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New insights from the MESA study: increased high-sensitivity troponins as a cardiovascular risk factor

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The most recent high-sensitivity assays for troponins I and T (hs-TnI and hs-TnT) have made it possible to detect blood concentrations up to 10 times lower than previous assays, making troponins detectable even in asymptomatic subjects without manifest cardiovascular disease. For this reason, hs-Tn, initially introduced as markers of myocardial damage in an acute setting, have also become possible markers of subclinical myocardial damage in baseline conditions. In fact, recent evidence suggests that hs-TnT and hs-TnI predict the risk of future cardiovascular events also in the context of primary prevention, and offer incremental information when added to current risk stratification models. The different association highlighted with different outcome measures, such as coronary heart disease, atherosclerotic cardiovascular disease, heart failure, and death from all causes, seems to indicate that the risk observed in asymptomatic subjects with high levels of hs-Tn is an expression of subclinical damage secondary to multiple pathophysiological mechanisms, and not only to atherothrombosis. However, the ability of hs-TnT and hs-TnI (until now used interchangeably), to provide differential predictive information, and not redundant with respect to more traditional factors, remains to be definitively clarified, both for the purpose of predicting specific outcomes and for the implementation of specific preventive strategies. To date, evidences available allow us to hypothesize their role more as markers than as risk factors.

Introduction

Troponins I and T are myofibrillar proteins of cardiac and skeletal muscles playing a role in muscle contraction. Specific amino acid sequences make it possible to distinguish the protein released into the circulation by the heart muscle from that coming from the skeletal muscles. This biochemical feature has been exploited to develop methods that specifically measure the circulating concentrations of cardiac isoforms I and T, which are therefore highly specific markers of myocardial damage.

The first assays for cardiac troponins (cTn) were introduced into clinical practice in the 1990s, but demonstrated low negative predictive value in ruling out the early diagnosis of acute myocardial infarction ('rule out'). Consequently, the development of highly sensitive assays was pursued. The high-sensitive assays both for troponin I and for T (hs-TnI and hs-TnT, respectively) made it possible to detect progressively lower serum concentrations, up to 10 times lower than the first marketed assay, with high precision. By 'high sensitivity', it is intended the ability of these assays to have a low limit of detection (LoD). By high precision, on the other hand, it is meant a high analytic quality, using as index of imprecision a coefficient of variation $<$10% measured at the 99th percentile of the normal reference population. In 2012, the Task Force of the International Federation of Clinical Chemistry defined a high-sensitivity assay as the one capable of measuring a cardiac troponin in more than 50% of healthy subjects and

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preferably in more than 95% of them. Assays that are capable of quantifying cTn at levels well below the lowest troponin concentrations found in healthy subjects are known as ultra-sensitive (*Figure 1*).[1,2] The choice to measure cardiac troponins I and T (cTnI or cTnT) is generally not clinically driven. Assays for cTnT come from a single manufacturer (Roche Diagnostics), while many manufacturers make assays for hs-cTnI (including Abbott, Siemens, and Beckman-Coulter).

On the one hand, the availability of highly sensitive assays has allowed for an earlier identification of myocardial damage in subjects with suspected acute coronary syndrome; on the other hand, it has introduced its potential use also as a marker for risk stratification in asymptomatic subjects without manifest cardiovascular disease.[3] In this latter regard, Willeit *et al.*[4] published in 2017 a meta-analysis with the aim of evaluating the association between hs-TnI concentrations and cardiovascular events in primary prevention studies. Specifically, 28 prospective studies were identified, for a total of 154 052 participants. Troponins were detectable in 80% of cases (hs-TnI in 82.6%; hs-TnT in 69.7% of cases). Comparing the highest with the lowest tertile of troponin values conferred a relative risk of 1.43 for cardiovascular events (95% confidence interval, 95% CI: 1.31–1.56); of 1.67 for fatal cardiovascular events (95% CI: 1.50–1.86); of 1.59 for coronary events (95% CI: 1.38–1.83); and 1.35 for stroke (95% CI: 1.23–1.48). Elevated troponin concentrations were, therefore, associated with an increased risk of cardiovascular events also in the general population.

This association was stronger for fatal cardiovascular events, and persisted after adjustment for conventional cardiovascular risk factors.[4] Several recent analyses conducted as part of the Multi-Ethnic Study of Atherosclerosis (MESA) confirm that high-sensitivity troponin assays may represent a potential new approach to the stratification of cardiovascular risk in subjects in primary prevention.[5,6]

High-sensitivity troponin and risk of cardiovascular events

The MESA study is a prospective epidemiological cohort study that enrolled 6814 individuals of both sexes, aged 45–84 years, belonging to four ethnic groups, with the aim of evaluating the prevalence and progression of subclinical cardiovascular disease. In an analysis of the study published in 2017, the researchers measured the concentrations of hs-TnT at baseline in 4986 participants. To understand the role of hs-Tn in cardiovascular risk stratification in primary prevention and the underlying pathophysiological mechanism, the study evaluated the possible association between blood concentrations of hs-TnT and myocardial fibrotic replacement. For this purpose, cardiac magnetic resonance imaging was performed at baseline, and repeated 10 years later in the 2831 participants who had not experienced cardiovascular events during the follow-up period. Overall, 1723 subjects received an analysis of the degree of myocardial fibrosis evaluated with late gadolinium enhancement (LGE). An LGE was identified in 6.5% of the 1723 participants. An ischaemic distribution

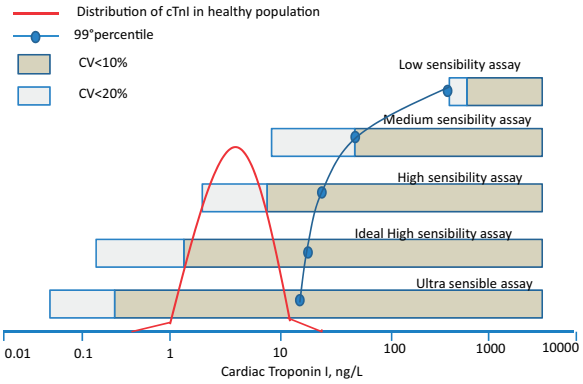

Figure 1 Sensitivity of the various assays for cardiac troponins. Schematic representation of the effect of the increased sensitivity of the assays for cardiac troponin I in the healthy population and measured for each assay together with 10% and 20% of the coefficient of variation (modified from Jarolim[1]). cTnI, cardiac troponin I; CV, coefficient of variation.

pattern was found in 33.6% of subjects, while a non-ischaemic distribution was found in the remaining 66.4% of cases. In subjects with higher hs-TnT values (>7.42 mg/L), there was a 2.5 times higher probability of fibrosis (odds ratio 2.41; 95% CI: 1.15–5.06) compared to subjects with undetectable hs-TnT (<3 ng/L, LoD after adjustment for age, sex, race, cardiovascular risk factors, left ventricular mass and ejection fraction). During a follow-up of 12.2 years, the authors analysed, in addition, the association of hs-TnT with the incidence of heart failure, coronary heart disease events, and cardiovascular mortality using Cox regression models. The incidence of heart failure and cardiovascular death were higher for the higher troponin levels at baseline (hs-TnT >8.81 ng/L) compared to subjects with undetectable troponin (hs-TnT <LoD) even after adjustment for various risk factors (hazard ratio, HR: 5.59; 95% CI: 2.97–10.68 and HR: 3.65; 95% CI: 1.73–7.75, respectively). A similar association, although weaker than that observed for heart failure and cardiovascular death, also emerged between hs-TnT concentrations at baseline and the risk of coronary events. Higher hs-TnT values were also associated with a greater likelihood of left ventricular mass increase, but not a decline in ejection fraction.[5]

High-sensitivity troponin and risk of atherosclerotic cardiovascular disease

In an analysis of 6749 participants of the MESA study published more recently in 2020, Sandoval *et al.*[6] evaluated the relationship between the baseline measure of hs-TnT (LoD 3 ng/L) combined with coronary calcium score with the risk of atherosclerotic cardiovascular disease (ASCVD). Coronary artery calcium (CAC) is a very well validated marker of subclinical atherosclerosis; specifically, a high CAC score (Agatston score >400) correlates with the presence of extensive coronary artery disease, while a CAC of zero has a high negative predictive power.[7] The event rate of ASCVD was therefore assessed in relation to outcome measurement of both biomarkers at baseline. Concentrations of hs-TnT were 'not measurable' (<3 ng/L)

in 32% of the participants; 'measurable' ($\geq$LoD and $\leq$99th percentile of the upper reference limit) in 60% of participants; and 'increased' (>99th percentile of the upper reference limit) in 9% of participants. Considering also the CAC score, baseline measures of the two biomarkers were both negative in 22% of cases, both positive in 40%, and discordant in the remaining 38%. A total of 1002 cardiovascular events of atherosclerotic nature were recorded during an average follow-up of 15 years. Specifically, the event rate for ASCVD was:

a. 2.8 per 1000 person-years in subjects with hs-TnT <LoD and CAC = 0;
b. 6.8 per 1000 person-years in subjects with hs-TnT $\geq$LoD and CAC = 0 [HR compared to group (a): 1.59; 95% CI: 1.17–2.16; $P = 0.003$];
c. 11.1 per 1000 person-years with hs-TnT <LoD and CAC = 0 (HR: 2.74; 95% CI: 1.96–3.83; $P < 0.00001$); and
d. 22.6 per 1000 person-years with hs-TnT $\geq$LoD and CAC > 0 (HR: 3.50; 95% CI: 2.60–4.70; $P < 0.00001$).

Subjects with hs-TnT $\geq$3 ng/L, therefore, had a higher risk of cardiovascular events of an atherosclerotic nature than those with hs-TnT <3 ng/L. In the latter group, the event rate was similar to those with a CAC score of zero (5.2 and 5.0 events per 1000 person-years, respectively). Furthermore, after Cox regression analysis, hs-TnT $\geq$3 ng/L was an independent risk factor for events related to ASCVD compared to hs-TnT <3 ng/L (15.4 and 5.2 events per 1000 person-years, respectively). The results were similar for both genders and among different ethnic groups.[6]

These data suggest a potential new approach to risk stratification for ASCVD in primary prevention. Indeed, higher hs-TnT concentrations would portend a higher risk of ASCVD than individuals with undetectable hs-TnT blood concentrations. Among subjects with CAC equal to zero, a detectable value of hs-TnT also identifies a subgroup at higher risk for ASCVD and, given the different rate of events for discordant results, it is likely that hs-TnT and CAC can provide complementary information, identifying different categories of patients with different risk profiles.

Indeed, from a pathophysiological point of view, CAC is a marker of subclinical atherosclerosis,[7] while hs-cTnT is an expression of subclinical myocardial damage secondary not only to coronary artery disease but also to other conditions, such as left ventricular hypertrophy.[8] The association with outcome measures is clearly greater when hs-cTnT and CAC are used together.[6] These data support the hypothesis that hs-TnT may represent a risk 'intensifier' for detectable serum concentrations, and a 'negative risk factor'—therefore a protection marker—for undetectable values.

High-sensitivity troponin in predictive risk stratification models

Risk factors for clinical coronary artery disease are used in prediction models for estimating the overall cardiovascular risk. The term 'risk factor', derived from statistical association measures, is often used to attribute a cause-and-effect relationship to the disease. In this sense, the definition of risk factor should, however, be reserved to associations with a clear aetiological role in an epidemiological relationship, and passes through the demonstration that its correction is associated with a reduction in the risk of disease. For risk factors that do not comply with these general criteria, it would be preferable to use the term 'risk marker', to emphasize its predictive value only. The very fact that a non-negligible percentage of subjects experience events despite being classified as low or intermediate risk according to predictive models currently in use highlights the need to identify new markers that can refine risk estimates. Of considerable importance are the results of an analysis of the Atherosclerosis Risk in Communities (ARIC) study, which demonstrate how, in a large cohort of subjects in primary prevention, the addition of hs-TnI to traditional risk factors improves the risk estimate. The ARIC investigators included 8121 subjects aged 54–74 with no baseline cardiovascular disease. The mean follow-up was 15 years, and detectable levels of hs-TnI were observed in 85% of the study population. The addition of hs-TnI to the risk factors used in the Pooled Cohort Equation (PCE) stratification model showed a modest but significant improvement in the estimation of risk for ASCVD, heart failure and global cardiovascular disease. Specifically, the net reclassification improvement resulting from the incorporation of hs-TnI into PCE models was 0.033 for ASCVD (95% CI: −0.0003 −0.086), 0.055 for cardiovascular disease (95% CI: 0.021–0.088), and 0.088 for heart failure hospitalizations (95% CI: 0.037–0.131). Furthermore, by also incorporating the hs-TnT assay into the model, a further improvement in risk estimation was highlighted, especially in relation to hospitalizations for heart failure.[9] It could therefore be hypothesized a system of risk stratification that includes the addition of hs-TnT and/or hs-TnI to conventional risk factors, thus refining the reclassification of low- or intermediate-risk subjects and identifying subjects in whom preventive and therapeutic strategies are most cost-effective (*Figure 2*).

Hs-TnI and hs-TnT: redundancy or complementarity in risk stratification?

Little is known about the possible different degree of association between hs-TnI and hs-TnT with overall cardiovascular risk. In a study published in 2018, Welsh *et al.*[10] measured hs-TnT and hs-TnI in 19501 subjects without overt cardiovascular disease of the Generation Scotland Scottish Family Health Study, evaluating their correlation and possible different association with traditional cardiovascular risk factors. The results showed that concentrations of both troponins were detectable in a different percentage of the population (53.3% for TnT and 74.8% for TnI). Furthermore, they were only weakly correlated after adjustment for age and sex ($R^2 = 9.5\%$), but both were higher in men and older subjects. Finally, important differences were observed in the association between the two troponins with the various traditional risk factors, although the association for both with the overall cardiovascular risk was similar. Specifically, after adjustment for various covariates, hs-TnI was more strongly associated with age, male

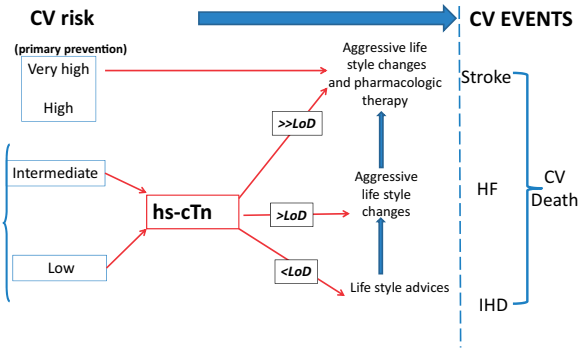

Figure 2 Stratification of cardiovascular risk in primary prevention as a guide for the implementation of more or less aggressive preventive strategies. Using cardiovascular risk stratification systems in primary prevention, subjects are classified into low, intermediate, high, and very high-risk categories. It is well known that many subjects, even if classified as low or intermediate risk, experience events. Adding troponin assays with high sensitivity (hs-TnT and/or hs-TnI) may refine the risk estimate of cardiovascular events by identifying truly low-risk subjects (hs-cTn <LoD) and reclassifying subjects towards higher risk categories when hs-cTn ≥LoD. This could represent a useful cost-effective approach to optimize the use of preventive therapeutic strategies. CV, cardiovascular; HF, heart failure; hs-cTn, high-sensitivity cardiac troponin; IHD, ischaemic heart disease; LoD, limit of detection.

sex, systolic blood pressure, and body mass index than hs-TnT ($P < 0.0001$), while hs-TnT had a higher degree of association with diabetes ($P < 0.0001$).[10] Moreover, the aforementioned analysis of the ARIC study conducted by Jia *et al.*[9] showed that in subjects without manifest cardiovascular disease there is only a modest correlation between the values of hs-TnI and hs-TnT (Spearman Rho = 0.47). Specifically, compared with those with low hs-TnI and higher hs-TnT, subjects with high hs-TnI but lower hs-TnT had more risk factors such as hypertension, dyslipidaemia, smoking, or left ventricular hypertrophy, but featured a better kidney function. The researchers also demonstrated that individuals with elevation of both troponins had a higher risk of cardiovascular events and higher all-cause mortality compared to individuals with only hs-TnI or hs-TnT elevation. Thus, in the general population hs-TnI and hs-TnT would appear to be only weakly correlated and differently associated with traditional risk factors. These assays could therefore provide distinct predictive information, proving complementary rather than redundant in primary prevention strategies. How much this may depend on a different kinetics of release and clearance from the circulation, or on a different performance of the assay needs to be investigated.

Additional considerations—specificity of cardiac troponin elevations

Can high-sensitivity troponins be considered a cardiovascular 'risk factor' today? To answer this question, it would be necessary to refer to the definition of risk factor mentioned above. On the basis of the available data, troponin levels are to be considered more as risk markers than as real risk factors. Levels of hs-Tn represent an early biochemical expression of subclinical cardiovascular disease and are confirmed as an independent predictor for the future incidence of heart failure, coronary and global cardiovascular events. Plasma concentrations are associated with the burden of myocardial fibrosis at cardiac magnetic resonance imaging, but without any specific association for an ischaemic or non-ischaemic pattern of LGE.[5] Also the different association demonstrated with various outcome measures, such as coronary artery disease, cardiovascular atherosclerotic disease, heart failure, and death from all causes seems to confirm that the risk observed in asymptomatic subjects with high levels of hs-Tn is the expression of a subclinical damage secondary to multiple pathophysiological mechanisms, and not necessarily to athero-thrombosis.[4,5] Some studies demonstrate an association between reversible ischaemia and elevation of cTn,[11] while others do not confirm this result.[12] Increased myocardial strain, as well as dysfunction of the coronary microcirculation, would also appear to be associated with higher blood levels of troponins.[8]

Moreover, a correct interpretation of the laboratory result should not be based on the assumption that detectable blood concentrations of troponin are uniquely related to myocardial necrosis. Several other mechanisms have in fact been called into question, such as normal cell turnover, apoptosis, proteolytic fragmentation, and increased cell permeability. Whether troponin is detectable or not depends on the overall effect of all these mechanisms, as well as on the sensitivity of the tests used.[8]

Conclusions

Considering hs-Tn as a risk marker to be added to current stratification models in primary prevention is a new potential approach for optimizing cardiovascular risk prediction. From the data available to date, it is plausible that individuals with detectable or elevated troponins can be considered targets of earlier preventive strategies, and that the presence of measurable or elevated concentrations of this novel risk marker leads to more aggressive corrective actions directed towards the proven risk factors with pathogenetic significance. However, even if simply defined as a 'marker', the high-sensitivity troponin assays remain an interesting new epidemiologic concept. Whether its inclusion in the current risk stratification algorithms can lead to prevention strategies different from current ones, however, still remains a hypothesis, and as such, requires appropriate testing.

Conflict of interest: none declared.

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