Biomarkers and cardiovascular events in patients with stable coronary disease in the ISCHEMIA Trials

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Importance Biomarkers may improve prediction of cardiovascular events for patients with stable coronary artery disease (CAD), but their importance in addition to clinical tests of inducible ischemia and CAD severity is unknown.

Objectives To evaluate the prognostic value of multiple biomarkers in stable outpatients with obstructive CAD and moderate or severe inducible ischemia.

Design and setting The ISCHEMIA and ISCHEMIA CKD trials randomized 5,956 participants with CAD to invasive or conservative management from July 2012 to January 2018; 1,064 participated in the biorepository.

Main outcome measures Primary outcome was cardiovascular death, myocardial infarction (MI), or hospitalization for unstable angina, heart failure, or resuscitated cardiac arrest. Secondary outcome was cardiovascular death or MI. Improvements in prediction were assessed by cause-specific hazard ratios (HR) and area under the receiver operating characteristics curve (AUC) for an interquartile increase in each biomarker, controlling for other biomarkers, in a base clinical model of risk factors, left ventricular ejection fraction (LVEF) and ischemia severity. Secondary analyses were performed among patients in whom core-lab confirmed severity of CAD was ascertained by computed cardiac tomographic angiography (CCTA).

Exposures Baseline levels of interleukin-6 (IL-6), high sensitivity troponin T (hsTnT), growth differentiation factor 15 (GDF-15), N-terminal pro-B-type natriuretic peptide (NT-proBNP), lipoprotein a (Lp[a]), high sensitivity C-reactive protein (hsCRP), Cystatin C, soluble CD 40 ligand (sCD40L), myeloperoxidase (MPO), and matrix metalloproteinase 3 (MMP3).

Results Among 757 biorepository participants, median (IQR) follow-up was 3 (2-5) years, age was 67 (61-72) years, and 144 (19%) were female; 508 had severity of CAD by CCTA available. In an adjusted multimarker model with hsTnT, GDF-15, NT-proBNP and sCD40L, the adjusted HR for the primary outcome per interquartile increase in each biomarker was 1.58 (95% CI 1.22, 2.205), 1.60 (95% CI 1.16, 2.20), 1.61 (95% 1.22, 2.14), and 1.46 (95% 1.12, 1.90), respectively. The adjusted multimarker model also improved prediction compared with the clinical model, increasing the AUC from 0.710 to 0.792 ($P < .01$) and 0.714 to 0.783 ($P < .01$) for the primary and secondary outcomes, respectively. Similar findings were observed after adjusting for core-lab confirmed atherosclerosis severity.

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Abbreviations: ASCVD, atherosclerotic cardiovascular disease AUC – Area under the receiver operating characteristics curve; CAD, coronary artery disease; CCTA, coronary computed tomography angiography; CI, confidence interval; eGFR, estimated glomerular filtration rate; GDF-15, growth/differentiation factor-15; HR, hazard ratio; hsCRP, High sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; IL-6, interleukin 6; Lp(a), Lipoprotein (a); LVEF, left ventricular ejection fraction; MI, Myocardial infarction; MACE, major adverse cardiovascular events; MMP3, matrix metalloproteinase 3; MPO, myeloperoxidase; NT-proBNP, N-terminal (NT)-pro hormone B-type natriuretic peptide (BNP); sCD40L, soluble CD-40 ligand.

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Conclusions and relevance Among ISCHEMIA biorepository participants, biomarkers of myocyte injury/distension, inflammation, and platelet activity improved cardiovascular event prediction in addition to risk factors, LVEF, and assessments of ischemia and atherosclerosis severity. These biomarkers may improve risk stratification for patients with stable CAD. (Am Heart J 2023;266:61–73.)

Keywords: Stable coronary artery disease; Biomarkers; Risk prediction; Coronary atherosclerosis; Inducible ischemia; ISCHEMIA trial

Key points

Question: Do biomarkers improve risk stratification for cardiovascular events among patients with stable coronary artery disease (CAD) when severity of ischemia and atherosclerosis are known?

Findings: In this substudy from the ISCHEMIA biorepository, a multimarker model of baseline high sensitivity cardiac troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), growth differentiation factor 15 (GDF-15) and soluble CD 40 ligand (sCD40L) was independently associated with and improved prediction of cardiovascular events after adjustment for clinical risk factors, ejection fraction, severity of ischemia and atherosclerosis.

Meaning: Biomarkers of myocyte injury/distension, inflammation, and platelet activity may improve risk stratification for patients with stable CAD in addition to assessments of ischemia and atherosclerosis severity.

Coronary artery disease (CAD) is the leading cause of death and disability worldwide, and affects over 18 million Americans, resulting in approximately 400,000 deaths annually.[1] Among patients with stable CAD, it remains challenging to predict who will have a cardiovascular event.[2-4] Contemporary risk assessment in stable CAD includes clinical risk scores,[5,6] stress testing, and assessment of coronary anatomy.[7,8] Even with these tools, an urgent need remains to improve risk stratification for among patients with stable CAD.[8] Biomarkers of processes underpinning the pathogenesis of CAD and cardiovascular events may provide important prognostic information.

Few studies have investigated multiple biomarkers simultaneously [8,9] or evaluated the prognostic value of biomarkers added to assessments of ischemia (i.e., stress testing) and atherosclerosis severity.[10-12] Prior studies are limited to patients undergoing angiography,[13,14] combined patients with stable and unstable syndromes,[15,16] or correlated biomarkers with ischemia or atherosclerosis.[12,17] Additionally, few if any patients with chronic kidney disease and CAD were included in prior studies. The ISCHEMIA (International Study of Comparative Health Effectiveness with Medical and Invsive Approaches) and ISCHEMIA-Chronic Kidney Disease (CKD) trials (collectively, the ISCHEMIA trials) randomized patients with stable CAD with moderate or severe ischemia to an initial invasive strategy of catheterization and revascularization with guideline-directed medical therapy compared with an initial strategy of guideline-directed medical therapy alone.[18,19] The objectives of this substudy were to test the hypotheses that one or more blood biomarkers would be associated with adjudicated cardiovascular events, and that addition of multiple biomarkers to traditional clinical risk factors and testing—including core-lab measured severity of ischemia and atherosclerosis—would improve prediction of cardiovascular events.

Methods

The design and primary results of ISCHEMIA trials have been reported.[18-20] ISCHEMIA enrolled patients with known or suspected CAD based on the finding of moderate or severe ischemia on stress imaging (echocardiography, nuclear perfusion, or cardiac magnetic resonance imaging), or severe ischemia on exercise electrocardiography.[20] Patients meeting criteria for ischemia severity with an estimated glomerular filtration rate [eGFR] ≥30 mL/kg/1.73 m^2 were enrolled in the main ISCHEMIA trial, and patients with an eGFR <30 mL/kg/1.73m^2 were enrolled in ISCHEMIA-CKD. Blinded coronary computed tomography angiography (CCTA) was performed in most (76%) ISCHEMIA patients with the goal of excluding patients with left main coronary stenosis ≥50% or no obstructive epicardial stenosis.[20,21] Participants with kidney impairment (eGFR <60 mL/min/1.73 m^2) or known coronary anatomy were not required to undergo a CCTA.[18,20] Overall, ISCHEMIA randomized 5,956 patients (5,179 from ISCHEMIA and 777 from ISCHEMIA CKD) to an invasive or a conservative approach, and tested the hypothesis that an initial invasive approach would improve clinical outcomes over

an initial conservative approach.[20,22] In ISCHEMIA and ISCHEMIA-CKD, the initial invasive approach did not reduce the risk of the primary or secondary endpoints.[19,23]

ISCHEMIA trials biorepository and sample selection and study outcomes

Venous blood samples were obtained from consenting participants at baseline within 6 weeks of enrollment and prior to receipt of assigned treatment strategy. Plasma was frozen in aliquots and stored at −70°C or colder until analysis. Details of biomarker analyses are provided in the Supplemental Methods and Supplemental Table 1. All biomarkers were measured at the Uppsala Clinical Research Center Laboratory at Uppsala University (Uppsala, Sweden), accredited to SS-EN ISO 15189.[24,25] We used the primary (cardiovascular death, MI, or hospitalization for unstable angina, heart failure, or resuscitated cardiac arrest) and secondary outcome (cardiovascular death or MI) from the ISCHEMIA trial.[20] In sensitivity analyses, we considered the individual endpoints of cardiovascular death, MI, and all-cause death.

Statistical methods

Clinical variables, stress testing, and CCTA findings are presented as median, 25th percentile, and 75th percentile for continuously measured variables and frequencies and percentages for categorical variables. We calculated 5-point descriptive summaries of the biomarker distributions and pairwise age- and sex-adjusted Spearman correlation coefficients between the biomarker variables. We compared baseline characteristics, stress testing, and CAD severity by CCTA across tertiles of biomarker distributions using the Kruskal-Wallis test and chi-square test for continuous and categorical variables, respectively.

We plotted the cumulative incidence of each study outcome by biomarker tertiles and used the Fine-Gray method to assess differences across groupings. Cox proportional hazards regression modeling was used to estimate cause-specific hazard ratios in separate models for each biomarker. We adjusted for 6 prespecified participant baseline characteristics (age, sex, diabetes, dialysis, eGFR among nondialysis patients, and LVEF),[19,23] in addition to ischemia severity. Biomarkers were entered by tertile to allow for nonlinear association and to facilitate clinical interpretation. We evaluated the added prognostic value of individual biomarkers and with multiple biomarkers modeled simultaneously and built Cox proportional hazards regression models with biomarkers measured continuously. Simulations in a Cox setting have shown that having at least 10 events per covariates is a prudent approach to avoiding estimation problems.[26] Therefore, to align with recommendations for the number of events per covariate (particularly when risk prediction is an objective),[26-28] we used a subset of biomarkers in the multiple marker model, modeled biomarker variables linearly, and used only the 6 prespecified baseline covariates and ischemia severity. Previous studies in patients with stable CAD[3,9,13,16,29-31] informed biomarker selection, along with data availability, biomarker variability, and correlations with other biomarkers. Hazard ratios for biomarkers reflect an increase from the 25th percentile to the 75th percentile, henceforth referred to as an "interquartile increase." Model accuracy and discrimination was estimated with a time-dependent Brier score and time-dependent area under the curve (AUC), respectively.[32,33] The time-dependent Brier score is a summary of predictive accuracy that simultaneously measures both calibration and discrimination. For a given time point, the Brier score is computed as the sum of the squared errors between the observed event status and estimated survival. Performance measures were computed accounting for the competing risk of noncardiovascular death with a cause-specific approach.[34] Each model was compared to a base model with only baseline covariates. Performance measures were computed within-sample and may be interpreted as an upper bound for the true predictive performance. A higher AUC and lower Brier score indicate a better model.

A sensitivity analysis was performed to compare performance between a base model with baseline characteristics (age, sex, diabetes, dialysis, eGFR among patients not on dialysis, LVEF and baseline ischemia severity), a base model + 2 biomarkers (hsTnT and NT-proBNP) and a base model + 4 biomarkers (hsTnT, NT-proBNP, GDF15 and sCD40L) among all participants in the ISCHEMIA biomarker biorepository.

To explore the importance of biomarkers when CAD severity is known, we replicated analyses among participants with a core-lab confirmed CCTA (508/757, 67%). Given the smaller sample size for analysis in which severity of CAD is known, covariate adjustment was limited to age, sex, LVEF and ischemia severity. We present only the estimated cause-specific hazard ratios because previous research has demonstrated that association studies may be less sensitive to the number of events per covariate compared to prognostic modeling.[27,28]

Treatment group was included as a stratum variable in Cox models to handle proportional hazards violation assumption by treatment strategy in ISCHEMIA.[19] All biomarkers were natural log-transformed to reduce skewness, and those with values below the detection limit were substituted with one-half the detection limit value.[9,35] We conducted analyses in R statistical software,[36] using the R package riskRegression.[37,38]

Results

A total of 1,064 ISCHEMIA Trials (ISCHEMIA and ISCHEMIA-CKD) participants consented for the biorepository. This nested cohort study included 757

participants with at least 9 of 10 biomarkers (Supplemental Figure 1). Sample characteristics are presented in Table 1. Baseline characteristics were similar between biorepository participants in this biomarker substudy (N = 757) compared with those excluded (N = 307) (Supplemental Table 2). Characteristics of participants in the combined ISCHEMIA Trials have been reported.[39]

The median (interquartile range, IQR) age was 67 years (61, 72), and median follow-up was 3 (2-5) years; 19% of participants were female (Table 1). Hypertension (85%), diabetes (45%), and obesity (45%) were common; 27% of participants had an eGFR <60 mL/min/1.73 m^2 and 6% of patients were on dialysis at baseline. Twenty-seven and 23% of participants had a prior percutaneous coronary intervention and MI, respectively, and 4% of the cohort had a history of heart failure. Ninety-one percent of participants in the biomarker substudy had at least moderate ischemia by stress testing. A CCTA was performed in 508 (67%) participants, 36% of whom had multivessel coronary artery disease ≥70% stenosis. Over a median follow-up of 3 years, there were 146 and 128 primary and secondary endpoints, respectively.

Biomarkers, participant characteristics, and clinical testing in the biorepository

Supplemental Table 3 summarizes biomarker distributions and percent below detection limits. Most biomarkers were detectable in all patients, with a maximum percent below detection of 7% for hsCRP. Eighteen percent (137/757) of IL-6 assays were missing due to insufficient sample volume; all others had <1% missing due to insufficient volume. The age- and sex-adjusted correlation between most biomarkers was weak to moderate, apart from GDF-15 and Cystatin-C (rho ≈ 0.7) (Supplemental Figure 2).

Supplemental Tables 4 and 5 present relationships between baseline characteristics, stress testing, and CCTA findings for a representative biomarker, hs-TnT. Participant risk factors and comorbidities including hypertension, diabetes requiring insulin, obesity, impaired renal function, baseline dialysis, history of heart failure, cerebrovascular disease and peripheral artery disease were generally more common across increasing biomarker tertiles (lowest to highest). This pattern was consistent for all biomarkers except Lp(a) and sCD40L, for which the distribution of risk factors and comorbidities was unchanged from tertile 1 to tertile 3 (data not shown). The proportion of participants with severe ischemia did not vary across tertiles of hsTnT (Supplemental Table 5) or any other biomarker (data not shown). In contrast, the proportion of patients with multivessel CAD (multivessel CAD ≥70%, multivessel CAD ≥50% stenosis) increased from tertile 1 to tertile 3 of hsTnT (Supplemental Table 5), NTproBNP and Lp(a) (data not shown). For all other biomarkers the proportion of multivessel CAD ≥70% was similar across tertiles (data not shown).

Table 1. Baseline characteristics of ISCHEMIA biorepository biomarker cohort

Characteristic	Study population no. (%)*
No.	757
Demographics	
Age at randomization, years	
No.	757
Median (Q1, Q3)	67 (61, 72)
Follow-up time, years	
No.	757
Median (Q1, Q3)	3 (2, 5)
Female sex	144/757 (19%)
Race	
White	634/755 (84%)
Black	84/755 (11%)
Asian	20/755 (3%)
Other or multiple ethnic groups	17/755 (2%)
Hispanic or Latino ethnicity	38/750 (5%)
Cigarette smoking	
Current smoker	85/757 (11%)
Former smoker	412/757 (54%)
Never smoker	260/757 (34%)
Randomized to invasive	368/757 (49%)
Randomized to conservative	389/757 (51%)
Clinical history	
Diabetes	344/757 (45%)
Insulin treated	141/341 (41%)
Noninsulin treated or diet controlled	200/341 (59%)
Hypertension	644/755 (85%)
BMI, kg/m^2	
No.	754
Median (Q1, Q3)	29 (26, 33)
Obese, BMI ≥30 kg/m^2	343/754 (45%)
eGFR, mL/min/1.73 m^2	
No.	757
Median (Q1, Q3)	74 (58, 90)
eGFR <60 mL/min/1.73 m^2	206/757 (27%)
Baseline dialysis	49/757 (6%)
Left ventricular ejection fraction	
No.	757
Median (Q1, Q3)	60 (54, 65)
Left ventricular ejection fraction <45%	52/756 (7%)
Prior myocardial infarction	175/754 (23%)
Prior PCI	203/757 (27%)
Prior CABG	43/757 (6%)
History of heart failure	34/757 (4%)
History of cerebrovascular disease	85/756 (11%)
History of peripheral artery disease	45/755 (6%)
Family history of premature CAD	209/645 (32%)
Ischemia severity by stress testing	
Severe	329/756 (44%)
Moderate	355/756 (47%)
Mild/none	69/756 (9%)
Uninterpretable	3/756 (0%)
CCTA findings	
CCTA performed	508/757 (67%)
Any obstructive disease ≥70% stenosis by CCTA	392/508 (77%)
Multivessel disease ≥70% stenosis by CCTA	185/508 (36%)
Vessels ≥70% stenosis by CCTA	
0	60/508 (12%)
1	134/508 (26%)
2	84/508 (17%)

(*continued on next page*)

Table 1. (*continued*)

Characteristic	Study population no. (%)*
3 or more	65/508 (13%)
Nonevaluable	165/508 (32%)

*Continuously measured variables are summarized with the median, 25th percentile (Q1), and 75th percentile (Q3).

Biomarkers and cardiovascular events

Figures 1A and 1B show the unadjusted cumulative incidence of the primary and secondary endpoint by biomarker tertiles (see Figure 2 for tertile cutoff values). The cumulative incidence of the primary and secondary outcome differed across tertiles for all biomarkers except MPO and Lp(a) (Figure 1A and 1B; Fine-Gray $P < .05$).

The 3-year unadjusted cumulative incidence of the primary and secondary outcome appeared greater across increasing tertiles of most biomarkers except Lp(a) and MPO (Figures 2A and 2B). After adjusting for age, sex, diabetes, dialysis, eGFR among those not on dialysis, LVEF and ischemia severity there was a significant increasing hazard for the primary (Figure 2a) and secondary (Figure 2b) outcome across biomarker tertiles. IL-6, hsTnT, GDF-15, NT-proBNP, Cystatin-C, and sCD40-L were each individually associated with the primary and secondary outcomes.

We next evaluated the potential contribution of biomarkers to improve risk prediction. For the primary and secondary outcome, in separate models by individual biomarker, Supplemental Table 6 presents cause-specific hazard ratios for an increase from the 25th to the 75th percentile ("interquartile increase") in a given biomarker distribution. For example, the IQR increase for NT-proBNP refers to an increase from 75 ng/L to 415 ng/L on the raw (un-transformed) scale. Each interquartile increase in hsTnT, NT-proBNP, or GDF-15 was associated with an approximately 2-fold greater hazard for the primary and secondary outcome; the hazards for the primary and secondary outcome per IQR increase in IL-6, Cystatin-C, sCD40L or MMP3 were more modest (Supplemental Table 6). The base model with clinical risk factors, LVEF and ischemia severity had an area under the receiver operating characteristics curve (AUC) of approximately 0.71 for the primary and secondary outcome (Supplemental Table 7). Compared to the base model, when considered individually hsTnT and GDF-15 significantly improved model discrimination for both composite outcomes (Supplemental Table 7). Predictive accuracy of both the primary and secondary outcome (as assessed by Brier score) was improved significantly by inclusion of hsTnT.

Table 2 presents adjusted cause-specific hazard ratios of biomarkers selected for multimarker modeling (hsTnT, NT-proBNP, GDF-15 and sCD40L) of the primary and secondary outcomes. Controlling for other biomarkers, participant characteristics and ischemia severity, an interquartile increase in each biomarker was individually associated with an approximately 50% (44%-61%) greater hazard of the primary and secondary outcome (Table 2). When included simultaneously, the addition of hsTnT, NT-proBNP, GDF-15 and sCD40L to the base model substantially improved model discrimination and predictive accuracy. The AUC increased from 0.711 to 0.791 ($P = .001$) for the 5-component primary outcome and from 0.712 to 0.783 ($P = .002$) for the secondary outcome of cardiovascular death or MI (Table 2, Figure 3A/B). Predictive accuracy as measured by the Brier score also significantly improved for both outcomes.

Sensitivity analysis demonstrated that compared to a clinical model with hsTnT and NT-proBNP, a clinical model with 4 biomarkers (hsTnT, NT-proBNP, GDF-15 and sCD40L) significantly improved discrimination of the primary and secondary outcomes by AUC but did not improve predictive accuracy (Supplemental Table 8). In an exploratory analysis with all 10 biomarkers entered simultaneously (adjusting for clinical covariates and ischemia severity), hsTnT, GDF-15, and sCD40L were each associated with an increased hazard of the primary and secondary outcome, while NT-proBNP was associated only with an increased hazard for the primary outcome (Supplemental Table 9).

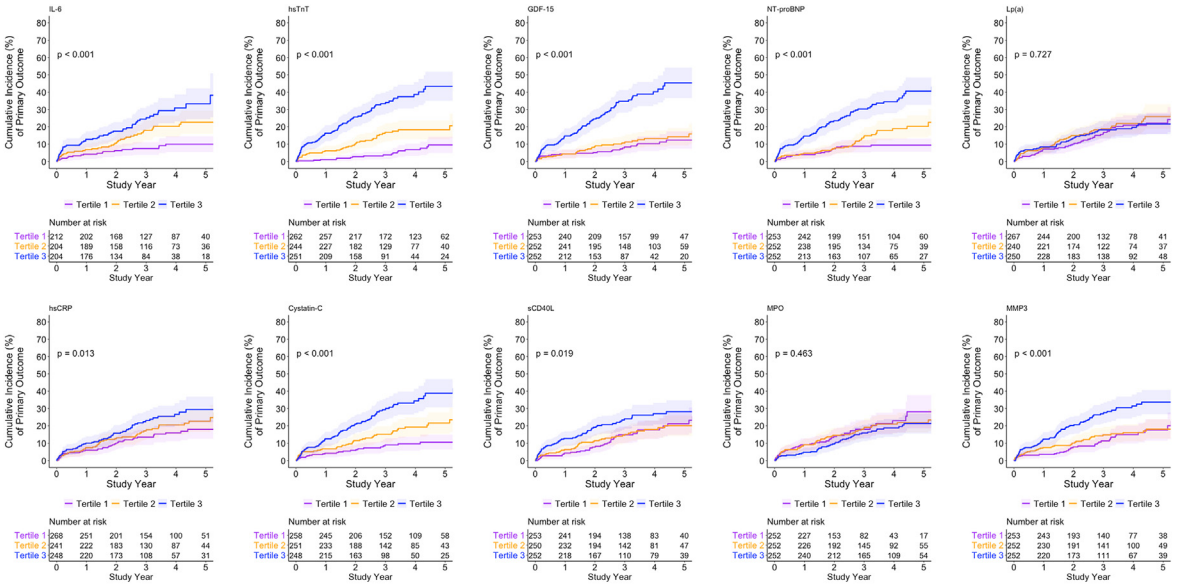
Analyses of the adjusted cause-specific hazard ratios between biomarkers and individual endpoints of MI, CV death, and all-cause death were largely consistent with the primary and secondary outcomes. HsTnT, NT-proBNP and GDF-15 were each associated with MI, CV death, and all-cause death. While not associated with the endpoint of MI, MMP3 and Cystatin C were associated with CV death and all-cause death. sCD40L was significantly associated only with MI (Supplemental Figures 3A-3C).

Biomarkers, severity of CAD, and cardiovascular events

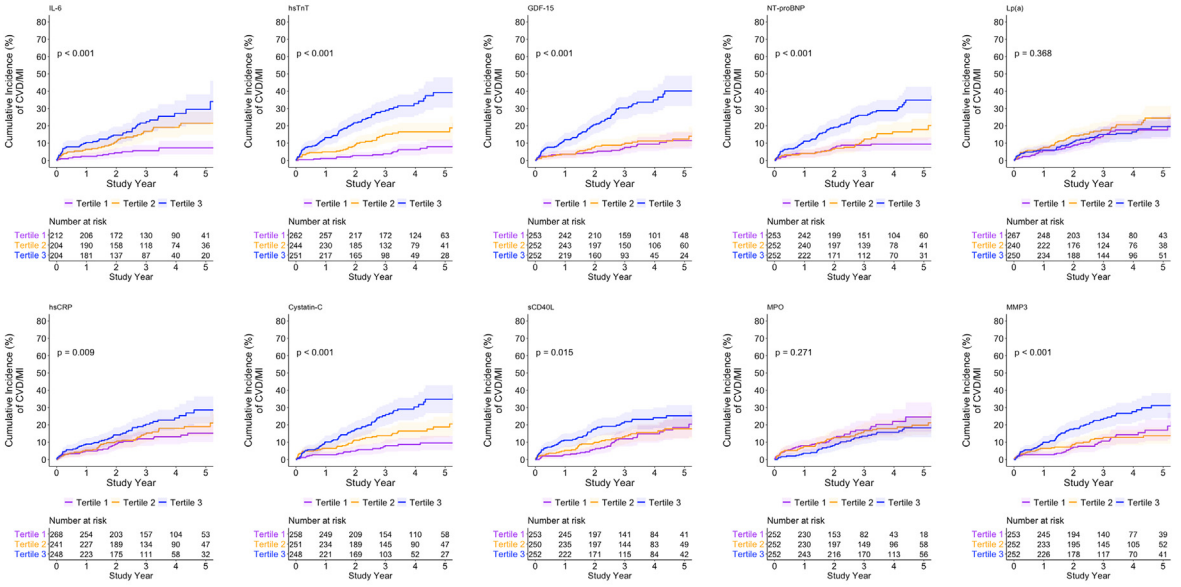
To characterize biomarker performance in addition to core-lab confirmed severity of CAD, we next performed analysis of biomarkers among ISCHEMIA biorepository substudy participants with available CCTA data. In comparison with the biomarker cohort (N = 757), participants with a CCTA (N = 508) had a lower burden of diabetes and better renal function, Supplemental Table 2.

We next explored associations with biomarkers after adjustment for CAD severity in the subset with core-lab confirmed atherosclerosis burden by CCTA. For the primary and secondary outcome, Supplemental Table 10 presents cause-specific hazard ratios for an interquartile increase in each biomarker. After adjusting for age, sex, LVEF, ischemia severity and core-lab confirmed multi-

Figure 1



(a)



(b)

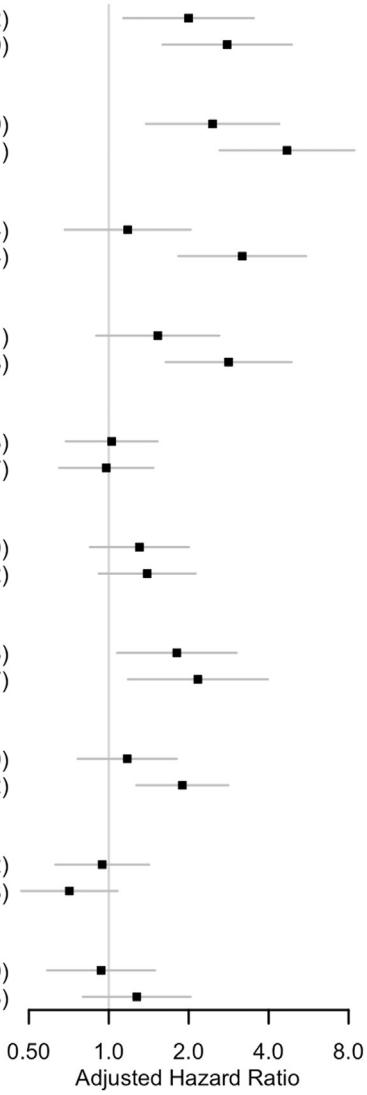
A. Cumulative incidence of the primary outcome (cardiovascular death, MI, hospitalization for unstable angina or heart failure, or resuscitated cardiac arrest) by tertiles[a] of biomarker distributions. *Footnotes:* [a] See Figure 2 for biomarker values of tertile cutoffs. Figure 1B. Cumulative incidence of the secondary outcome (cardiovascular death or MI) by tertiles[a] of biomarker distributions. *Footnotes:* [a] See Figure 2 for biomarker values of tertile cutoffs.

vessel CAD ≥70% stenosis, each IQR increase in IL-6, hsTnT, GDF-15, NT-proBNP, or sCD40L was individually associated with the primary and secondary outcome; hsCRP and Cystatin-C were associated with only the secondary outcome (Supplemental Table 10). For the primary and secondary outcomes, Supplemental Table 11 presents adjusted cause-specific hazard ratios of each biomarker selected for multimarker modeling (namely, hsTnT, NT-proBNP, GDF-15 and sCD40L), adjusting for other biomarkers, clinical characteristics, ischemia severity and multivessel CAD ≥70%. An interquartile increase in each of the selected biomarkers for the

Figure 2

(a)

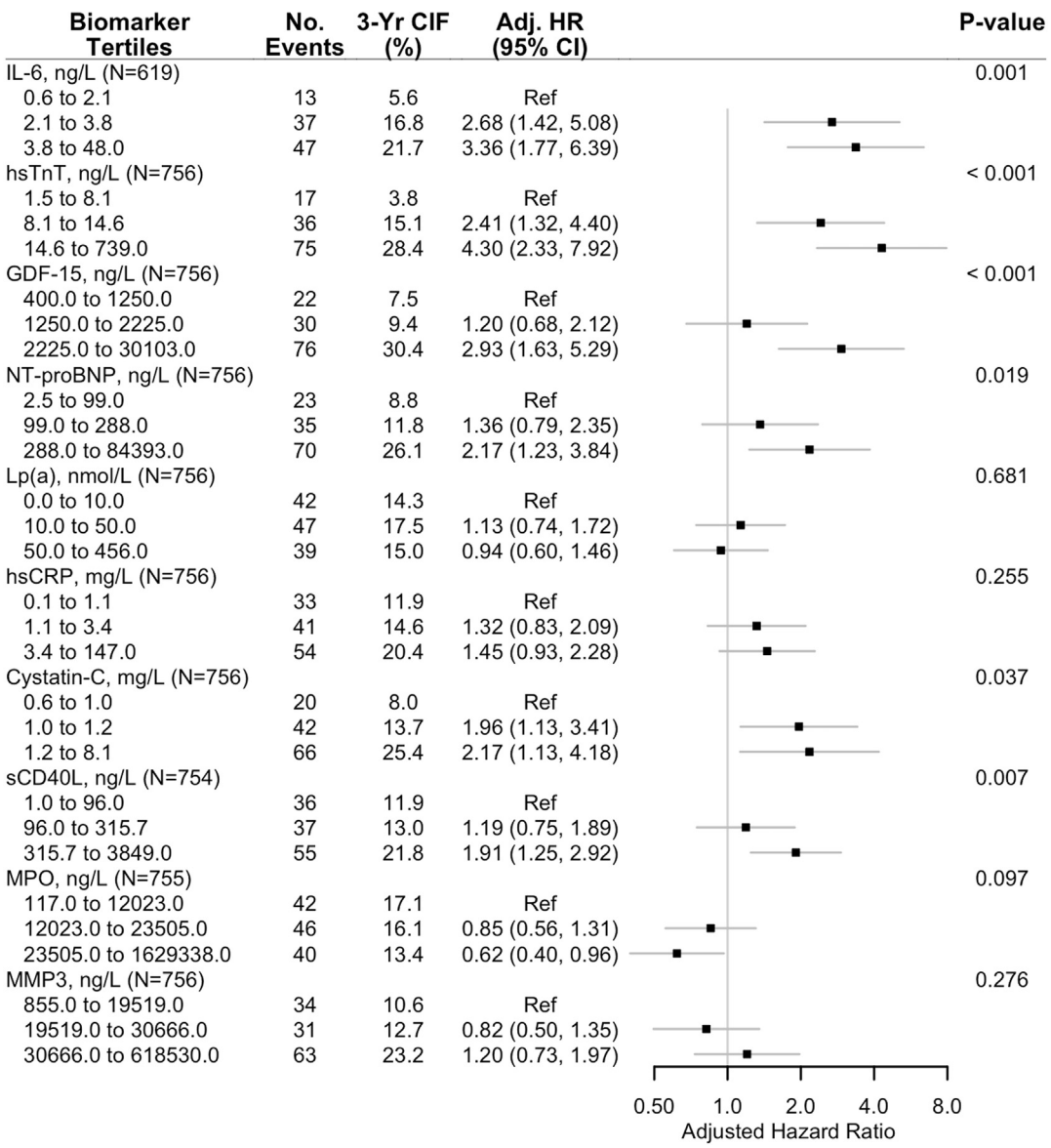
Biomarker Tertiles	No. Events	3-Yr CIF (%)	Adj. HR (95% CI)	P-value
IL-6, ng/L (N=619)				0.002
0.6 to 2.1	18	7.5	Ref	
2.1 to 3.8	39	18.0	2.00 (1.13, 3.52)	
3.8 to 48.0	55	24.6	2.79 (1.60, 4.89)	
hsTnT, ng/L (N=756)				< 0.001
1.5 to 8.1	18	3.8	Ref	
8.1 to 14.6	41	16.9	2.46 (1.38, 4.39)	
14.6 to 739.0	87	33.5	4.69 (2.61, 8.41)	
GDF-15, ng/L (N=756)				< 0.001
400.0 to 1250.0	24	8.4	Ref	
1250.0 to 2225.0	33	10.7	1.18 (0.68, 2.04)	
2225.0 to 30103.0	89	34.9	3.18 (1.83, 5.54)	
NT-proBNP, ng/L (N=756)				< 0.001
2.5 to 99.0	23	8.8	Ref	
99.0 to 288.0	39	14.2	1.53 (0.90, 2.61)	
288.0 to 84393.0	84	30.4	2.83 (1.64, 4.88)	
Lp(a), nmol/L (N=756)				0.974
0.0 to 10.0	49	16.2	Ref	
10.0 to 50.0	50	18.9	1.03 (0.69, 1.53)	
50.0 to 456.0	47	18.3	0.98 (0.65, 1.47)	
hsCRP, mg/L (N=756)				0.273
0.1 to 1.1	39	13.5	Ref	
1.1 to 3.4	47	17.1	1.30 (0.85, 2.00)	
3.4 to 147.0	60	23.2	1.40 (0.92, 2.12)	
Cystatin-C, mg/L (N=756)				0.034
0.6 to 1.0	23	9.1	Ref	
1.0 to 1.2	46	15.2	1.80 (1.08, 3.03)	
1.2 to 8.1	77	29.5	2.17 (1.18, 3.97)	
sCD40L, ng/L (N=754)				0.004
1.0 to 96.0	42	14.9	Ref	
96.0 to 315.7	43	14.6	1.17 (0.76, 1.80)	
315.7 to 3849.0	61	24.0	1.89 (1.27, 2.82)	
MPO, ng/L (N=755)				0.221
117.0 to 12023.0	45	18.2	Ref	
12023.0 to 23505.0	53	18.6	0.94 (0.63, 1.42)	
23505.0 to 1629338.0	47	15.9	0.71 (0.47, 1.08)	
MMP3, ng/L (N=756)				0.340
855.0 to 19519.0	36	11.3	Ref	
19519.0 to 30666.0	38	14.8	0.94 (0.59, 1.49)	
30666.0 to 618530.0	72	27.1	1.28 (0.80, 2.03)	



A. Forest plot of the adjusted association of the primary outcome and biomarker tertiles in the ISCHEMIA biorepository biomarker substudy. *Footnotes:* [a] Adjusted cause-specific hazard ratios for the association of the primary outcome by tertiles of each biomarker (controlling for sex, age, diabetes, dialysis, eGFR among non-dialysis patients, left ventricular ejection fraction and ischemia severity). [b] Primary outcome was the composite of cardiovascular death, myocardial infarction, hospitalization for unstable angina or heart failure, or resuscitated cardiac arrest. *Abbreviations:* No., number, 3-year CIF, 3-year Cumulative Incidence Function, Adj. HR, adjusted hazard ratio. Figure 2B. Forest plot of the adjusted association of secondary outcome and biomarker tertiles, in the ISCHEMIA biorepository biomarker substudy. *Footnote:* [a] Adjusted cause-specific hazard ratios for the association of the primary outcome by tertiles of each biomarker (controlling for sex, age, diabetes, dialysis, eGFR among nondialysis patients, left ventricular ejection fraction and ischemia severity). [b] Secondary outcome was the composite of cardiovascular or myocardial infarction. *Abbreviations:* No., number, 3-year CIF, 3-year Cumulative Incidence Function, Adj. HR, adjusted hazard ratio.

Figure 2

(b)



Continued

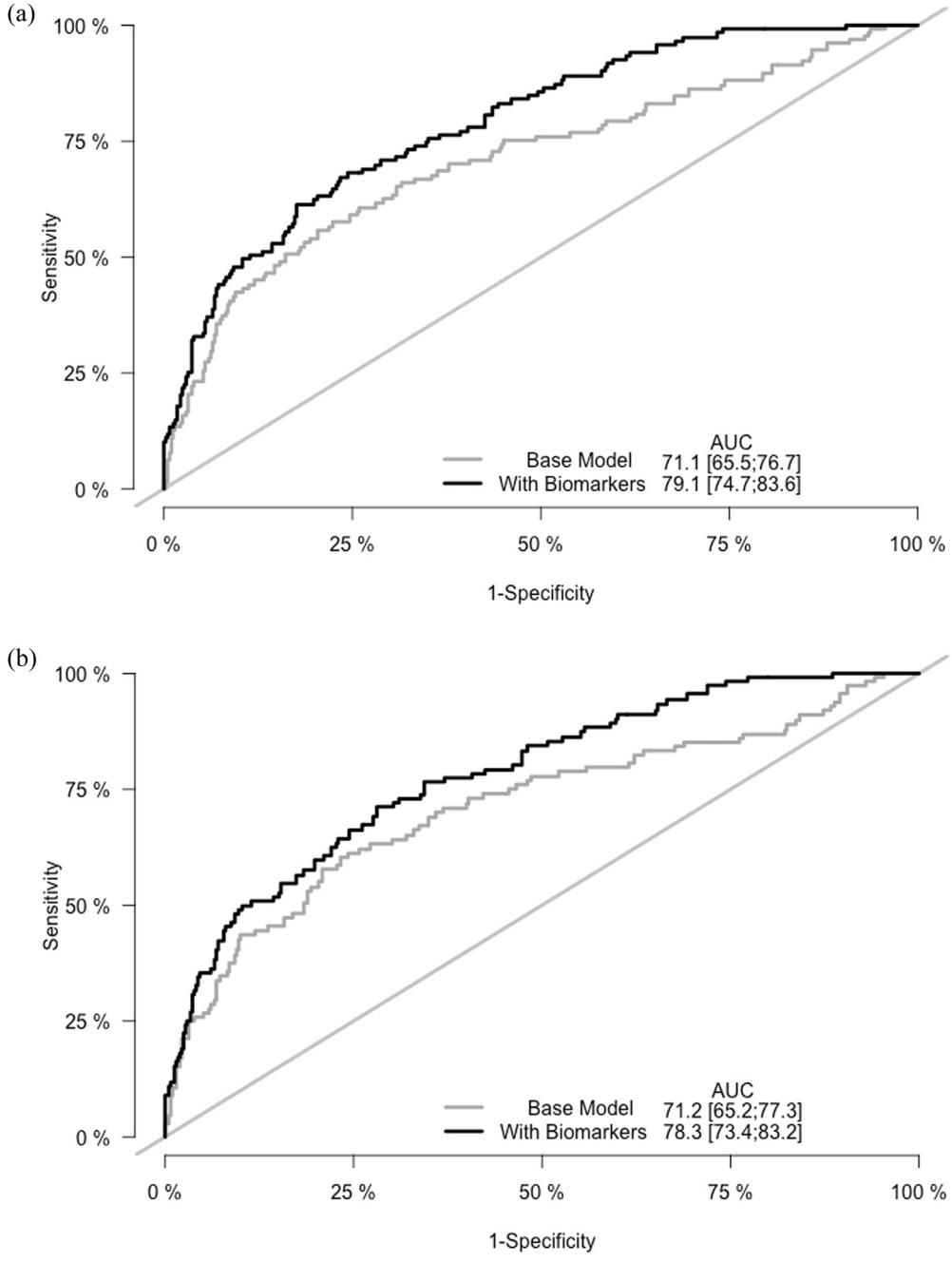
multimarker model was associated with an approximately 50% (42%-87%) greater hazard for the primary and secondary outcomes.

Discussion

In this analysis from the ISCHEMIA Trials biorepository, we found that biomarkers of myocardial injury/distension (hsTnT, NT-proBNP), inflammation (GDF-15), and platelet activity (sCD40L) were associated with and improved prediction of cardiovascular events after adjustment for clinical risk factors, LVEF, severity of ischemia and atherosclerosis. This suggests a clinical utility for biomarker measurement beyond current risk paradigms for stable CAD.

Landmark prospective cohort studies provided important data on the use of biomarkers to enhance cardiovascular risk prediction.[30,31,40-44] Data from the current analyses extend knowledge to CAD patients with core-lab confirmed ischemia in whom severity of CAD is known. Biorepositories embedded in randomized clinical trials present a unique opportunity to evaluate biomark-

Figure 3



Receiver operating curves at 3 years for the base model and selected biomarkers for the primary (3A) and secondary (3B) outcome in the ISCHEMIA biorepository biomarker substudy, N = *757*. *Footnote:* Base model includes adjustment for age, sex, diabetes, dialysis, eGFR among non-dialysis patients, ischemia severity and left ventricular ejection fraction. With biomarkers denotes the base model and 4 biomarkers: hsTnT, GDF-15, NT-proBNP, and sCD40L. Primary outcome: Cardiovascular death, MI, hospitalization for heart failure or unstable angina, or resuscitated cardiac arrest. Secondary outcome: CV death or MI.

Table 2. Multivariable adjusted multimarker association and prediction models in the ISCHEMIA biorepository biomarker cohort (N = 754)

Primary outcome*

Biomarker (IQR increase)	Adjusted HR‡ (95% CI)	Base AUC	New AUC	Δ AUC (95% CI)	*P* value	Base Brier Score	New Brier Score	Δ Brier score (95% CI)	*P* value
hsTnT, ng/L (7.0–18.4)	1.58 (1.22, 2.05)	0.711	0.791	0.080 (0.035, 0.126)	.001	0.129	0.117	-0.012 (-0.020, -0.005)	.001
GDF-15, ng/L (1086.0–2753.0)	1.60 (1.16, 2.20)								
NT-proBNP, ng/L (75.0–415.0)	1.61 (1.22, 2.14)								
sCD40L, ng/L (68.5–418.5)	1.46 (1.12, 1.90)								

Secondary outcome†

Biomarker (IQR increase)	Adjusted HR‡ (95% CI)	Base AUC	New AUC	Δ AUC (95% CI)	*P* value	Base Brier Score	New Brier Score	Δ Brier Score (95% CI)	*P* value
hsTnT, ng/L (7.0–18.4)	1.54 (1.17, 2.04)	0.712	0.783	0.071 (0.026, 0.116)	.002	0.116	0.107	-0.009 (-0.015, -0.002)	.008
GDF-15, ng/L (1086.0–2753.0)	1.57 (1.12, 2.20)								
NT-proBNP, ng/L (75.0–415.0)	1.44 (1.08, 1.94)								
sCD40L, ng/L (68.5–418.5)	1.51 (1.14, 2.00)								

Hazard ratios are expressed per increase in biomarker concentration from the 25th to the 75th percentile (termed IQR increase) of the distribution.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IQR, interquartile range. LVEF, left ventricular ejection fraction. Biomarker abbreviations as noted in the abbreviations list.

* Cardiovascular death, myocardial infarction, resuscitated cardiac arrest, hospitalization for unstable angina or heart failure.

† Cardiovascular death or myocardial infarction.

‡ Adjusted for age, sex, diabetes, dialysis, eGFR among patients not on dialysis, LVEF, and baseline ischemia severity.

ers alongside clinical testing and management. Of the 10,003 outpatients with stable chest pain randomized in the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) study,[45] 4,031 were included in a blood biorepository.[46,47] PROMISE analyses demonstrate that high-sensitivity troponin[46,47] and IL-6[48] were associated with CAD characteristics and cardiovascular events. However, PROMISE analyses have not evaluated if biomarkers associate with events when added to inducible ischemia or atherosclerosis severity.[45-48] The Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial compared the effect of an Lp-PLA$_2$ inhibitor (darapladib) with placebo on cardiovascular events in 15,828 patients with stable CAD, of whom 13,164 patients were included in a biorepository.[24,25] STABILITY demonstrated that NT-proBNP, hsTnT,[25] and IL-6[24] provide incremental predictive value when added to clinical testing.[25] However, severity of CAD and inducible ischemia were not core-lab confirmed prior to randomization and were not included in multivariable modeling.[24,25] Incorporation of atherosclerosis severity in modeling is important because without it, one cannot tell if the biomarker predicts atherosclerosis severity—a well-known prognostic indicator—or is independently associated with higher risk.

It is in the context of these landmark studies that we demonstrate that a multimarker model improves prediction of cardiovascular events in the setting of moderate-severe stress testing and core lab-confirmed severity of CAD by CCTA. Biomarkers identified represent complementary pathophysiological processes in stable CAD. Cardiac troponins are an integral part of myocardial contractile apparatus and are released into circulation following acute and chronic injury.[49] Naturietic peptides including NT-proBNP reflect myocardial dysfunction, wall stress and ventricular dysfunction.[50] GDF-15 is an stress responsive cytokine expressed and secreted in response to inflammation and oxidative stress,[51] and sCD40-L is an immunomodulator ligand with platelet activity.[52] We show that compared to a clinical model with hsTnT and NT-proBNP alone, the addition of GDF-15 and sCD40L significantly improves model discrimination but does not appreciably change predictive accuracy, as measured by the Brier score which takes into account both model discrimination and calibration. More broadly, this observation suggests that non-myocardial biomarkers, such as markers of inflammation or platelet activity, may have prognostic relevance for the care of patients with stable CAD.

Few, if any, patients with stable CAD and comorbid renal dysfunction have been included in prior biomarker

studies.[46] In contrast, more than one quarter (28%) of patients in the current analysis had an eGFR <60 ml/min/1.73 m^2. Our findings provide preliminary data on the use of biomarkers on the subset of patients with stable CAD and comorbid renal disease.

Our study has limitations. Analyses were conducted post-hoc on existing biorepository data and were unadjusted for multiple comparisons. Second, analyses were performed in a single cohort and caution is warranted for over-interpretation of predictive analyses. External validation is needed. Biomarkers were available in a subset of ISCHEMIA Trials participants in whom sample collection was allowed by country specific regulations. Analyses are limited to samples collected at baseline precluding analyses of change in biomarkers over time. Sites were encouraged to process and store the samples rapidly; however, delay in processing may have occurred and affected the values of some of the biomarkers reported. Analyses were based on hsTnT and recent data indicates high-sensitivity cardiac troponin I (hsTnI) may be more specific for cardiovascular outcomes than hsTnT.[53,54] Participants in ISCHEMIA were required to have moderate or severe ischemia prior to randomization.[19,20] Therefore, our results may not be applicable to patients without ischemia or with nonobstructive CAD. Finally, the subset of patients with inducible ischemia in whom CAD severity was available is a subset of the overall biorepository and does not include patients with CKD.

Conclusions

In this analysis from the ISCHEMIA Trials biorepository, biomarkers of myocyte injury/distension, inflammation, and platelet activity improved prediction of cardiovascular events. At a median follow-up of 3.5 years, high-sensitivity troponin T and NT-proBNP improved prediction of cardiovascular events in a high-risk population of patients with stable CAD when added to models including clinical risk factors, core lab-confirmed severity of CAD and inducible ischemia. Identified biomarkers will require prospective testing and external validation.

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Disclaimer

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahj.2023.08.007.

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