elevations of hs-cTn at ≥99th percentile cut-off without a significant rise or fall are common in patients with structural heart disease[28,46]. A stable pattern of elevated hs-cTn levels indicates chronic myocardial injury and may be caused by stable coronary artery disease, chronic heart failure, chronic kidney disease, chronic pulmonary hypertension, hypo- and hyper-thyroidism, diabetes, and other risk factors for coronary artery disease. Additionally, the association between hs-cTn elevation and increased all-cause and cardiovascular mortality was also observed in the general population[11,65-67].

There is strong evidence for increased total and cardiovascular mortality in patients with increased cTnI/T concentration in the course of pulmonary embolism, acute and chronic heart failure, sepsis, exacerbation of chronic obstructive pulmonary disease, stable coronary artery disease, and stroke, as well as in asymptomatic cTn-positive elderly patients[68-70].

An elevated level of hs-cTnI/T, not related to acute MI, may also result from: myocarditis, perimyocarditis, polymyositis, pulmonary embolism, acute heart failure, hypertension emergencies, cardiac procedures (e.g. percutaneous coronary intervention, coronary artery bypass grafting, ablation, cardioversion), tachy- and brachyarrhythmias, cerebral stroke or subarachnoid hemorrhage, sepsis, acute abdomen and acute gastrointestinal bleeding, acute kidney injury, along with some other less common causes. In the non-MI setting, elevated hs-cTn concentrations may result from overt or subclinical atherosclerotic cardiovascular disease in patients with stable coronary artery disease, elderly patients, and in those with cardiovascular risk factors. Other potential pathomechanisms of cTn release include: increased left or right ventricular

myocardial strain due to volume and/or pressure overload (e.g. acute and chronic heart failure, pulmonary embolism, pulmonary hypertension), left or right ventricular hypertrophy (e.g. arterial or pulmonary hypertension; aortic stenosis), myocardial necrosis due to increased oxygen demand (*e.g.*, atrial and ventricular tachyarrhythmias, sepsis), reduced coronary perfusion (*e.g.*, high-risk pulmonary embolism; septic or hemorrhagic shock; tachy- and bradyarrhythmias), disseminated intravascular coagulation (*e.g.*, sepsis), myocardial necrosis due to inflammatory infiltration (*e.g.*, myocarditis), myocardial apoptosis due to activation of the renin–angiotensin–aldosterone system, sympathetic overstimulation, inflammatory activation, reduced renal clearance of cTn (*e.g.*, chronic kidney disease), and thoracic trauma (e.g., cardiac contusion)[69,70].

8. Patients with chronic kidney disease

Elevated cTn levels in patients with chronic kidney disease (CKD) may be explained by cardiac injury associated with chronic structural heart disease (such as coronary artery disease or heart failure) rather than acute ischemia, especially when elevated levels do not change rapidly over time[71]. In patients with CKD the cardiospecific biomarkers cTnI, cTnT, BNP, and NT-proBNP are frequently elevated, reflecting impact of the heart besides kidneys. The tests remain accurate for acute MI and acute heart failure, albeit with a modest reduction in accuracy and often a need to consider higher cut-offs[72]. The ability of hs-cTn to rule-out acute MI in patients with CKD is well validated, however the rule-in of acute MI is more problematic[73-75]. Diagnosing acute MI in these patients is challenging as many have atypical ACS presentations and chronic hs-cTn elevations ≥99th percentile cut-off due to non-ischemic myocardial injury[73-77]. CKD patients with suspected ACS are often under-treated and that may, in part, be related to the difficulty in ruling-in acute MI in this patient group[78]. The etiology of persistent cTn elevation in patients with CKD remains incompletely explained[46].

A slight decrease of estimated glomerular filtration rate (eGFR) was associated with a significant increase of hs-cTn. For instance, Martens and coworkers showed that eGFR of 60 to 90 mL/min/1.73 m^2 compared with >90 mL/min/1.73 m^2 was associated with a 1.21 and 1.14 times higher hs-cTnT and hs-cTnI, respectively[79]. The best hs-TnT and NT-proBNP cut-offs tend to increase with declining renal function, but the use of eGFR-dependent cut-offs appeared to be very effective for risk stratification[80].

Should we use a unified cut-off value for hs-cTnI/T in patients with CKD admitted to the intensive care unit with suspected ACS? In a prospective longitudinal study a model for adjustment of cTn was developed. cTnI levels correlated with age and eGFR in males/females with age (r= 0.436/0.518) and with eGFR (r= −0.142/−0.207). In patients with renal dysfunction adjustment of cTnI showed no relevant loss of diagnostic information, as evidenced by comparable areas under the Receiver Operator Curves, to identify acute MI in males and females. Thus, the adjustments improved the diagnostic ability of cTnI to identify acute MI in elderly patients and in patients with renal dysfunction[77,81].

In a group of patients with CKD (70% hemodialysis) Buiten and colleagues measured cTnT and cTnI. The presence of coronary artery disease was significantly associated with a high cTnT (OR 4.70 p=0.02) but not with a high cTnI. Unlike cTnI, cTnT was associated with residual renal function. Thus, cTnT appeared to be better suited to detect coronary artery disease in patients with renal disease, than cTnI. In asymptomatic dialysis patients cTnI seemed to be superior to cTnT as a marker of left ventricular dysfunction[82].

Chenevier-Gobeaux and coworkers adapted the 99th percentile cut-off of the hs-cTnT assay by age and eGFR, regardless of the final diagnosis. hs-cTnT concentrations were slightly correlated with age and renal function. By adapting thresholds for hs-cTnT, the sensitivity and specificity for the diagnosis of acute MI or NSTEMI in elderly patients and in patients with renal failure improved[83].

9. Are individuals with cardiac troponin I or T cTnI/T levels ≤99th percentile of normal always normal?

Troponin levels have prognostic value even in individuals without cTn values >99th percentile of normal. In a group of patients having cTn concentrations within the normal reference range, coronary computed tomography angiography identified coronary artery disease in two-thirds of them. Although a cTn below the rule-out threshold of 5 ng/L did not exclude coronary artery disease, patients with intermediate cTn concentrations were 3 times more likely to have coronary artery disease and had a greater atherosclerotic plaque burden. These associations persisted after adjusting for age, sex, and cardiovascular risk factors, and remained present, regardless of whether patients had symptoms of angina[84]. It is now increasingly recognized that cTn concentrations within the normal reference range are a continuous marker of risk and can be used to improve risk stratification further[84-87]. In a randomized controlled trial patients with suspected ACS were tested with a conventional cTnI test and with a hs-cTnI test. Both tests had their own 99th percentile cut-off. Of 48282 patients enrolled in this study 10360 (21%) had cTn concentrations >99th percentile of normal when using the conventional cTn assay or the high sensitivity cTn assay. The hs-cTn assay identified 17% of these 10360 patients (1771 patients) with cTn values >99th percentile cutoff, whereas the contemporary assay failed to identify them as positive for MI. Thus, on the basis of hs-cTn results 1771 patients (17%) were reclassified as having acute MI. However, the incidence of MI and/or cardiovascular death at 1 year of these 1771 patients did not differ from that of the patients declared positive for MI by conventional assay. The authors question the current use of the 99th percentile cut-off values[88]. Once acute MI has been excluded, hs-cTn concentrations may be used to select further diagnostic procedures, such as coronary computed tomography angiography or echocardiography, to those who are most likely to benefit. A significant proportion will have unrecognized coronary artery disease or structural heart disease[89]. Instead of using hs-cTnI/T test results to guide clinical diagnosis and work-up in patients with suspected stable angina, the Scottish Computed Tomography of the Heart Trial (SCOT-HEART) used coronary computed tomography angiography for these purposes. This approach was successful in identifying patients with underlying coronary artery disease followed by more effective therapy, compared to patients who did not unwent coronary computed tomography angiography[90]. The comparison of this type of clinical diagnosis guidance with hs-cTnI/T results is lacking.

10. Protocols for rule-out and rule-in decisions: the 1 h difference, the 3 h difference, and the 6 h difference protocols

To interpret a rise or fall in biomarker concentrations, generally the reference change value (Δ) is used, meaning that the change in cTn concentrations must exceed the combined intra-individual biological variation and analytical variation to be clinically relevant[91]. The algorithm proposes a Δ value of ≥5 ng/L for Elecsys hs-cTnT and 6 ng/L for Architect hs-cTnI as an additional rule-in criterion[10]. An additional rule-out criterion is a Δ value of <3 ng/L for Elecsys and <2 ng/L for Architect, but is only valid for a small patient group with initial cTn values of 5–12 and 2–5 ng/L, respectively. For application of these rules, it is critical that the total analytical imprecision within the 1 h period is not larger than the imprecision in the core laboratories measuring cTn in the derivation/ validation studies that defined the Δ values[92].

Chronic myocardial injury frequently present with a stable elevation of cTn above the 99th percentile of normal. But for the diagnosis of acute MI, the Fourth Universal Definition of Myocardial Infarction prescribes a rise or fall of circulating cTnI/T, indicating an ongoing process of myocardial cell death[6]. Among

individuals with suspected ACS and two hs-cTnI measurements drawn within 1–7 h, the risk of death was highest in those with persistently elevated concentrations and lowest in persons with two normal concentrations. All-cause mortality was not affected by the relative change from first to second measurement in either of these two groups. However, individuals who went from a normal to an elevated hs-cTnI concentration, with a >50% relative rise, had an increased risk of death[93]. According to the European Society of Cardiology guidelines, a rapid rule-out of MI within 3 h is only feasible with the use of hs-cTn assays[94]. The proposed algorithms may fail to detect any subtle changes in very early NSTEMI presenters (*i.e.*, within 1 h of symptom onset, the "troponin-blind" interval) and in such patients an hs-cTn measurement should be repeated at 3 h.

Bjurman and coworkers evaluated the distribution of Δ intervals among 1178 patients with a final clinical diagnosis of NSTEMI. Importantly, the diagnostic standard for MI did not include a Δ criterion but rather was based on any hs-TnT >99th percentile cut-off in conjunction with a clinical history and other diagnostic testing consistent with myocardial ischemia[32].

Nowadays, most hs-cTnI/T assays use sex-specific cut-off values. When hs-cTnI/T values are elevated, the use of Δ values improves specificity but reduces sensitivity for the diagnosis of acute MI. A change of 50-80% is needed to be sure that one has exceeded the sum of biological and analytical variation. However, the use of large Δ values decreases sensitivity for detection of acute MI, whereas the use of very low Δ values as advocated by the 1 h algorithm diminishes specificity[40].

11. Should we use the absolute or relative difference of time-dependent troponin levels?

To facilitate the rule-in of acute MI, the cTn concentrations at presentation in addition with absolute or relative changes have become the recommended strategy. Earlier recommendations of the American National Academy for Clinical Biochemistry considered a Δ of ≥20% as significant, if initial cTn concentrations are elevated[95]. In patients in whom the initial cTn level is below the 99th percentile cut-off, an expert consensus committee of the European Society of Cardiology suggested a rise or fall of ≥50% as clinically significant[28]. The optimal Δ for the hs-cTnT assay has been shown to be an absolute change between two serial cTn concentrations of ≥7 ng/L, if the initial cTn concentration is <99th percentile of normal[96,97].

The area under the Receiver Operator Curve for diagnosing acute MI was significantly higher for 2-h absolute versus 2-h relative cTn changes (area under the curve [95% CI], hs-cTnT: 0.95 [0.92 to 0.98] versus 0.76 [0.70 to 0.83], p<0.001). The Receiver Operator Curve-derived cut-off value for 2-h absolute change was 7 ng/L for hs-cTnT and 20 ng/L for cTnI ultra (both cut-off levels are half of the 99th percentile of the respective cTn assay). Absolute changes were superior to relative changes in patients with both low and elevated baseline cTn levels[96].

Absolute Δ performed significantly better than relative Δ at 1–3 h (area under the Receiver Operator Curve 0.84 vs 0.69), 3–6 h (0.85 vs 0.73), and 6–9 h (0.91 vs 0.79). Current recommendations propose a Δ ≥20% within 3–6 h. Sensitivity results for absolute Δ at 1–3 h and 3–6 h (75.8%, 78.3%) were superior to those for relative Δ (48.0%, 61.3%). The negative predictive value (for rule out) was 99.6% when baseline TnI <30 ng/L and absolute Δ TnI <10 ng/L. Thus, absolute Δ performed significantly better than relative Δ at all time intervals. Baseline TnI and absolute Δ may be used in conjunction to estimate probability of acute MI[98].

In 2017, Apple and coworkers emphasized that: (i) there is no universal Δ cTn value for all hs-cTn assays; (ii) manufacturers or clinical studies need to determine specific Δ values for each hs-cTnI and hs-cTnT assay, and these values are not interchangeable; (iii) Δ values depend on time intervals (*i.e.*, the time between serial blood samplings, and the time

between the clinical presentation and serial blood samplings) used to perform the calculation; and (iv) the absence of a clear Δ does not exclude the possibility of acute MI in late presenters[34].

Absolute changes of cTn levels have a significantly higher diagnostic accuracy for acute MI than relative changes, and seem therefore to be the preferred standards to distinguish acute MI from other causes of cTn elevations[96].

12. Acute coronary syndrome scoring systems for risk stratification

For risk stratification of patients with suspected ACS a number of rapidly available scores have been developed. The HEART score includes the history, ECG, age, risk factors, and Tn information[22,99]. The history component was given 2 points for highly suspicious symptoms, 1 point for moderately suspicious symptoms, and 0 points for slightly suspicious symptoms. The ECG component was given 2 points in case of significant ST-segment depressions, 1 point in case of non-specific repolarization disturbances, a left bundle branch block or a ventricular paced rhythm, and 0 points in case of a normal ECG. The age component was given 2 points if the patient was ≥65 years, 1 point if the patient was 45 to 64 years and 0 points if the patient was <45 years. The risk factors component was given 2 points if the patient had a history of atherosclerotic disease (coronary revascularization, myocardial infarction, stroke, or peripheral artery disease) or three or more risk factors, 1 point if the patient had one or two risk factors and 0 points in the absence of risk factors. The risk factors that were scored were active or recent (<90 days) smoking, hypertension, diabetes mellitus, obesity (or body mass index >30 kg/m^2), hypercholesterolemia and positive family history. The Tn information contributes to the HEART score by 2 points (cTn ≥3x normal limit), 1 point (between >1 and <3x normal limit) or 0 points (≤1x normal limit). Implementation of routine pre-hospital point-of-care Tn data in the HEART score has been shown to effectively identify high-risk patients in Denmark, while pre-hospital HEART score assessment including a point-of-care Tn measurement has been shown to adequately identify low-risk patients in the Netherlands and the United States[22-24]. The Dutch ARTICA trial demonstrated that pre-hospital rule-out of NSTE-ACS in low-risk patients (with HEAR score ≤3) by a single point-of-care cTn measurement results in a significant reduction of healthcare costs in the first 30 days and first year, and that incidence of MACE is low in both strategies[19,20]. Ishak and coworkers integrated hs-cTnT results into the HEART score, measured from blood drawn in the ambulance, and therefore defined it as the 'modified HEART score' [99]. The sensitivity and negative predictive value of the modified HEART score for ruling out ACS in low-risk patients were 97.0 and 97.6%, respectively[100].

Antman and coworkers developed the Thrombolysis In Myocardial Infarction (TIMI) risk score for unstable angina/NSTE MI for the purpose of prognostication and therapeutic decision making[101]. Factors included in the TIMI risk score for unstable angina or NSTEMI are: age≥65 y, ≥3 risk factors for coronary artery disease, ST-deviation, severe angina complaints, use of aspirin in the last 7 days, and elevated serum cardiac markers. Strategies to identify low-risk patients with ACS incorporated TIMI risk scores =0 or ≤1. Thus, an early-discharge strategy using an hs-TnI assay and TIMI score ≤1 had similar safety as previously reported, with the potential to decrease the observation periods and admissions for ≈40% of patients with suspected ACS[102].

The HARS score was specifically developed for patients with acute ischemic chest pain without known coronary artery disease, without ST-segment deviation, and with normal hs-TnI levels, to help the clinician to assess cardiac event risk assessment and patient triage in the intensive care unit. The HARS score for acute chest pain patients with normal hs-TnI was calculated by: history of chest pain, age ≥60 years, ≥3 risk factors of coronary artery disease, and male sex[103].

A risk calculator for in-hospital mortality and mortality after 6, 12 and 36 months is the GRACE 2.0

Risk Calculator, that provides a modified algorithm incorporating renal failure and use of diuretics when Killip class and serum creatinine values are unknown[104,105].

13. The five phenotypes of myocardial infarction

In the Fourth Universal Definition of Myocardial Infarction MI is subclassified into five phenotypes that may or may not influence the diagnostic value of hs-cTnI/T levels[6]. Each definition has specific features regarding the type and thresholds of biomarkers used to define myocardial injury and the inclusion of supporting evidence of myocardial ischemia. Type 1 MI is defined by a spontaneous event related to the rupture of an atherosclerotic plaque, characterized by a (very) high concentration of cTn in blood. Type 2 MI is characterized as an instance of myocardial injury with necrosis due to an imbalance between myocardial oxygen supply and/or demand. This imbalance may be attributable to reduced myocardial perfusion in the context of fixed coronary atherosclerosis (without plaque disruption), coronary artery spasm, microvascular dysfunction, coronary embolism, dissection, or systemic causes such as hypoxemia, anemia, hypotension, or bradyarrhythmia, or increased myocardial oxygen demand attributable to tachyarrhythmia or severe hypertension. MI type 3 involves cardiac death due to MI, which MI was not, or could not be, diagnosed during life. MI type 4 involves periprocedural MI by percutaneous coronary intervention and MI type 5 involves MI by coronary artery bypass grafting. cTn concentrations are generally lower after type 2 MI than after type 1 MI. Thus, with conventional assays more type 2 events may be missed than type 1 events[106]. With hs-cTn assays Type 1 MI and Type 2 MI are not differentiated and, hence, integrating the results of hs-cTn measurements with robust clinical assessment remains the optimal approach. Currently, cTnI and cTnT cannot be used to classify the type of acute MI present[57].

14. The classification of myocardial infarction after intervention procedures

cTn is frequently elevated after PCI and almost universally elevated after coronary artery bypass grafting (CABG), with marked elevation in 20-40% of patients[107]. The classification of MI by the Fourth Universal Definition of Myocardial Infarction by types of MI that may occur after intervention procedures is also defined by the Academic Research Consortium-2 (ref 7) and by the Society for Cardiovascular Angiography and Interventions[8]. Each definition has specific features regarding the type and thresholds of biomarkers used to define myocardial injury and the inclusion of supporting evidence of myocardial ischemia. These differences largely influence the sensitivity and predictive ability of each definition, questioning which should be the one preferred in clinical practice[104]. According to the available literature, all three major international definitions of peri-operative MI are independently associated with an increased risk of all-cause mortality following percutaneous coronary intervention[108]. A recent discussion ("great debate") is devoted to the pros and cons of the definition of peri-operative MI, whether or not ECG changes should be included in the diagnosis, and the magnitude of cTn elevation chosen[109].

15. Troponin T or I and other biomarkers to diagnose acute coronary syndrome

Several studies addressed the question whether the hs-cTnI/T tests in patients with suspected ACS should be combined with other tests, such as tests of BNP, NT-proBNP and copeptin, as suspected ACS includes a wide variety of cardiac conditions that deserve proper diagnosis, particularly in the presence of comorbidities, such as chronic kidney disease. In patients with chronic kidney disease the blood concentrations of cardiac-specific biomarkers cTnI, cTnT, BNP, and NT-proBNP are frequently increased, typically reflecting both cardiac and renal impact.

Copeptin is a non-specific biomarker of endogenous stress and rises very early in acute MI, rapidly followed by a decline in the early period when cTn is still normal (the "troponin-blind" interval). In the AROMI study Pedersen and coworkers examined whether an accelerated dual-marker rule-out strategy combining prehospital copeptin and in-hospital hs-cTnT could reduce length of hospital stay. Employing this strategy, the length of hospital stay was reduced without increasing the cardiac event rate in the first 30 days[110]. Möckel and coworkers have used a single combined testing of cTn and copeptin in low-to-intermediate risk patients with suspected ACS to "rule out" acute MI. The combination of copeptin and Tn allowed for considerably larger numbers of patients to be discharged from the emergency department (67.6% vs. 12.0%), associated with substantial reduction in length of stay (4 h vs. 7 h). This randomized study in low risk chest pain patients suggests that the combination of copeptin with Tn may be useful to exclude acute MI safely in less time than when using the Tn assay alone[111].

Normal levels of cTn combined with low levels of copeptin at presentation allow rule out of acute MI in patients at low to intermediate risk[112]. The added value of copeptin is particularly high when less sensitive cTn assays are used[105].

Following a coronary occlusion, copeptin is rapidly released from the pituitary gland and appears in the blood in patients with an evolving MI, while cTn is still normal[113]. Copeptin concentrations decline rapidly to normal while cTn levels rise above the 99th percentile. Copeptin levels do not increase in patients with unstable angina. Copeptin not only improved a rapid rule-out of an emerging NSTEMI, but also helped to identify patients at higher risk for major adverse cardiac events, if initial copeptin levels were high[111].

In a cohort of patients with ACS the novel biomarker midregional proadrenomedullin was a good predictor of cardiovascular death, even better than cTnI. Blood levels of midregional proadrenomedullin added significant information regarding the outcome even after including important risk factors[114].

16. Interferences of the cardiac troponin test

The Tn test may undergo interference by biochemical factors such as anti-troponin I autoantibodies[4,115]. Chew-Harris and coworkers reviewed the literature on the options of interferences in immunoassays[116], leading to false-positive TnI attributed to a macrocomplex[117] and persistent increase of cTnI in plasma without evidence of cardiac injury[118]. Legendre-Bazydlo and coworkers sent a warning that a chronic stable cTnI/T elevation may indicate the presence of interfering substances, and alternative measurements should be sought if possible[118]. Van der Linden and colleagues paid attention to the possible presence of interferent factors in the blood, such as (auto) antibodies or proteases, that might modify cTnT or interfere with the assay[119]. These authors hypothesized that these factors may be ubiquitously present in the general population and might affect the measured concentrations of cTnT in individuals[119].

Bohner and coworkers discovered that false-negative immunoassay results for cTnI can be caused by circulating anti-troponin I autoantibodies[120] directed against the stable mid-fragment (aa30–110) of cTnI[121]. Eriksson and colleagues extended these findings by reporting that anti-troponin I autoantibodies can hamper the triage of patients with acute MI by interfering with cTnI detection and, at exceptionally high titres, can even totally mask the presence of cTnI in the bloodstream[122]. When this concept was tested on various cTnI assays, Tang and colleagues found that anti-troponin I autoantibodies may lead to considerable interference in five of the most commonly used cTnI immunoassay systems[123], although it may not affect assays that used antibodies against N- and C-terminal epitopes of cTnI[121]. Thus, in addition to a possible contribution towards pathological ventricular remodelling, anti-troponin I autoantibodies have the potential to prevent accurate diagnosis and appropriate treatment in a subset of patients with MI. Anti-TnT antibodies do not appear to interfere with cTnT immunoassays[120].

In 2023 The IFCC Committee on Clinical Applications of Cardiac Biomarkers published recommendations to recognize and assess antibody-mediated interferences of cTn assays[124].

Generally, false stable increases of cTn are mediated by IgG antibodies. Three types of mechanisms behind antibody-mediated cTn assay interferences are mentioned: (i) blocking anti-cTn antibodies, leading to falsely negative cTn values, (ii) heterophilic antibodies that crosslink antibodies used in the cTn assay, leading to falsely elevated cTn values, and (iii) macrotroponin which are patient's anti-cTn antibodies complexed to cTn, leading to long lived antibody-cTn complexes, also leading to falsely elevated cTn values. The Committee lists a number of laboratory procedures to examine whether the cTn test is flawed by interference and the methodology to investigate the type of this interference[124].

17. The unmet clinical need for a biomarker

Patients with suspected ACS undergo prehospital triage to identify low-risk patients and avoid hospitalisation, or admit (very) high risk patients to the emergency department for further clinical assessment, including anamnesis, physical examination, ECG and biomarkers, and later angiography and echocardiography. In this complex setting many situations occur that raise questions which deserve answers. Here is the unmet need for a biomarker. At several points in the clinical pathway the intended use of a biomarker should add impact on management decisions[125].

The clinical performance characteristics of the current sensitive and reliable hs-cTnI/T assays are such that quite rapidly (preferably within 1 h) test results are guiding clinical diagnostic steps dependent of magnitude and time-course of myocardial necrosis and/or injury. Together with newly developed ACS scoring systems the hs-cTnI/T results should support management decisions, such as avoid hospitalization, early discharge from hospital, or further clinical assessment such as angiography and echocardiography.

18. Conclusion

The modern literature on the use of cTn tests in patients with suspected ACS, in patients with stable angina, in patients with comorbidities, and in elderly residents is extensive and shows that the increased sensitivity of the cTn tests comes with new challenges, a number of them being reviewed here. The cTn concentration value itself, cTn's upper limit of normal, the dynamics of cTn (the cTn curve in time), the combination of cTn with diagnostic and prognostic scores, the classification of MI into five phenotypes, and the influence of cTn's diagnostic power by comorbidities have called forth a multitude of clinical studies. Combining the results of these studies is made complex as different cTn test have different properties. Also the setting in which the cTnI/T test is requested, the intended use of the test requires a specific set of clinical practices with the purpose to select the optimal care for the particular patient. Thus, the result of the cTnI/T test may be used for "rule out" or "rule in" of acute MI, for risk assessment after MI or in the absence of MI, and for identification of myocardial damage after so far innocent situations, such as marathon running. When trying to combine published data, the selection of patients should be taken into account, as selection criteria as age >70 y, and presence of chronic kidney disease will influence the cTn values. The presence of factors that may interfere with the cTn test, and how to cope with these factors, is a subject that may be studied in more detail in the future. And the ECG? The ECG is the diagnostic cornerstone of ACS: rapidly available, and indispensible for further work-up followed by cTn tests and subdivision of suspected ACS into a variety of clinical entities.

Conflict of Interest:

None

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None

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