Release Kinetics of Inflammatory Biomarkers in a Clinical Model of Acute Myocardial Infarction

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Rationale: Inflammation in the setting of acute myocardial infarction (MI) has been linked to risk stratification; however, the release kinetics of inflammatory biomarkers in patients with acute MI has been difficult to establish.

Objective: The aim of this study was to determine the kinetics of changes in the levels of several biomarkers specifically linked to inflammation after transcoronary ablation of septal hypertrophy, a procedure that mimics acute MI.

Methods and Results: We analyzed release kinetics of C-reactive protein, high-sensitivity C-reactive protein, interleukin-6, soluble CD40 ligand, and peripheral blood leukocyte subsets in patients (n=21) undergoing transcoronary ablation of septal hypertrophy. Blood samples were collected before transcoronary ablation of septal hypertrophy and at various times after transcoronary ablation of septal hypertrophy. Serum levels of C-reactive protein were increased at 24 hours (1.0 mg/dL [interquartile range [IQR], 0.7–1.75] versus 0.2 mg/dL [IQR, 0.1–1.05] at baseline [BL]; P<0.001), whereas high-sensitivity C-reactive protein increased as early as 8 hours (2.68 mg/L [IQR, 1.23–11.80] versus 2.17 mg/L [IQR, 1.15–5.06] at BL; P=0.002). Interleukin-6 was significantly increased at 45 minutes (2.59 pg/mL [IQR, 1.69–5.0] versus 1.5 pg/mL [IQR, 1.5–2.21] at BL; P=0.002), and soluble CD40 ligand was significantly decreased at 60 minutes (801.6 pg/mL [IQR, 675.0–1653.5] versus 1750.0 pg/mL [IQR, 1151.0–2783.0] at BL; P=0.016). Elevated counts of polymorphonuclear neutrophils were detectable at 15 minutes, with a significant increase at 2 hours (641.5 cells/μL [IQR, 5288–7827] versus 4697 cells/μL [IQR, 2892–5620] at BL; P=0.004). Significant monocytosis was observed at 24 hours (729 cells/μL [IQR, 584–1344] versus 523 cells/μL [IQR, 369–701] at BL; P=0.015).

Conclusions: Interleukin-6 and neutrophil granulocytes showed a continuous rise at all prespecified time points after induction of MI. Our results provide valuable additional evidence of the diagnostic value of inflammatory biomarkers in the setting of early acute MI. (Circ Res. 2015;116:867-875. DOI: 10.1161/CIRCRESAHA.116.304653.)

Key Words: acute myocardial infarction ■ inflammation ■ leukocytes

Cardiovascular disease, including acute myocardial infarction (AMI), is the leading cause of death in Western countries.1 Prolinflammatory cytokines like interleukin-6 (IL-6) and acute-phase proteins such as C-reactive protein (CRP) are upregulated in patients with AMI.2,3 Studies have suggested that using data on levels of biomarkers such as soluble CD40 ligand (sCD40L) in combination with cardiac troponins might helpful in the diagnosis of patients with acute coronary syndromes.4,5 Several studies have also reported that inflammation is associated with long-term outcome in patients with acute coronary syndrome.7,8 IL-6 has an effect on cardiac function, acting through various mechanisms including the induction of cardiomyocyte apoptosis.10 In addition, IL-6 concentrations are affected by circadian rhythm and physical exercise.11,12 Furthermore, data are emerging that support an association between CRP elevation and culprit lesion plaque rupture in acute coronary syndrome,13,14 which is triggered by inflammatory responses and plaque degradation.15

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In this context, high-sensitivity (hs) assays for CRP have been developed that provide both diagnostic and prognostic information.2,15,17 Studies have shown that hs assays detect CRP concentrations with the required precision even in patients...
with stable coronary artery disease.\textsuperscript{18} Hs-CRP is known to be an independent predictor of recurrent events, including myocardial infarction (MI), restenosis after percutaneous coronary intervention, and death.\textsuperscript{15,18}

However, thus far there have been no studies published that have addressed the differences in inflammation kinetics in patients with AMI. Several animal studies provided information about the release kinetics of inflammatory biomarkers during the course of AMI\textsuperscript{19}; however, these findings cannot be directly extrapolated to patients because of different physiological parameters (eg, metabolism, release, interindividual variability) or proinflammatory effects of surgical procedures performed to induce myocardial infarction.\textsuperscript{20–23} Because of the imprecise definition of the exact time point of the beginning of myocardial ischemia and the patient-related delay before presentation to the hospital, the early release kinetics of hs-CRP and IL-6, and sCD40L as well as the initial kinetics of circulating leukocyte subsets after human AMI are entirely unknown. Therefore, the objective of the present study was to characterize the time course of inflammatory biomarkers and early shifts of circulating myeloid cell subsets in patients undergoing transcoronary ablation of septal hypertrophy (TASH) as a model for patients with AMI.\textsuperscript{24}

### Methods

#### Study Design

From January 2010 to June 2011, 21 consecutive patients with hypertrophic obstructive cardiomyopathy undergoing TASH were included in the study. Pre- and postprocedural management of the patients has been published recently.\textsuperscript{24,25} In brief, clinical history, physical examination, 12-lead ECG, laboratory tests, echocardiography, and coronary angiography for all patients were assessed. The final diagnosis of hypertrophic obstructive cardiomyopathy was made according to the current guidelines based on severe symptoms during physical activity, asymmetrical septal hypertrophy >15 mm, systolic movement of the anterior mitral valve leaflet, and an intraventricular pressure gradient of 30 mm Hg at rest and >50 mm Hg after provocation by the Valsalva maneuver.\textsuperscript{26} All patients received analgesic and anxiolytic pretreatment. None of the patients showed clinical signs of infectious diseases or liver insufficiency. TASH was performed according to standard clinical practice with temporary septal branch occlusion for selective therapeutic injection of 96% ethanol. Postprocedural management included monitoring in the intensive care unit for 48 hours. All patients provided written informed consent for their participation in the study, and approval of the ethics board (FF 43/2010) was obtained. Twenty patients without any evidence of coronary artery disease or inflammatory disorder undergoing coronary angiography without percutaneous coronary intervention served as the control group. All patients from the control group provided written informed consent for their participation in the study, and approval of the local ethics board (FF 43/2010) was obtained. The investigation conforms to the principles outlined in the Declaration of Helsinki.

#### Blood Sample Collection and Processing

Venous blood samples for determination of CRP, IL-6, and sCD40L were collected in plain gel-filled tubes without additives and in EDTA-filled tubes (Sarstedt, Germany) before treatment and at 15, 30, 45, 60, 75, 90, and 105 minutes and at 2, 4, 8, and 24 hours after induction of myocardial infarction. In the control group, venous blood samples for determination of CRP and IL-6 were collected in gel-filled tubes without additives and in EDTA-filled tubes before and at 2, 4, and 24 hours after coronary angiography. Sera were processed immediately and frozen at –80°C until assay.

#### CRP and Interleukin Measurements

CRP was measured in serum with the high-sensitivity electrochemiluminescence immunoassay (hs-CRP assay; Elecsys Analyzer 2010, Roche Diagnostics, Mannheim, Germany). The lower detection limit for the hs-CRP assay is 0.15 mg/L and the highest concentration measurable is 20.0 mg/L. The lowest concentration measurable with a coefficient of variation <10% for this assay is 0.3 mg/L. CRP was also measured using a commercial 1-step electrochemiluminescence immunoassay (CRP assay third generation; Elecsys 2010, Roche Diagnostics, Mannheim, Germany). The lower detection limit of this assay is 0.3 mg/dL and the highest concentration measurable is 350.0 mg/dL. The lowest concentration measurable with a coefficient of variation <20% is 0.6 mg/dL.

IL-6 was measured in serum using a chemiluminescent microparticle immunoassay (IL-6 assay; Elecsys Analyzer 2010, Roche Diagnostics, Mannheim, Germany). The lower detection limit of this assay is 1.5 pg/mL, the 95th percentile is 7.0 pg/mL, and the lowest concentration measurable with a coefficient of variation <10% is 5.0 pg/mL.

The sCD40L concentration was measured in plasma using a quantitative sandwich immunoassay (human sCD40L assay; Quantikine, R&D Systems, Inc, MN). According to the manufacturer, the mean minimum detectable concentration of CD40 ligand is 4.2 pg/mL.

#### Determination of Peripheral Blood Leukocyte Subsets

Absolute counts of peripheral blood granulocyte and monocyte subpopulations were determined using BD TruCount flow cytometry assay (Cat. No. 340334, BD Biosciences). Briefly, 50 μL of freshly drawn peripheral EDTA-treated blood was added into TruCount tubes using reverse pipetting and was then stained with mixed antibody conjugates, including anti–CD14–AlexaFluor488, anti–CD66b–PerCP-Cy5.5, anti–CD16–APC (BD Biosciences), and anti–CD11b–AlexaFluor700 (BD) monoclonal antibodies. After a 20-minute incubation at room temperature in the dark, erythrocytes were lysed for 15 minutes with 1000 μL ammonium chloride-based lysis buffer (BD PharmLyse, Cat. No. 555899, BD Biosciences). Data acquisition was performed on BD FACS Verse flow cytometer using FACS Suite software (BD Biosciences). A defined number (10,000) of CD66b-positive granulocytes were counted for the stop gate. Spectral overlap between different channels was calculated automatically by the FACS Diva software after measuring single-color compensation controls. Optimal compensation was achieved using antibody capture beads (Anti-Mouse Ig, k CompBeads, Cat. No. 552843 BD Biosciences) and the corresponding conjugated antibodies. Data were analyzed using FACS-Diva Software and absolute cell counts per microliter peripheral blood were calculated according to the manufacturer’s protocol.

#### Statistical Analysis

All data for continuous variables are expressed as mean±SD or as median and interquartile range, as appropriate. Categorical variables are reported as number and percentage. Continuous variables were compared using the Wilcoxon signed-rank test. Within-subject comparisons were made across repeated observations without correction for multiple comparisons using Friedman’s test for the overall analysis and the Mann–Whitney U test for comparison of 2 groups. The relative change in biomarkers was calculated as a percentage of the
Results

Clinical and procedural characteristics of all patients (13 men; 8 women; mean [SD] age, 59.0 [13.29] years) as well as enrolled in the study are shown in Table 1 and have been described previously in detail. All TASH procedures were performed in a single-session procedure using a single septal branch occlusion. During the procedure, the mean (SD) volume of ethanol administered was 1.77 (0.59) mL. The median occlusion time was 20.0 minutes (interquartile range [IQR], 14.5–31.0 minutes). Creatine kinase serum concentrations were significantly increased 1 day after TASH compared with baseline values (maximal postprocedural creatine kinase, 935.0 U/L [545.5–1115.0] versus baseline creatine kinase, 80.0 U/L [63.5–109.0]; P<0.001).

Inflammatory Biomarkers

Measurement of serum CRP concentrations by the third generation CRP assay first showed a significant increase 24 hours after MI was initiated (0.9 mg/dL; IQR, 0.65–1.7 mg/dL) as compared with the median baseline concentration (<0.3 mg/dL; IQR, <0.3–1.05 mg/dL; P<0.001; Figure 1A). Measurement of serum CRP concentrations by the hs assay revealed a significant increase 8 hours after induction of MI, with a continuous rise at 24 hours (2.68 mg/dL; IQR, 1.23–11.8 mg/L) as compared with the median baseline concentration (2.17 mg/L; IQR, 1.15–5.06 mg/L; P=0.002; Figure 1B). The levels of CRP as measured by the third generation and hs assays increased in every patient during the first 24 hours. In the control group, CRP concentrations showed no significant difference during the prespecified time points after coronary angiography as compared with median baseline concentration (1.8 mg/dL; IQR, 0.58–3.1 mg/dL at 24 hours versus 1.2 mg/dL; IQR, 0.53–2.2 mg/dL at baseline; P=0.10; Online Figure IA).

All patients showed IL-6 concentrations below the 95th percentile at baseline. IL-6 concentrations increased significantly 45 minutes after induction of MI (2.59 pg/mL; IQR, 1.69–5.0 pg/mL) as compared with the median baseline concentrations (1.5 pg/mL; IQR, 1.5–2.21 pg/mL; P=0.002). We observed an increase of IL-6 concentrations in every patient during the first 2 hours with a continuous rise at all prespecified time points (Figure 1C). In the control group, IL-6 concentrations showed a significant difference at the first prespecified time point after coronary angiography as compared with median baseline concentration (3.08 pg/mL; IQR, 2.1–4.47 pg/mL at 120 minutes versus 2.63 pg/mL; IQR, <1.5–3.84 pg/mL at baseline; P=0.003; Online Figure IB). We further compared the slope of the increase of CRP and IL-6 between the TASH group and the control group. The increase of CRP concentrations (P=0.026) and the increase of IL-6 concentration (P<0.001) had a steeper slope and the concentrations were significantly higher in the TASH group compared with that of the control group (Online Figure IIA and IIB). When comparing median percentage changes versus baseline for CRP and IL-6, the relative maximum increase observed during the first 24 hours

Table 1. Baseline Characteristics of 21 Patients Undergoing TASH and 20 Patients Undergoing Coronary Angiography (Control Group)

<table>
<thead>
<tr>
<th>Variable</th>
<th>TASH Group (n=21)</th>
<th>Control Group (n=20)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>59.0 (13.3)</td>
<td>66.8 (7.4)</td>
<td>0.13</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>13 (61.9)</td>
<td>11 (55.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>Body mass index, mean (SD), kg/m²</td>
<td>30.2 (6.9)</td>
<td>27.8 (4.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Cardiovascular risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>10 (47.6)</td>
<td>6 (30.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (61.9)</td>
<td>15 (75.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>6 (28.6)</td>
<td>10 (50.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (28.6)</td>
<td>5 (25.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Family history</td>
<td>6 (28.6)</td>
<td>8 (40.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>Current medication, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blocker</td>
<td>7 (33.3)</td>
<td>14 (70.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>9 (42.9)</td>
<td>6 (30.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>ASA</td>
<td>4 (19.0)</td>
<td>8 (40.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Statins</td>
<td>2 (9.5)</td>
<td>7 (35.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Left ventricular EF, mean (SD)</td>
<td>63.6 (5.6)</td>
<td>51.9 (15.1)</td>
<td>0.018</td>
</tr>
<tr>
<td>Laboratory measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>68.6 (IQR 66.9–81.8)</td>
<td>68.9 (51.3–88.4)</td>
<td>0.77</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, mL/min per 1.73 m²</td>
<td>90.5 (IQR 79.0–113.7)</td>
<td>91.9 (67.7–113.9)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; ASA, acetylsalicylic acid; EF, ejection fraction; and TASH, transcoronary ablation of septal hypertrophy.
was significantly higher in the TASH group than in the control group (*P*=0.0001; Online Figure IIIA and IIIB).

The median serum sCD40L concentration first showed a significant change 60 minutes after initiation of myocardial infarction: 801.6 pg/mL, IQR 675.0 to 1653.5 pg/mL versus the median baseline concentration of 1750.0 pg/mL, IQR 1151.0 to 2783.0 pg/mL (*P*=0.016; Figure 1D). sCD40L concentrations remained decreased until ≥24 hours after induction of myocardial infarction. All patients had a significantly lower sCD40L concentration compared with the baseline concentration after 60 minutes.

No sex-specific differences in the rates of increase were observed for the biomarkers analyzed. There was also no correlation between smoking and diabetes mellitus and the biomarker kinetics. The IL-6, CRP, hs-CRP, and sCD40L concentrations at each of the different time points are shown in Table 2. The IL-6 and CRP concentrations in the control group at prespecified time points are shown in Online Table I.

Peripheral Blood Leukocyte Subsets

Elevated counts of polymorphonuclear neutrophils were first detectable at 15 minutes, with a significant increase starting at 2 hours (median, 6415 cells/μL [IQR, 5288–7827] versus 4697 cells/μL [IQR, 2892–5620] at baseline; *P*=0.004). In contrast, an absolute eosinophil granulocyte count showed a significant drop 8 hours after induction of MI. This decrease remained significant until 24 hours (Figure 2A and 2B). After the initial drop in cell count of monocytic cells, only CD14++CD16− (classical) monocytes (but not CD14++CD16++ nonclassical or CD14++CD16+ intermediate subsets) started to increase 8 hours after MI, and significant monocytosis developed at 24 hours (729 cells/μL [IQR, 584–1344] versus 523 cells/μL [IQR, 369–701] at baseline; *P*=0.015; Figure 2C). In the control group, no significant changes in granulocyte and monocyte subset counts were observed during the time course as compared with median baseline cell counts. The absolute cell counts of granulocyte and monocyte subsets at each of the different time points are shown in Online Tables II and III.

High-Sensitivity Cardiac Troponin T

The release kinetics of cardiac troponin T (cTnT) as measured by a hs assay (Roche hs assay) in this patient cohort were published recently.24 In brief, all patients showed a significant increase in hs-cTnT concentrations 15 minutes after induction of myocardial infarction compared with baseline: 21.4 ng/L (IQR, 13.3–39.7) versus 11.3 ng/L (IQR, 6.0–18.8; *P*=0.031). This increase was >50% higher than the baseline value (range of the percentage increase [min–max], 171.4%–257.5%; range of the absolute increase [min–max], 3.71–38.7 ng/L). At the 30-minute time point, the concentrations of hs-cTnT in

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**Figure 1.** Concentrations of biomarkers at baseline and prespecified time points after transcoronary ablation of septal hypertrophy. A to D, C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), interleukin (IL)-6, and soluble CD40 ligand (sCD40L) concentrations (median [interquartile range]) of all patients at baseline and throughout the study. *First time point with a significant increase (*P*<0.05) compared with the baseline value.
all patients were above the 99th percentile value. There were increases noted at all of the prespecified time points; the data are shown in Online Table IV.

The biomarker values of patients undergoing TASH, scaled as a percentage change (with the maximum values set to 100%) at the prespecified time points, are displayed in Figure 3. In

### Table 2. Concentrations of the Indicated Biomarkers in 21 Patients Undergoing Transcoronary Ablation of Septal Hypertrophy

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-6, pg/mL</th>
<th>CRP, mg/dL</th>
<th>hs-CRP, mg/L</th>
<th>sCD40L, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.5 (1.5–2.21)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.17 (1.15–5.06)</td>
<td>1750 (1151–2783)</td>
</tr>
<tr>
<td>15 min</td>
<td>1.5 (1.5–2.83)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>1.98 (1.14–5.07)</td>
<td>1608 (903.9–2066.0)</td>
</tr>
<tr>
<td>30 min</td>
<td>1.78 (1.5–3.69)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.09 (1.24–8.29)</td>
<td>1441.5 (448.9–2547.0)</td>
</tr>
<tr>
<td>45 min</td>
<td>2.59 (1.69–5.0)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.13 (1.15–8.16)</td>
<td>1032.9 (429.2–1587.5)</td>
</tr>
<tr>
<td>60 min</td>
<td>1.98 (1.71–4.88)</td>
<td>&lt;0.3 (&lt;0.3–1.0)</td>
<td>2.23 (1.16–9.33)</td>
<td>801.6 (675.0–1653.5)</td>
</tr>
<tr>
<td>75 min</td>
<td>2.12 (1.5–3.57)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.12 (1.15–8.97)</td>
<td>1001.5 (736.0–1403.3)</td>
</tr>
<tr>
<td>90 min</td>
<td>2.9 (1.6–2.3)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.19 (1.06–10.52)</td>
<td>1211.0 (639.6–1625.0)</td>
</tr>
<tr>
<td>105 min</td>
<td>3.17 (1.71–6.59)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.23 (1.15–9.89)</td>
<td>844.6 (398.5–2079.3)</td>
</tr>
<tr>
<td>120 min</td>
<td>3.09 (2.37–7.01)</td>
<td>&lt;0.3 (&lt;0.3–1.0)</td>
<td>2.31 (1.24–10.7)</td>
<td>971.2 (304.0–2349.8)</td>
</tr>
<tr>
<td>240 min</td>
<td>7.23 (3.83–11.89)</td>
<td>&lt;0.3 (&lt;0.3–1.05)</td>
<td>2.51 (1.20–11.82)</td>
<td>966.8 (546.9–1536.8)</td>
</tr>
<tr>
<td>480 min</td>
<td>7.52 (5.32–13.65)</td>
<td>0.3 (&lt;0.3–1.05)</td>
<td>2.68 (1.23–11.8)</td>
<td>751.1 (610.8–938.8)</td>
</tr>
<tr>
<td>1400 min</td>
<td>13.59 (9.98–35.06)</td>
<td>0.9 (0.65–1.7)</td>
<td>9.2 (7.3–13.7)</td>
<td>592.1 (359.9–961.8)</td>
</tr>
</tbody>
</table>

Values represent median (interquartile range). CRP indicates C-reactive protein; hs-CRP, high-sensitivity CRP; IL-6, interleukin-6; and sCD40L, soluble CD40 ligand.

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**Figure 2.** Peripheral blood leukocyte subsets at baseline and prespecified time points. **A**, Main myeloid leukocyte populations; **B** granulocyte populations; **C** monocyte subsets at baseline and throughout the study (mean±SEM); **D** subset immunophenotyping; **E** composition changes within the circulating monocyte compartment after transcoronary ablation of septal hypertrophy. *First time point with a significant increase (P<0.05) and #first time point with a significant decrease (P<0.05) compared with baseline values. MI indicates myocardial infarction.
addition, we performed a correlation analysis between the maximum hs-cTnT concentration, representing the extent of myocardial injury, and the other biomarker concentrations after induction of AMI. The concentrations of CRP ($r=0.815; P<0.001$), hs-CRP ($r=0.768; P=0.004$), and IL-6 ($r=0.945, P<0.001$) were highly correlated with hs-cTnT, whereas sCD40L ($r=-0.551; P=0.63$) did not show any significant correlation.

**Discussion**

Various prognostic biomarkers have been identified that predict poor outcome in the setting of acute coronary syndrome. Inflammatory markers such as CRP reflect the extent of myocardial necrosis and correlate with cardiac outcomes after AMI. CRP, mainly synthesized and secreted by hepatocytes, has been described to increase 6 hours after an acute stimulus. Recent studies demonstrated that CRP predicts the loss of left ventricular ejection fraction and infarct size measured by magnet resonance imaging 3 months after AMI. CRP has been the most widely studied inflammatory marker, but other biomarkers such as lipoprotein-associated phospholipase A2, myeloperoxidase, IL-6, sCD40 ligand, and pregnancy-associated plasma protein A have also shown promising results. In spite of these findings, however, less is known about the exact release kinetics of inflammatory biomarkers in patients with AMI.

The present study is the first to precisely describe the early release of IL-6 and CRP, measured with conventional and hs assays, as well as the kinetics of circulating leukocyte subsets in patients undergoing TASH.

In this study, the TASH procedure was assumed to be a valuable model of human AMI because of its associated well-defined chronological biomarker release after the induction of infarction. In contrast, we observed decreased sCD40L concentrations at the prespecified time points in the present study. We speculate that a reduction in the left ventricular outflow tract gradient points. Our study clearly shows that CRP release, as assessed by the hs assay, can be measured within 4 to 8 hours after MI and remains increased until 24 hours. The hs assay outperforms the third generation assay by virtue of its earlier detection of the first significant increase in CRP values (8 versus 24 hours).

Several pathophysiological mechanisms trigger the secretion of IL-6, including exhaustive exercise and emotional excitement. Previous studies demonstrated the induction of local and systemic inflammatory reactions with production of IL-6 as early as 6 hours after musculoskeletal surgical trauma. In addition, IL-6 shows a distinctive circadian biphasic variation, with concentrations peaking at 4:00 PM and 4:00 AM. Furthermore, experimental studies have shown that short periods of myocardial ischemia followed by reperfusion trigger proinflammatory reactions with the production of cytokines such as IL-6. Studies have shown that IL-6 may be of additional diagnostic value in the risk assessment of enzyme-negative patients with precordial chest pain of recent onset. Irrespective of higher concentrations of IL-6 in patients with left ventricular outflow tract obstruction, which indicates active proinflammatory processes, our data clearly show that IL-6 is released quickly, within the first minutes after the induction of myocardial infarction, and increases during all prespecified time points. Although there was an increase in IL-6 levels at several time points in the control group, we demonstrated that the increase in patients after induction of myocardial infarction was clearly steeper and significantly greater.
and improvement in patient hemodynamics affect platelet activation, which is different from the situation with plaque-ruptured AMI.

The TASH model provides a unique opportunity to compare the release kinetics of different biomarkers after MI. We were able to demonstrate a highly significant correlation between the maximum concentrations of hs-CtTnT, which reflect the extent of myocardial necrosis, and the inflammatory markers CRP and IL-6.

We further present for the first time the exact time-course kinetics of systemic myeloid leukocyte response after MI. Whereas previous studies have already described an early, sequential mobilization and systemic release of the main myeloid subsets in a mouse model of coronary artery ligation, no study thus far has reported the earliest shifts of granulocyte and monocyte subsets in the onset of human AMI. Neutrophil blood counts have been shown previously to be an independent predictor of short- and long-term mortality after AMI. Neutrophilia is also suggested to serve as a potential additive predictor of short- and long-term mortality after AMI. Our results clearly demonstrate a rapid occurrence of neutrophilia within the first hour after induction of ischemia. Estimating the precise early kinetics of bone marrow polymophonuclear neutrophil release may further help to elucidate clinical significance and diagnostic value of neutrophil count in AMI.

Based on the animal models of MI, monocyte subsets have been assumed to orchestrate not only the initiation and resolution of cardiac inflammation but also of healing processes after MI. In particular, persistently elevated levels of CD14++CD16−, classical monocytes, have been associated with the impairment of myocardial salvage and adverse left ventricular remodeling in patients after MI. Here, we show a detailed time course of CD14++CD16− monocytes within 24 hours after induction of ischemia. The isolated increase of classical monocytes after ischemia could be explained by ≥2 phenomena. First, rapid mobilization of splenic reservoirs of classical monocytes in the course of AMI is largely angiotensin II dependent. Furthermore, the early release of several chemoattractant cytokines, including monocyte chemoattractant protein 1, induces monocyte migration in vitro and plays a critical role in mononuclear cell trafficking to sites of sterile inflammation. Classical monocytes express high levels of CC-chemokine receptor 2, and they exit the bone marrow in a CC-chemokine receptor 2−dependent manner and are recruited in the myocardium through CC-chemokine receptor 2/monocyte chemoattractant protein-1 interactions.

We think that this is the first study that precisely describes release kinetics of CRP, IL-6, and circulating leukocyte subsets in patients with hypertrophic obstructive cardiomyopathy undergoing TASH. Understanding the time frame of the increase of these inflammatory markers in correlation with patient symptoms, ECG information, and imaging studies is important for individual risk stratification and individualized therapy in patients with suspected AMI.

There are some limitations of our study, however, that must be considered. The patients studied were without significant coronary artery disease and therefore without the possible phenomenon of inflammatory preconditioning, which may influence the effect of plaque rupture-related CRP release. The important role of inflammatory processes in plaque stability supports the possible benefits of lifestyle modification and drug therapy with statins and antioxidants. This may prevent vulnerable plaque rupture. Furthermore, the kinetics of CRP and IL-6 release as well as the time course of leukocyte subsets after alcohol ablation might be different from the release by the stuttering thrombotic occlusion of an epicardial coronary artery where the vessel dynamically opens and closes during the early period of MI. Nevertheless, our data clearly demonstrate a significant increase in hs-CRP at 8 hours, IL-6 at 45 minutes, and polymophonuclear neutrophils at 2 hours with a continuous rise during all prespecified time points after induction of myocardial infarction.

In conclusion, our study presents the precise kinetics of the systemic myeloid leukocyte response after MI. The data also clearly demonstrate the early release of IL-6 within the first hour after induction of MI. These results provide valuable additional evidence of the diagnostic value of inflammatory biomarkers in the setting of AMI.

Acknowledgments
We thank Heike Wagner, Sigrun Sass, and Nora Staubach, Kerckhoff Heart and Thorax Center, Bad Nauheim, Germany, for their laboratory expertise. We also acknowledge the assistance of Elizabeth Martinson in editing and preparation of the article. We are grateful to the William G. Kerckhoff-Stiftung, Bad Nauheim, Germany, for research funding.

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Disclosures
None.

References

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Liebeträub et al Inflammatory Biomarker Kinetics in AMI 873


Novelty and Significance

**What Is Known?**
- Inflammatory biomarkers in patients with acute myocardial infarction (AMI) have been linked to risk stratification in terms of long-term morbidity and mortality.
- The release kinetics of inflammatory biomarkers in the setting of human AMI has been difficult to establish.
- There are several animal models of AMI but these cannot always be directly extrapolated to the situation with humans.

**What New Information Does This Article Contribute?**
- Transcatheter ablation of septal hypertrophy, an exceptional model of human myocardial infarction, provides a unique opportunity to outline the acute inflammatory response to myocardial ischemia in man.
- The present study is the first to precisely describe the early release of interleukin-6 and C-reactive protein, measured with conventional and highly sensitive assays, as well as the kinetics of circulating leukocyte subsets in patients undergoing transcatheter ablation of septal hypertrophy.
- Interleukin-6 and neutrophil granulocytes show a continuous rise after induction of myocardial infarction via transcatheter ablation of septal hypertrophy. Our results provide valuable additional evidence for the diagnostic value of inflammatory biomarkers in the setting of early AMI.

Deciphering the individual kinetics of interleukin-6, C-reactive protein, and myeloid leukocyte responses after transcatheter ablation of septal hypertrophy provides valuable additional evidence of the diagnostic value of inflammatory biomarkers in the setting of early AMI. Understanding the time frame of the increase of these inflammatory markers in correlation with patient symptoms, ECG information, and imaging data is important for early diagnosis, individual risk stratification, and new individualized therapeutic strategies in patients with suspected AMI.
Release Kinetics of Inflammatory Biomarkers in a Clinical Model of Acute Myocardial Infarction

Christoph Liebetrau, Jedrzej Hoffmann, Oliver Dörr, Luise Gaede, Johannes Blumenstein, Hannes Biermann, Lukas Pyttel, Peter Thiele, Christian Troidl, Alexander Berkowitsch, Andreas Rolf, Sandra Voss, Christian W. Hamm, Holger Nef and Helge Möllmann

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Supplemental Table I. Concentrations of the IL-6 and CRP in 20 patients undergoing coronary angiography (controls).

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-6 (pg/mL)</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (IQR)</td>
<td>median (IQR)</td>
</tr>
<tr>
<td>baseline</td>
<td>2.63 (&lt;1.5-3.84)</td>
<td>1.2 (0.53-2.2)</td>
</tr>
<tr>
<td>120 min</td>
<td>3.08 (2.1-4.47)</td>
<td>1.25 (0.53-2.2)</td>
</tr>
<tr>
<td>240 min</td>
<td>4.41 (3.03-5.81)</td>
<td>1.2 (0.5-2.2)</td>
</tr>
<tr>
<td>1400 min</td>
<td>6.65 (4.87-12.6)</td>
<td>1.8 (0.58-3.1)</td>
</tr>
</tbody>
</table>

Supplemental Table II. Absolute cell counts of the indicated leukocyte subsets in 10 patients undergoing transcoronary ablation of septal hypertrophy (TASH).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neutrophils (cell/µL)</th>
<th>Eosinophils (cell/µL)</th>
<th>Classical (cell/µL)</th>
<th>Non-Classical (cell/µL)</th>
<th>Intermediate (cell/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (IQR)</td>
<td>median (IQR)</td>
<td>median (IQR)</td>
<td>median (IQR)</td>
<td>median (IQR)</td>
</tr>
<tr>
<td>baseline</td>
<td>4697 (2892-5620)</td>
<td>232.4 (89-377.6)</td>
<td>523.1 (368.9-700.7)</td>
<td>58.4 (34.1-66.4)</td>
<td>30.2 (24.6-63.8)</td>
</tr>
<tr>
<td>15 min</td>
<td>5361 (2524-6472)</td>
<td>190.5 (60.2-319.0)</td>
<td>504.1 (284.1-779.9)</td>
<td>51.4 (32.9-75.9)</td>
<td>21.9 (14.4-37.4)</td>
</tr>
<tr>
<td>30 min</td>
<td>5505 (2597-6070)</td>
<td>228.9 (57.3-375.1)</td>
<td>539.2 (291-743.4)</td>
<td>35.4 (23.3-121.5)</td>
<td>25.3 (11.5-59.1)</td>
</tr>
<tr>
<td>45 min</td>
<td>5334 (2838-6870)</td>
<td>267.9 (61.1-453.1)</td>
<td>490 (296.9-779)</td>
<td>44.1 (17.3-130.9)</td>
<td>35.2 (10.7-62.8)</td>
</tr>
<tr>
<td>60 min</td>
<td>5340 (4000-6689)</td>
<td>255.7 (74.3-466.7)</td>
<td>389.6 (279.9-818)</td>
<td>40.6 (17.9-113.6)</td>
<td>19.8 (7.8-49.9)</td>
</tr>
<tr>
<td>120 min</td>
<td>6415 (5288-7827)</td>
<td>131.6 (49.7-473.1)</td>
<td>344.7 (218.3-609.2)</td>
<td>35.7 (9.4-61)</td>
<td>10.1 (8.5-23.7)</td>
</tr>
<tr>
<td>240 min</td>
<td>6366 (5858-9921)</td>
<td>61.4 (15.6-186.9)</td>
<td>196.3 (56.7-530.7)</td>
<td>22.9 (9.9-48.7)</td>
<td>13.6 (11.1-25.9)</td>
</tr>
<tr>
<td>360 min</td>
<td>6100 (4977-9412)</td>
<td>31.0 (8.3-228.5)</td>
<td>181.8 (25.9-612.5)</td>
<td>27.5 (15.4-45.1)</td>
<td>17.3 (7.6-23.3)</td>
</tr>
<tr>
<td>480 min</td>
<td>6615 (5988-11895)</td>
<td>16.5 (5.5-166.5)</td>
<td>118 (23.3-371.8)</td>
<td>17.9 (2.9-47.8)</td>
<td>10.3 (5.1-16.5)</td>
</tr>
<tr>
<td>720 min</td>
<td>7701 (6659-10644)</td>
<td>12.5 (4.1-258.6)</td>
<td>336.9 (137.7-838.3)</td>
<td>30.4 (7.4-44)</td>
<td>11 (6.5-35.4)</td>
</tr>
<tr>
<td>1400 min</td>
<td>9290 (8053-11154)</td>
<td>19.2 (8.0-270.4)</td>
<td>728.8 (583.7-1344)</td>
<td>39.3 (13.7-71.5)</td>
<td>37.1 (11.5-162)</td>
</tr>
</tbody>
</table>
Supplemental Table III. Absolute cell counts of the indicated leukocyte subsets in 18 patients undergoing coronary angiography (controls).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neutrophils (cell/µL) median (IQR)</th>
<th>Eosinophils (cell/µL) median (IQR)</th>
<th>Classical (cell/µL) median (IQR)</th>
<th>Non-Classical (cell/µL) median (IQR)</th>
<th>Intermediate (cell/µL) median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>3848 (3102-4545)</td>
<td>124.4 (62.5-152.7)</td>
<td>441.5 (331.5-548.4)</td>
<td>29.8 (25-35.4)</td>
<td>25.2 (17-34.6)</td>
</tr>
<tr>
<td>120 min</td>
<td>3859 (3165-4545)</td>
<td>139.5 (60.5-192)</td>
<td>380.4 (307.4-457.6)</td>
<td>22.7 (15.2-28.4)</td>
<td>17.8 (12.6-34.2)</td>
</tr>
<tr>
<td>240 min</td>
<td>3566 (2786-4803)</td>
<td>146.6 (63-193.7)</td>
<td>495.5 (56.7-530.7)</td>
<td>30.2 (17.3-47.6)</td>
<td>18.3 (14-33.2)</td>
</tr>
<tr>
<td>1400 min</td>
<td>4502 (3609-5306)</td>
<td>156.2 (83.4-251.6)</td>
<td>579.3 (439.2-675.6)</td>
<td>38.1 (20.1-47.1)</td>
<td>28.5 (16.7-40.9)</td>
</tr>
</tbody>
</table>
Supplemental Table IV. High-sensitivity cardiac troponin T concentrations (pg/mL) and myoglobin concentrations (µg/L) in 21 patients undergoing transcoronary ablation of septal hypertrophy (TASH).*

<table>
<thead>
<tr>
<th>Variable</th>
<th>hs-cTnT (pg/mL)</th>
<th>hs-cTnT (pg/mL)</th>
<th>myoglobin (µg/L)</th>
<th>myoglobin (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (IQR)</td>
<td>min-max</td>
<td>median (IQR)</td>
<td>min-max</td>
</tr>
<tr>
<td>Baseline</td>
<td>11.3 (6.3-18.8)</td>
<td>&lt;3.0-54.2</td>
<td>38.0 (28.0-56.0)</td>
<td>25.0-76.0</td>
</tr>
<tr>
<td>15 min</td>
<td>21.4 (13.3-39.7)</td>
<td>7.7-92.9</td>
<td>104.0 (64.0-143.0)</td>
<td>37.0-265.0</td>
</tr>
<tr>
<td>30 min</td>
<td>51.3 (36.7-146.9)</td>
<td>32.9-443.9</td>
<td>188.0 (154.0-233.0)</td>
<td>62.0-383.0</td>
</tr>
<tr>
<td>45 min</td>
<td>103.5 (78.4-201.6)</td>
<td>54.9-449.6</td>
<td>232.0 (191.5-327.5)</td>
<td>114.0-358.0</td>
</tr>
<tr>
<td>60 min</td>
<td>194.3 (115.3-294.2)</td>
<td>86.9-526.5</td>
<td>275.0 (221.0-384.0)</td>
<td>130.0-563.0</td>
</tr>
<tr>
<td>75 min</td>
<td>218.2 (143.8-316.3)</td>
<td>96.8-584.0</td>
<td>292.0 (235.0-361.5)</td>
<td>142.0-531.0</td>
</tr>
<tr>
<td>90 min</td>
<td>321.2 (215.1-517.3)</td>
<td>181.0-767.7</td>
<td>311.0 (230.0-379.0)</td>
<td>142.0-714.0</td>
</tr>
<tr>
<td>105 min</td>
<td>351.9 (202.8-467.7)</td>
<td>156.9-933.4</td>
<td>297.5 (230.0-374.5)</td>
<td>157.0-828.0</td>
</tr>
<tr>
<td>120 min</td>
<td>429.4 (234.4-547.6)</td>
<td>173.4-1275.0</td>
<td>294.0 (256.0-348.0)</td>
<td>169.0-794.0</td>
</tr>
<tr>
<td>240 min</td>
<td>687.7 (472.8-1040.0)</td>
<td>242.7-2194.0</td>
<td>284.5 (258.3-361.3)</td>
<td>148.0-454.0</td>
</tr>
<tr>
<td>480 min</td>
<td>1314.0 (1033.2-1953.5)</td>
<td>821.0-4584.0</td>
<td>301.5 (165.8-392.8)</td>
<td>62.0-520.0</td>
</tr>
<tr>
<td>1400 min</td>
<td>2239.0 (1831.5-2832.0)</td>
<td>1568.0-4128.0</td>
<td>98.0 (54.5-145.5)</td>
<td>50.0-529.0</td>
</tr>
</tbody>
</table>

*Data from Liebetrau et al. 24
Online Figure I: CRP (A) and IL-6 (B) concentrations (median [IQR]) of all patients undergoing coronary angiography (controls) at baseline and throughout the study. An asterisk (*) indicates the first time point with a significant increase (p<0.05) compared with the baseline value.
Online Figure II: Comparison of the slope of the increase in CRP (A) and IL-6 (B) concentrations during the pre-specified time points between the TASH group and the control group.
Online Figure III: Comparison of the percent increase (% baseline) of CRP (A) and IL-6 (B) concentrations during the first 24 hours between the TASH group and the control group.