Markers of Plaque Instability in the Early Diagnosis and Risk Stratification of Acute Myocardial Infarction

Nora Schaub,† Tobias Reichlin,† Christophe Meune, Raphael Twerenbold, Philip Haaf, Willibald Hochholzer, Nadine Niederhauser, Piet Bosshard, Claudia Stelzig, Michael Freese, Miriam Reiter, Joachim Gea, Andreas Buser, Alexandre Mebazaa, Stefan Osswald, and Christian Mueller

BACKGROUND: Plaque erosion and plaque rupture occur early in the pathophysiology of acute myocardial infarction (AMI). We hypothesized that markers of plaque instability might be useful in the early diagnosis and risk stratification of AMI.

METHODS: In this multicenter study, we examined 4 markers of plaque instability, myeloperoxidase (MPO), myeloid-related protein 8/14 (MRP-8/14), pregnancy-associated plasma protein-A (PAPP-A), and C-reactive protein (CRP) in 398 consecutive patients presenting to the emergency department with acute chest pain and compared them to normal and high-sensitivity cardiac troponin T (cTnT and hs-cTnT). The final diagnosis was adjudicated by 2 independent cardiologists. Primary prognostic endpoint was death during a median follow-up of 27 months.

RESULTS: The adjudicated final diagnosis was AMI in 76 patients (19%). At emergency department presentation, concentrations of all 4 biomarkers of plaque instability were significantly higher in patients with AMI than in patients with other diagnoses. However, their diagnostic accuracy as quantified by the area under the ROC curve (AUC) was low (MPO 0.63, MRP-8/14 0.65, PAPP-A 0.62, CRP 0.59) and inferior to both normal and high-sensitivity cardiac troponin T (cTnT 0.88, hs-cTnT 0.96; P < 0.001 for all comparisons). Thirty-nine patients (10%) died during follow-up. Concentrations of MPO, MRP-8/14, and CRP were higher in nonsurvivors than in survivors and predicted all-cause mortality with moderate accuracy.

CONCLUSIONS: Biomarkers of plaque instability do not seem helpful in the early diagnosis of AMI but may provide some incremental value in the risk stratification of patients with acute chest pain.

© 2011 American Association for Clinical Chemistry

Acute myocardial infarction (AMI) is a major cause of death and disability worldwide. Rapid and accurate identification and risk stratification of AMI is critical to initiate appropriate treatment. Patient history, electrocardiography (ECG), and cardiac troponin T (cTnT) form the diagnostic and prognostic cornerstones of the clinical assessment of chest pain patients, but they often leave diagnostic uncertainty. Inflammation plays a key role in atherosclerotic plaque formation, plaque destabilization and plaque disruption. As AMI is caused by vulnerable plaques, biomarkers of plaque instability and inflammation may provide important diagnostic and prognostic information. Myeloperoxidase (MPO), for example, is a leukocyte-derived enzyme that catalyzes the formation of reactive oxidants and is thought to be a potential participant in plaque formation and plaque rupture. MPO has been shown to be increased in patients with stable coronary artery disease (CAD) and acute coronary syndrome (ACS). Recent studies that documented that therapeutic use of heparin induces preanalytical bias and error in MPO measurements have made it difficult to interpret promising initial data regarding the diagnostic use of MPO.
Myeloid-related protein 8/14 (MRP-8/14), also termed calprotectin, is secreted by activated monocytes and neutrophils, has proinflammatory properties, and is expressed in atherosclerotic lesions (12). Pilot studies suggested a possible prognostic role for MRP-8/14 in ACS patients (13, 14). Pregnancy-associated plasma protein A (PAPP-A) is a protease mainly produced by the placenta during pregnancy, but also by fibroblasts, osteoblasts, and vascular smooth muscle cells. Some studies reported PAPP-A to be highly expressed in unstable plaques (15, 16). PAPP-A has been suggested as a prognostic marker in patients with stable CAD as well as ACS (17–19). C-reactive protein (CRP) is a widely used biomarker of inflammation. Many prospective studies demonstrated a significant and independent association between CRP concentrations and future cardiovascular events in healthy subjects as well as in patients with established ACS (20–23).

We performed a large multicenter study to examine the value of commercially available biomarkers of plaque instability for early diagnosis of AMI and for predicting prognosis in unselected heparin-naive patients presenting to the emergency department with acute chest pain.

**Methods**

**STUDY DESIGN AND POPULATION**

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) is an ongoing prospective international multicenter study designed and coordinated by the University Hospital Basel, Switzerland (24–26). From April 2006 to May 2008, 689 consecutive patients presenting to the emergency department with symptoms suggestive of AMI, such as acute chest pain and angina pectoris, were recruited. Concentrations of all 4 markers of plaque instability (MPO, MRP-8/14, PAPP-A, CRP) as well as cTnT and high-sensitivity cTnT (hs-cTnT) were available in 398 (58%) patients. Patients with terminal kidney failure requiring dialysis were excluded. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients.

**ROUTINE CLINICAL ASSESSMENT**

All patients underwent an initial clinical assessment including clinical history, physical examination, 12-lead ECG, pulse oximetry, standard blood tests, and chest radiography. Standard cardiac troponin was measured at presentation and thereafter as long as clinically indicated. Treatment of patients was left at the discretion of the attending physicians.

**ADJUDICATED FINAL DIAGNOSIS**

To determine the final diagnosis for each patient, adjudication of final diagnoses was performed for all patients centrally in the core laboratory (University Hospital Basel) as follows. Two independent cardiologists blinded to the measured concentrations of the 4 markers and hs-cTnT reviewed all available medical records (including patient history, physical examination, results of laboratory testing including local cardiac troponin values, radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography) pertaining to the patient from the time of emergency department presentation through 60-day follow-up. In situations of diagnostic disagreement (16%), cases were reviewed and adjudicated in conjunction with a third cardiologist.

AMI was defined and cardiac troponin concentrations interpreted as recommended in current guidelines (6, 27–29). In brief, AMI was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Cardiac necrosis was diagnosed by at least 1 value of the local standard cardiac troponin above the 99th percentile (or above the 10% imprecision value if not fulfilled at the 99th percentile). In the absence of uniformly accepted published guidelines, a significant rise and/or fall was defined as a change of at least 30% from the 99th percentile (or the 10% CV level) within 6–9 h (6, 27–29). The following cTn assays were used for the adjudication of the final diagnosis on site: Abbott AxSYM cTnI ADV, Beckmann Coulter Accu cTnI, and Roche cTnI fourth generation. All 3 are well-validated current standard cardiac troponin assays with comparable performance in the diagnosis of AMI (see Supplemental Methods, which accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue1 for details on use of each of the local cardiac troponin assays for final diagnosis adjudication) (28, 29). Unstable angina (UA) was diagnosed in patients with normal cardiac troponin concentrations and typical angina at rest, a deterioration of a previously stable angina (clinical diagnosis without further tests, n = 7), in cases of typical angina and a positive cardiac exercise test (n = 16), or cardiac catheterization with coronary arteries found to have a stenosis of 70% or greater (50% or greater for left main disease) (n = 44), and in ambiguous cases in which follow-up information revealed AMI (n = 1) or a sudden, unexpected cardiac death within 60 days (n = 1). Further, predefined diagnostic categories included cardiac but not coronary symptoms (e.g., perimyocarditis, tachyarrhythmias) and noncardiac symptoms. If AMI was excluded in the emergency department, but insufficient further diagnostic proce-
percentile of the 10% CV was reported at 0.013 μg/L. Interassay imprecision of 4.4% at 5.1 mg/L, minimal detectable concentration of 3 mg/L, and functional sensitivity (level of 15% imprecision) of 0.56 mg/L (31). PAPP-A was measured in serum using a 1-step enzyme immunoassay based on electrochemiluminescence technology (Architect MPO, Abbott Diagnostics). According to the manufacturer, this assay had a dynamic range of 0–10 μmol/L with an analytical sensitivity of <0.01 μg/L, and a CV of <10% at 0.035 μg/L. hs-cTnT was similarly measured on the Elecsys 2010 (Roche Diagnostics). For hs-cTnT, limit of blank (LoB) and limit of detection (LoD) have been determined to be 0.003 μg/L and 0.005 μg/L. An imprecision corresponding to 10% CV was reported at 0.013 μg/L, and the 99th percentile of a healthy reference population at 0.014 μg/L (30). MPO was measured in EDTA plasma using a chemiluminescent automated microparticle immunoassay technology (Architect MPO, Abbott Diagnostics). According to the manufacturer, this assay had a dynamic range of 0–10 μmol/L with an analytical sensitivity of <5 pmol/L and a functional sensitivity of 70 pmol/L (total CV of 10%). MRP-8/14 was measured in serum using ELISA (Bühlmann Laboratories). Performance characteristics of this assay include intraassay imprecision of 4.8% at 3.4 mg/L, interassay imprecision of 4.4% at 5.1 mg/L, minimal detectable concentration of 0.3 mg/L, and functional sensitivity (level of 15% imprecision) of 0.56 mg/L (31). PAPP-A was measured in serum using a 1-step enzyme immunoassay based on electroluminescence technology (Elecsys 2010, Roche Diagnostics). This assay measures all forms of PAPP-A, the heterodimer as well as the free form. The lower limit of detection is 4 mIU/L and the 95th percentile in healthy individuals has been reported at 7.15 mIU/L. CRP was measured in heparin plasma by a particle-enhanced turbidimetric immunoassay (Dade Behring Inc.). The lower detection limit of this test is 3 mg/L. Glomerular filtration rate was calculated using the abbreviated Modification of Diet in Renal Disease formula (32).

FOLLOW-UP AND CLINICAL ENDPOINTS

After hospital discharge, patients were contacted after 3, 12, 24, and 36 months by telephone or in writing. Information regarding death was furthermore obtained from the national registry on mortality, the hospital’s diagnosis registry, and the family physician’s records. The primary prognostic endpoint was all-cause mortality, and secondary prognostic endpoints were cardiovascular death in all patients as well as a composite endpoint of all-cause mortality or first AMI in chest pain patients without AMI as the index event.

STATISTICAL ANALYSIS

Continuous variables are presented as mean (SD) or medians with interquartile range (IQR) and categorical variables as numbers and percentages. Comparisons between groups were made using χ² method for categorical and Mann–Whitney U-test for continuous variables. Correlations among the different markers were assessed with the use of the Spearman rank-correlation coefficient. ROC curves were constructed and areas under the curve (AUCs) calculated to assess the sensitivity and specificity throughout the concentrations of the biomarkers of plaque instability and cTnT to compare their individual or combined ability to diagnose AMI and to predict the probability of all-cause death and AMI during follow-up. Comparison of AUCs was performed as recommended by DeLong (33). Kaplan–Meier analysis was performed for survival, and log-rank values were used to assess statistical significance. Cox proportional hazard analysis was used to compute hazard ratios and 95% CIs for the markers assessed to predict mortality. All 4 markers were tested in a univariable model and furthermore in a multivariable model adjusted for clinical risk factors (age, arterial hypertension, dyslipidemia, diabetes mellitus, smoking, history of CAD, history of AMI, history of renal failure) and presentation values of hs-cTnT. Net reclassification improvement (NRI) compares the proportions moving up or down in clinical categories in survivors and nonsurvivors. We chose risk categories to obtain clinically meaningful results. For further verification, we used integrated discrimination improvement (IDI) analysis, which is not dependent on certain risk groups because probability differences are used instead of categories (34). All hypothesis testing was 2-tailed, and a P value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows 15.0 (SPSS Inc.) and MedCalc 11.2.1.0 (MedCalc Software).

Results

CHARACTERISTICS OF THE PATIENTS

Baseline characteristics of the 398 patients are shown in Table 1. The adjudicated final diagnosis was AMI in 76 patients (19%), unstable angina in 69 (17%), cardiac but noncoronary symptoms in 47 (12%), noncardiac symptoms in 175 (44%), and symptoms of unknown origin in 31 (8%).
CONCENTRATIONS OF THE MARKERS OF PLAQUE INSTABILITY IN DIFFERENT GROUPS OF CHEST PAIN

Concentrations of the markers of plaque instability at presentation are displayed in Fig. 1. The concentrations of all 4 biomarkers of plaque instability were significantly higher in patients with AMI than in patients with other diagnoses (median (IQR) MPO 117 (81–316) vs 91 (63–139) pmol/L, P < 0.001; MRP-8/14 4.9 (3.4–7.3) vs 3.9 (2.7–5.3) mg/L, P < 0.001; PAPP-A 4.6 (4.0–9.3) vs 4.0 (4.0–5.6) mIU/L, P < 0.001; CRP 3.3 (3.0–11.2) vs 3.0 (3.0–5.0) mg/L, P = 0.01).

Patients with AMI had higher MPO (P < 0.001), MRP-8/14 (P < 0.001), and CRP (P = 0.01) concentrations at presentation than patients with unstable angina, but no significant difference in the concentration of PAPP-A (P = 0.17) was found between these 2 groups. No significant differences were found for MPO, PAPP-A, or CRP values in patients

### Table 1. Baseline characteristics of the patients.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>AMI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>398</td>
<td>76</td>
<td>322</td>
</tr>
<tr>
<td>Age, years</td>
<td>64 (51–76)</td>
<td>73 (63–81)</td>
<td>62 (49–75)</td>
</tr>
<tr>
<td>Male sex</td>
<td>262 (66)</td>
<td>55 (72)</td>
<td>207 (64)</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>235 (59)</td>
<td>55 (72)</td>
<td>180 (60)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>161 (41)</td>
<td>33 (43)</td>
<td>128 (40)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>70 (18)</td>
<td>16 (21)</td>
<td>54 (17)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>100 (25)</td>
<td>21 (28)</td>
<td>79 (25)</td>
</tr>
<tr>
<td>History of smoking</td>
<td>135 (34)</td>
<td>22 (29)</td>
<td>113 (35)</td>
</tr>
<tr>
<td>History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>143 (36)</td>
<td>30 (40)</td>
<td>113 (35)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>107 (27)</td>
<td>24 (32)</td>
<td>83 (26)</td>
</tr>
</tbody>
</table>
| Previous revascularization (PCI, lysis, ACB)
| Peripheral artery disease | 32 (8)       | 9 (12)    | 23 (7)   | 0.18   |
| Previous stroke          | 28 (7)       | 8 (11)    | 20 (6)   | 0.19   |
| Vital status             |              |           |         |
| Heart rate, bpm          | 75 (65–88)   | 78 (62–92)| 75 (65–86)| 0.59   |
| Systolic blood pressure, mmHg | 141 (127–159) | 134 (119–154)| 142 (130–159)| 0.01   |
| Diastolic blood pressure, mmHg | 86 (78–95)   | 84 (76–96)| 87 (78–95)| 0.13   |
| Body mass index*         | 26 (24–29)   | 26 (24–29)| 26 (24–29)| 0.98   |
| Electrocardiographic findings |              |           |         |
| Left bundle branch block | 15 (4)       | 17 (9)    | 8 (3)    | 0.01   |
| ST-segment elevation      | 31 (8)       | 25 (33)   | 6 (2)    | <0.001 |
| ST-segment depression     | 40 (10)      | 12 (16)   | 28 (9)   | 0.06   |
| T-wave inversion          | 22 (6)       | 11 (15)   | 11 (3)   | <0.001 |
| No relevant ECG finding   | 290 (73)     | 21 (28)   | 269 (84) | <0.001 |
| Laboratory assessment     |              |           |         |
| cTnT, µg/L                | 0.01 (0.01–0.01) | 0.07 (0.01–0.27) | 0.01 (0.01–0.01) | <0.001 |
| Creatine kinase, U/L      | 100 (74–156) | 150 (93–242)| 97 (70–136)| <0.001 |
| Creatine kinase MB fraction, U/L | 3.8 (2.9–5.2) | 7.1 (4.4–13.8) | 3.6 (2.9–4.9) | <0.001 |
| Myoglobin, µg/L           | 42 (31–65)   | 83 (55–232)| 39 (30–56) | <0.001 |
| Glomerular filtration rate, mL/min/1.73m^2 | 95 (75–111) | 83 (60–103) | 97 (77–113) | <0.001 |

* Data are median (IQR) or n (%).*
* PCI, percutaneous coronary intervention; ACB, aortocoronary bypass.
with ST-elevation MI (STEMI) and non-ST-elevation MI (NSTEMI) \( (P = 0.73, P = 0.63, P = 0.06) \), but patients with STEMI had significantly higher concentrations of MRP-8/14 than patients with NSTEMI \( [5.8 \ (4.1–8.4) \ vs \ 4.5 \ (3.0–7.1) \ mg/L, \ P = 0.02] \).

Kinetics of the 4 markers of plaque instability in patients with AMI were analyzed by comparing early presenters (presenting \( \leq 3 \) h after the onset of symptoms) with those presenting 4–10 h after the onset of symptoms and the late presenters (\( > 10 \) h after the onset of symptoms) (online Supplementary Fig. 1). No differences in the presentation concentrations of MPO \( (P = 0.66) \), MRP-8/14 \( (P = 0.45) \), or PAPP-A \( (P = 0.53) \) were found. However, there was a statistically significant rise and fall observed for CRP \( (P = 0.03) \).

Overall, the 4 markers of plaque instability showed only a low to modest correlation with each other as well as with the extent of myocardial necrosis as quantified by concentrations of hs-cTnT (Table 2).

Coronary angiography was performed in 117 patients (29%). Differences of borderline significance in PAPP-A and CRP were observed for patients with and without at least 1 high-grade lesion with a stenosis >95% (median 4.0 vs 4.6 mIU/L and median 3.0 vs 3.0, \( P = 0.05 \) for both), whereas no differences in concentrations of the other 2 markers were found \( (P = 0.47 \) and 0.98 for MPO and MRP-8/14). Furthermore, a significant difference in concentrations of PAPP-A was observed between patients with and without 3-vessel disease (median 4.0 vs 4.85 mIU/L, \( P = 0.03 \)) whereas the other 3 markers showed no differences in levels
MARKERS OF PLAQUE INSTABILITY IN THE DIAGNOSIS OF AMI

The diagnostic accuracy of all 4 markers of plaque instability at presentation for the diagnosis of AMI as quantified by the area under the ROC curve was low (AUC MPO 0.63, MRP-8/14 0.65, PAPP-A 0.62, CRP 0.59) and inferior to that of both cTnT (AUC 0.88) and hs-cTnT (AUC 0.96) (P < 0.001 for all comparisons). Use of the markers of plaque instability in combination with either cTnT or hs-cTnT did not increase the diagnostic accuracy provided by hs-cTnT alone (Table 3).

Subgroup analysis restricted to patients presenting within the first 3 h after the onset of symptoms revealed similar findings: the diagnostic accuracy of all 4 markers of plaque instability was low and inferior to both cTnT and hs-cTnT (all P < 0.001). Again, the combination of the markers with cTnT or hs-cTnT did not improve the diagnostic accuracy of hs-cTnT alone (all P > 0.05).

MARKERS OF PLAQUE INSTABILITY IN THE DIAGNOSIS OF ACS

Poor diagnostic performance of the 4 studied markers of plaque instability was also observed in acute coronary syndrome (i.e., AMI and unstable angina), with an AUC of 0.54 for MPO, 0.55 for MRP-8/14, 0.61 for PAPP-A, and 0.52 for CRP, all inferior to cTnT and hs-cTnT (AUC 0.70 and 0.84, P < 0.001 for all comparisons). The combination of the markers of plaque instability and hs-cTnT similarly did not improve the diagnostic accuracy compared to hs-cTnT alone (data not shown).

PROGNOSTIC VALUE OF MARKERS OF PLAQUE INSTABILITY FOR THE PREDICTION OF ALL-CAUSE DEATH

During a median follow-up time of 27 months, there were 39 deaths in the whole cohort (9.8%). Median concentrations of MPO, MRP-8/14, and CRP in deceased patients were significantly higher than those in survivors (MPO 136 (87–294) vs 92 (64–141) pmol/L, P < 0.001; MRP-8/14 6.0 (4.0–10.2) vs 3.9 (2.8–5.4) mg/L, P < 0.001; CRP 9.3 (4.8–28.8) vs 3.0 (3.0–4.8) mg/L, P < 0.001). There was no significant difference in PAPP-A levels in deceased patients and survivors [4.0 (4.0–8.1) vs 4.0 (4.0–5.9) mIU/L, P = 0.47].

The outcome of chest pain patients in terms of survival was significantly worse in patients in higher tertiles of MPO, MRP-8/14, and CRP concentrations at presentation (Fig. 2). Cumulative 24-month all-cause mortality rates were 3.2%, 8.2%, and 15.7% in patients in tertiles of MPO (P = 0.002); 4.9%, 5.3%, and 16.8% in tertiles of MRP-8/14 (P < 0.001); and 2.3%, 3.3%, and 20.7% in tertiles of CRP (P < 0.001). Tertiles of PAPP-A did not predict 24-month all-cause mortality rates (7.6%, 7.1%, 12.0%; P = 0.59).

Prediction of all-cause death during follow-up as assessed by ROC curve analysis showed similar prognostic accuracy for MPO, MRP-8/14, and CRP (AUC 0.67, 0.70, and 0.78) compared to hs-cTnT (0.74, P >

---

**Table 2. Correlation among the different markers of plaque instability as well as with the extent of myocardial necrosis as quantified by hs-cTnT.**

<table>
<thead>
<tr>
<th></th>
<th>MRP-8/14</th>
<th>PAPP-A</th>
<th>CRP</th>
<th>hs-cTnT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>r = 0.36, P &lt; 0.001</td>
<td>r = 0.31, P &lt; 0.001</td>
<td>r = 0.20, P &lt; 0.001</td>
<td>r = 0.18, P &lt; 0.001</td>
</tr>
<tr>
<td>MRP-8/14</td>
<td>r = 0.00, P = 0.99</td>
<td>r = 0.35, P = 0.49</td>
<td>r = 0.23, P &lt; 0.001</td>
<td>r = 0.18, P &lt; 0.001</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>r = 0.35, P &lt; 0.001</td>
<td>r = 0.35, P &lt; 0.001</td>
<td>r = 0.35, P &lt; 0.001</td>
<td>r = 0.35, P &lt; 0.001</td>
</tr>
<tr>
<td>CRP</td>
<td>r = 0.18, P &lt; 0.001</td>
<td>r = 0.18, P &lt; 0.001</td>
<td>r = 0.18, P &lt; 0.001</td>
<td>r = 0.18, P &lt; 0.001</td>
</tr>
</tbody>
</table>

---

**Table 3. Diagnostic accuracy of all biomarkers for the diagnosis of AMI.**

<table>
<thead>
<tr>
<th></th>
<th>AUC (95% CI)</th>
<th>AUC for combination with cTnT (95%)</th>
<th>P value</th>
<th>AUC for combination with hs-cTnT (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-cTnT</td>
<td>0.955 (0.932–0.977)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cTnT</td>
<td>0.877 (0.820–0.933)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>0.634 (0.585–0.681)</td>
<td>0.895 (0.861–0.923)</td>
<td>0.42</td>
<td>0.951 (0.924–0.970)</td>
<td>0.12</td>
</tr>
<tr>
<td>MRP-8/14</td>
<td>0.645 (0.595–0.692)</td>
<td>0.907 (0.874–0.933)</td>
<td>0.20</td>
<td>0.950 (0.924–0.970)</td>
<td>0.29</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>0.619 (0.570–0.667)</td>
<td>0.891 (0.856–0.919)</td>
<td>0.53</td>
<td>0.952 (0.926–0.971)</td>
<td>0.42</td>
</tr>
<tr>
<td>CRP</td>
<td>0.589 (0.539–0.638)</td>
<td>0.889 (0.854–0.918)</td>
<td>0.58</td>
<td>0.952 (0.926–0.971)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

6 Clinical Chemistry 58:1 (2011)
0.05 for all comparisons). PAPP-A showed lower prognostic accuracy (0.53) with a significant inferiority compared to hs-cTnT, MPO, MRP-8/14, and CRP ($P < 0.001$, $P = 0.008$, $P = 0.01$, $P < 0.001$).

By Cox proportional hazards analysis, values at presentation in the highest tertile of MPO, MRP-8/14, and CRP, but not PAPP-A, predicted all-cause mortality in univariable analysis (Table 4). This was also true after adjustment for clinical risk factors (age, arterial hypertension, dyslipidemia, diabetes mellitus, smoking, history of CAD, history of AMI, history of renal failure) and hs-cTnT in multivariable analysis. When all markers of plaque instability were entered into the model simultaneously, only CRP remained an independent predictor of death.

Reclassification tables for predicting all-cause mortality based on the clinical risk factors and hs-cTnT and after 1 of the 4 markers was added yielded a significant improvement in NRI (0.19, $P = 0.04$) and IDI (0.11, $P = 0.004$) when CRP was added and in NRI (0.14, $P = 0.03$) but not IDI (0.05, $P = 0.16$) when MRP-8/14 was added. The addition of MPO and PAPP-A resulted in no reclassification improvement (online Supplemental Table 1).

![Fig. 2. Kaplan–Meier curves for the cumulative risk of all-cause death in all chest pain patients (n = 398) according to the concentrations of the 4 markers of plaque instability.](image)
**Table 4.** Cox proportional hazard analysis for prediction of all-cause death during follow-up.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO &gt;121.7 pmol/L</td>
<td>2.71 (1.44–5.11)</td>
<td>0.002</td>
<td>2.46 (1.24–4.88)</td>
<td>0.01</td>
<td>1.58 (0.72–3.46)</td>
<td>0.25</td>
</tr>
<tr>
<td>MRP-8/14 &gt;4.84 mg/L</td>
<td>3.48 (1.82–6.63)</td>
<td>&lt;0.001</td>
<td>3.72 (1.78–7.75)</td>
<td>&lt;0.001</td>
<td>1.64 (0.72–3.74)</td>
<td>0.24</td>
</tr>
<tr>
<td>PAPP-A &gt;4.9 mlU/L</td>
<td>1.29 (0.68–2.46)</td>
<td>0.44</td>
<td>0.86 (0.43–1.72)</td>
<td>0.75</td>
<td>0.64 (0.30–1.36)</td>
<td>0.24</td>
</tr>
<tr>
<td>CRP &gt;3.8 mg/L</td>
<td>8.98 (4.13–19.56)</td>
<td>&lt;0.001</td>
<td>7.25 (3.21–16.38)</td>
<td>&lt;0.001</td>
<td>5.45 (2.24–13.26)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* cRF, clinical risk factors (age, arterial hypertension, dyslipidemia, diabetes mellitus, current smoking, history of coronary artery disease, history of myocardial infarction, history of renal failure).

**Prognostic Value of Markers of Plaque Instability for the Prediction of Cardiovascular Death**

Of the 39 deaths during follow-up, 19 (49%) were deaths from cardiovascular cause. Higher levels of MRP-8/14 (P = 0.01) and CRP (P < 0.001), but not MPO (P = 0.44) or PAPP-A (P = 0.65), were associated with increased risk for cardiovascular death (online Supplementary Fig. 2).

**Prognostic Value of Markers of Plaque Instability for the Prediction of Death or AMI**

The ability of markers of plaque instability to predict survival free of AMI was assessed in patients without AMI at index presentation (n = 322). There were 22 (7%) deaths and 23 (7%) first AMIs during follow-up, resulting in a combined endpoint of death or AMI in 39 (12%) patients. In Kaplan–Meier analysis, only tertiles of CRP significantly predicted survival free of AMI (P = 0.001), whereas this was not true for MPO, MRP-8/14, and PAPP-A (P = 0.99, P = 0.95, P = 0.56). In ROC curve analysis, the prognostic accuracy for survival free of AMI was low for all 4 markers of plaque instability (AUC MPO 0.49, MRP-8/14 0.52, PAPP-A 0.48, and CRP 0.63).

**Discussion**

This prospective international multicenter study examined the clinical utility of 4 markers of plaque instability (MPO, MRP-8/14, PAPP-A, CRP) in the early diagnosis and risk stratification of AMI in 398 consecutive patients presenting to the emergency department with acute chest pain. We report 4 major findings.

First, concentrations of all 4 biomarkers of plaque instability taken at emergency department presentation were significantly higher in patients with AMI than in patients with other diagnoses. Second, the diagnostic accuracy of these markers was low and inferior compared to both cTnT and hs-cTnT. None of the 4 markers improved diagnostic accuracy when used in combination with either cTnT or hs-cTnT. Third, MPO, MRP-8/14, and CRP, but not PAPP-A, predicted all-cause mortality with moderate accuracy and provided independent prognostic information over clinical risk factors and hs-cTnT. Using all markers together, CRP emerged as the strongest predictor of the 4 markers. Fourth, the markers of plaque instability showed only a low correlation with each other as well as with the extent of myocardial necrosis as quantified by hs-cTnT.

**Diagnostic Value of Markers of Plaque Instability**

These findings have implications for the clinical use of these 4 markers of plaque instability and extend both previous clinical and experimental studies (7, 8, 12, 13, 15, 21). The exact mechanisms leading to plaque instability, plaque fissure, and plaque rupture are incompletely understood. Histopathological studies have consistently indicated the central role of inflammation. MPO, MRP-8/14, PAPP-A, and CRP have all been shown to be expressed in atherosclerotic plaques and to contribute to vascular inflammation (7, 12, 15, 21). As such, they were proposed as new markers for early diagnosis of AMI, since their release may precede myocardial necrosis and could therefore identify patients with unstable coronary plaques who are at risk for AMI. Despite a sound pathophysiological hypothesis, our data highlight the limited diagnostic value of currently available peripheral blood biomarkers of plaque instability in the diagnosis of AMI. The following 2 aspects may at least partly account for this finding. First, inflammation is also common in many disease entities resulting in other cardiac or noncardiac causes of acute chest pain, including pleuritis, pneumonia, and gastroesophageal reflux. The pathophysiological processes quantified by the inflammatory bio-
markers studied may therefore be rather unspecific. Second, many patients with AMI had normal concentrations of the markers of plaque instability in peripheral blood. The magnitude of release from the coronary arteries may have been too small to substantially alter systemic concentrations. And third, 3 recent studies have raised serious concerns regarding the validity of most previous studies on MPO and PAPP-A by documenting that the use of heparin induces preanalytical bias and error in MPO and PAPP-A measurements. Heparin seems to mobilize both vessel-bound MPO and PAPP-A, thereby artificially increasing the concentrations of the 2 markers. Since we performed our study in heparin-naive patients, this may explain the difference in the diagnostic accuracy of MPO and PAPP-A compared to previous studies.

**PROGNOSTIC VALUE OF MARKERS OF PLAQUE INSTABILITY**

Discovery of reliable markers for short- and long-term risk stratification of a heterogeneous group of patients with chest pain remains an unmet need. In patients with ACS, cTnT is the most established marker for prediction of short- and long-term adverse cardiac events. However, increased cTnT already implies myocardial cell death. An ideal prognostic marker would identify patients at a stage where preventive or therapeutic measures might influence the course of the disease. Therefore, biomarkers that identify unstable plaques would be an excellent tool for risk stratification. In addition to the diagnostic findings, our study extends and corroborates previous studies evaluating the prognostic use of markers of plaque instability in patients with acute chest pain.

Several studies have shown the association between high MPO concentrations and stable CAD and unfavorable outcome in ACS. Concerning the prediction of all-cause mortality, the present study confirms the prognostic role of MPO in patients with ACS and extends previous findings by demonstrating the prognostic value of MPO also in an unselected cohort of patients with acute chest pain. However, higher concentrations of MPO were associated with neither increased risk for cardiovascular death nor AMI during follow-up.

The prognostic value of MRP-8/14 has not been studied prospectively yet. A case-control study among 237 patients enrolled in the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction (PROVE IT-TIMI 22) trial showed an increased risk of recurrent cardiovascular events and death with increasing quartiles of MRP-8/14 after adjustment for standard risk factors and CRP during a follow-up of 24 months. In our study, the prognostic accuracy of MRP-8/14 for the prediction of all-cause death was modest (AUC 0.70) and remained significant also after multivariable adjustment.

PAPP-A has been suggested as a prognostic marker in patients with stable CAD and in ACS. Iversen et al. showed in 415 patients admitted with chest pain and both normal ECG and normal biomarkers that high levels of PAPP-A predicted the risk for death and also AMI. Our results did not confirm these findings; in our unselected cohort of chest pain patients, there was no significant difference in PAPP-A concentrations in deceased patients and survivors, and PAPP-A accordingly did not predict all-cause mortality.

Many prospective studies have demonstrated a significant and independent association between CRP concentrations and future cardiovascular events in healthy individuals as well as in patients with ACS. We could affirm the prognostic value of CRP for all-cause as well as cardiovascular mortality, and in our data, CRP—the most established, most widely available and most inexpensive biomarker—had the highest prognostic accuracy.

Potential limitations of the current study merit consideration. First, given that increasing concentrations of MPO, MRP-8/14, PAPP-A, and CRP were linked to factors that are also linked to increased morbidity and mortality, residual confounding may possibly be present in the reported data. Second, it is unknown yet how an adverse outcome could be best avoided in patients identified at high risk based on increased concentrations of the markers of plaque instability. Third, we cannot comment on the diagnostic and prognostic accuracy among patients with terminal kidney failure requiring dialysis, since such patients were excluded from our study. Fourth, with further research regarding the mechanisms of plaque rupture, other markers of plaque instability with greater clinical value may be detected in the future.

In conclusion, measurement of currently available biomarkers of plaque instability in the peripheral blood does not seem to be helpful in the early diagnosis of AMI. These markers, however, may provide some incremental information in the risk stratification of patients with acute chest pain.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures or Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:
Employment or Leadership: A:S. Buser, Director, Blood Transfusion Centre, Swiss Red Cross.

Consultant or Advisory Role: C. Mueller, Roche, Brahms.

Stock Ownership: None declared.

Honoraria: T. Reichlin, Brahms, Roche; C. Mueller, Brahms, Roche, Abbott, Alero, Siemens.

Research Funding: T. Reichlin, research grants from the Swiss National Science Foundation (P304P3-136995), Swiss Heart Foundation, Department of Internal Medicine at University Hospital Basel, Alere.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


10 Clinical Chemistry 58:1 (2011)
Plaque Instability Markers in AMI


