Journal of the American Heart Association

ORIGINAL RESEARCH

Risk Stratification of Acute Chest Pain in Patients With High-Sensitivity Troponin T Below the 99th Percentile: A Long-Term Cohort Study Assessing the Incremental Value of Necrosis and Non-necrosis Biomarkers to Clinical Risk Scores

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BACKGROUND: Approximately 90% of patients presenting to the emergency department with suspected acute coronary syndrome have acute myocardial infarction excluded, but they remain at risk of major adverse cardiovascular events. This study assessed the long-term outcomes of such patients, focusing on the incremental value of multiple biomarkers combined with clinical risk scores for risk stratification.

METHODS: In this prospective observational study, 487 patients with suspected acute coronary syndrome but excluded acute myocardial infarction were recruited from a large urban hospital, with a median follow-up of 5.8 years. The primary end point was major adverse cardiovascular events, including adjudicated type 1 myocardial infarction, unstable angina requiring urgent revascularization, and cardiovascular death. Biomarkers assessed were hs-cTnT (high-sensitivity cardiac troponin T), hs-cTnI (high-sensitivity cardiac troponin I), HFABP (heart-type fatty acid binding protein), GDF-15 (growth differentiation factor 15), NT-proBNP (N-terminal prohormone of brain naturetic peptide), galectin-3, and hs-CRP (high-sensitivity C-reactive protein (). Statistical methods included receiver operating characteristic analysis, Kaplan–Meier curves, net reclassification index, and Cox proportional hazards models to evaluate biomarker utility independently and combined with Thrombolysis in Myocardial Infarction and History, ECG, Age, Risk Factors, Troponin scores.

RESULTS: Of the 487 patients (median age 56 years; 44% female), 9.9% experienced major adverse cardiovascular events. Annualized major adverse cardiovascular events rates for patients with troponin levels below the limit of detection were 0.5% (hs-cTnT) and 0.7% (hs-cTnI), with no cardiovascular deaths over 5 years. Both hs-cTnT and hs-cTnI levels modestly enhanced risk stratification when added to Thrombolysis in Myocardial Infarction or History, ECG, Age, Risk Factors, Troponinscores. Of the non-necrosis biomarkers, only GDF-15 demonstrated independent prognostic value in multivariable models.

CONCLUSIONS: Hs-cTnT, hs-cTnI, and GDF-15 improve the long-term risk stratification of the History, ECG, Age, Risk Factors, Troponinand Thrombolysis in Myocardial Infarction scores in patients with suspected acute coronary syndrome and acute myocardial infarction excluded. Hs-cTn of either type at or below limit of detection was associated with excellent short- and long-term outcomes.

REGISTRATION: URL: https://clinicaltrials.gov; Unique identifier: NCT03628586.

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This article was sent to Timothy C. Wong, MD, MS, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.040590

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

Key Words: acute coronary syndrome ■ biomarkers ■ cohort study

CLINICAL PERSPECTIVE

What Is New?

- High-sensitivity troponin levels, even within the normal range, are associated with gradations of long-term cardiovascular risk.
- Troponin levels at or below the limit of detection identify a subgroup with particularly favorable long-term outcomes.
- GDF-15 (growth differentiation factor 15) adds independent prognostic value beyond troponin and clinical risk scores such as History, ECG, Age, Risk Factors, Troponin and Thrombolysis in Myocardial Infarction.

What Are the Clinical Implications?

- Troponin concentrations <99th percentile can inform long-term risk stratification following acute myocardial infarction exclusion.
- Very low troponin levels may help identify patients at low risk who require less intensive follow-up, and detectable low-range values could signal those who might benefit from targeted prevention.
- Combining troponin with biomarkers like GDF-15 may offer new opportunities for improving long-term cardiovascular outcomes through earlier risk modification.

Nonstandard Abbreviations and Acronyms

Cox PH	Cox proportional hazards
GDF-15	growth differentiation factor 15
HFABP	heart-type fatty acid binding protein
LOD	limit of detection
MACE	major adverse cardiovascular events
TIMI	Thrombolysis in Myocardial Infarction score
UA	unstable angina
YI	Youden's Index

Most patients presenting to the emergency department with suspected acute coronary syndrome (ACS) ultimately have acute myocardial infarction (AMI) excluded.[1–3] However, a subset of these patients harbor significant coronary disease, putting them at increased risk of future AMI and cardiovascular death. Although long-term cardiovascular outcomes in this population remain poorly studied, emerging evidence suggests that even low levels of detectable high-sensitivity troponin, <99th percentile, are linked to an increased risk of future events.[4,5] Despite the substantial resource use, including cardiac imaging, for short- and medium-term risk stratification of major adverse cardiovascular events (MACE), the utility of this approach remains unclear.[6–9] In this study, we examined the long-term prognosis of patients with suspected ACS who had AMI excluded, investigating different methods of risk stratification and the incremental value of multiple biomarkers.

OBJECTIVES

To evaluate the long-term prognosis of patients with suspected ACS who have AMI excluded and to assess the incremental value of multiple necrosis and non-necrosis biomarkers in addition to validated clinical risk scores, History, ECG, Age, Risk factors, Troponin (HEART) and Thrombolysis in Myocardial Infarction (TIMI).

METHODS

The authors declare that all supporting data are available within the article (Data S1).

Study Population and Design

A prospective observational study of 487 patients recruited between January 2011 and February 2013 in a large urban university teaching hospital in the northwest of England (University Hospital Aintree NHS Foundation Trust). Consenting adults who presented with suspected ACS were included if their hs-cTnT (high-sensitivity cardiac troponin T) levels measured as part of routine clinical care (at least 6 hours post symptom onset) were <99th percentile. Patients with a history of chronic atrial fibrillation or chronic heart failure were excluded. Baseline epidemiological characteristics, medical history, medications, treatments, clinical risk scores (HEART/TIMI) were assessed and calculations performed by a single physician researcher (J.J.) using a combination of case notes and patient interview. This study adhered to Strengthening the Reporting of Observational Studies guidelines. Details of the risk score calculations and software used are provided in Data S1.

Biomarker Analysis

Hs-cTnT was measured using the Roche Cobas Troponin T HS electrochemiluminescence standard and STAT

assays on the Roche Elecsys 2010 Immunoassay and Roche Cobas 8000 e602 Module Analyzers. In-house quality control at Liverpool Clinical Laboratories demonstrated a coefficient of variation of 11% for hs-cTnT at low levels (<10 ng/L). Monthly external quality assurance by the United Kingdom National External Quality Assessment Services reported an interhospital coefficient of variation of 10% (SD 0.5) for low-level hs-cTnT values. Remaining serum was aliquoted and stored at −80 °C for batch analysis of investigative biomarkers, which were subsequently analyzed in accordance with the manufacturers' guidelines. HFABP (heart-type fatty acid binding protein; automated immunoturbidimetric immunoassay from Randox Laboratories) and GDF-15 (growth differentiation factor 15; quantitative sandwich enzyme immunoassay technique using the Quantikine ELISA Human GDF-15 kit from R&D Systems) were measured using local research and clinical laboratories. Galectin-3, hs-CRP (high-sensitivity C-reactive protein), NT-proBNP (N-terminal prohormone of brain natriuretic peptide) and hs-cTnI (high-sensitivity cardiac troponin I) were determined using ARCHITECT immunoassays (Abbott Diagnostics) conducted by Abbott Laboratories, Illinois, USA. Specialist courier services ensured secure transport of samples on dry ice, with temperatures continuously monitored and dry ice replenished throughout transit.

Study Outcomes

The primary end point was the occurrence of MACE, defined as a composite of cardiovascular death, adjudicated nonfatal AMI, and unstable angina (UA) requiring revascularization. Definitions of MACE are detailed in Data S1. Several methods were employed to identify patients who experienced an end point. Multiple electronic medical records were searched at the recruiting hospital, which are regularly updated via National Health Service Spine and are integrated into a regional network. This was cross-referenced with the regional tertiary cardiology centre, where all regional surgical and percutaneous revascularization procedures are performed. A 6-month interval after the conclusion of follow-up was allowed to ensure adequate time for complete data upload into electronic records.

Statistical Analysis

Continuous variables are presented as the median with interquartile range. Categorical variables are reported as counts with percentages. Baseline characteristics were compared using the Mann–Whitney *U* test for continuous variables and either Pearson's chi-square test or Fisher's exact test for categorical variables, as appropriate. Receiver operating characteristic curves were generated for each biomarker and Youden's Index (YI) was calculated. Relative risk (RR) and Kaplan–Meier analyses were performed using YI and recognized thresholds. Biomarkers were natural-logarithmically transformed for subsequent analysis. The ability for biomarkers to improve risk stratification beyond TIMI and HEART scores was evaluated using the category-free net reclassification index and Cox proportional hazards (Cox PH) analysis. Correlations between biomarkers were assessed using Spearman's correlation test. Cox PH regression models were constructed after verifying proportionality assumptions, providing hazard ratio estimates with 95% CIs. The additional prognostic value of biomarkers was assessed using multivariable Cox PH models, with significance between models evaluated through the likelihood ratio test. Statistical significance was set at $P<0.05$. All statistical analyses were conducted using SPSS version 25 and R Version 4.4.0.

Sample Size Calculations

At the time of study design, there were no published data regarding outcomes in patients with high-sensitivity troponin levels <99th percentile, making sample size calculations challenging. A sample size of ≈500 patients was proposed, aligning with the rule of thumb for covariate analysis in regression, which suggests a 1:10 ratio between covariates and end points. Assuming a significance level of 0.05 and that 15% of the cohort would have at least 1 elevated biomarker, this sample size was estimated to provide 84% power to detect an RR of 2.3% and 91% power to detect an RR of 2.5.

Ethics and Clinical Trials Registration

This article conforms to the International Committee of Medical Journal Editors Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. Ethical approval was obtained from the local National Health Service ethics board (United Kingdom: North-West – Greater Manchester Research Ethics Committee), and this study complied with the principles of the Declaration of Helsinki. All subjects provided informed consent. The study was registered on ClinicalTrials.gov (NCT03628586).

RESULTS

A total of 487 patients consented and were included in the analysis, with a median follow-up of 5.8 years. The median age was 56 years (interquartile range, 49–66 years), and 56% were male. Smoking rates were comparable between patients who experienced MACE and the total cohort, whereas other traditional risk factors were more prevalent in patients with long-term MACE (see Table 1). Overall, 48 patients (9.9%) experienced MACE, comprising 16 cases of UA requiring revascularization, 28 cases of adjudicated AMI, and 4 cases

Table 1. Baseline Characteristics

	Total 487	Long-term MACE 48 (months)	Significance
Demographics			
Age, y	56 [11.8]	61 [12.2]	
Male sex	273 (56)	31 (64.6)	NS
Smoker	152 (31.2)	16 (30)	NS
Background			
Diabetes	79 (16.2)	16 (30)	0.002
Hypertension	232 (47.6)	33 (68.8)	0.002
Angina	134 (27.5)	32 (60.0)	<0.001
Myocardial infarction	102 (20.9)	21 (43.8)	<0.001
Percutaneous coronary intervention	67 (13.8)	19 (39.6)	<0.001
Coronary artery bypass graft	25 (5.1)	2 (4.2)	NS
Baseline meds			
Aspirin	172 (35.3)	33 (68.8)	<0.001
Clopidogrel/prasugrel	58 (11.9)	11 (22.2)	0.013
Betablocker	116 (23.8)	22 (45.8)	<0.001
Angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker	161 (33.1)	30 (62.5)	<0.001
Statin	222 (45.6)	34 (70.8)	<0.001

Age is presented as the median with SD in square brackets. Binary variables are displayed as absolute numbers with percentage of group in rounded parenthesis, and significance is assessed by Pearson's chi-square/Fisher's exact test. MACE indicates major adverse cardiovascular event.

of cardiovascular death. Thirty-four patients who did not experience MACE died of all-cause mortality during follow-up. Figure 1 illustrates the cumulative incidence of MACE over time using Kaplan–Meier curves. Table S1 shows the temporal pattern of MACE, indicating that no cardiovascular deaths occurred within 2 years and two thirds of all MACE occurred late (beyond 30 days).

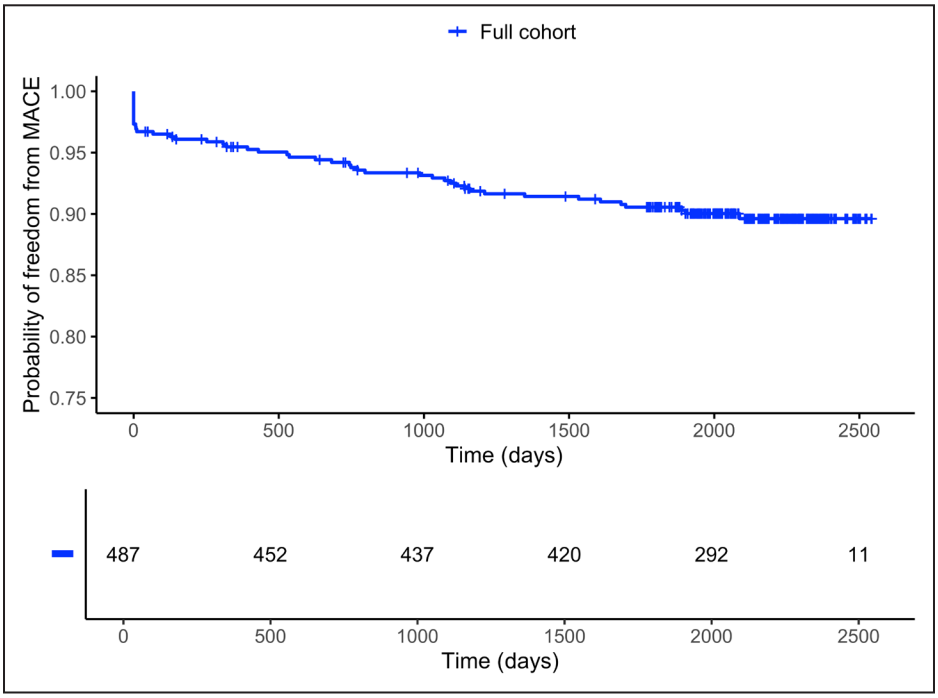


Figure 1. KM curve for full cohort over long-term follow-up.
KM curve showing probability of freedom from MACE for full cohort over time. Censoring is due to noncardiovascular system mortality and end of follow-up. KM indicates Kaplan–Meier; and MACE, major adverse cardiovascular event.

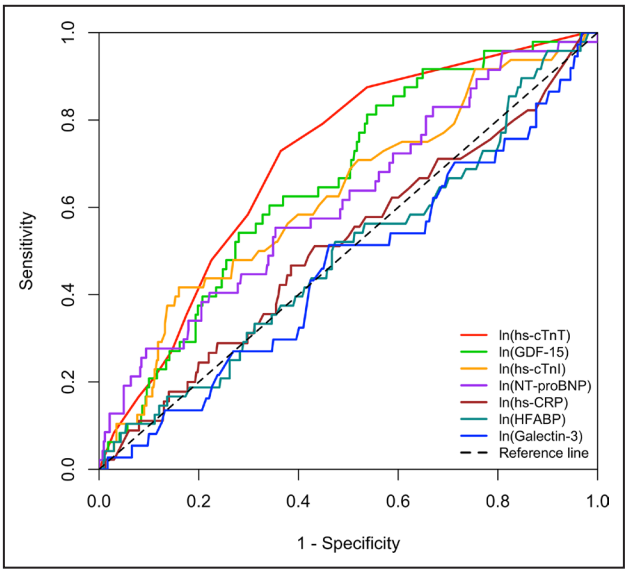


Figure 2. ROC curves of individual biomarkers and MACE.
ROC curve for the natural log of each biomarker according to long-term MACE. GDF-15 indicates growth differentiation factor 15; HFABP, heart-type fatty acid binding protein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; MACE, major adverse cardiovascular event; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; and ROC, receiver operating characteristic.

Biomarkers

HFABP and GDF-15 were measured in all patients. Hs-cTnI was measured in 479 patients (98.4%), NT-proBNP in 471 (96.7%), hs-CRP in 445 (91.4%), and galectin-3 in 386 (79.3%). Table S2 presents the median values and interquartile range of each biomarker. Figure 2 shows the receiver operating characteristic curve analysis for each biomarker in isolation, with C statistics reported in Table 2. Receiver operating characteristic curve analysis indicated that both high-sensitivity troponin assays, GDF-15, and NT-proBNP demonstrated predictive ability for future MACE, with hs-cTnT being the most predictive. In contrast, HFABP, hs-CRP, and galectin-3 did not have the same predictive ability.

Table 2. C Statistics

Biomarker	MACE long term
HFABP	0.528 (0.426–0.630) *P*=0.577
GDF-15	0.665 (0.583–0.746) *P*=0.001*
NT-proBNP	0.644 (0.541–0.746) *P*=0.004*
hs-CRP	0.482 (0.388–0.576) *P*=0.719
Galectin-3	0.538 (0.439–0.638) *P*=0.444
hs-cTnT	0.705 (0.625–0.785) *P*<0.001*
hs-cTnI	0.623 (0.525–0.721) *P*=0.014*

C statistic of each biomarker HFABP, GDF-15, hs-CRP, NT-proBNP, hs-cTnI and hs-cTnT derived from receiver operating characteristic curve analysis. 95% CIs are displayed in parentheses. GDF-15 indicates growth differentiation factor 15; HFABP, heart-type fatty acid binding protein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; MACE, major adverse cardiovascular event; and NT-proBNP, N-terminal prohormone of brain natriuretic peptide.
*P values <0.05.

Receiver operating characteristic optimized cutoffs by YI were calculated, and values are detailed in Table 3 along with the associated RR for having a biomarker level below this cutoff value. Kaplan–Meier curves using these optimized cutoffs can be visualized in Figure 3A through 3G. Whereas Kaplan–Meier show divergent curves for HFABP, GDF-15, NT-proBNP, hs-CRP, and both high-sensitivity troponins around the YI cutoff, only GDF-15, NT-proBNP, and the high-sensitivity troponins reached statistical significance. RR for novel biomarkers based on 99th percentile values of a normal population/recognized thresholds were less informative (Table S3), but cutoffs using limit of detection (LOD) for high-sensitivity troponins were of significance (Figure 4A and 4B).

Category-free net reclassification index was calculated for each biomarker and risk score (Table 4). This analysis suggested that only hs-cTnT had a significant improvement in the prediction of both events and nonevents.

Notably, patients with very low high-sensitivity troponin levels exhibited low annualized MACE rates with no cardiovascular deaths observed over more than 5 years of follow-up. Of the 209 patients with hs-cTnT values at or below limit of blank (≤3 ng/L) there were only 6 patients with MACE (4 UA requiring revascularization and 2 late AMI, giving an annualized MACE rate of 0.5% for hs-cTnT) and of the 69 patients with hs-cTnI at or below LOD (≤1.7 ng/L) there were only 3 MACE (2 UA requiring revascularization and 1 AMI), giving an annualized MACE rate of 0.7%.

Univariable Cox Proportional Hazards Regression

Significant predictors of MACE in univariable analysis include ln(NT-proBNP), ln(GDF-15), ln(hs-cTnT), ln(hs-cTnI), TIMI score, and HEART score. Due to a moderate correlation between ln(hs-cTnT) and ln(hs-cTnI), these were not assessed in the same model. Investigative biomarkers significant in univariable analysis (Tables S3 and S4) were evaluated in various models using risk scores and troponin levels.

Multivariable Cox Proportional Hazards Regression

Multivariable Cox PH regression models (Models 1A and 1B) evaluated the HEART score in combination with ln(GDF-15) and ln(NT-proBNP) alongside each high-sensitivity troponin. Backward selection excluded ln(NT-proBNP) in both models (Table 5). Incorporating

Table 3. RR Associated With Biomarker Level Under ROC Optimized Cutoff and Long-Term MACE

Biomarker	Biomarker cutoff according to YI	RR (95% CI)
Heart-type fatty acid binding protein	2.44 ng/mL	0.936 (0.874–1.004)
Growth differentiation factor 15	245.83 pg/mL*	0.894 (0.847–0.945)*
N-terminal prohormone of brain natriuretic peptide	167.35 mg/dL*	0.918 (0.856–0.983)*
High-sensitivity C-reactive protein	0.395 ng/mL	0.980 (0.917–1.048)
Galectin-3	13.65 pg/mL	0.980 (0.918–1.047)
High-sensitivity cardiac troponin T	5.5 ng/L*	0.858 (0.801–0.921)*
High-sensitivity cardiac troponin I	6.8 ng/L*	0.835 (0.744–0.937)*

RR for each biomarker according to the cutoff derived using YI from ROC curve analysis. The RR associated with a biomarker below this cutoff is displayed. MACE indicates major adverse cardiovascular event; ROC, receiver operating characteristic; RR, relative risk; and YI, Youden's index.
*P values <0.05.

ln(GDF-15) and the respective high-sensitivity troponin with the HEART score significantly improved the prediction of MACE. For each unit increase in ln(GDF-15), patients are 1.5 times more likely to experience MACE when HEART score and ln(hs-cTnT) were kept constant and 1.7 times when HEART score and ln(hs-cTnI) were kept constant.

Multivariable Cox PH regression models (Models 2A and 2B) assessed the TIMI score in combination with ln(GDF-15) and ln(NT-proBNP) alongside each high-sensitivity troponin. Again, backward selection excluded ln(NT-proBNP) from both models, and ln(GDF-15) was excluded only on multivariable analysis with the model containing ln(hs-cTnT). There was a significant improvement in TIMI models when incorporating high-sensitivity troponin levels, and for ln(GDF-15) combined with ln(hs-cTnI). For each unit increase in ln(GDF-15), patients were 1.627 times more likely to experience MACE when TIMI score and ln(hs-cTnI) were kept constant.

Cardiovascular and All-Cause Mortality

Consistent with previous reports, noncardiovascular mortality was far greater than cardiovascular death, with only 4 dying from cardiovascular causes as opposed to 38 deaths due to a variety of noncardiovascular causes. Table S5 reveals univariate Cox regression results for all-cause mortality. As biomarkers, quantitative values of hs-cTnT and GDF-15 provided the highest hazard ratios for noncardiovascular death in this population. As demonstrated in Table S5, we also reveal a significant risk from comorbidities as illustrated by the Charlson comorbidity index.

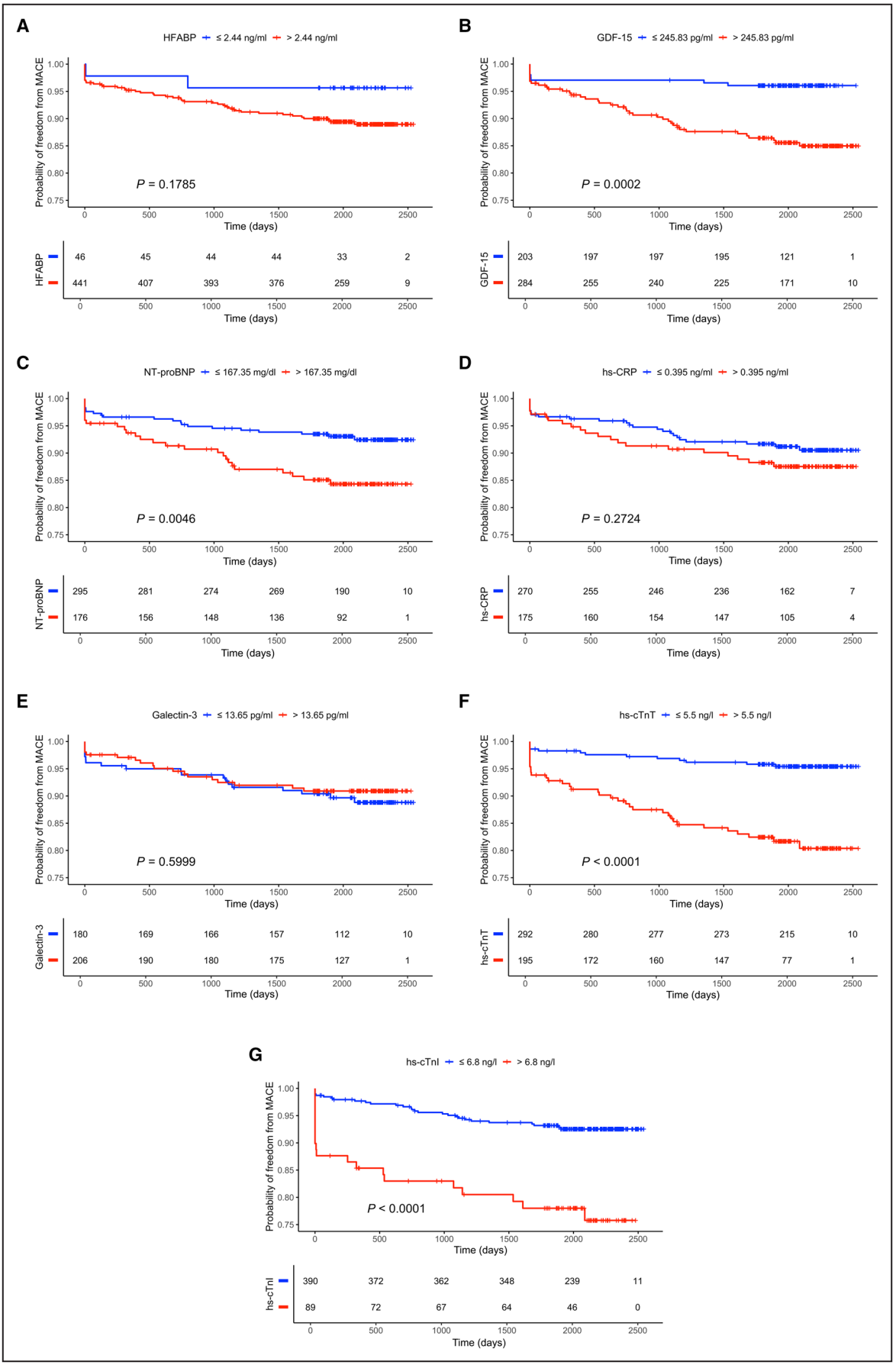
A more detailed analysis of much larger data sets is required to explain this phenomenon further.

DISCUSSION

Patients with suspected ACS account for approximately 10% of all presentations to the emergency department.[1] Most patients will have AMI excluded, and although outpatient investigations are often arranged, their yield and clinical benefit remain uncertain.[6–8] This study aimed to evaluate the long-term prognostic value of risk scores and biomarkers in patients with suspected ACS who had AMI excluded.

This population exhibits a high prevalence of cardiovascular risk factors, with additional undiagnosed or newly diagnosed risks further compounding their vulnerability. Although the short-term event rate was low, the long-term event rate, at 9.9% over a median of 5.8 years, highlights the ongoing cardiovascular risk in this cohort.

Figure 3. KM curves for biomarkers according to YI optimized cutoff.
A, KM curve for HFABP according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker HFABP levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **B**, KM curve for GDF-15 according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker GDF-15 levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **C**, KM curve for NT-proBNP according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker NT-proBNP levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **D**, KM curve for hs-CRP according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker hs-CRP levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **E**, KM curve for galectin-3 according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker galectin-3 levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **F**, KM curve for hs-cTnT according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker hs-cTnT levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. **G**, KM curve for hs-cTnI according to YI optimized cutoff. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by biomarker hs-cTnI levels using thresholds determined by YI. Censoring is due to noncardiovascular system mortality and the end of follow-up. GDF-15 indicates growth differentiation factor 15; HFABP, heart-type fatty acid binding protein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; KM, Kaplan–Meier; MACE, major adverse cardiovascular event; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; and YI, Youden's Index.



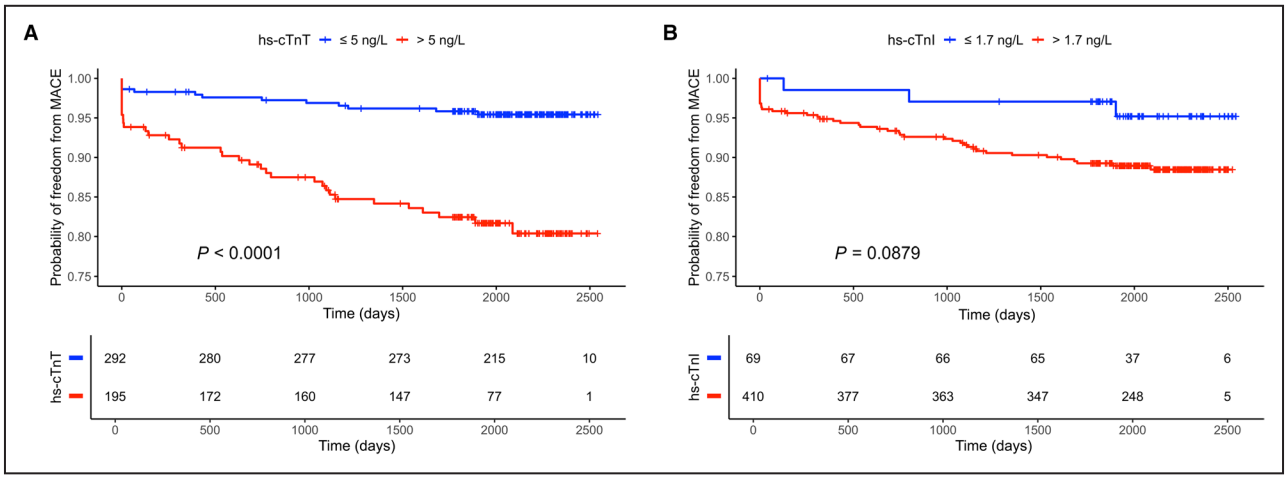


Figure 4. KM curves for troponins according to limit of detection.
A, KM curve for hs-cTnT according to LOD. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by hs-cTnT levels using thresholds determined by the LOD. Censoring is due to noncardiovascular system mortality and the end of follow-up. **B**, KM curve for hs-cTnI according to LOD. KM curves showing the probability of freedom from MACE for the full cohort over time, stratified by hs-cTnI using thresholds determined by the LOD. Censoring is due to noncardiovascular system mortality and the end of follow-up. hs-cTnI indicates high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; KM, Kaplan–Meier; LOD, limit of detection; and MACE, major adverse cardiovascular event.

We report several key findings from this prospective cohort study. GDF-15 was the only non-ecrosis biomarker that independently predicted long-term MACE. The absolute values of high-sensitivity troponins, <99th percentile but in the quantifiable range, modestly predicted MACE with slight improvement when incorporated into HEART or TIMI scores. Finally, patients with hs-cTnT or hs-cTnI ≤LOD had excellent long-term outcomes, with no cardiovascular deaths over 5 years of follow-up. In contrast, patients with hs-cTnT levels above the LOD but <99th percentile faced a significantly higher risk of future MACE.

These findings are consistent with prior studies,[11,12] including Cyon et al.,[11] who observed very low event

Table 4. Net Reclassification Index

Models	Net reclassification index (95% CI)	Improvement in the prediction of events (NRI_e)	Improvement in the prediction of nonevents (NRI_{ne})
History, ECG, Age, Risk Factors, Troponin score+biomarker			
HFABP	0.037 (−0.121 to 0.346)	0.348 (−0.422 to 0.550)	−0.310 (−0.352 to 0.422)
GDF-15	0.336 (−0.111 to 0.667)	−0.198 (−0.395 to 0.209)	0.534 (−0.345 to 0.637)
NT-proBNP	0.270 (−0.075 to 0.544)	−0.342 (−0.578 to 0.330)	0.612 (−0.381 to 0.790)
hs-CRP	−0.344 (−0.622 to 0.708)	0.496 (−0.612 to 0.697)	−0.653 (−0.665 to 0.751)
Galectin3	−0.060 (−0.172 to 0.495)	0.086 (−0.199 to 0.393)	−0.146 (−0.228 to 0.341)
hs-cTnT	0.558 (0.275 to 0.884)*	0.211 (0.004 to 0.458)*	0.347 (0.199 to 0.464)*
hs-cTnI	0.306 (−0.113 to 0.653)	−0.154 (−0.364 to 0.315)	0.460 (−0.357 to 0.610)
Thrombolysis in Myocardial Infarction score+biomarker			
HFABP	0.033 (−0.140 to 0.358)	0.348 (−0.417 to 0.564)	−0.315 (−0.356 to 0.418)
GDF-15	0.321 (−0.051 to 0.681)	−0.199 (−0.394 to 0.209)	0.520 (−0.342 to 0.633)
NT-proBNP	−0.093 (−0.569 to 0.439)	−0.395 (−0.564 to 0.473)	0.302 (−0.367 to 0.643)
hs-CRP	0.339 (−0.203 to 0.897)	0.683 (−0.632 to 0.802)	−0.344 (−0.622 to 0.708)
Galectin3	−0.034 (−0.166 to 0.460)	0.089 (−0.184 to 0.342)	−0.123 (−0.165 to 0.281)
hs-cTnT	0.683 (0.367 to 0.921)*	0.294 (0.015 to 0.501)*	0.389 (0.295 to 0.476)*
hs-cTnI	0.302 (−0.136 to 0.616)	−0.195 (−0.434 to 0.339)	0.497 (−0.389 to 0.631)

Table shows individual risk scores in combination with individual biomarkers HFABP, GDF-15, hs-CRP, NT-proBNP, hs-cTnI, and hs-cTnT. The NRI shows the total NRI with 95% CIs. NRI_e denotes the net proportion of events assigned as higher risk. NRI_{ne} denotes the net proportion of nonevents assigned a lower risk. GDF-15 indicates growth differentiation factor 15; HFABP, heart-type fatty acid binding protein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; and NRI, net reclassification index.
*P values <0.05.

Table 5. Models Incorporating HEART and TIMI Scores

Model	AUC	Significance
Baseline HEART score	0.725 (0.653–0.798)	…
Model 1A (HEART, hs-cTnT, GDF-15)	0.783 (0.719–0.848)*	<0.001*
Model 1B (HEART, hs-cTnI, GDF-15)	0.761 (1.690–0.831)*	0.003*
Baseline TIMI score	0.733 (0.652–0.814)	…
Model 2A (TIMI score, hs-cTnT)	0.788 (0.722–0.854)*	<0.001*
Model 2B (TIMI score, hs-cTnI, GDF-15)	0.768 (0.696–0.840)*	<0.001*

Table showing AUC with 95% CIs and significance for HEART and TIMI-based Cox models with biomarkers GDF-15, hs-cTnI, and hs-cTnT. Significance from baseline HEART and TIMI score model calculated by likelihood ratio test. AUC indicates area under the curve; GDF-15, growth differentiation factor 15; HEART, History, ECG, Age, Risk Factors, Troponin; HFABP, heart-type fatty acid binding protein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; and TIMI, Thrombolysis in Myocardial Infarction.

*P values <0.05.

rates among patients with hs-cTnT < LOD, with older patients demonstrating lower event rates than their age- and sex-matched controls in the general population. Our cohort, although considerably smaller than national data sets, benefited from detailed population characterization and adjudicated outcomes using the 4th Universal Definition for AMI.[13] Importantly, we extended our observations to include patients with hs-cTnI, enhancing the generalisability of our study. Although high-sensitivity troponin values improved risk stratification, their predictive ability was modest. Only hs-cTnT, in addition to the risk scores, achieved an net reclassification index that effectively predicted events and nonevents. Understanding optimum risk stratification, with meaningful improvement in outcome, in the suspected population with ACS who have AMI rule-out remains a challenge, and this study highlights the need for this research.

Our findings differ from historical reports regarding HFABP's prognostic value[14,15] in patients with UA or nonelevated high-sensitivity troponins, suggesting a diminished role in the era of high-sensitivity troponin assays. Delayed sampling, given HFABP's early release after myocardial necrosis, may have also reduced its predictive value.[15] Although GDF-15 added value to risk prediction (particularly safety with lower levels of GDF-15), further studies are required to establish the cost–benefit of this approach. Similarly, although natriuretic peptides are well-established predictors in confirmed ACS,[16,17] they failed to independently predict long-term MACE in our cohort. NT-proBNP demonstrated significant differences in MACE outcomes using YI, but it was excluded as an independent predictor in multivariable models. This suggests that its role may be context-specific and requires further exploration. Most of the previous studies used non-high-sensitivity troponins.

Extensive research has focused on early chest pain management and timely discharge. However, there is also a critical need to identify individuals at elevated long-term MACE risk. The acute hospital encounter should be viewed as a pivotal opportunity not only to screen for cardiovascular risk factors but also to initiate or intensify aggressive management of modifiable risks. Ensuring that patients at high risk receive comprehensive evaluation and targeted interventions during this period could significantly impact their long-term cardiovascular outcomes. Conversely, reassurance for low-risk patients without further investigation could optimize health care resource allocation. Our findings extend prior observations[8,18] by confirming that hs-cTnT at or below the LOD identifies a low-risk population, even in the longer term and question the value of further risk stratification by noninvasive imaging.

Limitations

During the recruitment process, Roche Diagnostics issued a technical bulletin indicating a downward shift in the standardization against the master lot, leading to the underestimation of hs-cTnT levels, particularly in samples with very low concentrations. Adjustments were made according to the manufacturer's guidelines to address this issue.[10] There was a limitation in the availability of remaining serum, which resulted in not all patients having the full set of biomarkers analyzed, particularly galectin-3. In terms of follow-up, our data capture was by regional databases covering a population of 2.3 million in the northwest of England. It is conceivable that patients could have suffered an ACS or undergone revascularization outside the northwest of England. However, in several cohort studies with national Hospital Episode Statistics using *International Classification of Diseases, Tenth Revision* (*ICD-10*) codes, encompassing all of England, we did not discover any presentations with MACE outside the northwest region.[2]

We have used HEART score for long-term risk stratification but note that the HEART score has been almost exclusively studied short term, so its utility in long-term risk stratification is not known.

CONCLUSIONS

Absolute values of hs-cTnT and hs-cTnI < 99th percentile significantly improve risk modeling for cardiovascular outcomes in patients presenting to the emergency department with chest pain and AMI excluded. Among the other biomarkers studied, only GDF-15 retained independent prognostic value. Very low levels of

high-sensitivity troponins are associated with excellent short- and long-term outcomes, suggesting less of a value in additional risk stratification with noninvasive imaging.

ARTICLE INFORMATION

Received December 12, 2024; accepted April 30, 2025.

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Sources of Funding

This work was supported by the Aintree Cardiology Group, Abbott Diagnostics, Dragon's Den award, University of Aintree Research Department.

Disclosures

HFABP (Randox Laboratories Ltd) and GDF- 15 (R&D Systems) assays were purchased by the research team and analysed by local NHS/University laboratories. Additional investigative biomarkers were analysed by Abbott Laboratories under a research agreement, at no cost to the research team. None of the commercial entities involved had access to the results or contributed to the data analysis or manuscript preparation. JJ - Attended educational event organised by Randox, with travel and accommodation provided. Completed online course (123sonography) subsidised by Janssen. EH - Travel grant (Abbott Medical). RD - Speaker fees: Bristol Myers Squibb, Novartis, AstraZeneca, Eisai, Ipsen, Servier. RA - Speaker fees: Abbott. TH - Honoraria: Gilead Pharmaceuticals. PC - Associate Editor: Journal of Applied Laboratory Medicine (ADLM); Honoraria: Siemens Healthineers; Advisory boards: Psyros Diagnostics, Radiometer; Previous advisory roles: Siemens Healthineers, LumiraDx; Consultant: IFCC Committee on Clinical Applications of Cardiac Biomarkers (unpaid). AK - Honoraria: Bayer, Daiichi Sankyo, AstraZeneca, Menarini, St. Jude, Abbott Vascular; Research funding: Bayer Medical, Menarini, Dragons Den awards (Liverpool University Hospitals); Research contracts: Abbott Diagnostics; Director: Northwest Educational Cardiac Group (non-profit); Course sponsorship: Bayer, AstraZeneca, Genzyme (Sanofi), Daiichi Sankyo, Circle Cardiovascular, Menarini; Clinical Champion: Northwest Coast Innovation Agency (personal and transformation funding).

Supplemental Material

Data S1
Tables S1–S5

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