Relationship of platelet reactivity and inflammatory markers to recurrent adverse events in patients with ST-elevation myocardial infarction

Krishma Adatia MB ChB BSc[1] *, Mohamed F Farag MB BCh MSc PhD [1,2] *, Ying X Gue MB ChB[1,2], Manivannan Srinivasan MB BS MD[1], Diana A Gorog MB BS MD PhD[1,2,3]

1. East and North Hertfordshire NHS Trust, Hertfordshire, United Kingdom
2. University of Hertfordshire, United Kingdom
3. National Heart and Lung Institute, Imperial College, London, United Kingdom

* these authors contributed equally to the manuscript.

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Correspondence to:

Prof. Diana A Gorog
National Heart and Lung Institute
Imperial College
Dovehouse Street
London SW3 6LR
United Kingdom
Tel +44 (0)207 034 8934
Fax +44 (0)207 034 8935
d.gorog@imperial.ac.uk

Abstract

Background

Patients with ST-elevation myocardial infarction (STEMI) exhibit prothrombotic and pro-inflammatory states. Markers of enhanced platelet reactivity and inflammation are predictive of adverse outcome. However, the relationship between these biomarkers, and their combined usefulness for risk stratification, is not clear.

Methods

In a prospective study of 541 patients presenting with STEMI, blood samples were taken on arrival to measure high-sensitivity CRP (hs-CRP), neutrophil/lymphocyte ratio (NLR), and platelet reactivity using the point-of-care Global Thrombosis Test. These biomarkers, alone and in combination, were related to the occurrence of major adverse cardiovascular events (MACE, defined as composite of cardiovascular death [CVD], myocardial infarction [MI], and cerebrovascular accident [CVA]) at 30 days and 12 months.

Results

Platelet reactivity and hs-CRP, but not NLR, were weakly predictive of MACE at 30 days and 12 months. The combination of enhanced platelet reactivity and raised hs-CRP was strongly predictive of MACE at 30 days (HR 3.46, [95% CI 1.81-6.62], $p<0.001$) and 12 months (HR 3.46, [95% CI 1.81-6.63], $p<0.001$). Combination of all three biomarkers (NLR, hs-CRP and platelet reactivity) provided the best prediction of MACE at 30 days (HR 3.73 [95% CI 1.69-8.27], $p<0.001$) and 12 months (HR 3.85 [95% CI 1.72-8.60], $p<0.001$) and improved the prediction of MACE when added to TIMI score (net reclassification index 0.296, $p<0.001$).

Conclusion

A combination of three easy to measure biomarkers on arrival, namely hs-CRP, NLR and platelet reactivity, can help identify STEMI patients at high risk of recurrent adverse events over the subsequent year.

Word count: 245

Keywords

STEMI; neutrophil; lymphocyte; platelet reactivity; inflammation

Summary table

What is known on this topic?

- Acute coronary syndromes (ACS) are associated with prothrombotic and proinflammatory states, which contribute to adverse outcomes.
- Individually, neutrophil/lymphocyte ratio (NLR), hs-CRP and platelet reactivity can predict adverse outcomes following ACS.

What does this paper add?

- NLR, hs-CRP and point-of-care platelet reactivity are easy to measure by clinicians, without the need for specialist expertise.
- Results of these biomarkers, used in combination, yield greater predictive value for the event-free survival following STEMI than any individual biomarker alone.
- Use of all three biomarkers together provides the strongest predictor of short- and medium-term major adverse events in patients with STEMI.

Abbreviations

ACS: Acute coronary syndrome

CAD: Coronary artery disease

CVA: Cerebrovascular accident

CVD: Cardiovascular death

DAPT: Dual anti-platelet therapy

GTT: Global Thrombosis Test

hs-CRP: High-sensitivity C-reactive protein

MACE: Major adverse cardiovascular event

MI: Myocardial infarction

NLR: Neutrophil/lymphocyte ratio

OT: Occlusion time

PPCI: Primary percutaneous coronary intervention

STEMI: ST-segment elevation myocardial infarction

Introduction

Following an acute coronary syndrome (ACS), a number of patients experience recurrent major adverse cardiovascular events (MACE), despite treatment with primary percutaneous coronary intervention (PPCI) and dual antiplatelet therapy (DAPT)[1]. Inflammation[2] and platelet hyperreactivity[3] are key mechanisms underlying the pathogenesis of atherosclerosis and arterial thrombus formation, and are important drivers for ongoing cardiovascular events. Various biomarkers of inflammation[4] and of enhanced platelet reactivity[3] have received increasing attention for their use as prognostic indicators in patients with ACS. Risk stratification using such biomarkers can help guide therapeutic management in these patients; more aggressive therapy may be reserved for those at high-risk, in order to minimise the consequences of aggressive antithrombotic therapy-associated bleeding in those at lower risk.

High-sensitivity C-reactive protein (hs-CRP) and neutrophil/lymphocyte ratio (NLR) are both readily available inflammatory biomarkers, accessible from routine blood tests carried out on hospital admission. Previous studies have demonstrated that both hs-CRP and NLR are good predictors of heart failure[5-8], stent thrombosis[7,9,10], short-[6,7,10-12] and long-term[4,7, 8,12, 13] mortality, and short-[7,12,14,15] and long-term[4,7,12,16,17] adverse outcomes following ACS. They are both positively associated with severity of coronary artery disease (CAD)[7,18], and infarct size in myocardial infarction (MI)[19]. NLR is a better predictor of future MI in patients with CAD[20], and 12-month outcome following ACS[14], compared to white cell, neutrophil, and lymphocyte counts. The comparative predictive values of hs-CRP and NLR, in contrast, is unclear, though Shin *et al.* have demonstrated that their combined use is a stronger predictor of two-year all-cause mortality than either value alone[21].

Quantitative assessment of platelet reactivity may be performed using complex laboratory assays, such as light transmission aggregometry and vasodilator stimulated phosphoprotein

phosphorylation, or point-of-care assays, including VerifyNow, PFA-100, and the Global Thrombosis Test[3]. Prior studies have shown a strong association between platelet reactivity and stent thrombosis[22-24], short-[25,26] and long-term[27] MACE, and all-cause mortality[28-30] in patients with ST-segment elevation MI (STEMI) undergoing PPCI. Whilst prior studies have demonstrated the combination of platelet reactivity and hs-CRP to have a greater predictive value for cardiac mortality than either biomarker alone[16,31], the combined predictive value of NLR and platelet reactivity using a point-of-care technique has not previously been investigated.

Although hs-CRP, NLR, and platelet reactivity have been studied extensively for their individual prognostic abilities in ACS, little is known about their comparative and combined predictive value. The aim of this study was to evaluate the predictive value of these biomarkers, alone and in combination, for the prediction of short- and medium-term clinical outcomes in patients with STEMI.

Methods

Study design and population

We conducted a prospective, observational study in 550 adults presenting to our heart attack centre with STEMI for emergency PPCI. The study was approved by the National Research Ethics Service and the UK Health Research Authority. All patients gave written informed consent.

Inclusion and exclusion criteria

Patients were eligible for inclusion if aged ≥18 years, and presenting with STEMI with a view to PPCI, based on clinical presentation and ECG criteria. Exclusion criteria included patients receiving oral anticoagulation, those with known coagulopathy, those unable to take DAPT, those with sepsis, platelet count $<100 \times 10^9$/L, haemoglobin <80 g/L, active malignancy, or those previously enrolled in the study.

Blood sampling

Antiplatelet therapy, consisting of oral aspirin 300 mg and ticagrelor 180 mg, was administered upon diagnosis, either in the ambulance or emergency department prior to blood sampling, but heparin was not given until after blood draw. Non-fasting blood samples were taken on arrival, and prior to PPCI, from a 6-French radial or femoral sheath, flushed only with non-heparinized saline before insertion. A two-syringe technique was employed: the

first 5ml of blood was used for routine blood tests, and the second 5ml for platelet reactivity assessment.

Assessment of platelet reactivity

Platelet reactivity was assessed using the point-of-care Global Thrombosis Test (GTT) (Thromboquest Ltd, London, UK)[32]. A 4ml non-anticoagulated blood sample was introduced into the instrument within 15 sec of blood withdrawal, and after that the measurement is fully automated. The instrument assesses the time taken for occlusive platelet thrombus formation to occur in whole blood, in response to high shear-induced platelet activation. Platelet reactivity is reported as occlusion time (OT, sec), where platelet reactivity represents the inverse of OT. Shorter OT thus reflects greater platelet reactivity.

Data collection and follow-up

During the index admission, patient demographics, blood results, medication and treatment-related information were recorded. Patients were followed-up by telephone and by accessing case notes.

Study endpoints

The primary endpoint was the occurrence of MACE at 30 days and 12 months. MACE was defined as the composite of cardiovascular death (CVD), non-fatal MI, including stent thrombosis (defined according to the Academic Research Consortium criteria), and

cerebrovascular accident (CVA). For all endpoints, source documents were obtained, and diagnoses verified by two independent clinicians, blinded to blood results.

Statistical analysis

This study aimed to assess the predictive values of NLR, hs-CRP and platelet reactivity for short- and long-term MACE. Based on an effect size (hazard ratio) of 2.7 from previous data[21], assuming a two-sided alpha value of 0.01, MACE rate of 10% and an attrition rate of 10% over the follow-up period, using the Cox proportional hazard model, we calculated that at least 527 patients would be required to achieve 80% power; allowing for drop out we calculated an optimal sample size of 550 patients. Mean values ± standard deviation are reported for normally distributed data, and median [interquartile range] for non-normally distributed data. Baseline characteristics for patients with and without MACE at 12 months were compared were compared using χ^2 test for continuous variables or Fisher's exact test for categorical variables. Correlations were analysed using Pearson's method. Receiver operating characteristic (ROC) curves were used to set optimal cut-points for NLR, hs-CRP and platelet reactivity, based on highest specificity and sensitivity; optimal cut-points were used to divide patients into groups of low/high biomarker combinations. Kaplan-Meier analysis and log-rank test were used to compare survival between groups. Univariate and multivariable Cox hazards models were used to investigate the relationship of NLR, hs-CRP and platelet reactivity with MACE. All the study variables were first analysed with univariate analysis and those that showed a significant interaction ($p<0.05$) were entered into the multivariate analysis. Net reclassification improvement (NRI) was used to assess the additive predictive value of the study biomarkers for the prediction of MACE, in addition to the well-established

Thrombolysis in Myocardial Infarction (TIMI) risk score. Analyses were performed with Stata V.15 (StataCorp, College Station, TX, USA).

Results

A total of 550 patients were recruited and 9 withdrew or were lost to follow up, such that 541 patients were included in the analysis. Baseline patient demographics and clinical characteristics are shown in Table 1, and clinical outcomes in Table 2.

Individual predictive values of NLR, hs-CRP and platelet reactivity

Figure 1 shows the ROC curves for 12-month MACE for NLR, hs-CRP, and platelet reactivity. The c-statistics for individual biomarkers were as follows: NLR 0.568 (95% confidence interval [CI] 0.477-0.659), with optimal cut-point of 5.634 (sensitivity 48%, specificity 68%); hs-CRP 0.648 (95% CI 0.552-0.743), with a cut-point of 8.0mg/l (sensitivity 62%, specificity 73%); and platelet reactivity (inverse of OT) 0.667 (95% CI 0.579-0.754), with cut-point 302s (sensitivity 69%, specificity 68%). NLR was weakly but significantly related to pain-to-door time (Pearson correlation coefficient=0.1214, p=0.011). Patients taking aspirin pre-admission had lower OT than patients not taking aspirin (383±177 vs. 344±168, p=0.045). There was a weak correlation between hs-CRP and NLR (r=0.25, p<0.001), and hs-CRP and platelet reactivity (inverse OT) (r=0.14, p=0.003), but no correlation between platelet reactivity and NLR.

The predictive value of each biomarker at 30 days and 12 months is shown in Table 3 and of all baseline characteristics in Table 4. Platelet reactivity and hs-CRP were significantly predictive of 30-day and 12-month MACE, using both univariate and multivariate Cox regression models. NLR did not predict 30-day MACE in either univariate or multivariate analysis, but did predict 12-month MACE in univariate, but not multivariate analysis. The addition of hs-CRP or platelet reactivity to the TIMI score improved the prediction of MACE compared to the TIMI score alone (Supplementary Table 1).

Combined predictive value of two biomarkers

Patients were stratified into quartiles, based on combinations of two biomarkers (Figure 2).

Patients with two high biomarkers had significantly lower 12-month MACE-free survival in (log-rank test p=0.002 for high NLR/high hs-CRP group; p<0.001 for both high platelet reactivity/high hs-CRP and high platelet reactivity/high NLR groups) (Figures 2-5).

On multivariate Cox regression analysis, combinations of two high biomarkers were significantly predictive of MACE at 30 days (high NLR/high hs-CRP: HR 2.50 [95% CI 1.32-4.76], p=0.005; high platelet reactivity/high hs-CRP: HR 3.46 [95% CI 1.81-6.62], p<0.001; high platelet reactivity/high NLR: HR 2.24 [95% CI 1.16-4.32], p=0.016) and 12 months (high NLR/high hs-CRP: HR 2.62 [95% CI 1.38-4.99], p=0.003; high platelet reactivity/high hs-CRP: HR 3.46 [95% CI 1.81-6.63], p<0.001; high platelet reactivity/high NLR: HR 2.23 [95% CI 1.15-4.30], p=0.017). The predictive probability of the ROC model was 0.66 when both hs-CRP and NLR were included, 0.76 for platelet reactivity and CRP, and 0.66 for platelet reactivity and NLR. The addition of the combination of NLR and platelet reactivity, or the combination of hs-CRP and platelet reactivity, to the TIMI score improved the prediction of MACE compared to the use of the TIMI score alone (Supplementary Table 1).

Combined predictive value of three biomarkers (NLR, hs-CRP and platelet reactivity)

Patients were divided into four groups, based on the number of biomarkers above the optimal cut-off values, as follows: all biomarkers low (n=140), one high biomarker (n=237), two high biomarkers (n=138), three high biomarkers (n=26). Patients with all three biomarkers above the cut-off had lower 12-month event-free survival (log-rank test p<0.001), with significantly higher rate of CVD (p<0.001), MI (p<0.001), CVA (p=0.004), and MACE (p<0.001),

compared to patients with all three biomarkers below the cut-off (Figures 4 and 5). The combination of high NLR, high hs-CRP, and high platelet reactivity, was significantly predictive of MACE at 30 days (HR 3.73 [95% CI 1.69-8.27], p<0.001) and 12 months (HR 3.85 [95% CI 1.72-8.60], p<0.001). ROC models including NLR, hs-CRP, and platelet reactivity improved predictive value to 0.72 compared to any biomarker alone.

NRI showed that the inclusion of NLR, hs-CRP and platelet reactivity to a model containing TIMI score significantly added to the model effectiveness (NRI estimate 0.296, p<0.001) (Supplementary Tables 1 and 2). The extended prognostic model including all 3 biomarkers helped reclassify patients without MACE events from medium to low risk group, and patients with MACE from medium to high risk group (Supplementary Table 2).

Discussion

In this prospective study of patients with STEMI, both hs-CRP and platelet reactivity, but not NLR, were predictive of MACE over 30 days and 12 months. Combinations of hs-CRP and platelet reactivity provided additive predictive value over either marker alone, but using all three biomarkers (NLR, hs-CRP and platelet reactivity) was the strongest predictor of MACE.

An acute inflammatory response and platelet hyperreactivity are known biomarkers associated with the development of recurrent adverse cardiovascular events following ACS[2,4,7,12,16,18]. Our study showed a weak relationship between inflammation, as measured by hs-CRP, and platelet reactivity. The pathophysiology underlying the association between platelet activation and inflammatory pathways has previously been demonstrated by Gori *et al.,* who showed that inflammatory mediators induce platelet activation and platelets, in turn, are able to induce an inflammatory response[33]. In keeping with this mechanism, we have shown that the combined presence of high inflammatory biomarkers (hs-CRP or NLR) and high platelet reactivity, provides a stronger predictor of MACE at 30 days and 12 months, than either of these biomarkers alone. The combination of hs-CRP and platelet reactivity, however, was a better predictor than NLR and platelet reactivity combined and may be beneficial in identifying STEMI patients with at high risk of MACE. Marcucci *et al.* have previously shown the combined use of hs-CRP and platelet reactivity, assessed using light transmission aggregometry, to be a better predictor of long-term cardiovascular mortality than either alone[31]. However, light transmission aggregometry is not easy for clinicians to perform and not suitable as a routine screening test.

A positive relationship between hs-CRP and NLR was seen in our study, and the combination of both biomarkers was a better predictor of outcome than either biomarker alone. The

positive relationship between hs-CRP and NLR has been demonstrated in prior studies[21,34,35]; correlation coefficients in the range of 0.245-0.71[21,34,35] have been reported, which is comparable to that seen in our study.

Optimal cut-offs for hs-CRP and NLR in our study were 8mg/l and 5.6, respectively. Previous studies have demonstrated that hs-CRP levels above 0.3-12mg/l[11,15,31,34,38] are associated with short-term MACE[33] and mortality[5, 14, 36, 37] in STEMI patients undergoing PPCI[13]. Meanwhile, NLR cut-offs in the range of 3.3-6.5[10, 18, 34, 39, 40] have been shown to predict short-[32,39] and long-[18, 40] term all-cause mortality, and long-term MACE in STEMI patients.

Prior studies have shown NLR to be a better predictor of 12-month MACE following ACS than white cell, neutrophil and lymphocyte counts[14]. In our study, however, hs-CRP and platelet reactivity had a higher sensitivity at predicting 12-month MACE compared to NLR. Although our study did not show NLR to be a predictor of either short- or long- term MACE, this may be attributed to the time of NLR measurement. The optimal time for NLR measurement is unclear; Park *et al.* reported that NLR at 24 hours was superior to admission NLR in predicting mortality[40], in contrast to Azab *et al.*[41], who reported that mean NLR over the entire hospital stay provided the best predictor of outcome compared to admission, discharge, or maximum NLR.

The GTT assesses the rate of shear-induced thrombus formation, and the OT is reflective of more than just platelet reactivity. Earlier studies have shown that thrombotic occlusion occurs due to platelet activation and downstream aggregation. Unlike some other platelet function tests, like the VerifyNow (Accriva Diagnostics, San Diego, CA, USA) which measures the platelet response to a particular platelet agonist, such as ADP to measure the effect of $P2Y_{12}$ receptor inhibition, the GTT measures global platelet response. Furthermore, since it employs

non-anticoagulated blood, it assesses the important contribution of thrombin release from activated platelets (which does not occur to any significant degree in anticoagulated blood). This is crucial, as thrombin is the most potent stimulus for thrombosis and plays a critical role in STEMI. Furthermore, optimisation of platelet reactivity based on the results of the VerifyNow $P2Y_{12}$ assay has not translated into a reduction in clinical events[42], perhaps at least in part because such a strategy does not address other (non-$P2Y_{12}$ receptor dependent) platelet activation. Furthermore, the high shear environment in the GTT closely resembles the milieu in a severely narrowed vessel, that may exist in subjects with STEMI prior to PPCI.

The GTT also measures lysis time (LT), a measure of endogenous fibrinolysis. Whilst previous studies have shown that impaired fibrinolysis (lysis time, LT) was a predictor of MACE in patients with STEMI[43], there is an inverse relationship between OT and LT (Pearson correlation $r= -0.26$, $p<0.001$) and therefore we did not seek to assess LT contribution in this analysis as it was not independent of OT and would compound interpretation. Furthermore, pathophysiologically, there is a well-established relationship between platelet reactivity and inflammation, so we chose to look at the combination of 3 available markers that assess these inter-related pathways.

A platelet count $<100 \times 10^9$/L was an exclusion criterion, because earlier studies, using other tests of platelet function, have shown that platelet function testing is unreliable in low platelet ranges ($20–100 \times 10^9$/L platelet range)[44]. This is the case with light transmittance aggregometry, multiple electrode aggregometry, thromboelastrography, PFA 100 and VerifyNow. Whilst we do not have data specifically for the Global Thrombosis Test at low platelet counts, based on the results of other platelet function tests, we used similar cut off, to minimise the risk of unreliable results[44-49]. We also excluded patients with haemoglobin <80 g/L, because earlier studies, using other platelet function test, reported that haematocrit significantly influenced the results of tests including the VerifyNow $P2Y_{12}$ assay, PFA-100

and light transmittance aggregometry[44, 50-53]. Most studies have shown that a haematocrit of at least 0.25 L/L (equivalent to a haemoglobin of 80 g/L) is needed for reliable results[44]. Since haemoglobin levels are closely correlated with haematocrit[54], we excluded individuals with haemoglobin level <80 g/L in case this interfered with platelet function testing. Patients taking aspirin pre-admission had lower OT than those not taking aspirin. Whilst this may be the play of chance (since only 20% of patients were on aspirin), it is also possible that patients already on aspirin had established cardiovascular disease and had background enhanced platelet reactivity that was not overcome by low dose maintenance aspirin treatment.

Limitations

All variables were measured only at a single timepoint, on admission, and not repeated on admission or over time. Furthermore, the treatment for STEMI with standard dual antiplatelet therapy and statins likely favourably modulated both platelet reactivity and inflammation, although all patients received these. Further, NLR increased with time from presentation, and this means it may just be a marker of delayed presentation. However, although the level of all three biomarkers are known to decrease with time from infarction, all three have nevertheless been shown to be predictive of outcome. It is for this reason, that we assessed all markers at presentation, before the introduction of pharmacotherapy and the resolution of the infarct, which would have improved the biomarkers.

Conclusion

In a relatively large cohort, we show that the combination of three easy to measure biomarkers on arrival, namely hs-CRP, NLR and platelet reactivity, can help identify STEMI patients at high risk of recurrent adverse event, over and above the TIMI risk score.

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Table 1. **Demographics, comorbidities and clinical characteristics of patients at baseline**

Values given as mean±SD or n (%). Renal insufficiency was defined as creatinine levels >177 μmol/L. Family history of premature IHD was defined as a diagnosis of IHD in a first-degree relative under the age of 60. Abbreviations: MACE: major adverse cardiovascular event, BMI: body mass index, CABG: coronary artery bypass grafting, CVA: cerebrovascular accident, IHD: ischaemic heart disease, MI: myocardial infarction, PCI: percutaneous coronary intervention, PVD: peripheral vascular disease.

Table 2. Clinical outcomes at 30 days and 12 months

Abbreviations: CVD: cardiovascular death, MI: myocardial infarction, ST: stent thrombosis, CVA: cerebrovascular accident, MACE: major adverse cardiovascular event.

Table 3. Predictive value of each biomarker and biomarker combination for 30-day and 12-month MACE using univariate and multivariate Cox regression analysis

Multivariate analysis adjusted for age, creatinine, prior stroke, prior aspirin use, prior statin use, number of diseased vessels, and LV function. Abbreviations: MACE: major adverse cardiovascular event, hs-CRP: C-reactive protein, NLR: neutrophil/lymphocyte ratio.

Table 4. Predictive value of baseline characteristics for 30-day and 12-month MACE using univariate and Cox regression analysis

Renal insufficiency was defined as serum creatinine >177 μmol/L. Family history of premature IHD was defined as a diagnosis of IHD in a first-degree relative under the age of 60. Abbreviations: MACE: major adverse cardiovascular event, BMI: body mass index, CABG: coronary artery bypass grafting, CVA: cerebrovascular accident, IHD: ischaemic heart disease, MI: myocardial infarction, PCI: percutaneous coronary intervention, PVD: peripheral vascular disease, EF: ejection fraction.

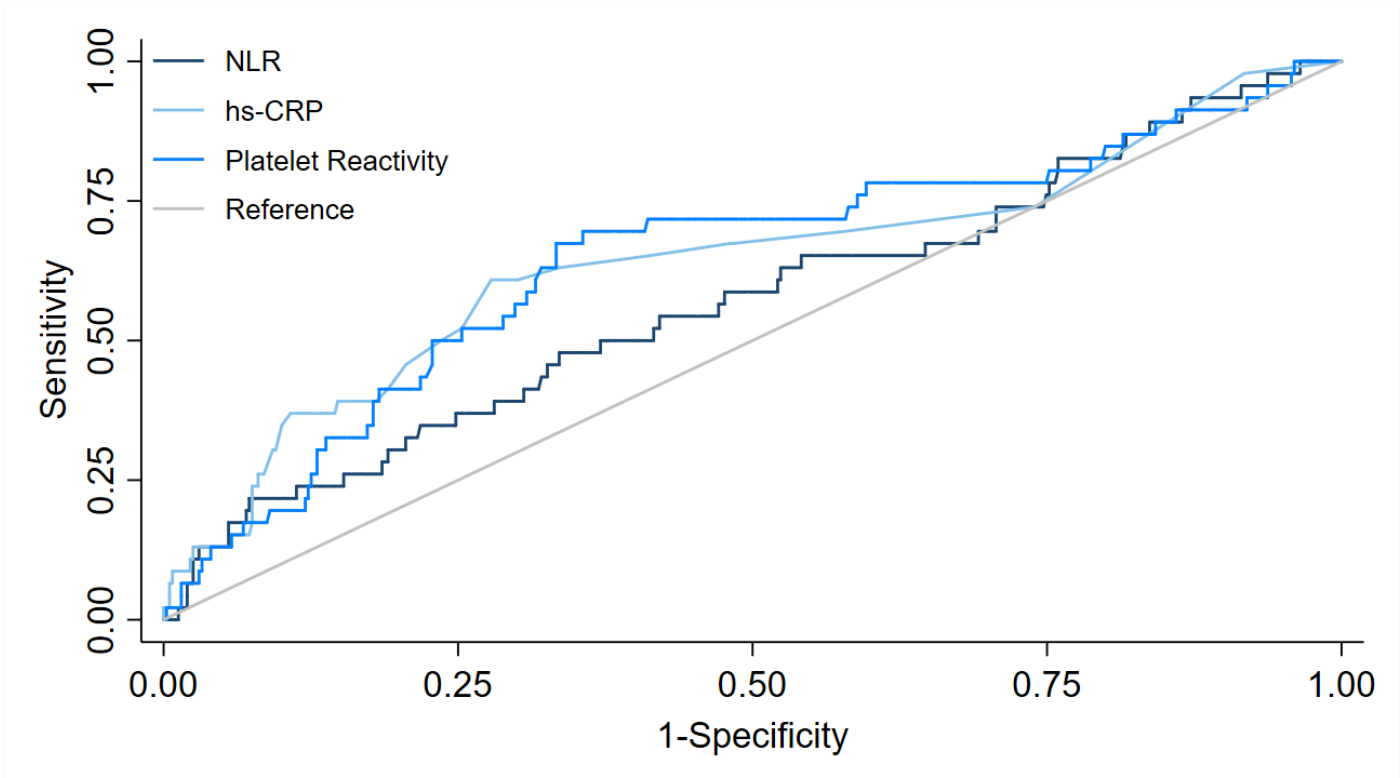
Demographic variables	All Patients (n=541)	MACE free at 12 months (n=491)	MACE within 12 months (n=50)	p value
Age, years	64±13	63±13	71±13	**<0.001**
Male	420 (78)	386 (79)	34 (68)	0.086
Caucasian	488 (90)	444 (90)	44 (88)	0.849
BMI, kg/m^2	27.4±5	27.5 ± 4.87	26.8 ± 5.29	0.402
TIMI score	3±3	3 ± 2	6 ± 4	**<0.001**
GRACE score	123±45	119 ± 40	164 ± 63	**<0.001**
Diabetes mellitus	108 (20)	94 (19)	14 (28)	0.14
Active Smoker	183 (34)	172 (35)	11 (22)	0.17
Hypertension	264 (49)	239 (49)	25(50)	0.86
Family history of premature IHD	218 (40)	202 (41)	16 (32)	0.21
Prior MI	65 (12)	52 (11)	13 (26)	**0.001**
Prior PCI	58 (11)	47 (10)	11 (22)	**0.007**
Prior CABG	6 (1)	3 (1)	3 (6)	**0.001**
Renal Insufficiency	12 (2)	7 (1)	5 (10)	**<0.001**
PVD	22 (4)	19 (4)	3 (6)	0.47
Prior CVA	24 (4)	18 (4)	6 (12)	**0.006**
Prior statin use	143 (26)	121 (25)	22 (44)	**0.003**
Prior aspirin use	99 (18)	81 (17)	18 (36)	**0.001**
Prior P_2Y_{12} inhibitor use				
Clopidogrel	16 (3)	12 (2)	4 (8)	0.051
Ticagrelor	7 (1)	5 (1)	2 (4)	0.130
Door to first device time, min	35±26	35±26	39±30	0.407
Call to first device time, min	110±51	110±48	112±75	0.787
Pain to first device time, min	206±146	203±143	237±178	0.168
Pain to door time, min	173±143	170±139	205±178	0.155
LV function				
Normal (EF ≥55%)	194 (37)	186 (38)	8 (17)	**0.002**
Mildly impaired (EF 45-54%)	143 (27)	131 (27)	12 (25)	0.739
Moderately impaired (36-44%)	135 (25)	124 (26)	11 (23)	0.732
Severely impaired (EF≤35%)	59 (11)	43 (9)	16 (34)	**<0.001**
Baseline blood results				
Haemoglobin, g/L	138±18	140±17	128±21	**<0.001**
Platelets, $x10^9/L$	248±77	246±74	267±101	0.065
Fibrinogen, g/L	4.6±1.3	4.6±1.3	5.0±1.4	**0.029**
Creatinine, µmol/L	88±30	86±28	101±38	**0.001**
Peak Troponin T, ng/L	299±861	267±818	604±1169	**0.020**

Clinical outcome	30 days	12 months
CVD	20	22
MI	17	21
Acute ST (<24 hours)	7	7
Sub-acute ST (24 hours – 30 days)	4	4
Late ST (30 days – 1 year)	0	2
CVA	5	7
MACE	42	50

	30-day MACE				12-month MACE			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	Hazard ratio (95% CI)	**p**	**Hazard ratio (95% CI)**	**p**	**Hazard ratio (95% CI)**	**p**	**Hazard ratio (95% CI)**	**p**
Platelet Reactivity	1.004 (1.002-1.006)	**<0.001**	1.004 (1.002-1.006)	**<0.001**	1.004 (1.002-1.006)	**<0.001**	1.004 (1.002-1.006)	**<0.001**
Hs-CRP	1.008 (1.005-1.012)	**<0.001**	1.01 (1.00-1.01)	**0.016**	1.008 (1.004-1.012)	**<0.001**	1.01 (1.00-1.01)	**0.014**
NLR	1.05 (0.99-1.12)	0.16	1.02 (0.96-1.09)	0.55	1.07 (1.01-1.13)	**0.035**	1.02 (0.96-1.09)	0.53
High NLR, hs-CRP, and platelet reactivity	5.10 (2.48-10.51)	**<0.001**	3.73 (1.69-8.27)	**0.001**	5.33 (2.59-10.99)	**<0.001**	3.85 (1.72-8.60)	**0.001**

	30-day MACE		12-month MACE	
	Hazard ratio (95% CI)	**p**	**Hazard ratio (95% CI)**	**p**
Age	1.05 (1.03-1.07)	**<0.001**	1.05 (1.03-1.07)	**<0.001**
Sex	1.70 (0.93-3.06)	0.083	1.72 (0.95-3.12)	0.074
Race	1.14 (0.72-1.80)	0.567	1.13 (0.72-1.79)	0.586
BMI	0.96 (0.92-1.04)	0.417	0.97 (0.92-1.03)	0.392
TIMI Score	1.33 (1.24-1.43)	**<0.001**	1.33 (1.24-1.43)	**<0.001**
Grace Score	1.01 (1.01-1.02)	**<0.001**	1.01 (1.01-1.02)	**<0.001**
Diabetes mellitus	1.62 (0.87-3.00)	0.126	1.63 (0.88-3.02)	0.121
Active smoker	0.54 (0.28-1.05)	0.072	0.54 (0.28-1.05)	0.071
Hypertension	1.04 (0.60-1.83)	0.867	1.04 (0.60-1.82)	0.881
Family history of premature IHD	0.68 (0.38-1.24)	0.21	0.68 (0.37-1.23)	0.199
Prior MI	2.69 (1.43-5.07)	**0.002**	2.67 (1.42-5.02)	**0.002**
Prior PCI	2.47 (1.26-4.81)	**0.008**	2.43 (1.23-4.76)	**0.009**
Prior CABG	7.13 (2.22-22.9)	**0.001**	7.28 (2.26-23.4)	**0.001**
Renal insufficiency	5.50 (2.18-13.9)	**<0.001**	5.43 (2.15-13.7)	**<0.001**
PVD	1.51 (0.47-4.87)	0.486	1.50 (0.47-4.81)	0.497
CVA	3.12 (1.33-7.33)	**0.009**	3.19 (1.36-7.50)	**0.008**
Prior statin use	2.27 (1.30-3.97)	**0.004**	2.26 (1.29-3.94)	**0.004**
Prior aspirin use	2.64 (1.48-4.70)	**0.001**	2.63 (1.48-4.69)	**0.001**
Prior P_2Y_{12} inhibitor use	2.10 (1.19-3.70)	**0.010**	2.10 (1.19-3.71)	**0.010**
LV function	1.84 (1.39-2.43)	**<0.001**	1.88 (1.42-2.49)	**<0.001**
Haemoglobin	0.97 (0.96-0.98)	**<0.001**	0.97 (0.96-0.98)	**<0.001**
Platelets	1.00 (1.00-1.01)	0.050	1.00 (0.99-1.01)	0.051
Fibrinogen	1.23 (1.02-1.49)	**0.029**	1.24 (1.03-1.49)	**0.026**

Creatinine	1.01 (1.00-1.01)	**0.001**	1.01 (1.00-1.01)	**0.001**
Peak troponin T	1.00 (1.00-1.00)	**0.027**	1.00 (1.00-1.00)	**0.026**


NLR
hs-CRP
Platelet Reactivity
Reference
Sensitivity
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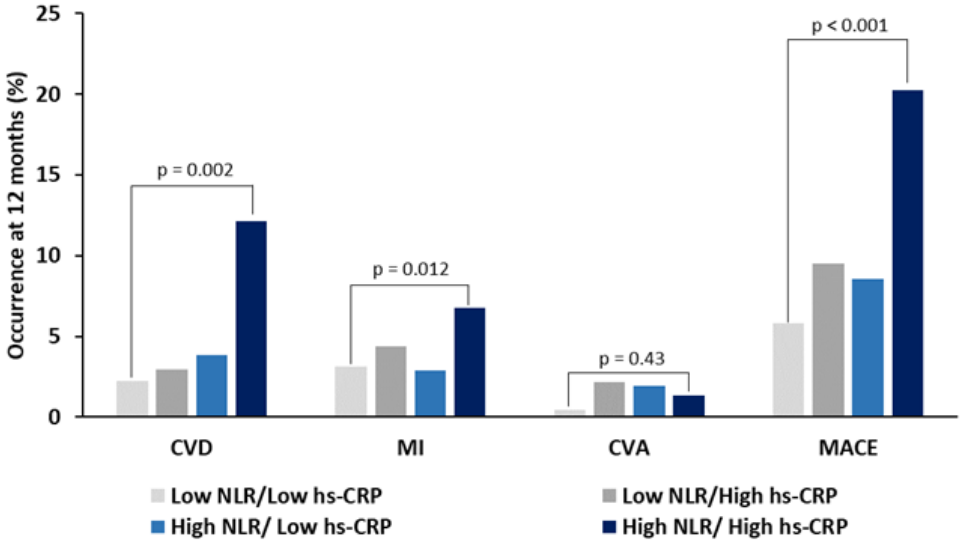
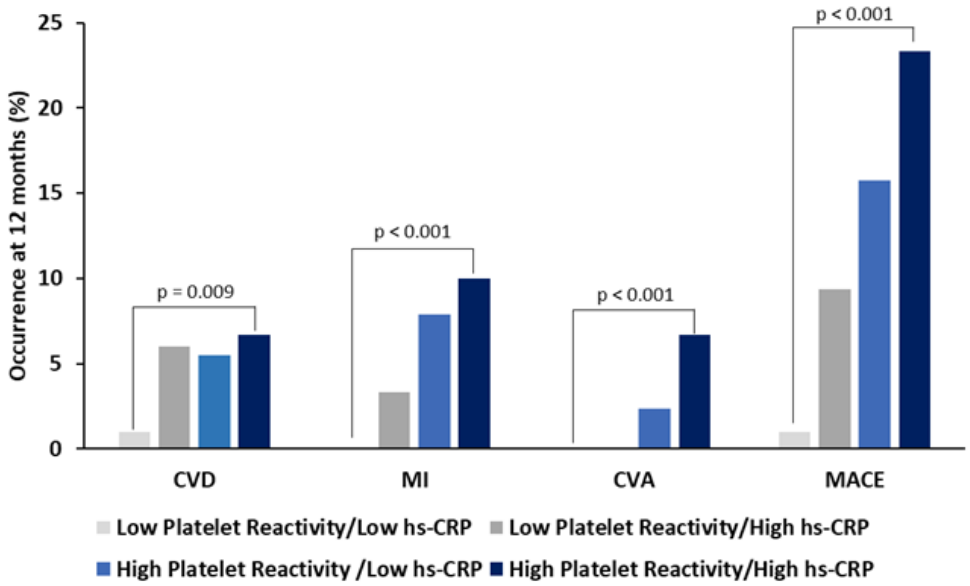
0.25
0.50
0.75
1.00
1-Specificity

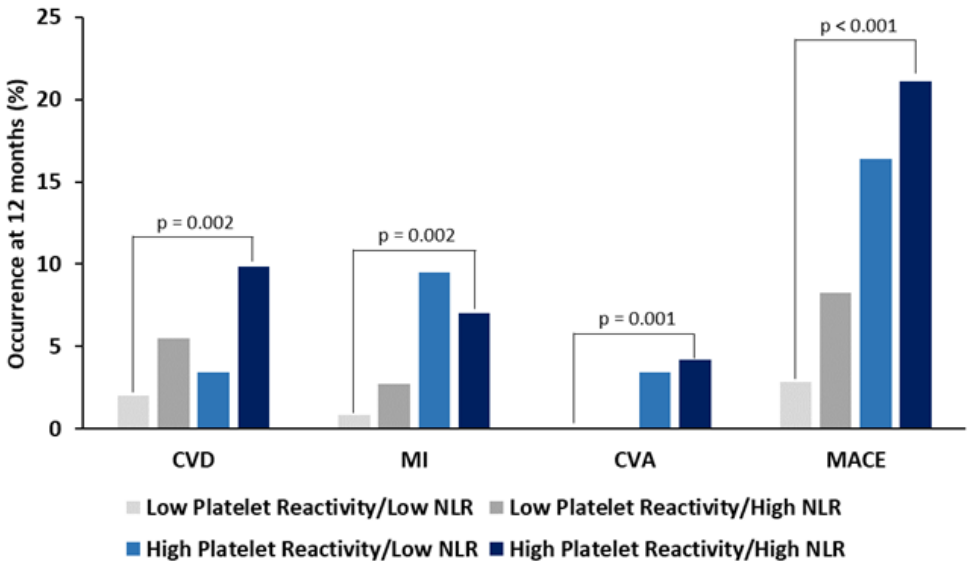
A


Occurrence at 12 months (%)
25
20
15
10
5
0
p = 0.002
p = 0.012
p = 0.43
p < 0.001
CVD
MI
CVA
MACE
Low NLR/Low hs-CRP
Low NLR/High hs-CRP
High NLR/ Low hs-CRP
High NLR/ High hs-CRP

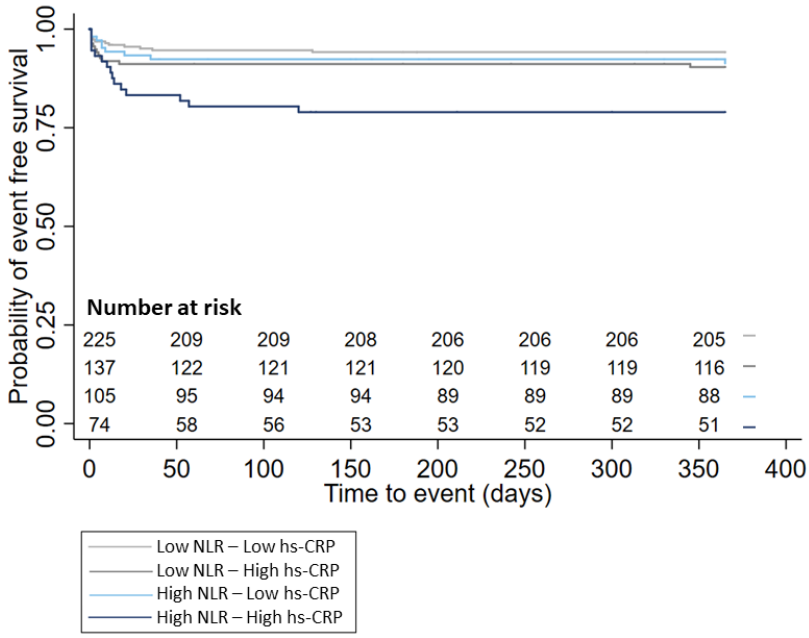
B


Occurrence at 12 months (%)
25
20
15
10
5
0
p = 0.009
p < 0.001
p < 0.001
p < 0.001
CVD
MI
CVA
MACE
Low Platelet Reactivity/Low hs-CRP
Low Platelet Reactivity/High hs-CRP
High Platelet Reactivity /Low hs-CRP
High Platelet Reactivity/High hs-CRP

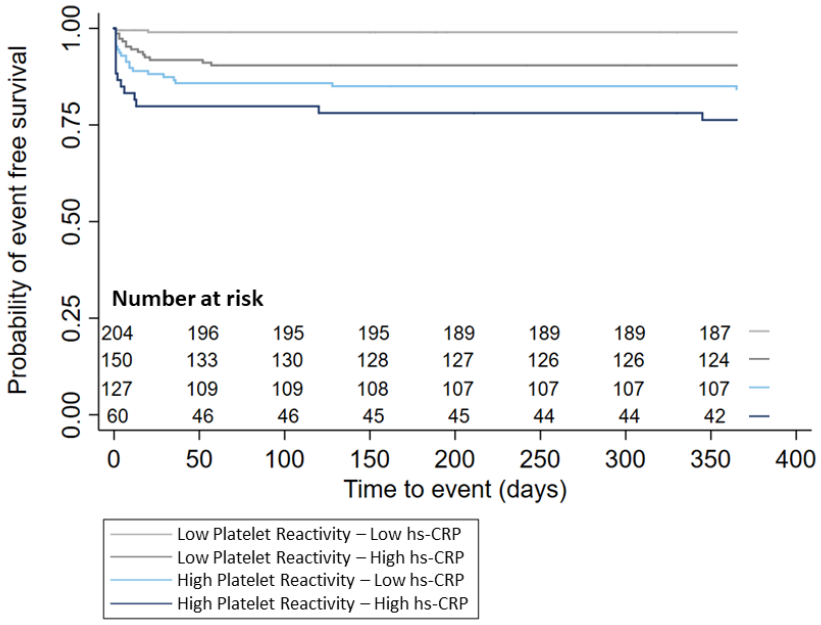
C


Occurrence at 12 months (%)
25
20
15
10
5
0
p = 0.002
p = 0.002
p = 0.001
p < 0.001
CVD
MI
CVA
MACE
Low Platelet Reactivity/Low NLR
Low Platelet Reactivity/High NLR
High Platelet Reactivity/Low NLR
High Platelet Reactivity/High NLR

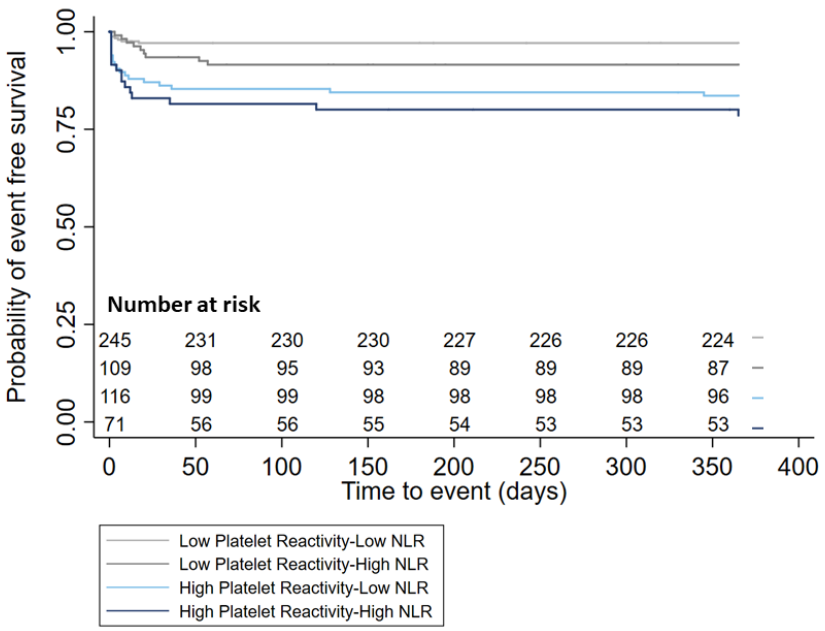
A

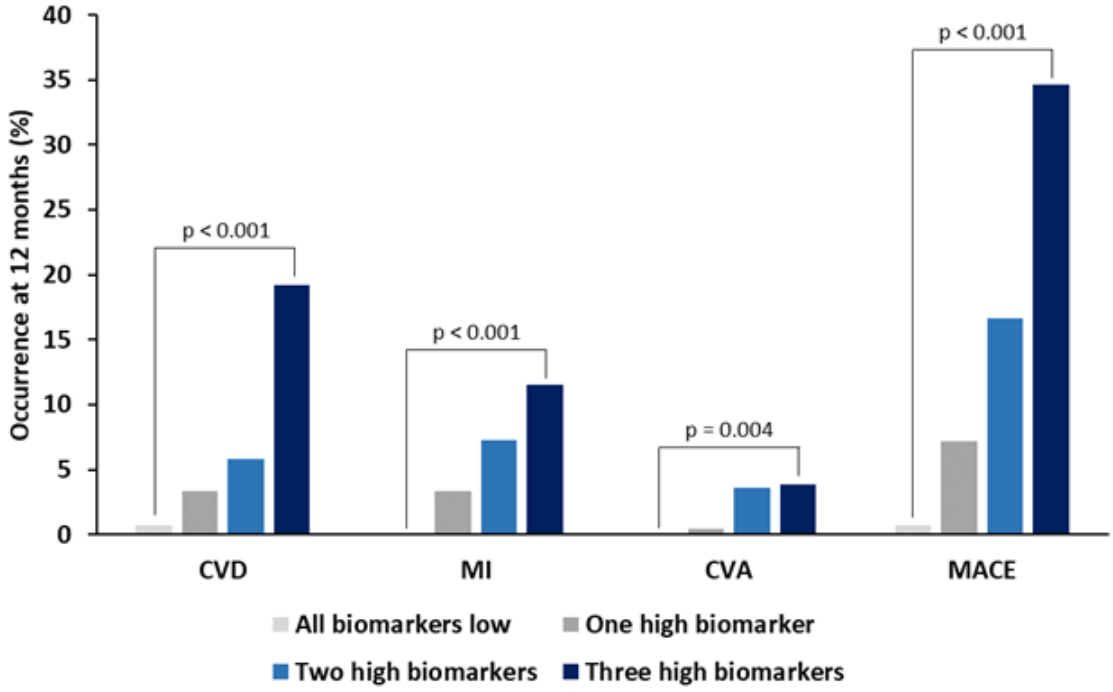

Probability of event free survival
0.00
0.25
0.50
0.75
1.00
Number at risk
225 209 209 208 206 206 206 205
137 122 121 121 120 119 119 116
105 95 94 94 89 89 89 88
74 58 56 53 53 52 52 51
0 50 100 150 200 250 300 350 400
Time to event (days)
Low NLR – Low hs-CRP
Low NLR – High hs-CRP
High NLR – Low hs-CRP
High NLR – High hs-CRP

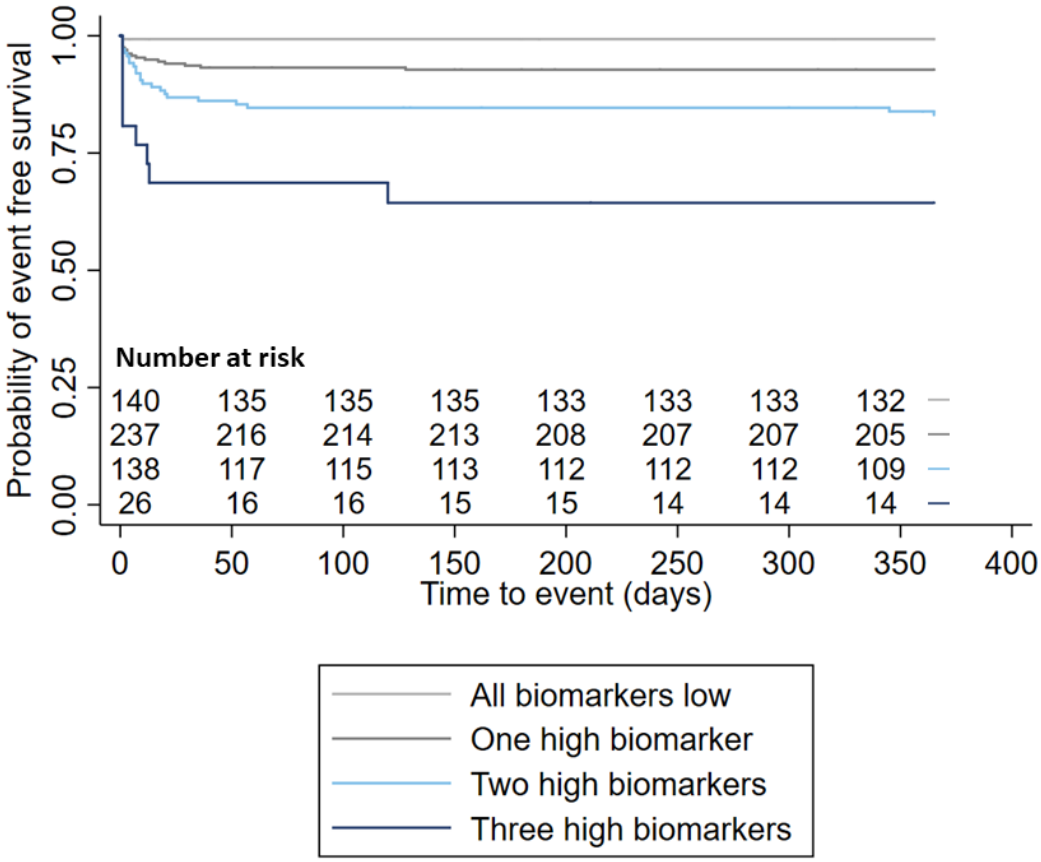
B


Probability of event free survival
0.00
0.25
0.50
0.75
1.00
Number at risk
204 196 195 195 189 189 189 187
150 133 130 128 127 126 126 124
127 109 109 108 107 107 107 107
60 46 46 45 45 44 44 42
0 50 100 150 200 250 300 350 400
Time to event (days)
Low Platelet Reactivity – Low hs-CRP
Low Platelet Reactivity – High hs-CRP
High Platelet Reactivity – Low hs-CRP
High Platelet Reactivity – High hs-CRP

C


Probability of event free survival
0.00
0.25
0.50
0.75
1.00
Number at risk
245 231 230 230 227 226 226 224
109 98 95 93 89 89 89 87
116 99 99 98 98 98 98 96
71 56 56 55 54 53 53 53
0 50 100 150 200 250 300 350 400
Time to event (days)
Low Platelet Reactivity-Low NLR
Low Platelet Reactivity-High NLR
High Platelet Reactivity-Low NLR
High Platelet Reactivity-High NLR


Occurrence at 12 months (%)
40
35
30
25
20
15
10
5
0
p < 0.001
p < 0.001
p = 0.004
p < 0.001
CVD
MI
CVA
MACE
All biomarkers low
One high biomarker
Two high biomarkers
Three high biomarkers


Probability of event free survival
0.00
0.25
0.50
0.75
1.00
Number at risk
140 135 135 135 133 133 133 132
237 216 214 213 208 207 207 205
138 117 115 113 112 112 112 109
26 16 16 15 15 14 14 14
0 50 100 150 200 250 300 350 400
Time to event (days)
All biomarkers low
One high biomarker
Two high biomarkers
Three high biomarkers

Supplementary Tables

Table 1. Net reclassification index (NRI) showing the effect of adding individual biomarkers, alone and in combination, to TIMI score for the prediction of major adverse cardiovascular events.

Value	NRI estimate	P Value
NLR	-0.031	0.168
hs-CRP	0.091	0.019
hs-CRP & NLR	0.059	0.287
Platelet reactivity	0.215	0.029
NLR & platelet reactivity	0.209	0.022
hs-CRP & platelet reactivity	0.227	0.006
All 3 biomarkers (NLR, hs-CRP and platelet reactivity)	0.296	<0.001

Table 2: Extended prognostic model including all 3 biomarkers (NLR, hs-CRP and platelet reactivity) added to TIMI score.

The risk cut-offs are arbitrary values of 5% and 20%; namely low (0-5%), medium (5.1-20%) and high (20.1-100%) risk.

		Extended model			
	Risk groups	Low	Medium	High	Total
NO EVENT					
Baseline model	Low	196 (84%)	38 (16%)	0	234
	Medium	103 (44%)	108 (46%)	25 (10%)	236
	High	0	7 (33%)	14 (67%)	21
	Total	299	153	39	491
EVENT					
Baseline model	Low	2 (29%)	5 (71%)	0	7
	Medium	2 (7%)	17 (63%)	8 (30%)	27
	High	0	1 (6%)	15 (94%)	16
	Total	4	23	23	50