

FASTTRACK Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes

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Plaques

Aims We investigated whether myeloid-related protein 8/14 complex (MRP8/14) expressed by infiltrating monocytes and granulocytes may represent a mediator and early biomarker of acute coronary syndromes (ACS).

Methods and results Immunohistochemistry of coronary thrombi was done in 41 ACS patients. Subsequently, levels of MRP8/14 were assessed systemically in 75 patients with ACS and culprit lesions, with stable coronary artery disease (CAD), or with normal coronary arteries. In a subset of patients, MRP8/14 was measured systemically and at the site of coronary occlusion. Macrophages and granulocytes, but not platelets stained positive for MRP8/14 in 76% of 41 thrombi patients. In ACS, local MRP8/14 levels [22.0 (16.2–41.5) mg/L] were increased when compared with systemic levels [13.4 (8.1–14.7) mg/L, $P = 0.03$]. Systemic levels of MRP8/14 were markedly elevated [15.1 (12.1–21.8) mg/L, $P = 0.001$] in ACS when compared with stable CAD [4.6 (3.5–7.1) mg/L] or normals [4.8 (4.0–6.3) mg/L]. Using a cut-off level of 8 mg/L, MRP8/14 but not myoglobin or troponin, identified ACS presenting within 3 h from symptom onset.

Conclusion In ACS, MRP8/14 is markedly expressed at the site of coronary occlusion by invading phagocytes. The occurrence of elevated MRP8/14 in the systemic circulation prior to markers of myocardial necrosis makes it a prime candidate for the detection of unstable plaques and management of ACS.

Introduction

Plaque rupture and erosion lead to coronary thrombosis and acute coronary syndromes (ACS). The use of troponin has facilitated the management of ACS.¹ However, although troponin is a sensitive and specific marker of myocardial necrosis, it provides only indirect information about unstable plaques. Indeed, troponin reflects myocardial necrosis due to micro-embolization of atherothrombotic material, which is a late event in ACS. This may explain why even some patients presenting with chest pain and negative troponin do experience cardiac events within the next 30 days.²

Early diagnosis and risk stratification of patients with non-cardiac chest pain, stable coronary artery disease (CAD), or ACS is of utmost importance. Markers of myocardial necrosis

only rise 2–3 h after symptom onset. As inflammation is an initiating event of plaque instability,³ markers of inflammation may provide insight into cellular processes linked to plaque instability and thrombus formation before myocardial necrosis ensues.

We hypothesized that myeloid-related protein 8/14 complex (MRP8/14), a marker of phagocyte activation,⁴ might identify ruptured plaques at an early stage. MRP8/14, also termed calprotectin, leukocyte protein L1 complex or cystic fibrosis antigen, is a heterodimer of two calcium-binding proteins (S100A8 and S100A9, also referred to as MRP8 and MRP14, or calgranulin A and B) involved in calcium-dependent signalling, cell differentiation, cell cycle progression, and cytoskeleton–membrane interactions.⁵ MRP8 and MRP14 are mainly expressed in cells of myeloid origin, particularly in monocytes and neutrophils.⁶ Upon phagocyte activation, MRP8 and MRP14 form the MRP8/14 complex, which translocates to the cytoskeleton and plasma

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membrane, where it is secreted.⁷⁻⁹ This is an early event during transendothelial migration and interaction of MRP-expressing neutrophils and monocytes with endothelium.¹⁰⁻¹³ Hence, MRP8/14 reflects phagocyte activation¹⁴ and recruitment which is involved in plaque destabilization.

Therefore we investigated whether in ACS, MRP8/14 is elevated at the site of coronary occlusion and expressed in coronary thrombi. To confirm this, we compared circulating levels of MRP8/14 in patients with ACS, stable CAD, or normal coronary arteries.

Methods

Study populations

The hypothesis was generated from immunohistochemical analysis of thrombi harvested from the site of unstable plaques in 41 patients with ACS and validated in a subsequent series of 75 patients, stratified according to clinical presentation and angiographic findings. In seven of these patients, and in an additional series of 10 patients, all with ACS, selected biomarkers were assessed simultaneously at the site of coronary occlusion and in the systemic circulation. According to this step-wise approach, the data acquisition covered a period from December 2004 to March 2006. The institutional Ethics Committee approved the protocol. Written informed consent was obtained from all patients.

Atherothrombotic material from the site of coronary occlusion

In 41 patients undergoing primary percutaneous coronary intervention (PCI) for ST-segment elevation myocardial infarction ($n = 35$; STEMI), or non-ST-segment elevation myocardial infarction ($n = 6$; NSTEMI), respectively, coronary thrombi were removed using an occlusion and aspiration system (PercuSurge™ GuardWire™).¹⁵ Clinical definitions were applied according to the Joint European Society of Cardiology-American College of Cardiology Committee for the Redefinition of Myocardial Infarction.¹⁶

Validation population

Patients were categorized into three groups based on clinical presentation and angiographic findings. The angiograms were reviewed by two independent observers blinded for the history.

- (i) *Normal coronary arteries.* This included 14 patients with angiographically normal coronary arteries. They underwent diagnostic angiography prior to valve surgery or due to atypical chest pain.
- (ii) *Stable CAD.* All 22 patients had angiographically confirmed CAD as defined by any stenosis of $\geq 50\%$ in any coronary artery. Patients presented either as stable angina or troponin T negative unstable angina pectoris (UAP) in the absence of an identifiable culprit lesion (see below).
- (iii) *Patients with ACS.* Thirty-nine patients presenting with STEMI ($n = 30$; 77%), NSTEMI ($n = 7$; 18%), or UAP ($n = 2$; 5%) and at least one culprit lesion, with at least two of the five angiographic criteria were included: (a) Ambrose lesions type IIb;^{17,18} (b) lesions with exulcerations or irregularities; (c) signs of thrombus formation (i.e. acute total occlusion, filling defects, haziness in the absence of calcifications, inhomogeneous opacification, contrast staining); (d) flow impairment distal to the lesion; or (e) impairment of the wall motion in the supplied territory. UAP was defined as typical chest pain at rest with the last episode occurring ≤ 24 h before admission and either a history of CAD or ischaemic ECG changes defined as ST-segment depression >0.05 mV, transient ST-segment

elevation exceeding 0.05 mV, or T-wave inversion >0.2 mV in two contiguous leads. Exclusion criteria were C-reactive protein (CRP) ≥ 10 mg/L, myocarditis, pericarditis, transient left ventricular apical ballooning, left ventricular ejection fraction $\leq 35\%$, or overt congestive heart failure.

Procedures

All patients underwent coronary angiography. Patients without or with stable CAD received conservative, interventional, or surgical treatment. Patients with ACS underwent PCI. Blood samples were taken prior to angiography and during PCI in the subgroup with local sampling.

Systemic and local blood sampling

Arterial samples were obtained from the femoral sheath. In the 17 ACS patients with local sampling, an additional aortic sample prior to PCI and a local sample from the site of coronary occlusion were retrieved using a balloon-based temporary occlusion and aspiration system as it has been described previously.¹⁵ Patients with local sampling all presented with STEMI, acute total occlusion, and poor flow (\leq TIMI grade I)¹⁹ after wire passage.

Parameters and biochemical assays

The following markers were assessed: MRP8/14, albumin, myoglobin, creatine kinase (CK), MB fraction of CK (CK-MB), troponin T, C-reactive protein, as well as PAPP-A, and sCD40-ligand (separate series with local vs. systemic analysis only, $n = 10$). MRP8/14 was detected in serum using a sandwich ELISA (Bühlmann Laboratories, Schönenbuch, Switzerland) with an inter-assay variation of 7.2% and a detection limit of 3.0 $\mu\text{g/L}$. The specific monoclonal antibody for the MRP8/14 heterodimer (mAb 27E10) was used as primary antibody and a polyclonal antibody coupled with horseradish peroxidase was used as secondary antibody. The antibody is specific for the MRP8/14 heterodimer or higher order complexes and does not bind MRP8 or MRP9 monomers.^{20,21} Albumin, myoglobin, CK, and troponin T were analysed on a Roche Modular Analytics System (Roche Diagnostics, Rotkreuz, Switzerland) with an inter-assay variation of 2.8%, 4.4%, 1.0%, and 3.7%, respectively. Corresponding cut-off values of myoglobin, troponin T, and CK-MB levels were >72 $\mu\text{g/L}$, >0.03 $\mu\text{g/L}$, and >24 U/L, respectively. C-reactive protein was determined on an Immulite 2000 chemiluminescence analyzer (Diagnostic Products Corporation, Los Angeles, USA) with an inter-assay variation of 6.3% and a detection limit of 0.1 mg/L. PAPP-A was measured by an immunometric assay based on time resolved amplification cryptate emission (Brahms, Henningsdorf, Germany) with a sensitivity of 0.004 IU/L, an intra- and inter-assay coefficient of variance $<2\%$, and a normal range <0.01 IU/L. sCD40L was determined by ELISA (Quantikine; R&D Systems) with a lower limit of detection <0.004 $\mu\text{g/L}$, an intra- and inter-assay coefficient of variance $<7\%$, and a normal range of 0.68–38.4 $\mu\text{g/L}$.

Immunohistochemistry

Solid material was kept in 4% buffered paraformaldehyde for 4–6 h and in 50% ethanol for 24 h prior to paraffin embedding (Leica EG1160) and xylol/ethanol fixation (Hypercenter XP, Shandon), and cutting with a microtome (Leica RM2035). Eosin-hematoxylin as well as Masson-Goldner's trichrome stain were performed in all cases for general orientation and identification of tissues and structures. For immunohistochemistry, the slides were treated with trypsin for 1 h and blocked with 4% fat-free milk in Tris-HCl pH 7.6 for 30 min. Monoclonal murine antibodies to human MRP8/14 (IgG1, clone 27E10, Acris Antibodies GmbH, Hiddenhausen, Germany)²¹ was used at a concentration of 1 $\mu\text{g/mL}$ and incubated with the slides at 4°C for 18 h. Murine IgG1 served as negative

control. After washing, biotinylated AffinityPure F(ab')₂ fragment goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratory, Cambridgeshire, UK) in 5% horse serum and 1% BSA were added to the slide and incubated for 30 min at 22–24°C. After washing, alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratory) was added and incubated for 30 min at 22–24°C. The staining was developed using the Dako Fast Red Substrate System (DakoCytomation, Glostrup, Denmark). For myeloperoxidase (MPO), polyclonal rabbit antibodies to human MPO (Dako Cytomation, A0398, Glostrup, Denmark) were used at a concentration of 1 µg/mL.

Data analysis and statistics

For each group the following calculations were performed: Number of cases, mean, standard deviation, standard error of the mean, minimum, maximum, and 95% confidence interval (CI) for the mean, medians [interquartile range (IQR)], Levene's test for homogeneity of variance, analysis of variance table, and robust tests of the equality of means for each dependent variable, *post hoc* range tests, and multiple comparisons to control the family-wise error rate (one-way ANOVA with *a posteriori* tests like Tamhane's T2, Dunnett's T3, Games-Howell, or Dunnett's C for significance level 0.05, equal variances not assumed). In case of doubts in the assumptions we used the Kruskal–Wallis test. To calculate the power (before and after the study) we used ANOVA with one of the following three multiple-comparison methods: Tukey's all pairs (MCA), comparisons with the best (MCB), and Dunnett's all vs. a control (MCC); and in case of parallel groups of patients with ACS and no ACS (=no CAD+stable CAD), one-/two-sided *t*-tests and one-sided ROC-tests, respectively. We used the Kolmogorov–Smirnov goodness-of-fit test to check the normality. ROC, χ^2 statistics with Bonferroni adjustment, CART, and C4.5 with 10-fold cross-validation were used to assess the quality of the discriminatory power of the test using sensitivity and specificity data and to identify optimal splits for MRP8/14 with 95% CI. In order to estimate the area under the ROC curve (AUC—a numerical measure of the accuracy of the model), we used the empirical (non-parametric) method by

DeLong *et al.* and validated this result by bootstrapping. Two-tailed Spearman's rank-correlation test was used to find associations between systemic arterial levels of MRP8/14 and C-reactive protein, as well as other continuous variables such as leukocyte count, and so on.

Results

Immunohistochemistry of atherothrombotic material from the site of coronary artery occlusion

Thrombi were obtained from patients with STEMI or NSTEMI and signs of coronary thrombosis on angiography (for details, see Supplementary data). In 31 (76%) of 41 patients, locally collected atherothrombotic material stained positive for MRP8/14. MRP8/14 co-localized with CD68-positive monocytes/macrophages and CD66b-positive granulocytes (Figure 1, see Supplementary data for details, including Supplementary material online, Figure S6). Atherothrombotic material was inhomogeneously structured, showing conglomerates of fibrin, erythrocytes, and white blood cells, consistent with red thrombus besides more amorphous areas. These sections with granular appearance and interposed layers of fibrin mainly represented platelet aggregates, and to some extent also plaque components, if there were additional less structured and more eosinophilic areas. The amorphous areas were surrounded by clusters of MRP8/14 positive cells, whereas single monocytes/macrophages and neutrophils scattered within red thrombi were mostly MRP8/14 negative. Similarly, the few CD3-positive lymphocytes stained negative for MRP8/14. Separate immunohistochemistry for MPO (see Supplementary material online, Figure S7) showed positive staining in most granulocytes and monocytes within the retrieved material ($n = 30$).

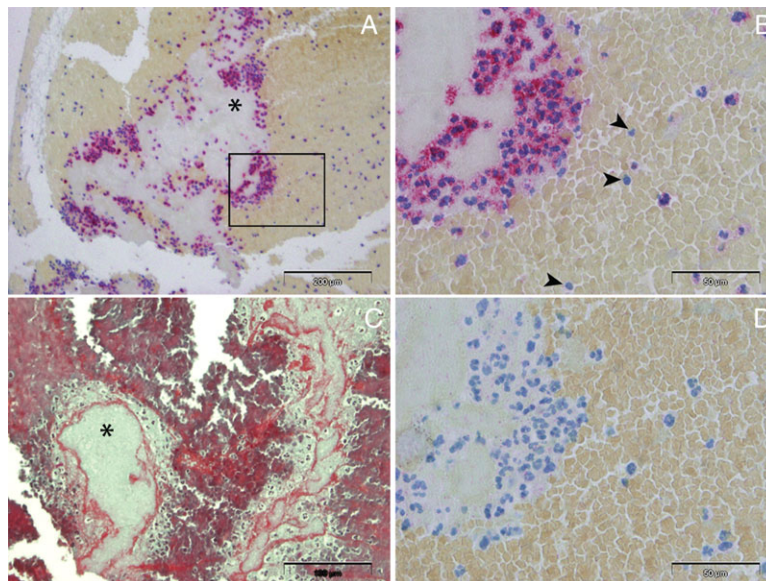


Figure 1 Myeloid-related protein 8/14 complex (MRP8/14) in the aspirate recovered from the site of coronary occlusion in acute coronary syndromes (ACS). Formation of the MRP8/14 complex was detected immunohistochemically by staining with a monoclonal murine antibody to human MRP8/14. Parts (A) and (B) show MRP8/14 positive monocytes/macrophages and granulocytes (red staining) forming clusters around amorphous areas (asterisk), mainly consisting of aggregated platelets, whereas single leukocytes, scattered within fibrin- and erythrocyte-rich sections (red thrombus) predominantly were MRP8/14 negative (arrows). In Part (C), components of recovered atherothrombotic material are visualized by trichrome stain (Masson–Goldner), rendering fibrin red, erythrocytes grey, and platelet aggregates greyish. Part (D) shows a negative control stained with murine IgG1 of the corresponding section shown in (B). Magnification was $\times 100$ in (A) and (C), and $\times 400$ in (B) and (D), respectively.

Patient characteristics of the validation population

Baseline characteristics of 75 consecutive patients stratified according to clinical presentation are summarized in Table 1. In ACS, troponin T levels peaked at 5.1 (1.7–9.6) µg/L. Culprit lesions were total occlusions in 27 (69%) patients, whereas in seven (18%) a non-occlusive thrombus with either an Ambrose lesion type IIb or flow impairment was seen. Five (13%) patients had exulcerations/irregularities or Ambrose type II lesions with flow impairment or wall motion abnormalities of the corresponding segment. Median time from the index clinical event to angiography was 299 (204–523) min.

Local vs. systemic levels of myeloid-related protein 8/14 complex in patients with acute coronary syndromes

At the site of coronary occlusion, MRP8/14 levels were markedly increased compared with the systemic circulation [22.0 (16.2–41.5) vs. 13.4 (8.1–14.7) mg/L, $P = 0.03$; Figure 2]. In

contrast, local C-reactive protein levels tended to be decreased [1.5 (0.5–1.8) mg/L vs. 1.8 (0.5–1.8) mg/L; $P = 0.06$].

For comparison, serum levels of the recently suggested biomarkers sCD40L and PAPP-A did not differ significantly between the aorta and the site of coronary occlusion in patients with ACS [sCD40L 142.8 (62.8–851.9) vs. 145.8 (113.8–629.41) pg/mL, median (IQR), $P = 0.7$; PAPP-A 85.2 ± 45.2 mIU/L vs. 76.8 ± 31.9 mIU/L, mean \pm SD, $P = 0.6$; Figure 3]. This series ($n = 10$) was investigated separately from the validation study.

Systemic levels of myeloid-related protein 8/14 complex according to diagnosis

Patients with ACS showed a highly significant increase in systemic levels of MRP8/14 when compared with those having normal coronaries or stable CAD [median (IQR): 15.1 (12.1–21.8) vs. 4.8 (4.0–6.3) vs. 4.6 (3.5–7.1); $P = 0.001$, Figure 4; mean \pm SD: 19.2 ± 12.9 vs. 5.4 ± 2.5 vs.

Table 1 Baseline characteristics of patients in the validation study

Characteristic	No CAD ($n = 14$)	Stable CAD ($n = 22$)	ACS ($n = 39$)	P -value
Demographics				
Age (years)	60.4 \pm 13.9	66.1 \pm 12.4	58.4 \pm 12.0	0.07
Male, no. (%)	10 (71)	17 (77)	30 (77)	0.91
Risk factors				
Hypercholesterolaemia, no. (%)	3 (21)	15 (68) [†]	19 (49)	0.02
Hypertension, no. (%)	7 (50)	15 (68)	15 (38)	0.08
Diabetes mellitus, no. (%)	2 (14)	5 (23)	3 (8)	0.26
Family history, no. (%)	6 (43)	6 (27)	9 (23)	0.39
Smoking, no. (%)	1 (7)	7 (32)	19 (49) [†]	0.02
PROCAM-Score (%)	4.0 (1.0–17.0)	8.5 (4.0–16.0)	7.0 (2.5–13.0)	0.42
Prior medication				
Aspirin, no. (%)	9 (64)	18 (82)	5 (13)*	<0.001
Beta-blockers, no. (%)	8 (57)	17 (77)	7 (18)*	<0.001
Angiotensin converting enzyme-inhibitor/ATB, no. (%)	6 (43)	8 (36)	7 (18)	0.26
Statin, no. (%)	6 (43)	15 (68)	7 (18)*	<0.001
Clinical presentation				
Emergency, no. (%)	4 (29)	2 (9)	39 (100)*	<0.001
Electrocardiography				
Normal, no. (%)	8 (57)	7 (31)	1 (3)*	<0.001
ST-elevation, no. (%)	0	0	29 (74)*	<0.001
Other ST changes, no. (%)	6 (43)	15 (68) [‡]	9 (23)	0.003
Laboratory values				
Leukocytes ($\times 1000/\mu\text{L}$)	6.9 \pm 0.8	7.3 \pm 1.6	10.7 \pm 3.6*	<0.001
C-reactive protein (mg/L)	1.8 (1.1–3.3)	1.5 (0.9–3.6)	1.8 (0.8–4.1)	0.99
Creatinine ($\mu\text{mol/L}$)	87.6 \pm 12.2	94.2 \pm 15.9	87.9 \pm 15.9	0.25
Angiographic findings				
One-vessel disease, no. (%)	0	5 (23)	20 (51)*	0.001
Two-vessel disease, no. (%)	0*	9 (41)	10 (26)	0.02
Three-vessel disease, no. (%)	0*	8 (36)	9 (23)	0.04
Chronic occlusions, no. (%)	0	7 (32)*	3 (8)	0.008
Calcifications, no. (%)	0	14 (64)*	7 (18)	<0.001
Left ventricular ejection fraction (%)	69.5 (63.0–72.3)	62.0 (56.0–67.5)	53.5 (45.8–58.3)*	<0.001

No CAD: denotes patients without evidence of coronary artery disease. Stable CAD: includes patients with documented coronary artery disease (CAD) presenting with either stable angina or with acute chest pain in the absence of an identifiable culprit lesion. ACS: denotes patients with myocardial infarction or unstable angina pectoris and at least one clearly identifiable culprit lesion, as defined by morphological aspects, signs of coronary thrombosis, and flow impairment in the corresponding coronary artery ($n = 39$).

* $P < 0.05$ for comparison with the other two groups.

[†] $P < 0.05$ for comparison with the no CAD group.

[‡] $P < 0.05$ for comparison with the ACS group.

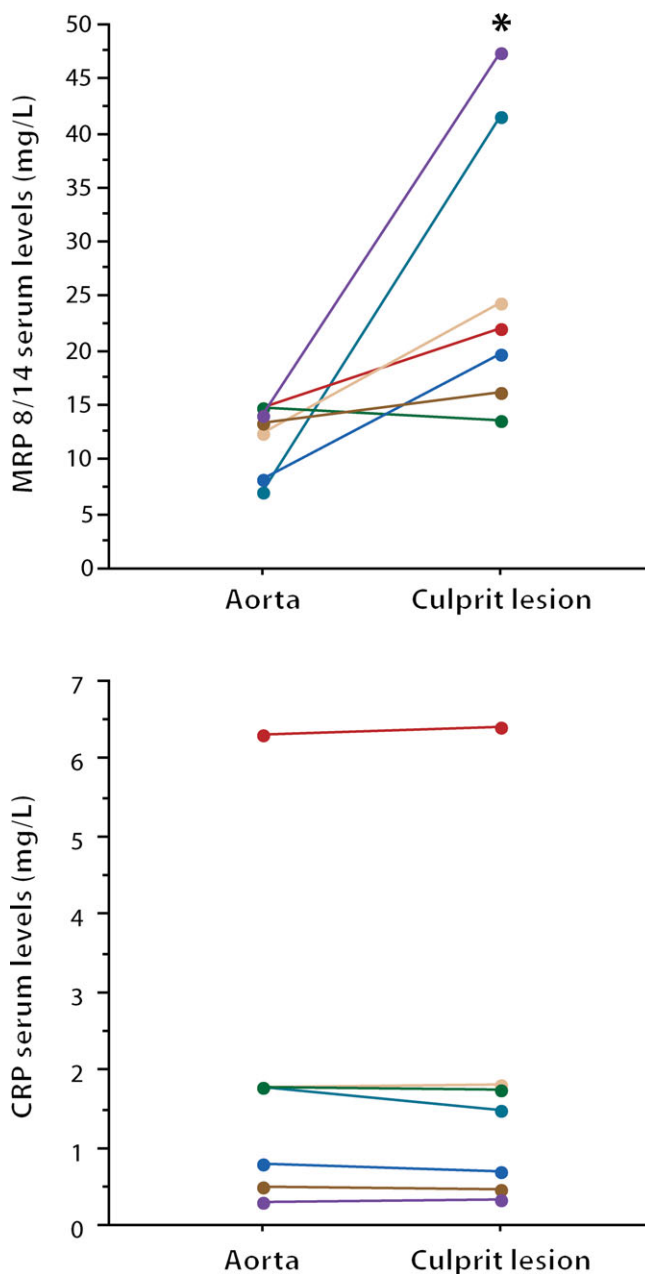


Figure 2 Serum concentrations of MRP8/14 (upper panel) and C-reactive protein (lower panel) at the site of the coronary occlusion (culprit lesion) compared with systemic values taken from the aorta in patients with acute coronary syndromes. Local levels of MRP8/14 are significantly elevated ($n = 7$; $*P = 0.03$).

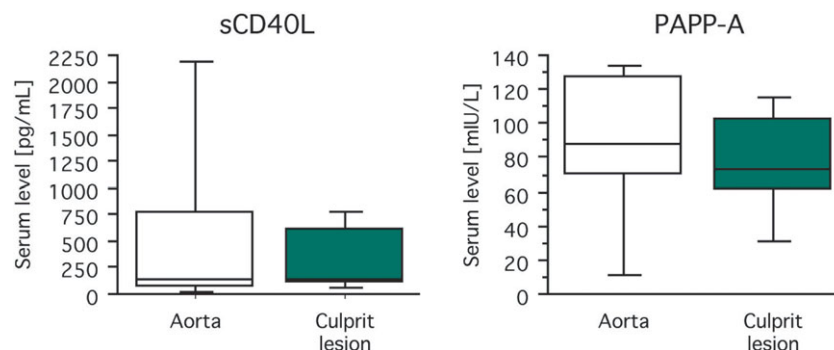


Figure 3 Serum concentrations of sCD40L (left panel) and PAPP-A (right panel) at the site of coronary occlusion (culprit lesion) compared with systemic values obtained from the aorta in 10 patients with myocardial infarction. For both biomarkers no significant differences were found.

5.4 ± 2.8 mg/L, $P = 0.001$]. Patients with normal coronaries showed no differences in the systemic levels of MRP8/14 compared with those having stable CAD ($P = 1.00$). The study achieved 100% power to detect the above differences (significance level 0.05). ACS patients were split into those with or without positive troponin T values at presentation. MRP8/14 both in troponin negative ($n = 17$) as well as troponin positive ($n = 22$) patients was elevated compared with the two other groups [ACS TnT negative: 13.0 (10.1–21.4) mg/L and ACS TnT positive: 17.0 (14.0–24.8) mg/L vs. 'no CAD': 4.8 (4.0–6.3) mg/L and 'stable CAD': 4.6 (3.5–7.1) mg/L; for all $P = 0.001$].

In ROC analysis, a cut-off value of 8.0 mg/L for MRP8/14 discriminated patients with ACS with a sensitivity of 95%, a specificity of 92%, and an area under the curve (AUC) of 0.97 (95% CI: 0.913, 0.989). Using bootstrap AUC estimation (3000 random curves) we obtained a bootstrap AUC of 0.96 (bootstrap 95% CI: 0.916, 0.994). χ^2 statistics with Bonferroni adjustment, CART, or C4.5 with 10-fold cross-validation delivered similar cut-off values for MRP8/14 (8.1, 8.0, and 7.8).

Myeloid-related protein 8/14 complex and symptom onset

Using a cut-off value ≥ 8.0 mg/L MRP8/14 identified all ACS patients presenting within 12 h after symptom onset, including those presenting within 0–3 h [0–3 h ($n = 4$), 3–6 h ($n = 11$), and 6–12 h ($n = 9$)]. In contrast, 0–3 h after symptom onset, markers of necrosis such as myoglobin, troponin T, and CK-MB were only positive in 75%, 25%, and 0% of the cases, respectively. Between 3 and 6 h after symptom onset, 66%, 44%, and 38% of the patients, respectively, showed elevated values of markers of necrosis (Figure 5).

Myeloid-related protein 8/14 complex in multivariate analysis

In multivariate analysis, MRP8/14 was the most important parameter to discriminate patients with ACS (adjusted $P = 0.001$). In contrast, C-reactive protein did not discriminate between ACS [1.8 (0.8–4.1) mg/L], stable CAD [1.5 (0.9–3.6) mg/L], or normal coronary arteries [1.8 (1.1–3.3) mg/L, adjusted $P = 1.000$]. There was no correlation between MRP8/14 and C-reactive protein (correlation coefficient $\rho = 0.048$; $P = 0.686$), a moderate correlation of MRP8/14 with leukocyte counts was observed in ACS ($\rho = 0.482$; $P = 0.001$), whereas CK-MB ($\rho = 0.237$;

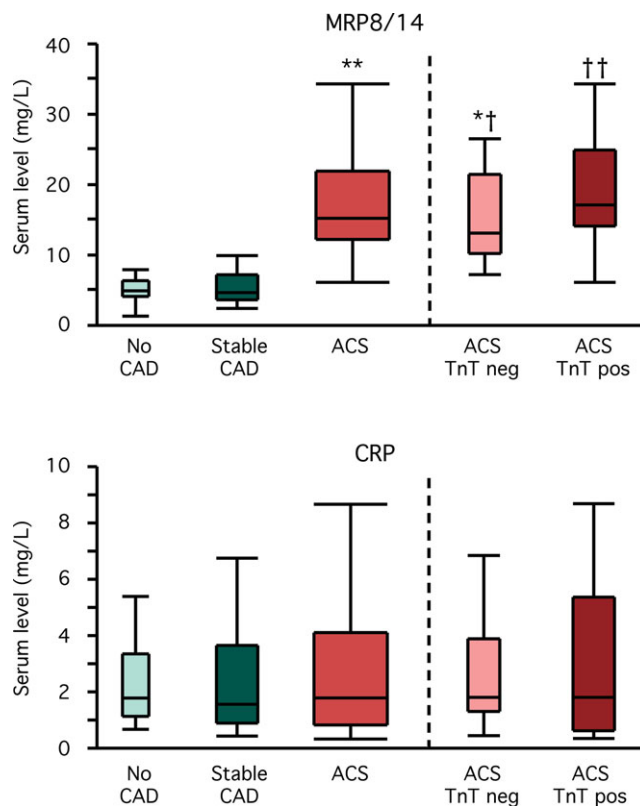


Figure 4 Systemic levels of MRP8/14 (upper panel) and C-reactive protein (lower panel) in three different groups of patients classified according to clinical presentation and coronary angiography. No CAD denotes patients without evidence of coronary artery disease ($n = 14$). Stable CAD stands for patients with documented coronary artery disease ($n = 22$). Patients with ACS ($n = 39$) show significantly elevated levels of MRP8/14 compared with the other two groups (** $P = 0.001$). At the right side of the dashed line, ACS patients are split into those with or without troponin T (referred to as 'ACS TnT negative', $n = 17$ and 'ACS TnT positive', $n = 22$). All ACS groups showed significantly elevated levels of MRP8/14 when compared with the two other groups ($P = 0.001$).

$P = 0.163$), myoglobin ($\rho = 0.261$; $P = 0.113$), and troponin T ($\rho = -0.260$; $P = 0.115$) did not correlate.

Discussion

This study for the first time demonstrates that MRP8/14, a heterodimeric protein complex expressed by activated monocytes and granulocytes, is elevated in ACS at the site of coronary occlusion and in the systemic circulation. Importantly, MRP8/14 was increased prior to necrosis markers such as myoglobin, CK-MB, and troponin, and separated patients with ACS from those with stable or no CAD. This makes this protein a prime candidate and a novel biomarker of unstable plaques in patients with chest pain.

Culprit lesions in ACS contain both plaque-derived tissue and thrombus. Accordingly, immunohistochemistry of atherothrombi collected at the site of coronary occlusion, consisted of platelets, fibrin, activated monocytes/macrophages, other blood cells, and plaque components. The vast majority of thrombi recovered from the coronary occlusion contained MRP8/14 positive cells. In addition, MRP8/14 was markedly elevated at the culprit lesion when compared with systemic levels. Thus, MRP8/14 appears to be produced locally at the site of coronary occlusion.

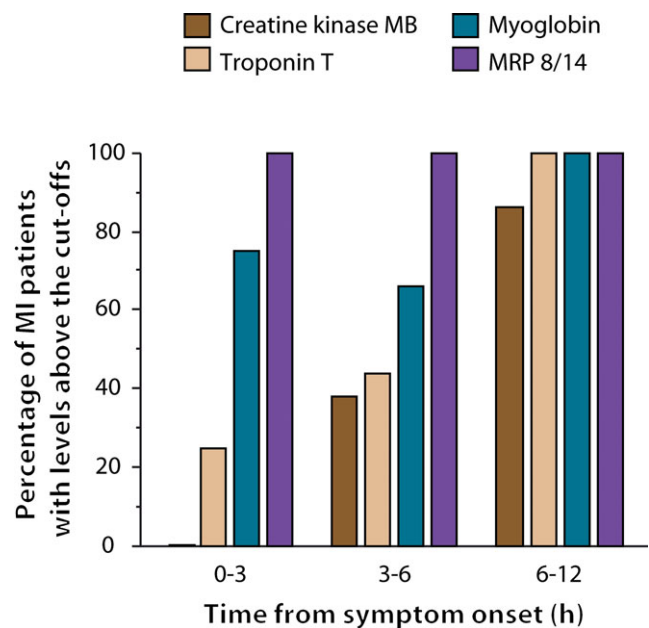


Figure 5 Percentage of patients with myocardial infarction (MI) and levels above the respective cut-offs for MRP8/14 and the classical markers of myocardial necrosis in relation to time (h; hours) from symptom onset to blood sampling. The number of patients for the three different time intervals are 4, 11, and 9, respectively. Patients presenting after 12 h are not shown.

MRP8/14 is expressed by myeloid-derived phagocytes, i.e. monocytes and neutrophils, which are activated in ACS. Cells positive for MRP8/14 in atherothrombi were predominantly activated CD68-positive monocytes/macrophages and CD66b-positive granulocytes. The triggers for induction of MRP8/14 synthesis and excretion in ACS remain to be determined, but may involve oxidative stress, cytokines, and acute phase proteins.²² MRP8/14 positive cells clustered around MRP8/14 negative platelet aggregates. In contrast, single monocytes and neutrophils scattered within the regions of aggregated erythrocytes were mainly MRP8/14 negative, as were CD3-positive lymphocytes. Others have found elevated transcripts of MRP14 (S 100A9) in circulating platelets of STEMI patients.²³ Our observation that platelets within coronary thrombi did not stain positive for the MRP8/14 complex suggests a specific local formation of the heterodimer by monocytes/macrophages and granulocytes, but not by platelets at the site of unstable plaques.

In line with the biological role of monocytes/macrophages in unstable plaques, secretion of MRP8/14 was an early event. Indeed, MRP8/14 was detectable immediately after symptom onset, while myoglobin, troponin, and CK-MB were negative in many patients. The late onset of established markers is explained by the fact that they are released once myocardial necrosis has occurred, while MRP8/14 appears to reflect initial cellular events. Detection of ACS prior to myocardial necrosis would be advantageous. Obviously, this has to be confirmed in large trials.

The diagnostic challenge in ACS lies in a persisting gap in the early and sensitive stratification of patients with chest pain. Many of them may have atypical chest pain, stable angina, or symptoms related to non-cardiac diseases. Therefore, mediators of inflammation, proteases implicated in plaque destabilization, and markers of platelet activation have been proposed.²⁴⁻²⁶ As reported,²⁵ C-reactive protein

did not separate patients with or without ACS also in our study. Thus, acute phase proteins are prognostic rather than diagnostic markers in this population.^{27,28} However, MRP8/14 exhibited an excellent diagnostic sensitivity and specificity. Importantly, MRP8/14 was increased at the site of coronary occlusion, indicating its involvement in this process. In contrast, neither was the C-reactive protein¹⁵ elevated at the site of the culprit lesion, nor were the recently suggested markers PAPP-A, which is expressed in unstable plaques,²⁵ or sCD40 ligand. This makes these markers unlikely mediators of plaque destabilization.

MPO is released after leukocyte activation²⁴ and predicts early risk of re-infarction.²⁹ As MRP8/14, MPO is systemically elevated prior to necrosis markers.^{29,30} We found it abundantly expressed in granulocytes within thrombi reflecting its constitutive expression (see Supplementary material online). Whether its levels at the site of plaque rupture are increased remains to be determined. So far, only MRP8/14 is expressed in activated phagocytes and present both in atherothrombi and in blood at the site of coronary occlusion. Hence, MRP8/14 is likely to reflect biological events in plaque destabilization. Independent of their diagnostic value, markers of ACS have been associated with prognosis. At least in healthy women, also MRP8/14 appears to predict the risk of future cardiovascular events independent of C-reactive protein and lipids.²³ The prognostic impact of MRP8/14 in unselected patients with chest pain as well as in the population as a whole, however, remains to be determined.

It is the strength of this study that all patients were characterized clinically, biochemically, and angiographically. Furthermore, all ACS underwent PCI. In contrast to previous observations,²³ patients with normal coronary arteries or stable CAD showed similar MRP8/14 levels, while they were markedly elevated in ACS. Differences in patient characteristics between this clinical study and the epidemiological cohort study²³ may account for this phenomenon. On the other hand, we acknowledge the limitations of the study (small number of patients, sub-protocols, and various patient subsets), which are characteristic for an observational study designed as first proof of a newly generated hypothesis—a phase I–II study in the architecture of diagnostic research.³¹ Further studies are required to elucidate the full diagnostic and prognostic potential of MRP8/14 in patients with chest pain.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Clinical vignette

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The Annexin code: revealing endocarditis

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A 54-year-old woman presented to our hospital after an episode of fever of unknown origin. The fever had occurred 6 weeks prior to presentation and had subsided spontaneously. The patient had no relevant prior medical history. At physical examination, a holosystolic murmur was heard on the apex. Echocardiography showed mitral regurgitation and a mass attached to the mitral valve (Panel A). Multiple blood cultures were taken at the time of admission.

We and other groups have shown the use of radiolabelled Annexin A5 imaging in detection of phosphatidylserine (PS) exposure. Several components in infective endocarditis may result in PS exposure, including activated macrophages, activated platelets, and programmed cell death. Therefore, we hypothesized that Annexin A5 imaging could be of help to image inflammatory activity in the heart.

One day after admission, using a dual isotope technique (thallium and tc99m-Annexin A5), nuclear imaging showed intense Annexin A5 uptake within the left ventricle in the area of the mitral valve. After 9 days of incubation, blood cultures showed *Streptococcus milleri*. In spite of adequate antibiotic treatment, patient developed increasing signs of congestive heart failure and was sent for mitral valve replacement 2 weeks after admission. Immunostaining of the valvular tissue showed abundant Annexin A5 binding (Panel B), which co-localized with CD68 positive cells staining, indicative of macrophages. In conclusion, this case suggests that molecular imaging of inflammatory activity using Annexin A5 may help to diagnose infective endocarditis before blood cultures become diagnostic (Panel C).

Panel A. Parasternal long-axis view of the left ventricle. Arrowhead indicates mass attached to the mitral valve. LV, left ventricle; Ao, Aorta.

Panel B. Top panel shows extensive binding of Annexin A5 to cells. Bottom panel shows co-localization with macrophages. Brown, Annexin; blue, macrophage marker (CD68).

Panel C. Dual isotope imaging. Top row: thallium images. Short-axis view of the left ventricle at the level of the mitral valve. Note the absence of thallium uptake in the septal part of the top right image, indicating insertion of the aorta. Bottom row: Annexin images. Arrowhead indicates the uptake of radiolabelled Annexin in the area of the mitral valve. L, liver; S, spleen.

