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

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In November, 2014, the average time from submission to first decision for all original research papers submitted to Circulation Research was 13.96 days.
ABSTRACT

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

Objective: The aim of this study was to determine the kinetics of changes in the levels of a number of biomarkers specifically linked to inflammation following transcoronary ablation of septal hypertrophy (TASH), a procedure that mimics AMI.

Methods and Results: We analyzed release kinetics of C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), interleukin 6 (IL-6), soluble CD40 ligand (sCD40L) and peripheral blood leukocyte subsets in patients (n=21) undergoing TASH. Blood samples were collected prior to TASH and at various times after TASH. Serum levels of CRP were increased at 24 h (1.0 mg/dL [IQR 0.7-1.75] vs. 0.2 mg/dL [IQR 0.1-1.05] at BL; p<0.001), whereas hs-CRP increased as early as 8 h (2.68 mg/L [IQR 1.23-11.80] vs. 2.17 mg/L [IQR 1.15-5.06] at BL; p=0.002). IL-6 was significantly increased at 45 min (2.59 pg/mL [IQR 1.69-5.0] vs. 1.5 pg/mL [IQR 1.5-2.21] at BL; p=0.002), and sCD40L was significantly decreased at 60 min (801.6 pg/mL [IQR 675.0-1653.5] vs. 1750.0 pg/mL [IQR 1151.0-2783.0] at BL; p=0.016). Elevated counts of polymorphonuclear neutrophils were detectable at 15 min, with a significant increase at 2 h (6415 cells/µL [IQR 5288-7827] vs. 4697 cells/µL [IQR 2892-5620] at BL; p=0.004). Significant monocytosis was observed at 24 h (729 cells/µL [IQR 584-1344] vs. 523 cells/µL [IQR 369-701] at BL; p=0.015).

Conclusions: IL-6 and neutrophil granulocytes showed a continuous rise at all pre-specified time points after induction of myocardial infarction. Our results provide valuable additional evidence of the diagnostic value of inflammatory biomarkers in the setting of early AMI.

Keywords:
Inflammation, leukocytes, acute myocardial infarction, TASH, septal ablation

Nonstandard Abbreviations and Acronyms:
ACS acute coronary syndrome
AMI acute myocardial infarction
CK creatine kinase
CRP C-reactive protein
CV coefficient of variation
HOCM hypertrophic obstructive cardiomyopathy
hs high sensitivity
IL-6 interleukin-6
PCI percutaneous coronary intervention
sCD40L soluble CD40 ligand
TASH transcoronary ablation of septal hypertrophy

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INTRODUCTION

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

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

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; however, these findings cannot be directly extrapolated to patients due to different physiological parameters (e.g. metabolism, release, inter-individual variability) or proinflammatory effects of surgical procedures performed to induce myocardial infarction. 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 following 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.

METHODS

Study design.

From January 2010 to June 2011, 21 consecutive patients with hypertrophic obstructive cardiomyopathy (HOCM) undergoing TASH were included in the study. Pre- and post-procedural management of the patients has been recently published. In brief, clinical history, physical examination, 12-lead ECG, laboratory tests, echocardiography, and coronary angiography for all patients were assessed. The final diagnosis of HOCM 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 mmHg at rest and/or >50 mmHg after provocation by the Valsalva maneuver. 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. Post-procedural management included monitoring in the intensive care unit for 48 h. All patients provided written informed consent for their participation in the study, and approval of the ethics board (FF 31/2010) was obtained. Twenty patients without any evidence of

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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, and IL-6, and sCD40L were collected in plain gel-filled tubes without additives and in EDTA-filled tubes (Sarstedt, Germany) prior to treatment before and at 15, 30, 45, 60, 75, 90, and 105 min and 2, 4, 8, and 24 h 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 prior to and 2, 4, and 24 h 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 (CV) < 10% for this assay is 0.3 mg/L. CRP was also measured using a commercial one-step electrochemiluminescence immunoassay (CRP assay 3rd 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 CV < 20% is 0.6 mg/dL.

Interleukin 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 CV < 10% is 5.0 pg/mL.

The sCD40L concentration was measured in plasma using a quantitative sandwich immunoassay (human soluble CD40 Ligand assay, Quantikine®, R&D Systems, Inc., MN, USA). 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 were added into TruCount tubes using reverse pipetting and were then stained with mixed antibody conjugates, including anti-CD14-AlexaFluor488, anti-CD66b-PerCpCy5-5, anti-CD16-APC (BD Biosciences), and anti-CD11b-AlexaFluor700 (BD) monoclonal antibodies. Following a 20-min incubation at room temperature in the dark, erythrocytes were lysed for 15 min 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 FACSuite 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 FACSDiva software after measuring single-color compensation controls. Optimal compensation was achieved using antibody capture beads (Anti-Mouse Ig, κ CompBeads, Cat. No. 552843 BD Biosciences) and the corresponding conjugated antibodies. Data were analyzed using FACSDiva Software and absolute cell counts per μl 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 two groups. The relative change in biomarkers was calculated as a percent of the baseline value. The maximum percent increase of CRP and IL-6 within 24 hours in TASH and control groups were compared using the Mann-Whitney U Test. Also, we used the Mann-Whitney U test for comparisons between the TASH group and the control group regarding the slope of the increase of CRP and IL-6 concentrations. Correlation analyses were performed with the use of the Spearman rank coefficient. All statistical tests were performed with SPSS software, version 19.0, and GraphPad Prism, version 5.0 for Macintosh. A two-tailed P value <0.05 was considered to be statistically significant.

RESULTS

Clinical and procedural characteristics of all patients (13 men, 8 women, mean [SD] age 59.0 [13.29] y) as well as enrolled in the study are shown in Table 1 and have been previously described 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 min (IQR, 14.5–31.0 min). Creatine kinase (CK) serum concentrations were significantly increased one day after TASH compared with baseline values (maximal post-procedural CK 935.0 U/L [545.5–1115.0] vs. baseline CK 80.0 U/L [63.5–109.0]; p<0.0001).

Inflammatory biomarkers.

Measurement of serum CRP concentrations by the third generation CRP assay first showed a significant increase 24 h 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 h after induction of MI, with a continuous rise at 24 h (2.68 mg/L, 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 h. In the control group, CRP concentrations showed no significant difference during the pre-specified time points after coronary angiography as compared with median baseline concentration (1.8 mg/dL [IQR 0.58-3.1 mg/dL] at 24 h vs. 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 min 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 h with a continuous rise at all pre-specified time points (Figure 1C). In the control group, IL-6 concentrations showed a significant difference at the first pre-specified time point after coronary angiography as compared with median baseline concentration (3.08 pg/mL [IQR 2.1-4.47 pg/mL]) at 120 min vs. 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 percent changes vs. baseline for CRP and IL-6, the relative maximum increase observed during the first 24 h 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 min after initiation of myocardial infarction: 801.6 pg/mL, IQR 675.0–1653.5 pg/mL vs. the median baseline concentration of 1750.0 pg/mL, IQR 1151.0–2783.0 pg/mL (p=0.016; Figure 1D). sCD40L concentrations remained decreased until at least 24 hours after induction of myocardial infarction. All patients had a significantly lower sCD40L concentration compared with the baseline concentration after 60 min.

No gender-specific differences in the rates of increase were observed for the biomarkers analyzed. There was also no correlation between smoking and/or diabetes and the biomarker kinetics. The IL-6, CRP, and 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 pre-specified time points are shown in Supplemental Table I.

Peripheral blood leukocyte subsets.

Elevated counts of polymorphonuclear neutrophils were first detectable at 15 min, with a significant increase starting at 2 h (median 6415 cells/µL [IQR 5288-7827] vs. 4697 cells/µL [IQR 2892-5620] at BL; p=0.004). In contrast, an absolute eosinophil granulocyte count showed a significant drop 8 h after induction of MI. This decrease remained significant until 24 h (Figure 2A and 2B). Following the initial drop in cell count of monocytic cells, only CD14++CD16- (“classical”) monocytes (but not CD14+CD16++ “non-classical” or CD14++CD16+ “intermediate” subsets) started to increase 8 h following MI, and significant monocytosis developed at 24 h (729 cells/µL [IQR 584-1344] vs. 523 cells/µL [IQR 369-701] at BL; 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 Supplemental Tables II and III.

High-sensitivity cardiac troponin T.

The release kinetics of cTnT as measured by a high-sensitivity assay (Roche hs-assay) in this patient cohort were recently published. In brief, all patients showed a significant increase in hs-cTnT concentrations 15 min after induction of myocardial infarction compared with baseline: 21.4 ng/L [IQR 13.3–39.7] vs. 11.3 ng/L [IQR 6.0–18.8] (p=0.031). This increase was more than 50% higher than the baseline value (range of the percent increase [min–max]: 171.4–257.5%; range of the absolute increase [min–max]: 3.71–38.7 ng/L). At the 30-min time point, the concentrations of hs-cTnT in all patients were above the 99th percentile value. There were increases noted at all of the pre-specified time points; the data are shown in Supplemental Table IV.

The biomarker values of patients undergoing TASH, scaled as a percent change (with the maximum values set to 100%) at the pre-specified time points, are displayed in Figure 3. In 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 following 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 due to its associated well-defined chronological biomarker release after the induction of infarction. One of the most important findings of our study is that blood levels of the inflammatory cytokine IL-6 and the signaling product CRP increased throughout the pre-specified time points. Our study clearly shows that CRP release, as assessed by the hs assay, can be measured within 4-8 hours following 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 h vs. 24 h).

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 p.m. and 4 a.m. Furthermore, experimental studies have shown that short periods of myocardial ischemia followed by reperfusion trigger pro-inflammatory 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 pro-inflammatory 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 pre-specified 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.

Reports in the literature concerning the diagnostic accuracy of sCD40L in patients with AMI have been controversial. Previous studies reported that this peptide is elevated in patients with AMI. Furthermore, sCD40L was shown to be elevated in patients with HOCM compared with healthy volunteers. In contrast, we observed decreased sCD40L concentrations at the pre-specified time points in the present study. We speculate that a reduction in the left ventricular outflow tract gradient 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 following AMI. We were able to demonstrate a highly significant correlation between the maximum concentrations of hs-cTnT, 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 following 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 previously shown to be an independent predictor of short- and long-term mortality after AMI. Neutrophilia is also suggested to serve as a potential additive diagnostic biomarker for AMI in emergency patients. Our results clearly demonstrate a rapid occurrence of neutrophilia within the first hour following induction of ischemia. Estimating the precise early kinetics of bone marrow polymorphonuclear 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 following 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 following AMI. Here, we show a detailed time course of CD14++CD16 monocytosis within 24 hours after induction of ischemia. The isolated increase of classical monocytes following ischemia could be explained by at least two phenomena. Firstly, rapid mobilization of splenic reservoirs of “classical” monocytes in the course of acute myocardial infarction is largely angiotensin II-dependent. Furthermore, the early release of several chemoattractant cytokines, including monocyte chemoattractant protein 1 (MCP-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 (CCR2), and they exit the bone marrow in a CCR2-dependent manner and are recruited in the myocardium through CCR2/MCP-1 interactions.

We believe 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, electrocardiogram 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/or 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 min, and PMN at 2 hours with a continuous rise during all pre-specified time points after induction of myocardial infarction.

In conclusion, our study presents the precise kinetics of the systemic myeloid leukocyte response following 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.
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DISCLOSURES
None.

REFERENCES


FIGURE LEGENDS

**Figure 1:** Concentrations of biomarkers at baseline and pre-specified time points following TASH. A through D. CRP, hs-CRP, IL-6, and sCD40L concentrations (median [IQR]) of all patients 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.

**Figure 2:** Peripheral blood leukocyte subsets at baseline and pre-specified 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 following TASH. An asterisk (*) indicates the first time point with a significant increase ($P<0.05$) and a pound symbol (#) indicates the first time point with a significant decrease ($P<0.05$) compared with baseline values.

**Figure 3:** Release kinetics of soluble CD40 ligand (sCD40L), C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), interleukin 6 (IL-6), high-sensitivity cardiac troponin T (hs-cTnT) and myoglobin. Biomarker values are shown as percent change for all patients during the pre-specified time points after induction of myocardial infarction, with the maximum value set to 100%. Data for hs-cTnT and myoglobin are from 24.
Table 1. Baseline characteristics of 21 patients undergoing transcoronary ablation of septal hypertrophy (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, years, mean (SD)</td>
<td>59.0 (13.3)</td>
<td>66.8 (7.4)</td>
<td>0.13</td>
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<tr>
<td>Male, n (%)</td>
<td>13 (61.9)</td>
<td>11 (55.0)</td>
<td>0.54</td>
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<tr>
<td>Body mass index, kg/m², mean (SD)</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>
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<tr>
<td>Hypertension</td>
<td>13 (61.9)</td>
<td>15 (75.0)</td>
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<td>Hypercholesterolemia</td>
<td>6 (28.6)</td>
<td>10 (10.0)</td>
<td>0.76</td>
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<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>
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<tr>
<td>Current medication, n (%)</td>
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<td></td>
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<tr>
<td>Beta-blocker</td>
<td>7 (33.3)</td>
<td>14 (70.0)</td>
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<td>ACE-Inhibitor</td>
<td>9 (42.9)</td>
<td>6 (30.0)</td>
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<td>ASA</td>
<td>4 (19.0)</td>
<td>8 (40.0)</td>
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<td>Statins</td>
<td>2 (9.5)</td>
<td>7 (35.0)</td>
<td>0.13</td>
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<tr>
<td>Left ventricular EF, mean (SD)</td>
<td>63.6 (5.6)</td>
<td>51.9 (15.1)</td>
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<tr>
<td>Laboratory measurements</td>
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<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>
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<tr>
<td>Estimated glomerular filtration rate (mL/min/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>
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Table 2. Concentrations of the indicated biomarkers in 21 patients undergoing transcoronary ablation of septal hypertrophy (TASH).

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-6 (pg/mL) median (IQR)</th>
<th>CRP (mg/dL) median (IQR)</th>
<th>hs-CRP (mg/L) median (IQR)</th>
<th>sCD40L (pg/mL) median (IQR)</th>
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<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>
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<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 (1.5-6.23)</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>
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 a number of animal models of AMI but these cannot always be directly extrapolated to the situation with humans.

What New Information Does This Article Contribute?

- Transcoronary ablation of septal hypertrophy (TASH), 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 IL-6 and CRP, measured with conventional and highly sensitive assays, as well as the kinetics of circulating leukocyte subsets in patients undergoing TASH.
- IL-6 and neutrophil granulocytes show a continuous rise after induction of myocardial infarction via TASH. Our results provide valuable additional evidence for the diagnostic value of inflammatory biomarkers in the setting of early AMI.

Deciphering the individual kinetics of IL-6, CRP and myeloid leukocyte responses following TASH 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, electrocardiogram information, and imaging data is important for early diagnosis, individual risk stratification, and new individualized therapeutic strategies in patients with suspected AMI.
Figure 1

A) CRP concentration (mg/dL) over time (minutes)

B) hs-CRP concentration (mg/L) over time (minutes)

C) IL-6 concentration (pg/mL) over time (minutes)

D) SCD40 Ligand concentration (pg/mL) over time (minutes)
Figure 2

A  Main myeloid subsets

B  Granulocyte subsets

C  Monocyte subsets

D  CD14$^+$CD16$^+$ "non-classical"
   CD14$^{++}$CD16$^+$ "intermediate"
   CD14$^{++}$CD16$^+$ "classical"

E  % total monocytes
Figure 3
Release Kinetics of Inflammatory Biomarkers in a Clinical Model of Acute Myocardial Infarction

Christoph Liebetrau, Jedrzej Hoffmann, Oliver Dörr, Luise Gaede, Johannes M Blumenstein, Hannes Biermann, Lukas P Pyttel, Peter Thiele, Christian Troidl, Alexander Berkowitsch, Andreas Rolf, Sandra Voss, Christian Hamm, Holger M 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) median (IQR)</th>
<th>CRP (mg/dL) median (IQR)</th>
</tr>
</thead>
<tbody>
<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) 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>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 (11-59.1)</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>489.6 (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 (5.6-166.5)</td>
<td>118 (22.3-371.8)</td>
<td>17.9 (2.9-47.8)</td>
<td>10.3 (5.6-16.5)</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 (7-44)</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 (311.5-548.4)</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) &lt;3.0-54.2</td>
<td>38.0 (28.0-56.0)</td>
<td>25.0-76.0</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>21.4 (13.3-39.7) 7.7-92.9</td>
<td>104.0 (64.0-143.0)</td>
<td>37.0-265.0</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>51.3 (36.7-146.9) 32.9-443.9</td>
<td>188.0 (154.0-233.0)</td>
<td>62.0-383.0</td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td>103.5 (78.4-201.6) 54.9-449.6</td>
<td>232.0 (191.5-327.5)</td>
<td>114.0-358.0</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>194.3 (115.3-294.2) 86.9-526.5</td>
<td>275.0 (221.0-384.0)</td>
<td>130.0-563.0</td>
<td></td>
</tr>
<tr>
<td>75 min</td>
<td>218.2 (143.8-316.3) 96.8-584.0</td>
<td>292.0 (235.0-361.5)</td>
<td>142.0-531.0</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>321.2 (215.1-517.3) 181.0-767.7</td>
<td>311.0 (230.0-379.0)</td>
<td>142.0-714.0</td>
<td></td>
</tr>
<tr>
<td>105 min</td>
<td>351.9 (202.8-467.7) 156.9-933.4</td>
<td>297.5 (230.0-374.5)</td>
<td>157.0-828.0</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>429.4 (234.4-547.6) 173.4-1275.0</td>
<td>294.0 (256.0-348.0)</td>
<td>169.0-794.0</td>
<td></td>
</tr>
<tr>
<td>240 min</td>
<td>687.7 (472.8-1040.0) 242.7-2194.0</td>
<td>284.5 (258.3-361.3)</td>
<td>148.0-454.0</td>
<td></td>
</tr>
<tr>
<td>480 min</td>
<td>1314.0 (1033.2-1953.5) 821.0-4584.0</td>
<td>301.5 (165.8-392.8)</td>
<td>62.0-520.0</td>
<td></td>
</tr>
<tr>
<td>1400 min</td>
<td>2239.0 (1831.5-2832.0) 1568.0-4128.0</td>
<td>98.0 (54.5-145.5)</td>
<td>50.0-529.0</td>
<td></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

**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.