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High-Sensitivity Troponin and The Application of Risk Stratification Thresholds in Patients with Suspected Acute Coronary Syndrome

Running Title: *Bularga et al.; High-Sensitivity Troponin for Risk Stratification*

Anda Bularga, MD[1]; Kuan Ken Lee, MD[1]; Stacey Stewart, MSc[1]; Amy V. Ferry, MSc[1]; Andrew R. Chapman, MD, PhD[1]; Lucy Marshall, MSc[1]; Fiona E. Strachan, PhD[1]; Anne Cruickshank, MD[2]; Donogh Maguire, MD, PhD[3]; Colin Berry, MD, PhD[4]; Iain Findlay, MD[5]; Anoop S.V. Shah, MD, PhD[1,6]; David E. Newby, MD, PhD[1]; Nicholas L. Mills, MD, PhD[1,6*]; Atul Anand, MD, PhD[1*]; on behalf of the High-STEACS Investigators[†]

[1]BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [2]Department of Biochemistry, Queen Elizabeth University Hospital, Glasgow, UK; [3]Emergency Medicine Department, Glasgow Royal Infirmary, Glasgow, UK; [4]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [5]Department of Cardiology, Royal Alexandra Hospital, Paisley, UK; [6]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK

[*] Contributed equally

[†] Listed at the end of the manuscript

Address for Correspondence:
Nicholas L. Mills, MD, PhD
BHF/University Centre for Cardiovascular Science
The University of Edinburgh
Chancellor's Building
49 Little France Crescent
Edinburgh EH16 4SB
United Kingdom
Tel: +44 (0) 131 242 6515
Fax: +44 (0) 131 242 6379
Email: nick.mills@ed.ac.uk

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Abstract

Background: Guidelines acknowledge the emerging role of high-sensitivity cardiac troponin for risk stratification and the early rule-out of myocardial infarction, but multiple thresholds have been described. We evaluate the safety and effectiveness of risk stratification thresholds in patients with suspected acute coronary syndrome.

Methods: Consecutive patients with suspected acute coronary syndrome (n=48,282) were enrolled in a multi-center trial across ten hospitals in Scotland. In a prespecified secondary and observational analysis, we compared the performance of the limit of detection (<2 ng/L) and an optimised risk stratification threshold (<5 ng/L) using the Abbott high sensitive troponin I assay. Patients with myocardial injury at presentation, with ≤2 hours of symptoms or with ST-segment elevation myocardial infarction were excluded. The negative predictive value (NPV) was determined in all patients and in subgroups for a primary outcome of myocardial infarction or cardiac death within 30 days. The secondary outcome was myocardial infarction or cardiac death at 12 months, with risk modelled using logistic regression adjusted for age and sex.

Results: In total, 32,837 consecutive patients (61±17 years, 47% female) were included, of whom 23,260 (71%) and 12,716 (39%) had cardiac troponin I concentrations <5 ng/L and <2 ng/L at presentation. The NPV for the primary outcome was 99.8% (95% confidence interval [CI] 99.7–99.8%) and 99.9% (95% CI 99.8–99.9%) in those with cardiac troponin I concentrations <5 ng/L and <2 ng/L, respectively. At both thresholds, the NPV was consistent in men and women and across all age groups, although the proportion of patients identified as low-risk fell with increasing age. Compared to patients with cardiac troponin I concentrations ≥5 ng/L but <99th centile, the risk of myocardial infarction or cardiac death at 12 months was 77% lower in those <5 ng/L (5.3% *versus* 0.7%; adjusted Odds Ratio [aOR] 0.23, 95% CI 0.19–0.28), and 80% lower in those <2 ng/L (5.3% *versus* 0.3%; aOR 0.20, 95% CI 0.14–0.29).

Conclusions: Use of risk stratification thresholds for high-sensitivity cardiac troponin I identify patients with suspected acute coronary syndrome and at least 2 hours of symptoms as low-risk at presentation irrespective of age and sex.

Clinical Trial Registration: URL: https://clinicaltrials.gov Unique Identifier: NCT01852123

Key Words: Myocardial infarction; acute coronary syndrome; risk stratification; troponin

Non-standard Abbreviations and Acronyms
AHA/ACC – American Heart Association/American College of Cardiology
ECG – Electrocardiogram
ESC – European Society of Cardiology
GFR – Glomerular Filtration Rate
IHD – Ischemic Heart Disease
MDRD – Modified Diet in Renal Disease
NPV – Negative Predictive Value

Clinical Perspective

What is new?

- In 32,837 consecutive patients with suspected acute coronary syndrome and at least 2 hours of symptoms, we evaluated the performance of two risk stratification thresholds for a high-sensitivity cardiac troponin I assay.

- An optimized risk stratification threshold of <5 ng/L identified twice as many patients at presentation as low-risk compared to the limit of detection (<2 ng/L), with an equivalent NPV for myocardial infarction or cardiac death at 30 days.

- Compared to the diagnostic threshold, patients with cardiac troponin I concentrations <2 ng/L or <5 ng/L were 80% and 77% lower risk of subsequent cardiac events at 12 months, respectively.

What are the clinical implications?

- The use of separate risk stratification and diagnostic thresholds for high-sensitivity cardiac troponin will improve the safety of our assessment of cardiovascular risk in patients with suspected acute coronary syndrome.

- Incorporating a risk stratification threshold into the early evaluation of these patients will enable the majority of patients to avoid unnecessary hospital admission with major benefits for patients and healthcare providers.

Introduction

The way in which cardiac troponin testing is used in clinical practice is evolving rapidly in parallel with major improvements in assay precision and sensitivity.[1,2] High-sensitivity cardiac troponin assays are essential for the diagnosis of acute myocardial infarction, but are increasingly also used in the assessment of cardiovascular risk to identify patients in the Emergency Department who are low-risk and could be directly discharged.[3-9] Given that fewer than one in ten patients with suspected acute coronary syndrome have myocardial infarction,[10] this application of high-sensitivity cardiac troponin testing has major potential to reduce unnecessary hospital admissions with benefits for patients and healthcare providers.

Whilst the Universal Definition of Myocardial Infarction recommends the use of sex-specific 99th centile or upper reference limits from a normal reference population as the diagnostic threshold for myocardial infarction,[3] there is less consensus on the optimal troponin threshold for the evaluation of cardiovascular risk.[4,5] The ideal risk stratification threshold would permit the greatest number of patients without myocardial infarction to be classified as low-risk, without compromising safety. The limit of detection has been proposed,[11-13] but assay performance at this level is variable, potentially reducing the consistency and effectiveness of this approach.[14-17] We previously defined the optimal risk stratification threshold as the highest troponin concentration that gave a negative predictive value for myocardial infarction or cardiac death at 30 days of at least 99.5%,[6] to maximise the number of patients identified as low-risk whilst maintaining safety. This was achieved using a high-sensitivity cardiac troponin I assay at a concentration <5 ng/L, which identified two-thirds of patients as low-risk at presentation and misclassified fewer than 1 in 200 patients. The only subgroup that did not meet this target for

safety were those who presented within 2 hours of symptoms onset, and guidelines now clearly state that serial testing is required in these early presenters.[3,7]

The use risk stratification thresholds in diagnostic pathways has been evaluated in retrospective analyses of cohort studies,[8,9] but have not been prospectively validated.[4,18] Many approaches have been proposed, often in small cohorts of selected patients attending a single centre, with a limited number of patients in high-risk subgroups. As such, there remains uncertainty as to the performance of these thresholds in practice, where patients are often older and more likely to have comorbidities. Our aim was to compare the diagnostic performance of an optimized risk stratification threshold with the limit of detection, in the patient population in whom risk stratification thresholds have been advocated by international guidelines.[3] In a pre-specified secondary and observational analysis of a multi-center trial of consecutive patients with suspected acute coronary syndrome, we evaluate diagnostic performance in patients presenting with at least 2 hours of symptoms by age and in subgroups to provide reliable estimates for clinical practice. In a sub-study of the trial population, we explore the generalisability of this approach by evaluating performace of these risk stratification thresholds across different high-sensitivity assays.

Methods

Transparency and openness promotion

The trial makes use of multiple routine electronic health care data sources that are linked, deidentified and held in our national safe haven, which is accessible by approved individuals who have undertaken the necessary governance training. Summary data and the analysis code can be made available upon request from the corresponding author.

Study population

*High-*Sensitivity *T*roponin in the *E*valuation of patients with suspected *A*cute *C*oronary *S*yndrome (*High-STEACS*) was a stepped-wedge cluster randomized controlled trial that evaluated the implementation of a high-sensitivity cardiac troponin I assay in consecutive patients presenting with suspected acute coronary syndrome across 10 secondary and tertiary hospitals in Scotland (ClinicalTrials.gov number, NCT01852123). The study design has been described in detail previously[19] and was conducted with the approval of the Scotland Research Ethics Committee in accordance with the Declaration of Helsinki. Individual patient consent was not sought. This approach ensured that consecutive patients presenting with suspected acute coronary syndrome were included without selection bias. All patients presenting to Emergency Departments between June 10th 2013 and March 3rd 2016 were screened by the attending clinician and prospectively included in the trial if cardiac troponin was requested for suspected acute coronary syndrome.

For this pre-specified secondary and observational analysis, we evaluate the performance of high-sensitivity cardiac troponin I in patients without evidence of myocardial injury at presentation (cardiac troponin concentrations below the sex-specific 99th centile), excluding those patients who presented early (≤2 hours from symptom onset to the initial blood draw), or those with a ST-segment elevation myocardial infarction.

Substudy population

To evaluate the generalisability of risk stratification thresholds we used stored samples from a substudy of the trial to compare the performance of different high-sensitivity cardiac troponin I assays (Abbott ARCHITECT$_{STAT}$ and Siemens Atellica, Siemens Healthineers) and high-sensitivity cardiac troponin T (Roche Elycsys, Roche Diagnostics). Participants provided

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informed consent for additional blood sampling and storage, as described previously.[20-22] The

analysis population was defined in the substudy using the same inclusion and exclusion criteria

as for the trial population.

Cardiac troponin testing

As previously described, cardiac troponin testing was performed at presentation and repeated 6

or 12 hours after the onset of symptoms at the discretion of the attending clinician in accordance

with national and international guidelines in use during enrollment.[19,23,24] In all patients during

both phases of the trial, cardiac troponin was measured using the ARCHITECT$_{STAT}$ high-

sensitive troponin I assay (Abbott Laboratories, Abbott Park, IL, USA). This assay has a limit of

detection of between 1.2 ng/L and 1.9 ng/L,[25] and for consistency with prior studies we defined

this as any concentration <2 ng/L.[26] For the purpose of this analysis, all patients with an

undetectable troponin concentration were assigned a value of 1.0 ng/L. The inter-assay

coefficient of variation is less than 10% at 4.7 ng/L and the sex-specific 99th centile diagnostic

thresholds are 16 ng/L for women and 34 ng/L for men.[27] High-sensitivity cardiac troponin I

concentrations were only disclosed to clinicians during the implementation phase of the trial, but

given risk stratification thresholds were not used to guide clinical decisions we pooled data from

both phases of the trial for the purpose of this analysis.

In the substudy, samples were also analyzed using the Siemens Atellica high-sensitivity

cardiac troponin I assay and Roche Elycsys high-sensitivity cardiac troponin T assays.[5,22] For

these assays the limit of detection is 1.6 ng/L and 5 ng/L respectively, and the limit of blank for

the cardiac troponin T assay is 3 ng/L. For all three assays, we evaluated performance of the risk

stratification threshold of 5 ng/L, the lower threshold of <2 ng/L for cardiac troponin I, and <3

ng/L for cardiac troponin T as these thresholds are equivalent to the limit of detection and limit of blank, respectively.

Adjudication of the diagnosis of myocardial infarction

Clinical information was collected from a standardised electronic patient record (TrakCare; InterSystems Corporation, Cambridge, MA, USA) linked to local and national datasets. Electrocardiograph (ECG) data including algorithmic interpretation was available by electronic capture in a sub-group of patients (MUSE, GE Healthcare). All unique interpretation codes generated by this system (n=4,291) were reviewed by a consensus panel who selected codes consistent with possible ischemia (n=180). Example ECGs featuring these codes were then reviewed independently by at least two physicians to determine reliability for clinically significant myocardial ischemia. The final list of 119 codes (*Supplementary Appendix*) were then applied to the study population with electronic ECGs to determine if myocardial ischemia was present for each patient.

Two physicians from our adjudication panel independently reviewed all clinical information to classify patients with any high-sensitivity cardiac troponin measurement >99th centile on serial testing during the index presentation in accordance with the third universal definition of myocardial infarction.[28] Myocardial infarction following discharge and all death outcomes were also independently adjudicated by two physicians blinded to study phase and any disagreements were resolved by a third physician.

Study outcomes

The primary safety outcome was type 1 or 4b myocardial infarction during the index presentation, or subsequent type 1 or 4b myocardial infarction or cardiac death within 30 days of the index presentation. The secondary safety outcome was subsequent type 1 or 4b myocardial

infarction or cardiac death at 12 months. Type 2 myocardial infarction was not included in the composite outcome, as by definition these patients present with alternative, often non-cardiac conditions that determine whether they require hospital admission or discharge.

The number and proportion of patients with high-sensitivity cardiac troponin concentrations less than 2 ng/L or 5 ng/L at presentation were measured to evaluate effectiveness of these risk stratification thresholds. Secondary outcomes of cardiac catheterization, coronary intervention and new medical therapy were collected from local and national databases as previously described.[19]

Statistical analysis

Baseline characteristics are summarized as percentages for categorical variables, mean (standard deviation) or median [interquartile range] as appropriate. The negative predictive value was determined using 2x2 tables to calculate the true and false negative rates for the primary outcome, comparing patients with cardiac troponin concentrations at presentation less than 2 ng/L and less than the risk stratification threshold of 5 ng/L. As we expected the negative predictive value to approach 100%, we estimated the proportion by sampling from a binomial likelihood distribution with a Jeffrey's prior, as such approaches have good coverage for proportions that approach 0 or 1 (ß distribution shape parameters both 0.5).[29] Analysis by stratification was used to compare performance in different sub-groups. For age, the negative predictive value was calculated for each integer age value between 20 and 90 years, and plotted with a line of best fit and 95% confidence intervals (CI). The negative predictive value was also determined separately in those with and without prior history of ischemic heart disease, diabetes mellitus, stroke, heart failure and renal impairment (estimated glomerular filtration rate [GFR]

<60 mL/min/1.73 m^2 determined by Modified Diet in Renal Disease [MDRD] equation) or myocardial ischemia on the ECG at presentation.

For the secondary outcome, the rates of myocardial infarction or cardiac death were compared in patients with cardiac troponin concentration at presentation less than 2 ng/L, less than 5 ng/L, and 5 ng/L to the sex-specific 99th centile. In a post-hoc analysis, we also compared the rates of myocardial infarction or cardiac death in patients with cardiac troponin concentrations between these risk stratification thresholds. Logistic regression modelling for the primary and secondary outcomes was performed using patients with cardiac troponin concentrations between 5 ng/L and the sex-specific 99th centile as a reference group. Odds ratios were adjusted for differences in age and sex. All analyses were performed using R (version 3.5.1).

Results

The trial enrolled 48,282 consecutive patients (61±17 years, 47% women) across ten hospitals in Scotland. A total of 32,837 patients (68%) remained in the analysis population (58±1 years, 47% women) after excluding those with cardiac troponin concentrations >99th centile at presentation (n=7,795), and those presenting ≤2 hours of symptom onset (n=6,469) or with ST-segment elevation myocardial infarction (n=925), and where the high-sensitivity cardiac troponin concentrations at presentation were missing (n=256; *Supplementary Figure 1*).

Proportion and characteristics of patients identified by risk stratification thresholds

In our analysis population, 23,260 (71%) had a cardiac troponin concentration below 5 ng/L, and 9,577 (29%) were between 5 ng/L and the 99th centile. There were 12,716 (39%) patients with cardiac troponin concentrations below 2 ng/L at presentation. Patients with cardiac troponin

concentrations below these risk stratification thresholds were younger, more likely to be female, and had fewer cardiovascular risk factors than those with troponin concentrations between 5 ng/L and the 99th centile (***Table 1***). Similarly, the use of anti-platelet agents and secondary prevention were half as frequent in patients with cardiac troponin concentrations below 5 ng/L compared to those above this threshold. In those below 2 ng/L, even lower rates of cardiovascular risk factors were observed amongst younger, predominantly female patients when compared to those with troponin concentrations 2–4 ng/L (***Supplementary Table 1***).

Diagnostic performance of risk stratification thresholds

In the analysis population, 1.6% (517/32,837) of patients experienced a primary outcome event of index myocardial infarction, or subsequent myocardial infarction or cardiac death within 30 days of presentation. This composite measure included 475 patients with an index myocardial infarction, and 78 and 49 patients with a subsequent myocardial infarction or cardiac death within 30 days, respectively. The majority of composite events occurred in those with cardiac troponin concentrations between 5 ng/L and the 99th centile where the event rate was 4.8% (462/9,577) at 30 days. There were 55 events in 23,260 patients (0.2%) with cardiac troponin concentrations less than 5 ng/L, and 15 events in the subgroup of 12,716 patients (0.1%) less than 2 ng/L. Of these composite events, cardiac death within 30 days occurred in 45 of 9,577 patients with troponin concentrations between 5 ng/L and the 99th centile (0.5%), 4 of 23,260 patients less than 5 ng/L (0.02%) and 1 patient from 12,716 below 2 ng/L (0.01%, ***Table 2***).

The negative predictive value for the primary outcome at 30 days in patients with cardiac troponin concentrations less than the risk stratification threshold of 5 ng/L at presentation was 99.8% (95% CI 99.7–99.8%). The negative predictive value in the subgroup of patients with cardiac troponin concentrations <2 ng/L was 99.9% (95% CI 99.8–99.9%). Although the

prevalence of the primary outcome varied between sites (range 0.8%–2.1%), the negative

predictive value remained consistent across all sites (***Supplementary Table 2***). In patients

presenting within 2 hours of symptom onset (n=6,469), the negative predictive value was lower

at both thresholds (99.0%, 95% CI 98.7–99.3% for those <5 ng/L and 99.6%, 95% CI 99.3–

99.8% for patients <2 ng/L, ***Supplementary Table 3***). Confusion matrices and other diagnostic

metrics for the trial and analysis populations are shown in ***Supplementary Tables 4 & 5***.

Diagnostic performance of risk stratification thresholds in subgroups

The proportion of patients with cardiac troponin concentration below the 5 ng/L and 2 ng/L

thresholds varied markedly by age, but the negative predictive value of these approaches to risk

stratification were identical across all age groups (***Figure 1***). The lower bounds of the 95%

confidence interval was >99.5% for both thresholds even in the oldest patients. In patients >65

years old (n=11,837), the proportion identified as low-risk with a high-sensitivity troponin

concentration below the 2 ng/L risk stratification threshold was diminished at just 11%

(1,303/11,837), compared to 46% (5,463/11,837) with cardiac troponin concentrations <5 ng/L.

Central estimates of negative predictive value were below 99.5% for both risk

stratification thresholds in patients with a prior history of ischemic heart disease, diabetes

mellitus, stroke, heart failure and renal impairment, although the upper bound of the 95%

confidence intervals crossed the pre-specified safety margin of 99.5% (***Figure 2***). In those with

available electronic ECGs and evidence of myocardial ischemia, the negative predictive value

was 99.6% (95% CI 99.3–99.9%) in those with cardiac troponin concentrations less than 5 ng/L

and 99.7% (95% CI 99.2–100.0%) in those below 2 ng/L.

The proportion of patients with cardiac troponin concentrations below both thresholds

differed widely in these sub-groups, but in every subgroup with prior cardiovascular disease, at

least twice as many patients were identified as low-risk using a risk stratification threshold of 5 ng/L compared to 2 ng/L (*Figure 3*). Invasive cardiac procedures and changes to preventative cardiac medications were rarely undertaken or initiated following Emergency Department assessment (*Supplementary Table 6*). Cardiac catheterization occurred in fewer than 1 in 100 patients below either threshold and new antiplatelet therapy was commenced in fewer than 1 in 25.

Secondary safety outcomes at 12 months

Subsequent myocardial infarction or cardiac death following discharge from hospital occurred in 2.0% (667/32,837) of patients at 12 months. Event rates were similar between patients with cardiac troponin concentrations at presentation below 2 ng/L and 5 ng/L (35/12,716 [0.3%] *vs.* 161/23,260 [0.7%], respectively), and were lower than those with cardiac troponin concentrations of 5 ng/L to the 99th centile at presentation (506/9,577 [5.3%]; *Table 2 and Figure 4*). Lower cardiac troponin concentrations were associated with fewer subsequent events at 12 months; patients with concentrations <2 ng/L had a lower event rate than those with concentrations between these thresholds (126/10,544 [1.2%], *Supplementary Figure 2*). When accounting for substantial differences in age and sex between these groups, the risk of subsequent myocardial infarction or cardiac death at 12 months was 80% lower in those below 2 ng/L (adjusted odds ratio [aOR] 0.20, 95% CI 0.14–0.29), and 77% lower in those less than 5 ng/L (aOR 0.23, 95% CI 0.19–0.28), compared to patients with troponin concentrations between 5 ng/L and the 99th centile. At both 30 days and 1 year, adjusted risk estimates of myocardial infarction and cardiac death were similar for those with cardiac troponin concentrations <2 ng/L and <5 ng/L, and for those patients with concentrations between these thresholds (aOR 0.30, 95% CI 0.24–0.36, *Supplementary Table 7*).

Diagnostic performance of risk stratification thresholds for different high-sensitivity assays

In our substudy, 1,185 patients presenting more than 2 hours from symptom onset were evaluated using the Siemens Atellica cardiac troponin I assay, and 1,042 patients evaluated using the Roche Elecsys troponin T assay (*Supplementary Table 8*). Using the Siemens assay, 55% and 15% of patients had a cardiac troponin I concentration <5 ng/L and <2 ng/L at presentation with a negative predictive value of 99.3% (95% CI 98.5–99.8) and 99.2% (95% CI 97.4–99.9), respectively. For the cardiac troponin T assay, 46% and 24% of patients were <5 ng/L and <3 ng/L at presentation, with a negative predictive value of 99.1% (95% CI 98.0–99.7) and 99.4% (95% CI 98.2–99.9) respectively (*Supplementary Table 9*).

Discussion

In this pre-specified secondary analysis from the High-STEACS trial, we have evaluated the use of risk stratification thresholds for high-sensitivity cardiac troponin I in 32,837 consecutive patients with suspected acute coronary syndrome. We report several important findings for clinicians managing patients with this common presentation. First, in patients with at least 2 hours of symptoms prior to testing, a cardiac troponin concentration below 5 ng/L identifies a group at very low risk of immediate or future cardiac events, with a negative predictive value greater than 99.5%. Second, this performance is maintained regardless of age, sex and the presence of myocardial ischemia on the electrocardiogram. Third, using a risk stratification threshold of 5 ng/L identifies twice as many patients as low-risk at presentation when compared to the limit of detection. Fourth, the negative predictive value of applying a risk stratification threshold of 5 ng/L is consistent across high-sensitivity cardiac troponin I and T assays. Fifth, patients with cardiac troponin concentrations above the risk stratification threshold of 5 ng/L, but

below the diagnostic threshold, represent a high-risk group with a 7-fold greater risk of subsequent myocardial infarction or cardiac death over 12 months compared to those below either risk stratification threshold. Taken together, we suggest the use of separate risk stratification and diagnostic thresholds for cardiac troponin, will substantially improve our ability to identify patients at risk compared to the binary approach used in practice today.

High-STEACS is the largest clinical trial to evaluate consecutive patients with suspected acute coronary syndrome reported to date.[19] This analysis of 32,837 patients is larger than the combined number of patients from 30 observational cohort studies, who were included in two recent major retrospective meta-analyses of risk stratification using high-sensitivity cardiac troponin I and T.[8,9] The negative predictive value of the risk stratification threshold of 5 ng/L for myocardial infarction or cardiac death at 30 days was found to be 99.5% (95% CI 99.3–99.6) across 19 of these cohorts using cardiac troponin I,[8] which is similar to the 99.8% (95% CI 99.7–99.8) observed here, and was 99.3% (95% CI 97.3–99.8) in 11 cohorts using cardiac troponin T.[9] Taken together these findings suggest that a single risk stratification threshold could be safely applied for both high-sensitivity cardiac troponin I and T assays.

The American Heart Association/American College of Cardiology (AHA/ACC) and European Society of Cardiology (ESC) guidelines for the management of acute coronary syndromes recommend the diagnostic threshold for myocardial infarction at the 99th centile as an appropriate limit for exclusion in patients except in early presenters.[3,30] Alternative approaches have been suggested, such as those described in the recent COMPASS-MI study, which uses a range of thresholds in combination with serial testing and change between two cardiac troponin measures to estimate the negative and positive predictive value for individual patients.[5] As demonstrated in our prior work, the gain in effectiveness from increasing the threshold above 5

ng/L is small, and the negative predictive value for our safety outcome is lower than 99.5% at higher concentrations.[19] Similarly, the 0/1 hour pathway recommended by the ESC uses multiple thresholds, but not the 99th centile to rule in and rule out myocardial infarction at presentation or at 1 hour.[30-32]

These varied approaches acknowledge that patients without myocardial injury at presentation are at risk of cardiovascular events; in the present study more than 1 in 20 patients with cardiac troponin measures between the risk stratification and diagnostic thresholds experienced a subsequent myocardial infarction or cardiac death within 12 months of presentation. Troponin is a continuous marker of cardiovascular risk and low concentrations can be used to estimate long-term cardiovascular risk.[33-35] This can be informative for clinical decision making, but results need to be interpreted in the context of the individual patient, and these thresholds have not been optimized for this purpose. However, what is clear is that those with intermediate troponin concentrations are at higher risk of future events, and the use of the 99th centile alone does not appear to be an appropriate threshold to risk stratify patients with suspected acute coronary syndrome.

There are a number of strengths to our study. The trial design avoided selection bias through the inclusion of consecutive patients ensuring our analysis population included both low-risk and high-risk individuals, an equal proportion of men and women, patients who presented outside routine hours, and those who were unlikely to survive. Enrollment was across ten hospitals in Scotland including both secondary and tertiary care centers. Despite differences in the prevalence of the primary outcome between sites, the proportion of patients identified as low risk and the safety of risk stratification with cardiac troponin was consistent across sites. Within our substudy, we have further explored the generalizibility of our findings, demonstrating

equivalent diagnostic performance of the same risk stratification threshold for other high-sensitivity cardiac troponin I and T assays. By using robust and established regional and national registries we ensured follow-up was complete in all patients who remained resident in Scotland through linkage of electronic health-care records.[36,37] Finally, all primary or secondary outcome events were adjudicated in accordance with the Universal Definition of Myocardial Infarction.

There are approximately 20 million presentations with suspected acute coronary syndrome to the Emergency Departments in the US and Europe every year.[38] The adoption of a safe and effective approach to rule out of myocardial infarction would have a considerable impact on healthcare provision. Using an optimized risk stratification threshold of 5 ng/L compared to the limit of detection (<2 ng/L) identifies twice as many low-risk patients. This is particularly relevant in older patients with established cardiovascular disease, where the clinical assessment of pre-test probability is more challenging. The optimized risk stratification threshold maintains an excellent safety profile across all age groups and identifies four-times as many patients >65 years old as low-risk. It is well recognised that cardiac troponin concentrations increase with age[39] where they reflect the presence and control of traditional cardiovascular risk factors, such as hypertension[40] and hypercholesterolemia,[25] the burden of coronary artery disease,[26,27] vulnerable plaque,[41] and left ventricular hypertrophy or myocardial fibrosis.[42,43] This property of cardiac troponin as a dynamic barometer of heart health[44] provides the pathophysiological basis to explain its powerful role in the risk stratification of patients with suspected acute coronary syndrome.[39]

Whilst the safety profile of both the 5 ng/L and 2 ng/L thresholds appear excellent, prospective trials in which patients are assessed and clinical decisions are guided using this approach are needed to ensure that the very low event rates observed here are not a consequence

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of hospital admission for further investigation and treatment. In our current analysis, we confirm

our previous findings in patients who present within 2 hours of symptoms onset, and suggest that

serial testing is required in early presenters to maintain the very high negative predictive value of

this approach in all patient groups (**Supplementary Table 3**).[6] In those presenting more than 2

hours from symptom onset, we further explored the performance of risk stratification thresholds

across subgroups. Despite our large sample size, it is possible we were underpowered to evaluate

safety in smaller subgroups, such as those with a prior history of ischemic heart disease, diabetes

mellitus, stroke, heart failure and renal impairment. In these sugroups, the central estimate, but

not the upper bound of the confidence interval for the negative predictive value, was below

99.5% for both risk stratification thresholds. There was evidence of heterogeneity between those

with and without prior ischemic heart disease. However, even in those with established risk

factors or cardiovascular conditions, all estimates of negative predictive value encompassed our

pre-specified safety margin of 99.5%. The safety and effectiveness of introducing risk-

stratification thresholds into clinical practice is currently being addressed in the *Hi*gh-*S*ensitivity

cardiac *T*roponin *O*n presentation to *R*ule out myocardial *I*nfar*C*tion (*HiSTORIC*) trial

(ClinicalTrials.gov identifier: NCT03005158), and in the *L*imit *o*f *D*etection of troponin and

*E*CG *D*ischarge (*LoDED* study, ISRCTN 86184521).[45]

There are some study limitations relevant to this analysis. We were unable to report use

of non-invasive diagnostic testing in our study population, and ECGs were only available for a

proportion of patients. However, our analysis shows that the negative predictive value of the

optimized risk stratification threshold and 2 ng/L was similar in the presence or absence of

myocardial ischemia. In the absence of ST-segment elevation, other abnormalities on the ECG

appear to be less important in patients who have very low cardiac troponin concentrations. This

analysis evaluates the risk stratification threshold of a single troponin assay, but we have provided evidence in our substudy of the consistency of this approach for other high-sensitivity cardiac troponin I and T assays. Recent reports also support the validity of this approach across differing high-sensitivity cardiac troponin I and T assays.[9,12,22,46] The assay's precision and analytical variation[16,17] at the risk stratification threshold is likely to influence the clinical utility of using very low cardiac troponin concentrations, and we have not evaluated assay performance or the implications of misclassification here. Whilst the trial was conducted across ten different hospitals in Scotland, all are part of a single healthcare system, and additional studies would be helpful in countries where less selective cardiovascular testing is performed.[47] However, we have previously observed similar safety and effectiveness in a meta-analysis of 19 different cohorts across 9 different countries.[8]

In conclusion, the use of a risk stratification threshold for high-sensitivity cardiac troponin I in the evaluation of patients with suspected acute coronary syndrome presenting at least 2 hours from symptom onset identifies the majority of patients at low-risk of immediate and future cardiovascular events. The use of an optimized risk stratification threshold of 5 ng/L compared to 2 ng/L, classifies twice as many patients as low-risk. Although the proportion identified as low-risk is reduced in older patients, the safety of this approach is maintained across patients irrespective of age or sex. The adoption of risk stratification thresholds in clinical practice has potential to improve both the effectiveness and safety of the evaluation of patients with suspected acute coronary syndrome with major benefits for patients and healthcare providers.

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Table 1. Baseline characteristics of participants stratified by presentation high-sensitivity cardiac troponin I (hs-cTnI).

		Presentation hs-cTnI			
	All	<2 ng/L	2 ng/L – 99th centile	<5 ng/L	5 ng/L – 99th centile
n	32837	12716	20121	23260	9577
Age	58.4 (17.1)	47.8 (13.9)	65.1 (15.4)	53.6 (15.7)	70.1 (14.5)
Males	17478 (53)	5620 (44)	11858 (59)	11519 (50)	5959 (62)
Presenting Complaint					
Chest pain	24085 (73)	9793 (77)	14292 (71)	17830 (77)	6255 (65)
Dyspnoea	1001 (3)	169 (1)	832 (4)	398 (2)	603 (6)
Palpitation	825 (3)	269 (2)	556 (3)	540 (2)	285 (3)
Syncope	1162 (4)	216 (2)	946 (5)	574 (3)	588 (6)
Other	1197 (4)	306 (2)	891 (4)	722 (3)	475 (5)
Past Medical History					
Ischemic heart disease	7467 (23)	1309 (10)	6158 (31)	3863 (17)	3604 (38)
Myocardial infarction	2537 (8)	432 (3)	2105 (11)	1287 (6)	1250 (13)
Stroke or TIA	1700 (5)	231 (2)	1469 (7)	735 (3)	965 (10)
Percutaneous coronary intervention	2416 (7)	461 (4)	1955 (10)	1351 (6)	1065 (11)
Coronary artery bypass grafting	477 (2)	58 (1)	419 (2)	207 (1)	270 (3)
Diabetes mellitus	1867 (6)	253 (2)	1614 (8)	782 (3)	1085 (11)
Heart failure	1956 (6)	130 (1)	1826 (9)	535 (2)	1421 (15)
Medications					
Aspirin	8277 (25)	1654 (13)	6623 (33)	4619 (20)	3658 (38)
Clopidogrel	2555 (8)	437 (3)	2118 (11)	1307 (6)	1248 (13)
Ticagrelor	225 (1)	43 (0.3)	182 (1)	129 (1)	96 (1)
Oral anticoagulant	1951 (6)	219 (2)	1732 (9)	753 (3)	1198 (13)
ACE inhibitor or ARB	9799 (30)	1969 (16)	7830 (39)	5470 (24)	4329 (45)
Beta-blocker	8398 (26)	1943 (15)	6455 (32)	4863 (21)	3535 (37)
Statin	12264 (37)	2594 (20)	9670 (48)	7002 (30)	5262 (55)
Loop diuretics	3420 (10)	356 (3)	3064 (15)	1176 (5)	2244 (23)
Laboratory Results					
Presentation hs-cTnI	2.4 [1.0, 5.7]	1.0 [1.0, 1.1]	4.5 [2.9, 8.7]	1.6 [1.0, 2.8]	9.0 [6.3, 14.0]
Peak hs-cTnI	2.7 [1.0, 6.0]	1.0 [1.0, 1.3]	5.0 [3.0, 9.7]	1.8 [1.0, 3.0]	10.0 [7.0, 15.5]
Serial hs-cTnI test	13554 (41)	4552 (36)	9002 (45)	8954 (39)	4600 (48)
GFR, mL/min/1.73 m^2	88 (24)	96 (19)	82 (25)	92 (21)	76 (27)

Data are number of patients (%), mean (SD) or median [IQR]; TIA = Transient Ischemic Attack, ACE = Angiotensin Converting Enzyme; ARB = Angiotensin Receptor Blocker; GFR = Glomerular Filtration Rate.

Table 2. Logistic regression modelling for safety outcomes at 30 days and 12 months stratified by presentation cardiac troponin I concentration

	5 ng/L - 99th centile (n=9,577)		<2 ng/L (n=12,716)			<5 ng/L (n=23,260)		
	Events	OR (Reference)	Events	OR (95% CI)	Adjusted OR	Events	OR (95% CI)	Adjusted OR
30 days								
Myocardial infarction	59 (0.6%)	1.00	3 (0.0%)	0.06 (0.02-0.17)	0.10 (0.02-0.28)	19 (0.1%)	0.13 (0.08-0.22)	0.17 (0.10-0.31)
Cardiac death	45 (0.5%)	1.00	1 (0.0%)	0.03 (0.00-0.15)	0.16 (0.01-0.76)	4 (0.0%)	0.04 (0.01-0.09)	0.10 (0.03-0.26)
MI or cardiac death	99 (1.0%)	1.00	4 (0.0%)	0.05 (0.02-0.13)	0.12 (0.03-0.29)	23 (0.1%)	0.09 (0.06-0.15)	0.16 (0.09-0.25)
12 months								
Myocardial infarction	282 (2.9%)	1.00	25 (0.2%)	0.11 (0.07-0.16)	0.20 (0.13-0.31)	105 (0.5%)	0.15 (0.12-0.19)	0.23 (0.18-0.30)
Cardiac death	253 (2.6%)	1.00	11 (0.1%)	0.06 (0.03-0.10)	0.19 (0.10-0.34)	60 (0.3%)	0.10 (0.07-0.13)	0.23 (0.16-0.31)
MI or cardiac death	506 (5.3%)	1.00	35 (0.3%)	0.09 (0.06-0.12)	0.20 (0.14-0.29)	161 (0.7%)	0.12 (0.10-0.15)	0.23 (0.19-0.28)

Data are number of myocardial infarctions or cardiac deaths excluding index events (%); MI = Myocardial Infarction. Odds Ratios (OR) are derived from logistic regression models comparing the group with presentation hs-cTnI <2 ng/L or <5 ng/L against the reference group 5 ng/L – 99th centile (95% confidence intervals). Adjusted OR includes age and sex in the logistic regression model
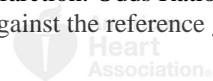
Figure Legends

Figure 1. Performance of cardiac troponin I risk stratification thresholds by age. Negative predictive value for the primary outcome of myocardial infarction or cardiac death at 30 days across a range of ages with 95% confidence intervals (shaded) for patients with cardiac troponin concentrations below 2 ng/L (grey) and 5 ng/L (red) at presentation. The negative predictive value was calculated for each integer age value between 20 and 90 years, and plotted with a line of best fit and 95% confidence intervals (CI). The bar chart shows the proportion of patients in each 5-year age band with cardiac troponin concentrations below each threshold.

Figure 2. Safety of cardiac troponin I risk stratification thresholds by sub-groups. Forest plot showing the number of patients in each sub-group, true negatives (TN) and false negatives (FN) with the negative predictive value for the primary outcome, stratified by patients with cardiac troponin concentrations below 2 ng/L (black) and below 5 ng/L (red). *ECG ischemia data available in 7,167/32,837 (22%) of patients.

Figure 3. Proportion of patients identified as low-risk at the <2 ng/L and <5 ng/L risk stratification thresholds by sub-groups. Proportion of patients in each sub-group with cardiac troponin concentrations below 2 ng/L (grey) or 5 ng/L (red) at presentation.
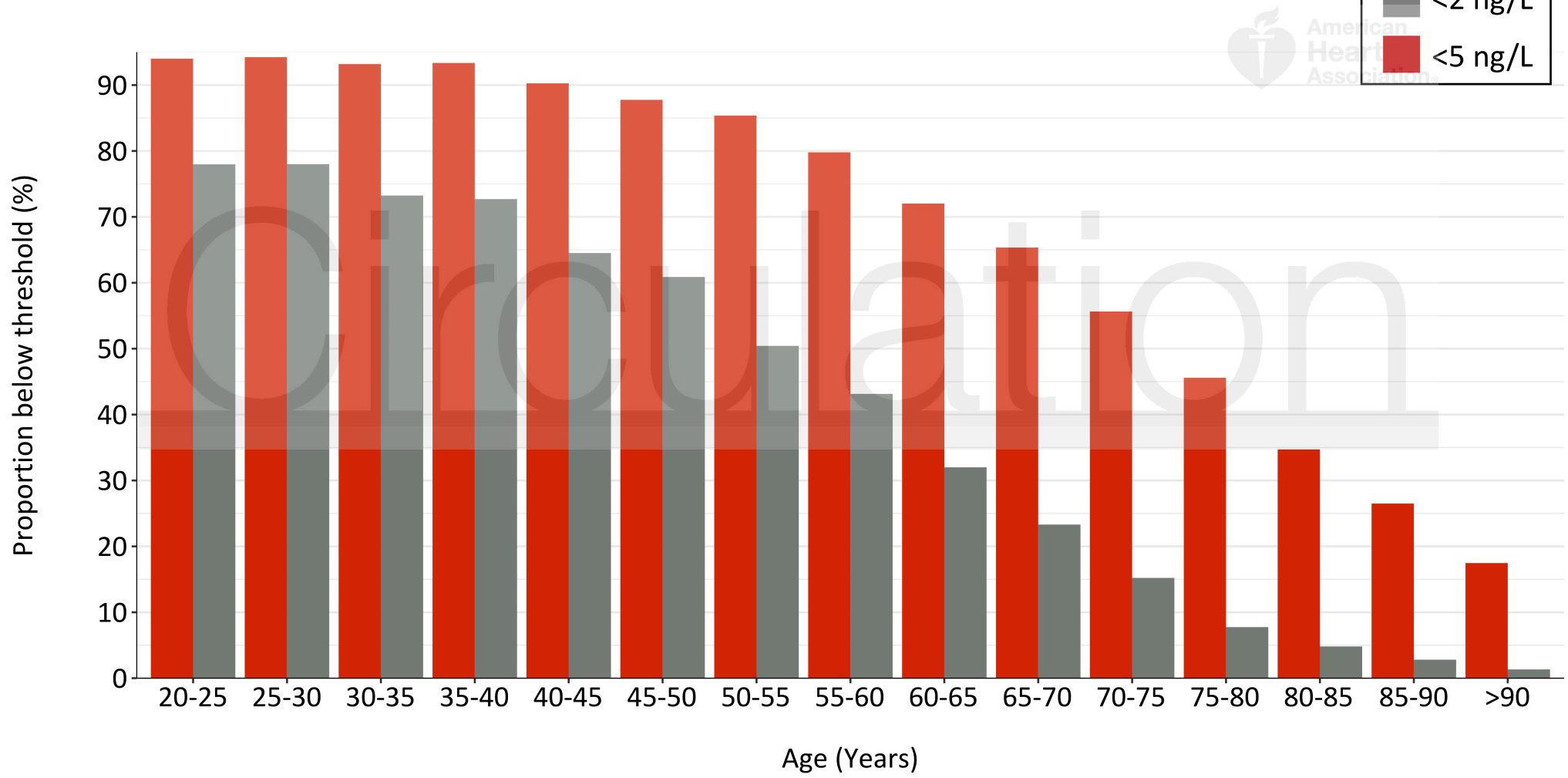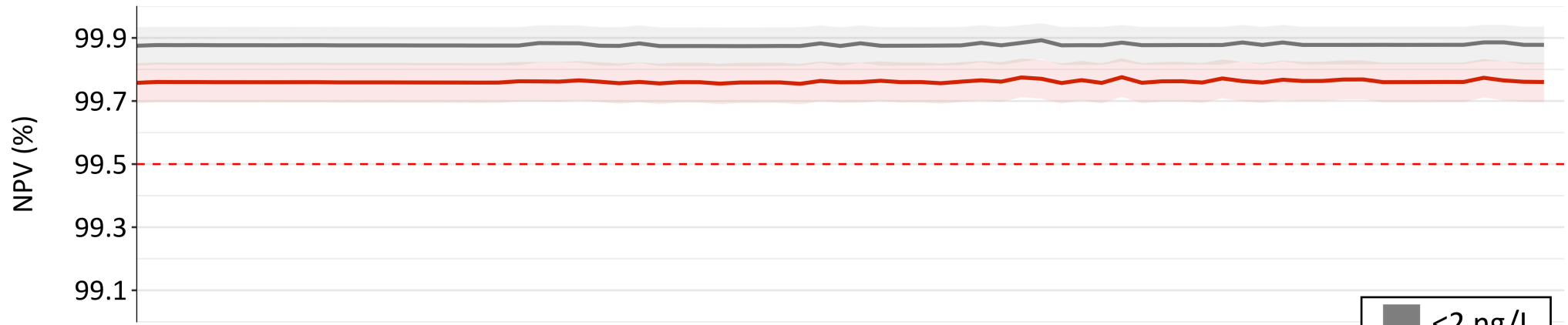
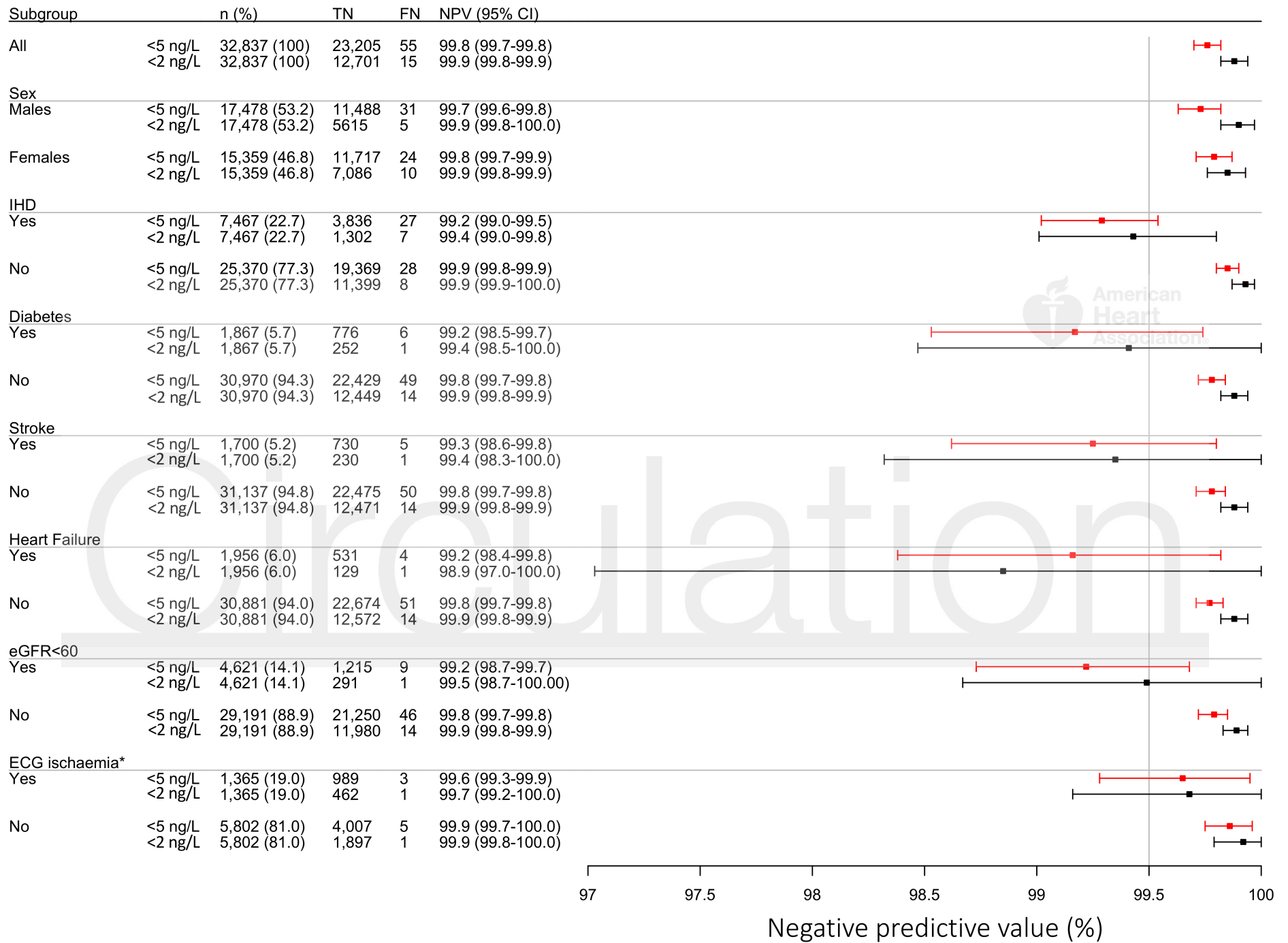**Figure 4. Cumulative incidence of myocardial infarction or cardiac death at 12 months.** Plots stratified by cardiac troponin concentration at presentation: (A) below 2 ng/L (grey) and

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between 2 ng/L and 99th centile (blue); (B) below 5 ng/L (red) and between 5 ng/L and 99th
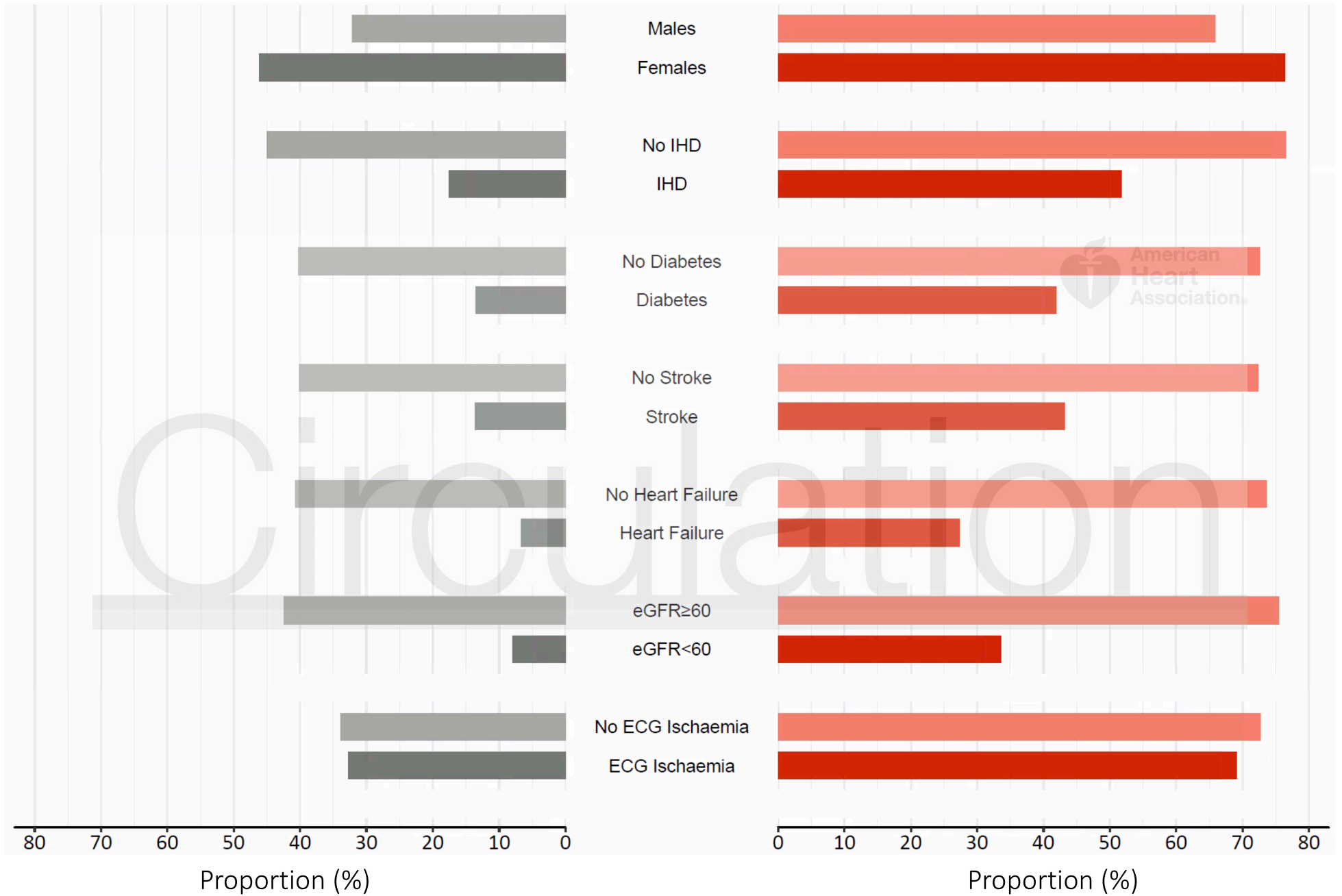
centile (blue).

Subgroup		n (%)	TN	FN	NPV (95% CI)
All	<5 ng/L	32,837 (100)	23,205	55	99.8 (99.7-99.8)
	<2 ng/L	32,837 (100)	12,701	15	99.9 (99.8-99.9)
Sex					
Males	<5 ng/L	17,478 (53.2)	11,488	31	99.7 (99.6-99.8)
	<2 ng/L	17,478 (53.2)	5615	5	99.9 (99.8-100.0)
Females	<5 ng/L	15,359 (46.8)	11,717	24	99.8 (99.7-99.9)
	<2 ng/L	15,359 (46.8)	7,086	10	99.9 (99.8-99.9)
IHD					
Yes	<5 ng/L	7,467 (22.7)	3,836	27	99.2 (99.0-99.5)
	<2 ng/L	7,467 (22.7)	1,302	7	99.4 (99.0-99.8)
No	<5 ng/L	25,370 (77.3)	19,369	28	99.9 (99.8-99.9)
	<2 ng/L	25,370 (77.3)	11,399	8	99.9 (99.9-100.0)
Diabetes					
Yes	<5 ng/L	1,867 (5.7)	776	6	99.2 (98.5-99.7)
	<2 ng/L	1,867 (5.7)	252	1	99.4 (98.5-100.0)
No	<5 ng/L	30,970 (94.3)	22,429	49	99.8 (99.7-99.8)
	<2 ng/L	30,970 (94.3)	12,449	14	99.9 (99.8-99.9)
Stroke					
Yes	<5 ng/L	1,700 (5.2)	730	5	99.3 (98.6-99.8)
	<2 ng/L	1,700 (5.2)	230	1	99.4 (98.3-100.0)
No	<5 ng/L	31,137 (94.8)	22,475	50	99.8 (99.7-99.8)
	<2 ng/L	31,137 (94.8)	12,471	14	99.9 (99.8-99.9)
Heart Failure					
Yes	<5 ng/L	1,956 (6.0)	531	4	99.2 (98.4-99.8)
	<2 ng/L	1,956 (6.0)	129	1	98.9 (97.0-100.0)
No	<5 ng/L	30,881 (94.0)	22,674	51	99.8 (99.7-99.8)
	<2 ng/L	30,881 (94.0)	12,572	14	99.9 (99.8-99.9)
eGFR<60					
Yes	<5 ng/L	4,621 (14.1)	1,215	9	99.2 (98.7-99.7)
	<2 ng/L	4,621 (14.1)	291	1	99.5 (98.7-100.00)
No	<5 ng/L	29,191 (88.9)	21,250	46	99.8 (99.7-99.8)
	<2 ng/L	29,191 (88.9)	11,980	14	99.9 (99.8-99.9)
ECG ischaemia*					
Yes	<5 ng/L	1,365 (19.0)	989	3	99.6 (99.3-99.9)
	<2 ng/L	1,365 (19.0)	462	1	99.7 (99.2-100.0)
No	<5 ng/L	5,802 (81.0)	4,007	5	99.9 (99.7-100.0)
	<2 ng/L	5,802 (81.0)	1,897	1	99.9 (99.8-100.0)

Negative predictive value (%)

97 97.5 98 98.5 99 99.5 100

<2 ng/L   **Subgroups**   **<5 ng/L**

Males
Females

No IHD
IHD

No Diabetes
Diabetes

No Stroke
Stroke

No Heart Failure
Heart Failure

eGFR≥60
eGFR<60

No ECG Ischaemia
ECG Ischaemia

Proportion (%)   Proportion (%)