Treatment strategies and risk stratification in acute coronary syndromes
Damman, P.

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MULTIPLE BIOMARKERS AT ADMISSION ARE ASSOCIATED WITH ANGIOGRAPHIC, ELECTROCARDIOGRAPHIC AND IMAGING CARDIOVASCULAR MECHANISTIC MARKERS OF OUTCOMES IN PATIENTS UNDERGOING PRIMARY PERCUTANEOUS CORONARY INTERVENTION FOR ACUTE ST-ELEVATION MYOCARDIAL INFARCTION


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ABSTRACT

Background: The multimarker risk score, based on estimated glomerular filtration rate (eGFR), glucose and N-terminal pro-brain natriuretic peptide (NT-pro-BNP), has been shown to predict mortality in patients with ST-segment elevation myocardial infarction ( STEMI) undergoing primary percutaneous coronary intervention (PPCI). In this study, we investigated the relation between the multimarker risk score and cardiovascular mechanistic markers of outcomes in STEMI patients undergoing PPCI.

Methods: Complete biomarkers were available in 197 patients with STEMI. Angiographic TIMI flow grade and myocardial blush grade at the end of the PPCI, electrocardiographic ST-segment resolution (STR) at the time of last contrast injection and 240 minutes after last contrast, and Cardiac Magnetic Resonance (CMR) left ventricular ejection fraction (LVEF) and infarct size (IS) at 4 to 6 months after the index event was available.

Results: In linear regression models, higher multimarker scores were associated with worse angiographic (P<0.01 for both outcomes), electrocardiographic (P<0.001 for the association with STR at last contrast, and P<0.01 for STR at 240 minutes), and CMR outcomes (P<0.01 for both).

Conclusions: The multimarker risk score is associated with angiographic, electrocardiographic and CMR mechanistic markers of outcomes. These data support the ability of the multimarker risk score to identify patients at high-risk of sub-optimal reperfusion and CMR outcomes and may aid in the early triage of patients who stand to benefit most of adjuvant treatments in STEMI.
INTRODUCTION

Patients presenting with ST-elevation myocardial infarction (STEMI) preferably undergo mechanical reperfusion with primary percutaneous coronary intervention (PPCI). Despite progress in reducing mortality over the past decade, subgroups at high risk for adverse events remain. The ability to differentiate patients at high risk for adverse events from patients at low risk may be a valuable tool to optimize the allocation of novel adjunctive therapies and post-PPCI strategies, which may improve outcomes. Recently we developed a multimarker risk score for rapid, early assessment of patient baseline risk. This score is based on blood glucose, N-terminal pro-brain natriuretic peptide (NT-pro-BNP), and estimated glomerular filtration rate (eGFR) measured immediately prior to PPCI. With this score, we were able to identify high-risk patients with a more than 20% all-cause mortality at 1-year follow-up.

In order to further explore the association between our multimarker risk score and higher mortality risk, we assessed the association between this score and several cardiovascular mechanistic markers of outcomes. Mechanistic markers are measurements or physical signs used as surrogates for clinical outcomes, and effects on these markers should associate with clinical outcomes. Several mechanistic markers of outcomes are associated with subsequent mortality, including angiographic Thrombolysis In Myocardial Infarction (TIMI) flow and myocardial blush grade (MBG), electrocardiographic ST-segment resolution (STR) and cardiovascular magnetic resonance (CMR) infarct size and left ventricular ejection fraction. In this study, we investigated the association between the multimarker risk score and cardiovascular mechanistic markers in STEMI patients undergoing PPCI. The additional value of the multiple biomarkers on top of established risk factors have been described previously.

METHODS

Source population

The current analysis is a substudy from the PRoximal Embolic Protection in Acute myocardial infarction and Resolution of ST-Elevation (PREPARE) study. The study design and main findings have been described previously. Briefly, patients with STEMI were randomized to primary PCI with combined proximal embolic protection and thrombus aspiration using the Proxis Embolic Protection System (St. Jude Medical, St Paul, MN, USA) or primary PCI solely. Patients were eligible for inclusion in the PREPARE trial if they experienced onset of symptoms of myocardial infarction less than 6 hours before presentation with electrocardiographic evidence of persistent ST-segment elevation of at least 200 µV in two or more contiguous leads and TIMI flow grade 0 to 1 on diagnostic angiography.

Multimarker risk score

The multimarker risk score is based on blood samples obtained at the time of arterial sheath insertion and calculated as follows: a glucose 8-9 mmol/l, NT-pro-BNP...
150-599 ng/l, and eGFR 60-90ml/min are assigned 2 points, a glucose ≥10 mmol/l, and NT-pro-BNP ≥600 ng/l are assigned 3 points, and an eGFR <60 ml/min is assigned 4 points. The multimarker low-risk group is defined as a score ≤4, the intermediate-risk group as score 5 or 6, and the high-risk group as score >6.

Mechanistic markers of outcomes
The mechanistic markers of outcomes for our current analysis are angiographic TIMI flow grade and myocardial blush grade at the end of the PPCI, electrocardiographic ST-segment resolution at the time of last contrast injection and 240 minutes after last contrast, and Cardiac Magnetic Resonance (CMR) left ventricular ejection fraction (LVEF) and infarct size (IS) at 4 to 6 months after the index event.

Data collection
Blood was collected in 4.5-mL, gel / lithium-heparine-coated tubes, centrifuged without undue delay, and stored individually at -70 °C until further analysis. NT-pro-BNP, Creatine kinase-myocardial band mass(CK-MB) and cardiac troponin T were measured using a Hitachi modular E-170 (Roche Diagnostics GmbH, Mannheim, Germany). Glucose, creatinine and C-reactive protein were measured on the Hitachi Modular P-800 unit (Roche Diagnostics GmbH). The eGFR was calculated according the Cockcroft and Gault formula. Coronary angiograms immediately after PPCI were acquired using digital technique (Philips Medical Systems, Best, The Netherlands). TIMI flow grade and myocardial blush grade were assessed visually at an independent core laboratory (University Medical Center Groningen, Groningen, The Netherlands). With the use of continuous digital 12-lead ECG/Holter monitoring (Northeast Monitoring 180+ Natick, Mass, USA), STR was assessed at the end of the PCI procedure following last contrast injection and 240 minutes after last contrast. All ST Holter data were analyzed at an independent core laboratory (Duke Clinical Research Institute – Duke University Medical Center, Durham, NC, USA). We chose early ST-segment recovery (at last contrast injection) and 240 min after last contrast injection as our surrogate outcomes. As part of an ancillary PREPARE study, patients underwent CMR at four to six months after index procedure. CMR imaging was performed with a 1.5T clinical scanner (Sonato/Avanto, Siemens, Erlangen, Germany). Functional assessment was studied with a standard cine steady-state free precession sequence, and late gadolinium enhancement (LGE) images were acquired after administration of gadolinium-based contrast agent (0.2 mmol/kg, Magnevist, Schering AG, Berlin, Germany). All functional and LGE images were analyzed as described previously 10. In patients with a previous MI, the old MI was identified by coronary territory and not included in the IS. The CMR data were analyzed by a single experienced physician (J.D.E.H.) using the MASS software (version 5.1, MEDIS Medical Imaging Systems, Leiden, The Netherlands).

Study population
The current analysis included 197 of the 206 patients who were enrolled in the PREPARE ancillary CMR study of whom biomarker data was available.
Statistical analysis
Normal-distributed continuous variables were presented as the mean with standard deviation and compared with the Student T-test, skewed-distributed were presented as the median with interquartile range (IQR) and compared with the Wilcoxon rank-sum test. Categorical variables were presented as frequencies and compared with the chi-square test. The association between the biomarker risk score groups and mechanistic markers of outcomes was investigated with the use of Chi-square trend tests for the angiographic and electocardiographic data, and with linear regression models for the CMR data. We merged the intermediate-risk and high-risk group because of a low patient frequency in the intermediate and high-risk groups. In an exploratory analysis, we repeated the analyses using the biomarker risk score as a continuous parameter. Patients who were lost to follow-up due to death received imputed values equal to the worst cardiac CMR parameters in our study population. Statistical analysis was performed using Statistical Package for Social Sciences software (SPSS 15.0 for Windows, SPSS Inc., Chicago, Illinois, USA). A P value <0.05 was considered statistically significant. The authors are solely responsible for the design and conduct of this study, all study analyses and drafting and editing of the paper.

Sources of funding
This study is supported by grants from St. Jude Medical and from the University of Amsterdam.

RESULTS
Patient population
A total of 197 patients from the PREPARE study were included in the current analysis. The baseline characteristics of the study patients are presented in Table 1. The mean age was 58 years and 84% were male. The median multimarker score was 2 (IQR 0-4), and respectively 156 patients were in the low-risk (score <5), 31 in the intermediate-risk (score 5-6) and 10 in the high-risk group (score >6). The intermediate-risk and high-risk groups were merged because of low patient numbers. Patients in the combined intermediate-high-risk group were significantly older, more often diabetic and less often current smokers.

Angiographic and procedural characteristics
Of all patients, as per protocol, 93% presented with a totally occluded artery at the initial angiography. Multiple vessel disease was observed in 31% of the patients. The right coronary artery was the most prevalent infarct-related artery (IRA). Although there was no difference in number of diseased vessels between the multimarker low-risk and intermediate-high-risk patients (P=0.66), the infarct-related artery was more frequently left in the higher risk category (P=0.03). Figure 1 presents the baseline angiographic findings according to the multimarker risk categories. Other angiographic and procedural characteristics are shown in Table 1.
### Table 1. Baseline and procedural characteristics of the study patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total study group (n=197)</th>
<th>Low-risk group (n=156)</th>
<th>Intermediate-high-risk group (n=41)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age – years, mean (SD)</td>
<td>58 (11)</td>
<td>57 (10)</td>
<td>64 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI – kg/m²</td>
<td>26.8 (3.6)</td>
<td>26.8 (3.5)</td>
<td>26.7 (3.7)</td>
<td>0.82</td>
</tr>
<tr>
<td>Male gender – no. (%)</td>
<td>166 (84%)</td>
<td>135 (87%)</td>
<td>31 (76%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Risk factors – no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>14 (7%)</td>
<td>7 (5%)</td>
<td>7 (17%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>42 (21%)</td>
<td>31 (20%)</td>
<td>11 (27%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>24 (12%)</td>
<td>18 (12%)</td>
<td>6 (15%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Current smoking</td>
<td>121 (61%)</td>
<td>104 (67%)</td>
<td>17 (42%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>History – no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>8 (4%)</td>
<td>6 (4%)</td>
<td>2 (5%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Percutaneous Coronary Intervention</td>
<td>8 (4%)</td>
<td>7 (5%)</td>
<td>1 (2%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Baseline angiographic data – no. (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>135 (69%)</td>
<td>109 (70%)</td>
<td>26 (63%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48 (24%)</td>
<td>37 (24%)</td>
<td>11 (27%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 (7%)</td>
<td>10 (6%)</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td>Infarct-related artery</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Left anterior descending artery</td>
<td>58 (29%)</td>
<td>40 (26%)</td>
<td>18 (44%)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex artery</td>
<td>22 (11%)</td>
<td>16 (10%)</td>
<td>6 (15%)</td>
<td></td>
</tr>
</tbody>
</table>
**Right coronary artery** | 117 (59%) | 100 (64%) | 17 (42%)  
**TIMI flow** | 0.85  
Grade 0 | 183 (93%) | 145 (93%) | 38 (93%)  
Grade 1 | 11 (6%) | 9 (6%) | 2 (5%)  
Grade 2-3 | 3 (1%) | 2 (1%) | 1 (2%)  

**Baseline biomarker data, median (IQR)**

| Biomarker       | Low Risk | Intermediate-High Risk | High Risk | P-value  
|-----------------|----------|------------------------|-----------|---------
| Troponin T      | 0.00 (0.00–0.07) | 0.00 (0.00–0.05) | 0.00 (0.09–0.22) | <0.001  
| CK-MB           | 5.0 (3.5 – 9.2) | 4.7 (3.3 – 7.8) | 8.8 (5.0 – 17.8) | <0.001  
| Glucose         | 7.9 (7.1 – 9.2) | 7.7 (6.8 – 8.6) | 9.7 (8.2 – 12.6) | -  
| Estimated GFR   | 107 (86 – 137) | 113 (95 – 142) | 82 (70 – 93) | -  
| C-reactive protein | 2.2 (1.2 – 4.6) | 2.1 (1.2 – 4.1) | 3.4 (1.6 – 7.7) | 0.04  
| NT-pro-BNP      | 85 (14 – 245) | 64 (14 – 136) | 417 (139–1236) | -  
| Multimarker score | 2 (0 – 4) | 2 (0 – 2) | 5 (5 – 7) | -  

**Procedural characteristics – no. (%)**

| Characteristic | Low Risk | Intermediate-High Risk | High Risk | P-value  
|----------------|----------|------------------------|-----------|---------
| Systolic blood pressure | 134 (28) | 134 (26) | 136 (34) | 0.70  
| Heart rate | 74 (20) | 73 (18) | 79 (24) | 0.08  
| Symptom onset to balloon | 158 (126 – 218) | 161 (128 – 218) | 150 (122 – 214) | 0.30  
| Glycoprotein IIb/IIIa receptor inhibitors | 76 (39%) | 54 (35%) | 22 (54%) | 0.03  
| Proximal embolic protection | 93 (47%) | 71 (46%) | 22 (54%) | 0.35  
| Visible thrombus | 141 (72%) | 108 (69%) | 33 (81%) | 0.16  

*P-value for comparison between low-risk and intermediate-high-risk groups. As by definition, biomarkers are significantly different between low risk and intermediate-high risk patient groups.

BMI : body mass index, GFR : glomerular filtration rate, IQR : interquartile range, SD : standard deviation, TIMI : Trombolysis In Myocardial Infarction
Multimarker risk score and mechanistic markers of outcomes

Table 2 shows the mechanistic markers of outcomes according to multimarker risk score categories. Angiographic TIMI flow grade 3 and myocardial blush grade 3 were less frequently observed in the intermediate-high-risk multimarker group when compared with the low-risk group.

Regarding early ST-segment resolution or 240 minutes post-procedure, 50-70% or ≥70% resolution was more often observed in the low-risk group. Finally, the mean IS as measured by MRI was significantly lower in the low-risk group (P=0.01), while the LVEF was significantly higher (P<0.01). In an exploratory analysis, we compared peak CK-MB and peak Troponin T which both approximate IS. The median CK-MB peak was 210 µg/l (IQR 135-392, n=92) in low-risk patients compared with 262 µg/l (IQR 161-494, n=92) in intermediate-high-risk patients (P=0.11). The median

![Figure 1. Multiple biomarker score and baseline angiographic characteristics. Shown are the number of diseased vessels (upper panel) and infarct-related artery (lower panel). P-values by the Chi-square test. RCA : right coronary artery, LAD : left anterior descending artery, RCX : ramus circumflexus.](image-url)
Troponin T peak was 2.91 µg/l (IQR 1.29-7.19, n=132) in low-risk patients compared with 5.17 µg/l (IQR 2.04-10.67, n=35) in intermediate-high-risk patients (P=0.02).

The association between the multimarker risk score as a continuous variable and the mechanistic markers of outcomes are shown in Table 3. Higher multimarker risk scores were significantly associated with lower TIMI flow grade and myocardial blush grade, less early ST-segment resolution at last contrast and at 240 minutes, larger IS and a smaller LVEF. The association between the continuous multimarker score and CMR outcomes is presented in Figure 2. Regarding CMR outcomes, the association remained after excluding the patients with imputed outcomes.

**DISCUSSION**

Several conclusions can be drawn from our current report. First, an intermediate to high multimarker risk score is associated with worse mechanistic markers.
Table 3. Association between multimarker risk score and mechanistic markers of outcomes.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Multimarker risk score (continuous)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-square test for trend</td>
</tr>
<tr>
<td>Angiography</td>
<td></td>
</tr>
<tr>
<td>TIMI flow grade</td>
<td>-</td>
</tr>
<tr>
<td>Myocardial blush grade</td>
<td>-</td>
</tr>
<tr>
<td>Electrocardiography</td>
<td></td>
</tr>
<tr>
<td>ST-segment resolution at last contrast</td>
<td>-</td>
</tr>
<tr>
<td>ST-segment resolution at 240 minutes</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linear regression models</th>
<th>Beta</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Resonance Imaging</td>
<td>Unstandardized</td>
<td>Standardized</td>
<td>95% CI</td>
</tr>
<tr>
<td>Infarct size (g/m²)</td>
<td>0.90</td>
<td>0.20</td>
<td>(0.28 – 1.51)</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>-1.12</td>
<td>-0.24</td>
<td>(-1.78 – -0.47)</td>
</tr>
</tbody>
</table>

of cardiovascular outcomes. These include the angiographic TIMI flow and myocardial blush grade, electrocardiographic early ST-resolution at last contrast and 240 minutes, and CMR IS and LVEF. Second, higher multimarker risk scores are more frequently associated with a LAD IRA.

**Angiographic mechanistic markers of outcomes**

Higher multimarker risk scores were inversely associated with TIMI flow grade, reflecting a worse epicardial flow after performing PPCI. This was mainly present in anterior MI (data not shown), which may be explained by the fact that the majority of TIMI flow grade 2 is observed in LAD related MIs, while the majority of TIMI flow grade 3 is observed in RCA related MIs. Besides worse epicardial flow, higher multimarker scores were also associated with worse myocardial perfusion as quantified by the myocardial blush grade. Regarding the relation between angiographic outcomes and mortality, the directionality of the relationship between infarct size and epicardial flow has not yet been demonstrated and successful restoration of epicardial flow is not always associated with successful “downstream” myocardial perfusion.

**Electrocardiographic mechanistic markers of outcomes**

ST-segment resolution reflects the quality of both epicardial and myocardial tissue reperfusion and complements the angiographic characteristics of
**Figure 2.** Association between multiple biomarker score and CMR outcomes. P-values from linear regression models.
reperfusion mentioned above. Analogous to the relation with the angiographic measurements, a higher multimarker score was associated with less frequent ST-segment resolution at both last contrast and 240 minutes.

**Cardiovascular magnetic resonance mechanistic markers of outcomes**

Infarct size is a strong predictor for prognosis after an acute MI, and is associated with late mortality. However, a reduction in IS may lead to a long-term improvement in LV remodelling, yet may not translate in short-term mortality. Although this provides a possible mechanism for increased (long-term) mortality with higher biomarker risk scores, it is still unclear whether the biomarker risk score is higher because of a larger IS or vice versa.

**Pathophysiological mechanisms between biomarkers and mechanistic markers of outcomes**

As mentioned earlier, the lack of myocardial reperfusion may be indicated by reduced myocardial blush grade, “upstream” epicardial TIMI flow grade (“no-reflow”), or absent ST-segment resolution. The lack of myocardial reperfusion in the STEMI setting, or reperfusion microvascular obstruction (MVO), results from both distal embolization from epicardial arteries and ischemia-reperfusion injury within ischemic tissue. Other mechanisms of MVO include external compression by edematous tissue, in situ thrombosis, vasospasm, and activation of inflammatory cascades with leukocyte stasis and extravasation. Of the biomarkers incorporated in the multimarker score, hyperglycemia has been associated with microvascular dysfunction, increased inflammation, and a prothrombotic state. Higher glucose levels are also associated with TIMI flow grade 0-1 before PCI. NT-pro-BNP has been identified as a predictor of ST-segment resolution. Moreover, an association between higher NT-pro-BNP levels and angiographic no-reflow has been shown previously. In an earlier report, we described the independent relation between NT-pro-BNP and LVEF among the patients with a non-anterior wall MI. NT-pro-BNP was also predictive for IS, but lost its significance after adjustment for admission troponin T.

Furthermore, it should be noted that the additional mechanisms not associated with the currently described mechanistic markers of outcomes can potentially explain the increased mortality with higher multimarker scores. As we described earlier, possible mechanisms by which renal dysfunction increase mortality risk are progressive renal decline, a high prevalence of coronary risk factors among patients with chronic kidney disease, and “therapeutic nihilism”. Nevertheless, previous studies have demonstrated the independence of renal dysfunction as a predictor of adverse outcomes, including mortality.

Altogether, the current analysis expands the previously shown association between the multimarker score and mortality with mechanistic insights. The risk score predictors might herald proclivity to specific mechanistic problems that interfere with the benefits of reperfusion. Thus we hypothesize that the multimarker risk score might assist in identifying high-risk STEMI patients who are ideal for studies of
novel therapies. Before the score can be used in clinical practice or research, further studies are needed to confirm whether the multimarker risk score identifies these mechanisms mediating poor clinical outcomes as a potential reversible target, or whether the risk score reflects other non-modifiable risk predictors such as age. Second, the score has to be validated in another cohort of patients.

Limitations
Several limitations of the current analysis deserve to be mentioned. First, because of the small patient sample, we were underpowered to investigate the clinical outcome mortality in the multimarker risk score groups. Moreover, due to the relatively small size of the study, we could not differentiate between the intermediate risk and high risk patients as assessed by the multimarker score. However, the association between the multimarker score and mechanistic outcomes remained if the multimarker score was entered as a continuous variable. Finally, we were underpowered to compare between the original randomized treatments and surrogate outcomes in the different multimarker risk score groups.

CONCLUSION
The multimarker risk score is associated with angiographic, electrocardiographic and CMR mechanistic markers of outcomes. These data support the ability of the risk score to identify patients at high-risk of sub-optimal reperfusion and CMR outcomes and may aid in the early triage of patients who stand to benefit most of adjuvant treatments in STEMI.

ACKNOWLEDGEMENTS
We thank all investigators and patients who participated in the PREPARE trial.

REFERENCE LIST


