Serial Measurements of Acute Phase Proteins in Patients with Acute Coronary Syndrome

ANTONIOS G. ZIAKAS*, KONSTANTINOS C. KOSKINAS*, EFTHIMIA SOULIOU, STAVROS GAVRILIDIS, GEORGE D. GIANNOGLOU, KONSTANTINOS GEIMITZIS, IOANNIS STYLIADIS

* The first two authors contributed equally to this work.

First Cardiology Department, AHEPA University Hospital, Aristotle University Medical School, Thessaloniki, Greece

Introduction: Levels of inflammatory markers increase in patients with acute coronary syndromes (ACS) and the magnitude of the inflammatory response has been related to clinical outcomes. The release patterns and, thereby, the time point of maximal increase for multiple inflammatory markers following an ACS are not fully defined. Our purpose was to serially measure three acute phase proteins (APPs) in patients with ACS.

Methods: We prospectively enrolled 74 consecutive patients (54 men, age 62.2 ± 9.8 years) with ACS: 38 with ST-elevation acute myocardial infarction (STEMI), and 36 with non-ST-elevation acute myocardial infarction (NSTEMI) or unstable angina (UA). Peripheral levels of alpha-1 antitrypsin (A1AT), alpha-1 acid glycoprotein (A1GP) and haptoglobin (HPT) were measured on admission, and 6, 12, 24, 48, 72 hours, 7 days and 6 months after the initial evaluation.

Results: Baseline levels of the APPs did not differ between the two groups. A1AT, A1GP and HPT all exhibited a similar time course among NSTEMI/UA patients, all reaching maximal values at 7 days. The markers showed an earlier increase and A1AT showed earlier peaking (at 72 hours) in STEMI patients. Peak levels were higher in patients with STEMI vs. NSTEMI/UA for all three APPs (p<0.01 for A1AT and HPT, p<0.05 for A1GP).

Conclusions: A1AT, A1GP and HPT levels increase significantly in patients with ACS and display different release curves in those with STEMI versus NSTEMI or UA. Knowledge of the release patterns of APPs may determine the optimal time point of measurement and thereby enhance their potential prognostic value in the setting of ACS.

Inflammation is a critical factor in the pathophysiology of atherosclerosis and its thrombotic complications.1,2 Population-based studies suggest that the assessment of circulating inflammatory markers may facilitate cardiovascular risk stratification among healthy individuals.3 Accumulating evidence has also established the role of inflammatory biomarkers in the prognosis of patients with acute coronary syndromes (ACS).4 Interest has primarily focused on C-reactive protein (CRP), a biomarker that independently predicts future vascular events. Although the assessment of multiple inflammatory markers has been shown to enhance their predictive value,5 the release patterns and the time point of maximal increase of other acute phase proteins (APPs) have been studied to a much lesser extent.

The inflammatory response triggered in the ACS setting is the cumulative result of preexisting low-grade inflammation in vulnerable atherosclerotic plaques, and the ongoing myocardial ischemic damage during the progression of an acute coronary event. Consistently, the magnitude of the inflammatory response, as reflected by the peripheral levels of inflammatory markers, is largely determined by the
temporal interval between symptom onset and the time point of biochemical measurement. Optimal interpretation of the elevated circulating levels of biomarkers would therefore require knowledge of their release curves and consideration of the time point of blood sampling. Furthermore, differences have been reported in circulating levels of APPs between patients with acute myocardial infarction (AMI) and unstable angina (UA), indicating the variability of inflammatory reactions according to the exact nature and extent of ischemic myocardial damage.

The purpose of the present study was to serially investigate the temporal changes in three APPs, haptoglobin (HPT), alpha-1 antitrypsin (A1AT) and acid alpha-1 glycoprotein (A1GP) in patients with ACS, determine the time points of their maximal increase, and compare the time course of these markers in the ST-elevation acute myocardial infarction (STEMI) vs. non-ST-elevation acute myocardial infarction (NSTEMI) or unstable angina (UA) setting.

Methods

Study population and follow up

Seventy-four consecutive patients (54 men, mean age 62.2 ± 9.8 years) who were admitted to the Coronary Care Unit of AHEPA University Hospital in Thessaloniki, Greece, with a diagnosis of ACS were recruited prospectively. Presentation within ≤6 hours after symptom onset was required. Thirty-eight patients (51.3%) were diagnosed with STEMI and 36 (48.7%) with NSTEMI or UA, according to established criteria. Patients with a history of systemic inflammatory diseases, such as infections or autoimmune disorders, neoplastic or hematological disease, administration of anti-inflammatory or immunosuppressive drugs, surgical procedures or trauma in the preceding 3 months, as well as patients with an equivocal or uninterpretable electrocardiogram (ECG), including left bundle branch block or persistent ST-segment elevation due to a previous myocardial infarction, were excluded from the study.

Therapeutic management during hospitalization was left to the discretion of the attending cardiologist, according to the patients’ clinical course and standard institutional protocols. All STEMI patients received thrombolytic treatment.

Witnessed informed consent was obtained from all patients prior to enrollment in the study, which was approved by the Institutional Ethics Committee.

Blood sampling and biochemical analyses

Serial venous blood samples were obtained at baseline, which was the time of admission, and after 6, 12, 24, 48, 72 hours and 7 days. Blood samples were also obtained on a follow-up evaluation 6 months (187 ± 21 days) after admission. Blood was collected in tubes containing EDTA and centrifuged, and plasma was frozen and stored at -70°C for subsequent analyses. HPT, A1AT, and A1GP levels were determined using rate nephelometry (ARRAY 360- BECKMANN-USA). The normal range for plasma concentrations was 0.16-2.0 g/L for HPT, 0.83-1.99 g/L for A1AT and 0.47-1.25 g/L for A1GP. The intra- and inter-assay coefficients of variation for the biochemical measurements were 7.9% and 11.3% for HPT, 4.4% and 7.1% for A1AT, and 5.9% and 8.5% for A1GP, respectively. All other biochemical measurements were performed in the biochemistry laboratory of our hospital from the samples obtained at baseline, using standard methods. All measurements were carried out by personnel blinded to the patients’ baseline characteristics and clinical outcomes.

Statistical analyses

Continuous variables are presented as mean ± standard deviation (SD) and categorical ones as actual numbers and percentages. Normality of distribution for continuous variables was assessed using the Kolmogorov-Smirnov test. Comparisons between categorical variables were performed using the chi-square test or Fischer’s exact test, as appropriate. Levels of the APPs over time were compared to baseline values using the paired-samples t-test or Wilcoxon Signed Rank test as required; comparisons between groups were carried out using Student’s t-test or the Mann-Whitney U-test according to the normality of distribution. Findings were considered statistically significant at the 0.05 level. All calculations were performed using the SPSS 14.0 software package (SPSS Inc, Chicago IL, USA).

Results

Baseline characteristics

The patients’ baseline characteristics are summarized in Table 1. A higher incidence of dyslipidemia (p<0.01), higher levels of triglycerides (p<0.05) and lower high-density lipoprotein (HDL) cholesterol (p<0.05) were observed in NSTEMI/UA compared to STEMI patients.
Temporal changes in HPT, A1AT and A1GP levels in patients with NSTEMI/UA and STEMI are presented in Table 2. Levels of all markers were within normal values at baseline in both groups. In NSTEMI/UA patients all markers exhibited a similar changing pattern: they were significantly increased at 48 hours compared to baseline and continued to rise, reaching their peak value at 7 days. In the STEMI group, all three markers started increasing at 24 hours, thereby exhibiting an earlier increase compared to the NSTEMI/UA group. Two of the markers, HPT and A1GP, reached maximal levels at 7 days in the STEMI group, similar to the NSTEMI/UA group, while A1AT peaked earlier, at 72 hours. All markers in both groups returned to normal levels at 6 months.

Baseline levels of all three markers did not differ between the two groups. Peak levels, i.e. levels at 7 days, and exceptionally at 72 hours for A1AT in the STEMI group, were significantly higher in the STEMI group compared to the NSTEMI/UA group. The magnitude of the increase was similar for all three markers.

In conclusion, the temporal changes in acute phase proteins observed in patients with NSTEMI/UA and STEMI are consistent with previous studies. The results suggest that the acute phase response is an important marker of disease severity and may be useful in predicting the prognosis of patients with acute coronary syndrome.
group, were significantly higher in patients with STEMI versus NSTEMI/UA for all three APPs (p<0.01 for A1AT and HPT, p<0.05 for A1GP) (Figure 1).

We also investigated the association between preadmission use of statins and the peak levels of the markers. There was no significant difference in peak levels between patients with (n=29) versus patients without statin treatment before admission (n=45) for A1GP (1.18 ± 0.24 vs. 1.26 ± 0.2, p=NS) and HPT (2.44 ± 0.63 vs. 2.62 ± 0.72, p=NS). In contrast, patients receiving statins at baseline exhibited lower peak levels of A1AT compared to statin-naïve patients (1.69 ± 0.41 vs. 2.12 ± 0.49 g/L, p<0.05).

Discussion

While the temporal changes of CRP have been extensively investigated in patients with ACS, limited data are available regarding the three inflammatory markers measured in the present study. We found that HPT, A1AT, and A1GP all increased in the setting of ACS, and showed different temporal patterns in patients with STEMI vs. NSTEMI/UA. Considering the potential prognostic value of increased inflammatory markers in patients with acute cardiac events, our results underscore the differential inflammatory response in different types of ACS, and the impact of the timing of biochemical measurements on the results recorded.

Acute phase proteins contribute to the restoration of homeostasis at sites of inflammation. HPT regulates iron homeostasis and is a critical component of the endogenous anti-oxidant system, due to its ability to counterbalance hemoglobin-induced oxidative damage.11 A1AT is a plasma proteinase inhibitor that counteracts the effects of neutrophil-derived proteolytic enzymes, thereby preventing the degradation of matrix proteins during inflammatory processes. A1GP, or orosomucoid, exerts immunomodulatory and antithrombotic effects, and also binds steroid hormones and lipophilic drugs.

Time course of acute phase proteins

We found that peripheral levels of APPs increased in patients with ACS and continued to rise for several days, a finding consistent with the well described acute phase reaction.12 Inflammatory reactions are critical determinants of the progression of clinical syndromes precipitated by atherosclerotic plaque disruption or erosion. Macrophage-derived pro-inflammatory cytokines interleukin (IL)-6 and IL-1 and tumor necrosis factor-a (TNF-a) activate the hepatic production of several inflammatory-sensitive proteins, including CRP, HPT and A1GP.13 The latter in turn further enhance the production of pro-inflammatory mediators by macrophages and endothelial cells, thereby sustaining a self-perpetuating circle of inflammation. Our findings are also in agreement with previously reported data from ACS patients showing a sustained increase and delayed peaking of CRP compared to IL-6, the main promoter of hepatic CRP production.14

The increase in plasma levels of APPs after ACS has been reported previously, although there is a paucity of data concerning the release curve of each marker. Crook et al reported elevated A1GP levels in patients with AMI compared to controls.15 A prolonged increase of HPT and A1GP levels was also demonstrated in ACS patients who underwent coronary stent implantation, starting at 48 hours and rising up to 7 days post stenting; however, these changes were attributed to the combination of the ACS and the coronary intervention.16

We identified different release curves of the measured APPs between STEMI and NSTEMI/UA patients. We found that patients with STEMI displayed higher peak levels, but not baseline levels of A1AT, A1GP and HPT, compared to patients with NSTEMI/UA. Brunetti et al reported higher levels of A1AT and HPT, obtained at one single time point within 12 hours of symptom onset, in patients with AMI compared to UA patients.8 Similar differenc-

Figure 1. Peak levels of haptoglobin (HPT), alpha-1 antitrypsin (A1AT), and alpha-1 acid glycoprotein (A1GP) in patients with non-ST-elevation myocardial infarction (NSTEMI) or unstable angina (UA), versus ST-elevation acute myocardial infarction (STEMI). **p<0.01; *p<0.05.
es have been found for CRP, in single, pre-discharge measurements of AMI vs. UA patients, as well as in serial measurements up to 96 hours from symptom onset in patients with Q-wave AMI vs. non Q-wave AMI or UA patients. Further, Kazmierczak et al reported earlier peaking of A1GP in patients with AMI compared to UA; however, unlike the results of our study, these authors reported a more marked increase of A1GP in UA patients, likely due to a more intense response to continuous inflammatory stimulation during the progression of unstable angina. Differences in the study population, the interval between symptom onset and the initial evaluation, and the time points of biochemical measurements may account for the discrepancy with the present study.

The underlying mechanisms of the variable activation of APPs in heterogeneous clinical entities defined as ACS have not been fully elucidated. The cumulative effect of preexisting chronic inflammation in the atherosclerotic coronary vasculature, acute phase reactions in highly-inflamed atherosclerotic plaques, and the inflammatory response to myocardial necrosis or ischemia reperfusion damage, may all contribute to the marked activation of APPs in AMI. In contrast, low-grade myocardial necrosis that occurs during the progression of UA may account for the less accelerated elevation, and the less pronounced peaking of A1AT, A1GP and HPT in our NSTEMI/UA compared to STEMI patients. The exact nature of the myocardial damage, as well as the time lapse between the initiation of the atherothrombotic event and blood sampling, clearly affect the magnitude of the recorded inflammatory activation, emphasizing the importance of the time point of the assessment of different biomarkers in different types of acute coronary events.

We also observed variable changing patterns of the measured markers within the STEMI group, consisting of an earlier peaking of A1AT compared to A1GP and HPT. The finding that the APPs measured in our study do not exhibit the same response to equal stimuli may support the need for a multi-marker approach rather than the quest for a unique “ideal” biomarker in patients with ACS. Indeed, the evaluation of multiple biomarkers has been shown to enhance their performance as cardiovascular risk predictors. Although in the present study we did not specifically investigate the effect of medications on the release of the assessed inflammatory markers, we determined the potential impact of statins, recognized anti-inflammatory agents, on the release patterns of these markers. While statin treatment has previously been associated with attenuation of the inflammatory response in the ACS setting, statins were related to the decrease of only one of the markers measured in this study, namely A1AT. This finding likely indicates the heterogeneity in the response of individual inflammatory mediators to the anti-inflammatory properties of statins.

In conclusion, the present study found a significant increase of three acute phase proteins during the progression of acute coronary syndromes. These markers exhibited variable release curves in patients with STEMI compared to NSTEMI or UA, emphasizing the differential variable release curves in patients with STEMI compared to NSTEMI or UA, emphasizing the differential inflammatory activation triggered by different types of myocardial damage. Increased levels of inflammatory markers predict adverse events in different manifestations of coronary atherosclerotic disease. Our present serial investigation adds to previous studies by showing that differences in the changing patterns among certain markers might determine the optimal timing for their measurement. Given the well established predictive role of CRP in the ACS setting, serial evaluation of less studied inflammatory mediators may further optimize individual risk stratification in ACS patients, and likely enable more aggressive management and targeted follow up of higher-risk patients.

**Study limitations**

The relatively small number of patients and the fact that CRP, a gold standard inflammatory marker, was not measured are acknowledged as limitations of the present study. Further studies with larger population sizes may be warranted to further assess the serial changes, and the potential role of acute phase proteins as risk predictors after acute coronary events.

**References**


17. Zebrack JS, Anderson JL, Maycock CA, Horne BD, Bair TL, Muhlestein JB. Usefulness of high-sensitivity C-reactive protein in predicting long-term risk of death or acute myocardial infarction in patients with unstable or stable angina pectoris or acute myocardial infarction. Am J Cardiol. 2002; 89: 145-149.


