



ORIGINAL ARTICLE

# Multimarker approach with cystatin C, N-terminal pro-brain natriuretic peptide, C-reactive protein and red blood cell distribution width in risk stratification of patients with acute coronary syndromes



Catarina Vieira\*, Sérgio Nabais, Vítor Ramos, Carlos Braga, António Gaspar, Pedro Azevedo, Miguel Álvares Pereira, Nuno Salomé, Adelino Correia

Department of Cardiology of Hospital de Braga, Braga, Portugal

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## KEYWORDS

Cystatin C;  
C-reactive protein;  
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natriuretic peptide;  
Multimarker  
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Acute coronary  
syndromes

## Abstract

**Introduction and aim:** Biomarkers have emerged as interesting predictors of risk in patients with acute coronary syndromes (ACS). The aim of this study was to determine the utility of the combined measurement of cystatin C (CysC), C-reactive protein (CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and red blood cell distribution width (RDW) in the risk stratification of patients with ACS.

**Methods:** In this prospective study including 682 patients consecutively admitted to a coronary care unit for ACS, baseline measurements of CysC, CRP, NT-proBNP and RDW were performed. Patients were categorized on the basis of the number of elevated biomarkers at presentation. The primary outcome was 6-month mortality.

**Results:** The number of biomarkers elevated on admission (study score) was an independent predictor of 6-month mortality; patients with four biomarkers elevated on admission had a significantly higher risk of 6-month mortality compared with patients with none or one. In addition, in patients with high risk defined by the GRACE score, our multimarker score was able to further categorize their risk of 6-month mortality.

**Conclusions:** A multimarker approach using CysC, NT-proBNP, CRP and RDW was an independent predictor of 6-month mortality and added prognostic information to the GRACE risk score in patients with ACS and high risk defined by GRACE, with increasing mortality in patients with a higher number of elevated biomarkers on admission.

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\* Corresponding author.

E-mail address: [katarinafvieira@gmail.com](mailto:katarinafvieira@gmail.com) (C. Vieira).

**PALAVRAS-CHAVE**

Cistatina C;  
 Proteína C reativa;  
 Pro-peptídeo  
 natriurético tipo B;  
 Estratificação  
 multimarcadores;  
 Síndromes coronárias  
 agudas

## Abordagem multimarcadores com a cistatina C, pro-peptídeo natriurético tipo B cerebral, proteína C reativa e índice de anisocitose na estratificação de risco dos doentes com síndrome coronária aguda

**Resumo**

**Introdução e objetivo:** Os biomarcadores têm surgido como interessantes preditores de risco em doentes com síndrome coronária aguda (SCA). O objetivo deste estudo foi definir a utilidade da combinação da cistatina C (CysC), da proteína C reativa (CRP), a porção N-terminal do peptídeo natriurético tipo B (NT-proBNP) e do índice de anisocitose (RDW) na estratificação de risco dos doentes com SCA.

**Métodos:** Estudo prospetivo, incluindo 682 doentes admitidos consecutivamente na nossa unidade coronária por SCA. À admissão foi feito o doseamento plasmático da CysC, CRP, NT-proBNP e RDW. Os doentes foram categorizados de acordo com o número de biomarcadores elevados à admissão. O evento primário estudado foi a mortalidade aos seis meses.

**Resultados:** O número de biomarcadores elevados à admissão (*score* estudado) foi preditor independente de mortalidade aos seis meses; os doentes com quatro biomarcadores elevados à admissão tiveram um risco de morte aos seis meses significativamente superior aos doentes sem nenhum ou com apenas um biomarcador elevado. Adicionalmente, nos doentes com alto risco definido pelo *score* GRACE, o nosso *score* multimarcadores teve a capacidade de os categorizar adicionalmente quanto ao risco de morte aos seis meses.

**Conclusão:** A abordagem multimarcadores baseada na CysC, NT-proBNP, CRP e RDW foi preditor independente de mortalidade aos seis meses e proporcionou informação prognóstica adicional nos doentes de alto risco definido pelo *score* GRACE, com maior da mortalidade nos doentes com maior número de biomarcadores elevados à admissão.

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**Abbreviations**

ACS	acute coronary syndrome
AUC	area under the receiver operating characteristic curve
BNP	brain natriuretic peptide
CG	Cockcroft-Gault equation
CI	confidence interval
CRP	C-reactive protein
cTnI	cardiac troponin I
CysC	cystatin C
GFR	glomerular filtration rate
hsCRP	high sensitivity C-reactive protein
MI	myocardial infarction
NT-proBNP	N-terminal pro-brain natriuretic peptide
LV	left ventricular
NSTE-ACS	non-ST-segment elevation acute coronary syndrome
OR	odds ratio
PCI	percutaneous coronary intervention
RDW	red blood cell distribution width
STE-ACS	ST-segment elevation acute coronary syndrome

**Introduction**

Various risk factors have been identified in patients with acute coronary syndrome (ACS) and incorporated into

scoring systems that enable the clinician to stratify risk and guide treatment.<sup>1</sup> In recent years, a deeper understanding of the pathobiology of atherothrombosis as the underlying mechanism of ACS has directed studies towards the evaluation of novel serum biomarkers as potential diagnostic tools for the clinical setting.<sup>2,3</sup> Recognition of the importance of including all the major mechanisms of ACS in risk stratification has increased the appeal of the multimarker approach. However, the ideal biomarkers and the ideal combination to improve risk stratification have not been defined.

The aim of this study was to assess the value of a multimarker approach with cystatin C (CysC), C-reactive protein (CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and red blood cell distribution width (RDW) in the risk stratification of patients with ACS.

Another objective of the study was to determine the hierarchy of these biomarkers in terms of predictive power for 6-month mortality.

**Methods****Study design and population**

Of a total of 902 consecutive patients admitted to our department's coronary care unit between July 1, 2009 and June 30, 2011 with a diagnosis of ACS, we prospectively studied 682 patients. The time between the onset of ACS symptoms and the time of blood sampling to assess biomarkers was <24 hours. The remaining 220 patients were excluded for two main reasons: more than 24 hours between

symptom onset and the time of blood sampling; or if at least one of the four laboratory predictors had not been assessed on admission (clotted blood sample or failure to request the laboratory study). The excluded patients were slightly older than the study population, but did not differ significantly in other baseline characteristics (Table 1).

The study was approved by the ethics committee and all patients provided written informed consent.

The patients included in the study presented a history of chest pain at rest or symptoms suggestive of ACS, the most recent episode being up to 24 hours before admission. Symptoms could be associated with persistent or transient ST-segment elevation, ST-segment depression, or T-wave inversion on the ECG, or elevated biomarkers of cardiac injury. The biomarkers used were cardiac troponin I (cTnI) and the MB fraction of creatinine kinase (CK-MB) and the 99th percentile of a reference population was employed as the diagnostic threshold. Patients with unstable angina and normal admission ECG were also included (n=24). Left ventricular (LV) ejection fraction was assessed by transthoracic echocardiography 1–2 days after admission. In patients with ST-segment elevation ACS (STE-ACS) echocardiography was performed after cardiac catheterization as long as a mechanical complication was not suspected. In patients with non-ST-segment elevation ACS (NSTEMI-ACS) echocardiography was performed before cardiac catheterization and usually within 24 hours of admission.

The four laboratory predictors were considered to be elevated when CysC >0.95 mg/l (normal 0.53–0.95); CRP >5.0 mg/l (<5); NT-proBNP >125 pg/ml (<125) and RDW >14% (11.6–14.0), which were the upper limit values of a normal population. Glomerular filtration rate (GFR) was estimated by the Cockcroft-Gault (CG) formula and renal dysfunction was defined as GFR <60 ml/min/1.73 m<sup>2</sup>.<sup>4</sup>

## Follow-up and adverse events

The study's primary outcome was cumulative all-cause mortality at six months. Follow-up and confirmation of adverse events were by telephone contact and by consultation of the hospital's databases and records. Six-month follow-up was achieved in 633 patients (92.8% of the study population). The majority of patients lost for follow-up had moved away or were from other areas of reference and so contact was not possible; their baseline characteristics did not differ significantly from those of the study population (Table 2).

## Statistical analysis

Patients were divided into five groups according to the number of elevated laboratory predictors on admission: G0 (n=24), in whom none of the four biomarkers was elevated at admission; G1 (n=158), in whom one biomarker was elevated at admission; G2 (n=281), in whom two biomarkers were elevated at admission; G3 (n=156), in whom three biomarkers were elevated at admission; and G4 (n=63), in whom all biomarkers were elevated at admission. The groups were compared in terms of baseline characteristics. Categorical variables were expressed as percentages and continuous variables as means  $\pm$  standard deviation or medians with ranges. Between-group differences were compared

using the Student's t test or the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate. Multivariate linear regression analysis was performed to determine which of the following variables were associated with a higher number of laboratory predictors elevated on admission: age and gender; history of diabetes, hypertension, smoking, myocardial infarction (MI), renal failure, stroke/transient ischemic attack, atrial fibrillation, or coronary artery bypass grafting (CABG); previous medication with aspirin, thienopyridines, beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and statins; Killip class, heart rate, GFR, reoperolobin and type of ACS on admission.

To assess the association between a higher number of biomarkers elevated on admission and 6-month mortality, multivariate logistic regression analysis was performed, the models being constructed with the number of predictors elevated on admission as a categorical variable and including four other covariables associated with 6-month mortality, given the small number of events in the study. The threshold for retaining a variable in the final model was set at  $p < 0.1$ . The variables included were age, Killip class on admission, LV systolic dysfunction, coronary revascularization and the number of elevated biomarkers on admission. To assess whether the study score (number of laboratory predictors elevated on admission) was a predictor of mortality independent of the GRACE score,<sup>5</sup> the GRACE score was included in a second multivariate logistic regression model which included the multimarker score, LV systolic dysfunction, coronary revascularization and GRACE score. Age and Killip class were not included in this second model because they are already included in the GRACE score.

Kaplan-Meier survival curves were constructed to compare event-free survival at six months according to the number of elevated laboratory predictors on admission, using the log-rank test. A value of  $p < 0.05$  was considered statistically significant. All statistical tests were two-sided. The statistical software used was Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, Ill).

The hierarchy of the biomarkers in terms of predictive power for 6-month mortality was assessed by comparing the areas under the receiver-operating characteristic curve (AUCs). This calculation and comparison was performed with MedCalc version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium).

Given the known value of the GRACE score in the risk stratification of patients with ACS, we conducted a stratified analysis of 6-month mortality according to the number of elevated biomarkers in patients with low, intermediate and high risk according to their GRACE score (in both NSTEMI-ACS and STE-ACS).

## Results

### Baseline characteristics

Of the 682 patients enrolled in the study, 356 (52.2%) had ST-segment elevation MI, 302 (44.3%) had non-ST-segment elevation MI, and 24 (3.5%) had a final diagnosis of unstable angina. On admission, 24 (3.5%) patients were in group

**Table 1** Differences in baseline characteristics between included and excluded patients.

	Included (n=682)	Excluded (n=220)	p
<i>Demographic</i>			
Age (years)	64±13	66±13	0.027
Male (%)	77.9	76.8	0.747
<i>CV risk factors (%)</i>			
Diabetes	26.4	27.3	0.797
Hypertension	63.5	68.2	0.206
Dyslipidemia	53.5	53.6	0.976
Active smoker	30.2	25.9	0.223
<i>CV history (%)</i>			
MI	15.2	17.7	0.382
Stroke	7.5	8.2	0.733
PAD	2.9	3.2	0.850
PCI	6.6	7.3	0.729
CABG	4.1	3.6	0.757
Chronic kidney disease	3.4	4.1	0.632
<i>Previous medication (%)</i>			
Aspirin	24.5	26.4	0.576
Thienopyridines	7.2	6.4	0.678
Beta-blockers	22.1	19.1	0.337
ACEIs/ARBs	45.0	43.6	0.721
Statins	34.2	36.8	0.006
<i>Clinical presentation</i>			
ST-segment elevation (%)	52.2	55.0	0.400
Killip class >I (%)	21.8	26.4	0.382
SBP (mmHg)	129±26	126±29	0.204
DBP (mmHg)	78±15	76±16	0.128
HR (bpm)	76±19	78±21	0.128
Hemoglobin (g/dl)	13.9±2.2	13.6±1.9	0.109

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CABG: coronary artery bypass grafting; CV: cardiovascular; DBP: diastolic blood pressure; HR: heart rate; MI: myocardial infarction; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention; SBP: systolic blood pressure.

G0, 158 (23.2%) in G1, 281 (41.2%) in G2, 156 (22.9%) in G3 and 63 (9.2%) in G4. The demographic characteristics of the study population according to the number of elevated biomarkers on admission are shown in Table 3. Patients with a higher number of elevated biomarkers on admission were older ( $p$  for trend <0.001), more likely to be female ( $p$ <0.001), and had a history of diabetes ( $p$ =0.001) and hypertension ( $p$ <0.001). These patients more often had a history of MI ( $p$ <0.001), CABG ( $p$ =0.021) and stroke ( $p$ <0.001), and on admission more often presented with NSTEMI-ACS ( $p$ <0.001), Killip class >I ( $p$ <0.001) and lower mean hemoglobin ( $p$ <0.001).

Table 4 shows patients' in-hospital treatment and angiographic and echocardiographic characteristics according to the number of laboratory predictors elevated on admission. Three-vessel and left main disease ( $p$ <0.001) and LV dysfunction ( $p$ <0.001) were more frequent in patients with a higher number of laboratory predictors elevated on admission, who were also less often revascularized ( $p$ <0.001). Furthermore, percutaneous coronary intervention (PCI) ( $p$ <0.0001) and thienopyridines ( $p$ <0.0001) and beta-blockers were used less often in these patients, whereas diuretics ( $p$ =0.003), aldosterone antagonists ( $p$ <0.0001) and intravenous inotropics ( $p$ <0.0001) were used more frequently.

## Outcomes

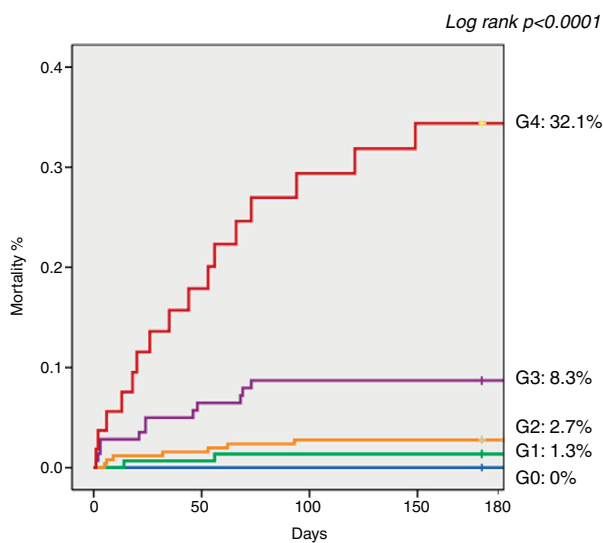
Sixteen (2.4%) patients died during initial hospitalization and 23 (3.4%) died within six months of hospital discharge. The primary outcome (all-cause mortality) was recorded in 39 (5.7%) patients during 6-month follow-up. Patients with a higher number of laboratory predictors elevated on admission had increased in-hospital ( $p$ <0.0001) and 6-month mortality ( $p$ <0.0001). Table 4 lists the in-hospital events and Figure 1 shows Kaplan-Meier curves for the primary outcome during follow-up according to the number of laboratory predictors elevated on admission.

In Table 5, the results of multivariate logistic regression analysis are presented for 6-month mortality according to the number of laboratory predictors elevated on admission. After adjustment for the previously mentioned possible confounding factors, patients with four laboratory predictors elevated on admission had a higher incidence of 6-month mortality compared to patients with none or one, with an odds ratio (OR) of 10.66 (95% confidence interval [CI], 2.11–53.93;  $p$ =0.004). When the GRACE risk score was included in the second model (Table 6), the number of biomarkers elevated on admission was still an independent predictor of 6-month mortality; patients with

**Table 2** Differences in baseline characteristics between patients with follow-up and those lost to follow-up.

	With follow-up (n=827)	Without follow-up (n=75)	p
<i>Demographic</i>			
Age (years)	64±13	63±14	0.794
Male (%)	78.0	75.5	0.681
<i>CV risk factors (%)</i>			
Diabetes	26.4	26.5	0.982
Hypertension	64.0	57.1	0.338
Dyslipidemia	54.2	44.9	0.209
Active smoker	29.7	36.7	0.301
<i>CV history (%)</i>			
MI	15.3	14.3	0.846
Stroke	7.7	4.1	0.348
PAD	2.8	4.1	0.621
PCI	7.0	2.0	0.182
CABG	4.1	4.1	0.993
Chronic kidney disease	3.3	4.5	0.661
<i>Previous medication (%)</i>			
Aspirin	25.6	10.2	0.016
Thienopyridines	7.4	4.1	0.383
Beta-blockers	22.3	20.4	0.762
ACEIs/ARBs	44.9	46.9	0.779
Statins	35.1	22.4	0.073
<i>Clinical presentation</i>			
ST-segment elevation (%)	52.0	55.1	0.673
Killip class >I (%)	21.8	26.4	0.303
SBP (mmHg)	129±26	130±27	0.666
DBP (mmHg)	78±15	81±15	0.172
HR (bpm)	76±19	73±15	0.315
Hemoglobin (g/dl)	14.0±2.2	13.4±2.0	0.092

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CABG: coronary artery bypass grafting; CV: cardiovascular; DBP: diastolic blood pressure; HR: heart rate; MI: myocardial infarction; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention; SBP: systolic blood pressure.

**Figure 1** Kaplan-Meier curves for 6-month mortality according to the number of biomarkers elevated on admission.

four laboratory predictors elevated on admission had almost 14 times greater risk of 6-month mortality than patients with none or one.

The AUCs for 6-month mortality of CysC, NT-proBNP, CRP and RDW (Figure 2) of all the patients included in the study were, respectively, 0.84 ( $p < 0.0001$ ), 0.83 ( $p < 0.0001$ ), 0.75 ( $p < 0.0001$ ) and 0.64 ( $p = 0.0036$ ). There was no statistically significant difference between the predictive power of CysC and NT-proBNP (differences in AUC); however, CysC and NT-proBNP had stronger predictive power than CRP and RDW, and CRP had stronger predictive power than RDW (Figure 2).

Among patients with a high GRACE score, our score was able to further stratify risk, in both patients with STE-ACS (Figure 3) and patients with NSTEMI-ACS (Figure 4). Patients with low and intermediate NSTEMI-ACS had a very low mortality in our population. In patients with NSTEMI-ACS and intermediate GRACE risk, those with four elevated biomarkers on admission had high mortality; in patients admitted for STE-ACS, all patients with four biomarkers elevated on admission were in the high risk group defined by the GRACE score (Figure 3), while in patients admitted for NSTEMI-ACS

**Table 3** Baseline patient characteristics according to the number of biomarkers elevated on admission.

	G0 (n=24)	G1 (n=158)	G2 (n=281)	G3 (n=156)	G4 (n=63)	p
<i>Demographic</i>						
Age (years)	53±11	58±12	62±13	68±13	76±9	<0.0001
Female (%)	8.3	15.2	18.1	32.7	36.5	<0.0001
<i>CV risk factors (%)</i>						
Diabetes	33.3	14.6	27.0	32.1	36.5	0.001
Hypertension	58.3	55.7	60.1	67.9	88.9	<0.0001
Dyslipidemia	66.7	52.5	54.4	53.2	47.6	0.607
Active smoker	50.0	37.3	35.2	17.9	12.7	<0.0001
<i>CV history (%)</i>						
MI	4.2	14.6	10.3	21.2	28.6	<0.0001
Stroke	0.0	4.4	3.9	10.3	27.0	<0.0001
PAD	4.2	1.3	3.6	4.5	4.8	0.579
PCI	12.5	8.2	4.6	7.1	6.6	0.400
CABG	0.0	1.9	2.8	7.7	7.9	0.021
<i>Previous medication (%)</i>						
Aspirin	16.7	23.4	19.2	28.8	42.9	0.001
Thienopyridines	0.0	3.2	6.8	12.8	7.9	0.010
Beta-blockers	12.5	18.4	17.8	28.8	38.1	0.001
ACEIs/ARBs	33.3	35.4	39.1	55.1	74.6	<0.0001
Statins	50.0	28.5	29.9	39.7	47.6	0.006
<i>Clinical presentation</i>						
ST-segment elevation (%)	45.8	58.2	58.4	45.5	28.6	<0.0001
Killip class >I (%)	0.0	6.3	18.9	34.0	52.4	<0.0001
SBP (mmHg)	131±19	127±24	128±25	129±28	137±29	0.098
DBP (mmHg)	82±12	78±13	79±15	76±17	79±16	0.222
HR (bpm)	72±17	74±18	76±18	77±19	83±23	0.030
Renal failure (%) <sup>a</sup>	4.2	6.3	11.7	35.9	74.2	<0.0001
Hemoglobin (g/dl)	15.0±1.4	14.2±1.5	14.2±1.7	13.6±3.2	12.1±2.3	<0.0001

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CABG: coronary artery bypass grafting; CV: cardiovascular; DBP: diastolic blood pressure; HR: heart rate; MI: myocardial infarction; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention; SBP: systolic blood pressure.

<sup>a</sup> Glomerular filtration rate <60 ml/min/1.73 m<sup>2</sup>.

and with low risk according to the GRACE score, there were no patients with four elevated biomarkers.

## Discussion

The present study demonstrates that a multimarker approach with CysC, NT-proBNP, CRP and RDW has value in the risk stratification of patients admitted with ACS. We found that a higher number of elevated biomarkers, measured on admission by the same laboratory, was progressively associated with an increased incidence of in-hospital and 6-month mortality in an unselected population of patients admitted for ACS. Moreover, in multivariate logistic regression analysis, the study score (number of biomarkers elevated on admission) was a predictor of mortality independently of other important variables, including the GRACE score; patients with four biomarkers elevated on admission had a significantly higher risk of 6-month mortality than those with none or one. When the population was divided by GRACE risk score into groups of low, intermediate

and high risk, our score was able to further stratify risk in both STE-ACS and NSTEMI-ACS. Patients with low risk had a very low mortality in our population. In patients with NSTEMI-ACS and intermediate GRACE risk, those with four elevated biomarkers on admission had high mortality. This may be taken into account in the risk stratification of these patients and help in clinical decision-making and management. Another important fact is that patients with four biomarkers elevated on admission were only at intermediate or high risk defined by GRACE, with none in the low risk category. Therefore, having four biomarkers elevated on admission indicates significantly higher risk and may be used in addition to conventional risk scores to complement risk stratification.

As in other registries, patients at higher risk (as reflected by higher biomarker scores) were treated less often with an invasive strategy, which goes against current guidelines. This 'treatment paradox' may have contributed to greater risk in patients with a higher score.

CysC and NT-proBNP were the strongest predictors, similar in terms of discriminatory power, followed by CRP and

**Table 4** Treatment, angiographic and echocardiographic characteristics, and in-hospital events according to the number of laboratory predictors elevated on admission.

	G0 (n=24)	G1 (n=158)	G2 (n=281)	G3 (n=156)	G4 (n=63)	p
<i>In-hospital medication (%)</i>						
(%)	100	100	100	100	100	
Thienopyridines	100	99.4	98.6	100	88.9	<0.0001
Beta-blockers	87.0	93.7	89.3	87.2	71.4	<0.0001
ACEIs/ARBs	91.3	87.3	89.0	90.3	84.1	0.710
Statins	100	100	99.3	100	99.6	0.420
Nitrates	17.4	22.2	34.3	33.3	20.0	0.003
UFH	41.7	40.5	40.6	27.6	27.0	0.024
LMWH	62.5	60.1	63.3	75.0	76.2	0.017
GP IIb/IIIa inhibitors	0.0	14.6	11.7	11.5	6.3	0.190
Revascularization	95.3	87.2	82.4	67.3	55.8	<0.0001
Diuretics	0.0	15.6	22.6	54.7	76.7	<0.0001
Aldosterone antagonists	0.0	6.3	15.7	28.0	33.3	<0.0001
IV inotropics	0.0	1.3	3.6	11.0	14.3	<0.0001
<i>Procedures (%)</i>						
Cardiac catheterization	100	100	99.3	96.2	87.3	<0.0001
PCI	81.0	71.8	65.5	59.3	41.0	<0.0001
CABG	14.3	15.4	16.9	8.0	14.8	0.167
Left main disease	0.0	6.3	8.9	7.3	23.2	<0.0001
Three-vessel disease	29.2	18.4	17.1	25.8	42.9	<0.0001
LVSD	9.1	44.9	58.5	59.4	63.3	<0.0001
<i>In-hospital events (%)</i>						
Killip class II-IV	0.0	12.0	28.8	48.1	71.4	<0.0001
Mortality	0.0	0.6	1.1	3.2	11.1	<0.0001
Reinfarction	0.0	2.5	3.6	1.9	3.2	0.771
Ischemic stroke	0.0	1.3	1.1	0.6	0.0	0.868
Hemorrhagic stroke	0.0	0.9	0.7	0.6	1.6	0.692

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CABG: coronary artery bypass grafting; CV: cardiovascular; GP: glycoprotein; IV: intravenous; LMWH: low molecular weight heparin; LVSD: left ventricular systolic dysfunction (ejection fraction <50% on echocardiography); PCI: percutaneous coronary intervention; UFH: unfractionated heparin.

finally RDW. This result may be also taken into account in clinical practice when stratifying risk.

In the present study, we included a marker of hemodynamic stress (NT-proBNP), one of inflammation (CRP), one of renal dysfunction (CysC), and RDW, which covers more than one mechanism (nutritional deficiencies, renal failure and inflammatory stress).<sup>6,7</sup> Various studies have shown that NT-proBNP, CRP, CysC, and RDW are also associated with adverse outcomes in patients with ACS independently of hemodynamic stress, inflammation and renal dysfunction.<sup>6-18</sup> In addition, all of these biomarkers are commonly used in clinical assessment of patients with ACS and are readily available, which is also an important factor when choosing a biomarker. This specific combination was chosen not only on the basis of the evidence in the above-mentioned studies, but also because they were used individually in the risk stratification of our population of ACS patients in other studies, in which they were good predictors of outcome.<sup>7</sup>

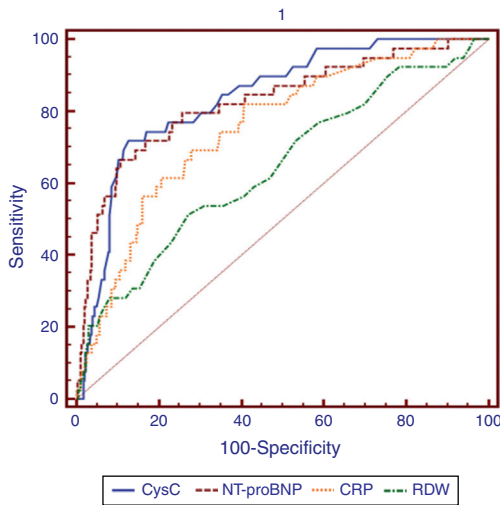
A large number of candidate biomarkers have been investigated in numerous studies, with the aim of further improving diagnosis and risk assessment in patients with ACS.<sup>6-31</sup>

Some studies have shown that a multimarker approach provides prognostic information in patients with ACS. To the best of our knowledge, this is the first study adopting a multimarker approach with these specific markers. James et al. made an important contribution to the growing evidence demonstrating that combining a biomarker

**Table 5** Independent predictors of 6-month mortality on multivariate logistic regression analysis.

Variable	OR (95% CI)	p
Age	1.06 (1.02-1.10)	0.005
LV dysfunction	0.80 (0.35-1.82)	0.593
Killip class on admission >1	2.54 (1.12-5.78)	0.026
Revascularization	0.80 (0.36-1.78)	0.582
<i>Laboratory predictors</i>		
G2/G0G1	1.38 (0.27-7.13)	0.703
G3/G0G1	2.90 (0.58-14.43)	0.193
G4/G0G1	10.66 (2.11-53.93)	0.004

CI: confidence interval; G0G1: groups 0 and 1; G3: group 3; G4: group 4 (see text); LV: left ventricular.

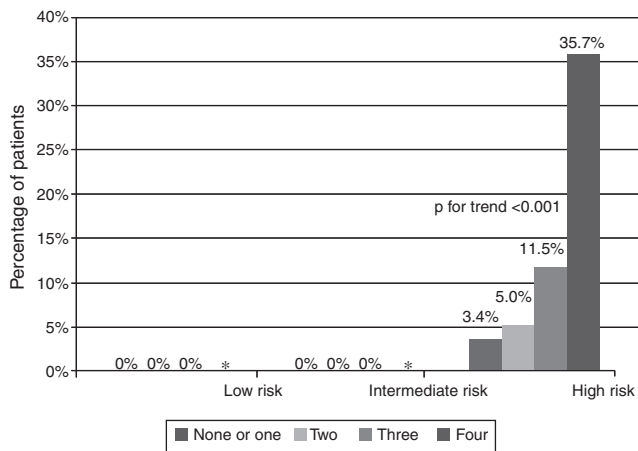


Laboratory predictor	AUC	95% CI	p
CysC	0.838	0.807-0.866	<0.0001
NT-proBNP	0.825	0.793-0.854	<0.0001
CRP	0.753	0.717-0.786	<0.0001
RDW	0.644	0.605-0.681	0.0036

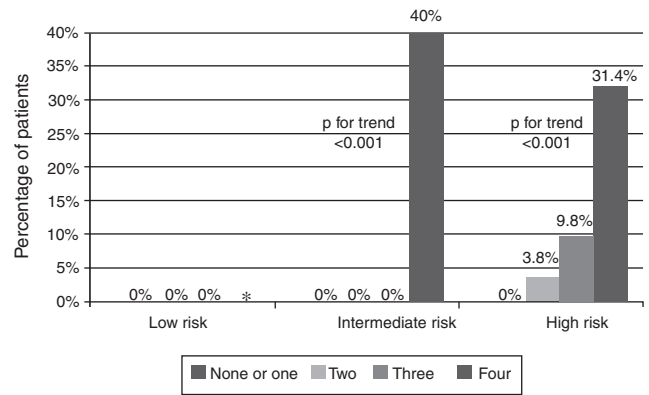
  

Comparison between laboratory predictors	Difference between AUCs	95% CI	p
CysC_NT-proBNP	0.0126	-0.0704-0.0957	0.7661
CysC_CRP	0.0848	0.0014-0.168	0.0046
CysC_RDW	0.194	0.104-0.285	<0.0001
NT-proBNP_CRP	0.0722	0.0195-0.164	0.0328
NT-proBNP_RDW	0.182	0.0698-0.294	0.0015
CRP_RDW	0.110	0.00510-0.224	0.0411

**Figure 2** Receiver-operating curves and AUCs of the four biomarkers for 6-month mortality. AUC: area under the receiver-operating characteristic curve for 6-month mortality; CRP: C-reactive protein; CysC: cystatin C; NT-proBNP: N-terminal pro-brain natriuretic peptide; RDW: red blood cell distribution width.



**Figure 3** Six-month mortality in ST-elevation acute coronary syndromes according to GRACE risk score and the number of biomarkers elevated on admission. \*No patients with four elevated biomarkers in these risk categories.



**Figure 4** Six-month mortality in non-ST-elevation acute coronary syndromes according to GRACE risk score and number of biomarkers elevated on admission. \*No patients with four elevated biomarkers in this risk category.

of hemodynamic stress (brain natriuretic peptide [BNP] or NT-proBNP) or of inflammation (high sensitivity C-reactive protein [hsCRP]) with a biomarker of necrosis (cTnI) enhances risk assessment in patients with ACS.<sup>32</sup> Another study, assessing the same biomarkers simultaneously (cTnI, CRP and BNP), showed that a multimarker approach improves the risk stratification of patients with NSTEMI-ACS.<sup>33</sup> Tello-Montoliu et al. found that a multimarker approach based on cTnI, CRP, and NT-proBNP, but not D-dimers, provides added information to the TIMI risk score in terms of ACS prognosis at six months.<sup>34</sup> Möckel et al. evaluated the importance of various biomarkers (cTnI, NT-proBNP, hsCRP, placental growth factor, lipoprotein-associated phospholipase A2 (Lp-PLA2), D-dimers, whole blood choline and plasma choline) in patients with ACS and aimed to define their hierarchy with respect to the prediction of major adverse cardiac events over a 42-day follow-up period.<sup>35</sup> They used logistic regression synergistically with classification and regression tree (CART) analysis to define a multimarker strategy and the cutoff values and sequencing needed to optimize risk stratification in a low- to moderate-risk emergency department population. They found that a combination of NT-proBNP, whole blood choline and Lp-PLA2 with cutoffs identified by CART analysis was optimal for risk stratification and superior to all other possible combinations of markers.

**Table 6** Independent predictors of 6-month mortality on multivariate logistic regression analysis.

Variable	OR (95% CI)	p
<i>LV dysfunction</i>	0.676 (0.29–1.55)	0.356
<i>Revascularization</i>	1.55 (0.67–3.57)	0.304
<i>Laboratory predictors</i>		
G2/G0G1	1.22 (0.23–6.41)	0.811
G3/G0G1	2.96 (0.60–14.61)	0.182
G4/G0G1	13.86 (2.83–67.97)	0.001
GRACE risk score	1.03 (1.02–1.04)	<0.0001

CI: confidence interval; G0G1: groups 0 and 1; G3: group 3; G4: group 4 (see text); LV: left ventricular.



## Limitations

Although our study was prospective in nature, it was a non-randomized observational study conducted in a single center, so the results may have been influenced by confounding factors, identified or not, and thus no causality can be determined. Besides, few events occurred, which may limit extrapolation of the results to the general population of patients with ACS. In addition, our analysis is based on only one measurement of all biomarkers, and other studies, perhaps using sequential samples, are warranted to explore whether the prognostic information changes over time.

## Conclusions

A multimarker approach using CysC, NT-proBNP, CRP and RDW was an independent predictor of 6-month mortality and added prognostic information to the GRACE risk score in patients with ACS and high risk defined by GRACE, with increasing mortality in patients with a higher number of elevated biomarkers on admission.

Individually, CysC and NT-proBNP are the strongest predictors.

Since the pathophysiology of ACS is complex, a combination of markers representing different mechanisms may be used in the future instead of a single marker. Other studies are needed to find combinations of ideal markers in addition to clinical risk factors.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

## Conflicts of interest

The authors have no conflicts of interest to declare.

## References

- de Araujo Goncalves P, Ferreira J, Aguiar C, et al. TIMI PURSUIT, and GRACE risk scores: sustained prognostic value and interaction with revascularization in NSTEMI-ACS. *Eur Heart J*. 2005;26:865–72.
- Nagesh CM, Roy A. Role of biomarkers in risk stratification of acute coronary syndrome. *Indian J Med Res*. 2010;132:627–33.
- Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation*. 2006;113:2335–62.
- National Kidney Foundation. *Kidney Disease Outcome Quality Initiative (K/DOQI) clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification*. *Am J Kidney Dis*. 2002;39 Suppl. 1:S1–266.
- Moscucci M, Fox KA, Cannon CP, et al. Predictors of major bleeding in acute coronary syndromes: the Global Registry of Acute Coronary Events (GRACE). *Eur Heart J*. 2003;24:1815–23.
- Tonelli M, Slacks F, Arnold M, et al. Relation between red blood cell distribution width and cardiovascular risk in people with coronary disease. *Circulation*. 2008;117:163–8.
- Nabais S, Losa N, Gaspar A, et al. Association between red blood cell distribution width and outcomes at six months in patients with acute coronary syndromes. *Rev Port Cardiol*. 2009;28:905–24.
- Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med*. 1996;335:1342–9.
- Anzai T, Yoshikawa T, Shiraki H, et al. C-reactive protein as a predictor of infarct expansion and cardiac rupture after a first Q-wave acute myocardial infarction. *Circulation*. 1997;96:778–84.
- Shishehbor MH, Bhatt DL, Topol EJ. Using C-reactive protein to assess cardiovascular disease risk. *Cleve Clin J Med*. 2003;70:634–40.
- de Lemos JA, Morrow DA, Bentley JH, et al. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med*. 2001;345:1014–21.
- Khan SQ, Narayan H, Ng KH, et al. N-terminal pro-B-type natriuretic peptide complements the GRACE risk score in predicting early and late mortality following acute coronary syndrome. *Clin Sci (Lond)*. 2009;117:31–9.
- Omland L, Persson A, Ng L, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. *Circulation*. 2002;106:2913–8.
- Khan SQ, Quinn P, Davies JE, et al. N-terminal pro-B-type natriuretic peptide is better than TIMI risk score at predicting death after acute myocardial infarction. *Heart*. 2008;94:40–3.
- Jernberg T, Lindahl B, James S, et al. Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. *Circulation*. 2004;110:2342–8.
- Wiviott SD, Morrow DA, Sabatine MS, et al. Baseline cystatin C measurement is a potent predictor of adverse cardiovascular outcomes following ACS: a PROVE IT – TIMI 22 analysis. *Circulation*. 2007;114:II.418.
- Acuna JG, Garcia EG, Gutierrez M, et al. Increased cystatin C serum levels improve risk stratification in patients with acute coronary syndrome and normal glomerular filtration rate. *Circulation*. 2008;118:S.763.
- Keller T, Mesow CM, Lubos E, et al. Cystatin C and cardiovascular mortality in patients with coronary artery disease and maintained renal function. *Circulation*. 2008;118:S.1131.
- Gitt AK, Wienbergen H, Towae F, et al. Impact of glomerular filtration rate on outcome in consecutive patients with STEMI in clinical practice: results of the ACOS-registry. *Circulation*. 2007;116:II.317.
- Mocatta TJ, Pilbrow AP, Cameron VA, et al. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol*. 2007;49:1993–2000.
- Kilcullen N, Viswanathan K, Das R, et al. Heart-type fatty acid-binding protein and identifies high-risk patients across the range of troponin values. *J Am Coll Cardiol*. 2007;50:2061–7.
- Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:60–8.
- Wollert KC, Kempf T, Peter T, et al. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation*. 2007;115:962–71.

24. Shimpo M, Morrow DA, Weinberg EO, et al. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation*. 2004;109:2186–90.
25. Khan SQ, Dhillon O, Struck J, et al. C-terminal pro-endothelin-1 offers additional prognostic information in patients after acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) Study. *Am Heart J*. 2007;154:736–42.
26. Khan SQ, O'Brien RJ, Struck J, et al. Prognostic value of midregional proadrenomedullin in patients with acute myocardial infarction: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. *J Am Coll Cardiol*. 2007;49:1525–32.
27. Khan SQ, Dhillon OS, O'Brien RJ, et al. C-terminal pro-vasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. *Circulation*. 2007;115:2103–10.
28. Matsui S, Ishii J, Kitagawa F, et al. Pentraxin 3 in unstable angina and non-ST-segment elevation myocardial infarction. *Atherosclerosis*. 2010;210:220–5.
29. Chan D, Ng LL. Biomarkers in acute myocardial infarction. *BMC Med*. 2010;8:34–45.
30. Weber M, Hamm C. Novel biomarkers – the long march from bench to bedside. *Eur Heart J*. 2008;29:1079–81.
31. Morrow DA, Braunwald E. Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. *Circulation*. 2003;108:250–2.
32. James SK, Lindahl B, Siegbahn A, et al. N-terminal pro-brain natriuretic peptide and other risk markers for the separate prediction of mortality and subsequent myocardial infarction in patients with non-ST elevation acute coronary disease: a Global Utilization of Strategies To Open occluded arteries (GUSTO)-IV substudy. *Circulation*. 2003;108:275–81.
33. Sabatine MS, Morrow DA, de Lemos JA, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I C-reactive protein, and B-type natriuretic peptide. *Circulation*. 2002;105:1760–3.
34. Tello-Montoliu A, Marín F, Róldan V, et al. A multimarker risk stratification approach to non-ST elevation acute coronary syndrome: implications of troponin T, CRP, NT pro-BNP and fibrin D-dimer levels. *J Intern Med*. 2007;262:651–8.
35. Möckel M, Danne O, Müller R, et al. Development of an optimized multimarker strategy for early risk assessment of patients with acute coronary syndromes. *Clin Chim Acta*. 2008;393:103–9.