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Pre-existing inflammation influences the outcome of acute coronary syndrome: a cross sectional study

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Abstract

Objectives

Inflammation is a well-established risk factor for the development of coronary artery disease (CAD) and acute coronary syndrome (ACS). However, less is known about its influence on the outcome of ACS. The aim of this study was to determine if blood biomarkers of inflammation were associated specifically with acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Design

Cross sectional study

Setting

Patients admitted to the coronary care unit, via the emergency room, at a central county hospital over a four-year period (1992-96).

Participants

From 5292 patients admitted to the coronary care unit, we identified 908 patients aged 30-74 years, who at discharge had received the diagnosis of either MI (527) or UA (381).

Main outcome measures

MI or UA

Results

When adjusted for smoking, age, sex, and duration of chest pain, concentrations of plasma biomarkers of inflammation (hsCRP>2mg/L (OR=1.40 (1.00-1.96) and

fibrinogen (p for trend=0.035)) analysed at admission were found to be associated with MI over UA, in an event of an ACS. A strong significant association with MI over UA was found for blood cell markers of inflammation, i.e. counts of neutrophils (p for trend<0.001), monocytes (p for trend<0.001), and thrombocytes (p for trend=0.021), while lymphocyte count showed no association. Interestingly, eosinophil count (p for trend=0.003) was found to be significantly lower in patients with MI compared to UA.

Conclusions

Our results show that in patients with an ACS the blood cell profile and degree of inflammation at admission was associated with the outcome. Furthermore, our data suggest that a pre-existing low-grade inflammation may dispose towards MI over UA.

Keywords: Inflammation, acute coronary syndrome, unstable angina, myocardial infarction

Abbreviations:

ACS; Acute Coronary Syndrome

MI; Myocardial Infarction

UA; Unstable Angina

CAD; Coronary Artery Disease

PCI; Percutaneous Coronary Intervention

CABG; Coronary Arterial Bypass Graft Surgery

hsCRP; High sensitivity C-Reactive Protein

SAA; Serum Amyloid A

OR; Odds Ratio

CI; Confidence Interval

What is already known on this subject:

- Inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions
- The role of inflammation for the outcome of an acute coronary syndrome (ACS) to either myocardial infarction or unstable angina is less established

What this study adds:

- A pre-existing inflammation is a risk factor for a more severe ACS outcome.
- The early inflammatory response predicts the outcome of an ACS.
- A distinct difference in blood cell profile is associated with ACS outcome.

Strengths and limitations of this study

Strengths:

- The patients were recruited before the introduction of PCI, CABG and modern antithrombotic drugs in the standard management of ACS. Thus, it was possible to identify progression to UA or MI as distinct outcome groups within the cohort, in the absence of interventions that would otherwise influence the thrombotic processes involved in ACS.
- The study was based in a single centre with the same two cardiologists evaluating and categorising all 5292 patients, using consistent criteria.

Limitations:

- Some of the UA cases would likely have been diagnosed as NSTEMI using the most recent criteria of MI.
- Treatments and risk factor profiles have partly evolved since the study was performed.

Introduction

The acute coronary syndrome (ACS) is usually initiated by an atherosclerotic plaque rupture or disruption of the overlying endothelial surface. Subsequent thrombosis formation can permanently occlude the lumen of a coronary artery, causing myocardial cell death and the induction of myocardial infarction (MI). However, in other cases it can be transient, or only partially occlude the vessel, resulting in unstable angina (UA) [1 2]. It is not known why some patients progress to the former, rather than the latter outcome. It is well established that a low-grade inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions [1 2]. A role for inflammatory mediators during the evolution of an ACS is indicated by the widespread coronary inflammation found during UA, throughout the entire coronary artery bed, not only the artery containing the culprit lesion [3 4]. To what extent ACS outcome is related to a concurrent inflammatory response or to the degree of pre-existing inflammation is less established [2 5].

The Carlscrona Heart Attack Prognosis Study (CHAPS) constitutes a patient cohort recruited before the introduction of percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) surgery and modern antithrombotic drugs in the management of patients with ACS. Thus, to our knowledge, this study is unique in that MI and UA could be identified as distinct groups within an ACS population. In a previous CHAPS report we demonstrated that smoking, or impaired glucose homeostasis, were acquired risk factors for a severe ACS outcome [6]. In the current study the aim was to determine if blood biomarkers of inflammation, e.g. high sensitivity CRP (hsCRP), serum amyloid protein A (SAA), plasma fibrinogen, and blood cell counts and indices are associated specifically with either acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Materials and Methods

Patient recruitment

The patient material has previously been described in detail [6]. In brief, in the Carlscrona Heart Attack Prognosis Study (CHAPS) we recruited 5292 consecutive patients admitted to the coronary intensive care unit with acute chest pain (indicative of a possible ACS) at Blekinge Hospital, Karlskrona, between January 26, 1992 and January 25, 1996. Of the total number of admittances, 2992 were between 30-74 years of age at admittance. In patients with multiple admittances, only the first classifying admittance was included as 'event' (UA or MI) in the analysis. Informed consent was obtained from all included patients and the study complies with the Declaration of Helsinki.

Acute coronary syndrome patients

As previously described [6] a diagnosis of ACS was confirmed in 908 of the eligible patients aged 30-74 years of age (644 men and 264 women). Two groups were identified: (i) patients experiencing at least one acute MI during the study (527) or (ii) patients experiencing no acute MI, but having at least one episode of UA during the study (381). Data on environmental and lifestyle factors, and blood samples, were collected on first admittance under the classifying diagnosis. The classifying diagnosis was set at discharge by one of two experienced cardiologists.

A diagnosis of acute MI was made when patients fulfilled at least two of the following criteria: (i) A history of chest pain of at least 15 min duration, (ii) an increase in activity of cardiac enzymes to at least twice the upper limit of normality, or (iii)

characteristic ECG changes for MI (typical sequence change of ST segment and/or of T-waves and/or appearance of new Q-waves). These criteria included both patients with ST-elevation MI (STEMI) and non-ST elevation MI (NSTEMI).

A diagnosis of UA was made when patients fulfilled all of the following criteria: (i) no evidence of MI, (ii) acute chest pain of increased/modified character to any previously experienced, during the preceding 48 h and (iii) angina pectoris diagnosed and medically treated before admission, or alternatively, angina pectoris ascertained by clinical evaluation, including a bicycle exercise test prior to discharge from the hospital[6]. Post-infarction angina and patients with secondary angina were not included.

Patients admitted to the coronary intensive care unit were initially treated with aspirin, and in case of on-going chest pain, also nitrates and morphine. In cases of clear diagnosis of ST elevation MI, thrombolysis with streptokinase was given (194 of 527 patients with MI). If the diagnosis of MI was based on cardiac markers only, thrombolysis was not given. Acute coronary artery intervention was not available at this hospital at the time of the study.

Ethical approval

Carlscrona Heart Attack Prognosis Study (CHAPS) was approved by the Regional Ethical Review Board, Lund, Sweden (EPN 2009/762 and LU 298-91).

Risk factors

Information on risk factors and medical history were recorded at admission from patient history and/or extracted from earlier medical files, and the diagnosis and

information were also verified at discharge from the hospital [6]. Smoking status was defined as current- or non-smoker. Patients who had quit smoking >1 month prior to admission were classified as non-smokers.

Laboratory analyses

Samples for laboratory analysis were collected at hospital admission. Haematological variables (blood cell count and indices) and plasma fibrinogen were analysed using routine diagnostic methods in fresh samples at time of admission. Blood cell count was analysed in EDTA whole blood by ADVIA 2120 (Siemens, Germany) and plasma fibrinogen in Sodium Citrate blood samples on a Trombotrack instrument (Nycomed, Norway). High sensitivity CRP (hsCRP) and Serum Amyloid A protein (SAA) were analysed in samples that had been stored at -80 and thawed. Both proteins were analysed by BN ProSpec (Siemens, Germany).

Statistical methods

STATA and IBM SPSS Statistics (version 21) were used for data analyses. Standard methods were used for descriptive statistics. Associations between categorical variables were examined using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (CI). Principal analyses were made with men and women combined in one group, but were repeated where men and women were analysed separately. Age was entered into the regressions in 10-year age groups. Duration of chest pain from onset to blood sampling upon admission to the Emergency Room (ER) was divided in ≥ 4 hours or < 4 hours. Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The tertiles were then entered into the regression as a linear variable to test for trend. Confounding was considered

by stratification and by multivariate regression models forcing age group, sex, current smoking, and duration ≥240 minutes into the same model. Individuals with a missing variable were excluded in the respective analysis. Two-way interaction terms were used to explore the association of sex and the major risk factors with ACS outcome.

Results

We included 908 patients with ACS (527 MI, 381 UA). In table 1 patient characteristics are shown. When analysing the plasma protein inflammatory biomarkers, adjusted for differences in age and gender, we found that high sensitivity CRP (hsCRP)>2 mg/L at hospital admission was significantly associated with MI over UA (OR=1.75 (1.31-2.35)). Also fibrinogen (p for trend = 0.01) and Serum Amyloid A (SAA) (p for trend = 0.005) were significantly associated with MI (Table 2).

To separate an inflammatory response to myocardial tissue necrosis in patients with MI from that of a possible pre-existing inflammation, we analysed hsCRP levels in relation to duration from onset of chest pain until blood sampling. Controlling for differences in age and sex we found a significant correlation of hsCRP with duration only in the MI patients that had ≥ 240 minutes duration since onset of symptoms (r=0.19, p=0.033) but not in MI patients with a shorter duration (r=0.02, p=0.777), or in UA patients with ≥ 240 minutes duration or shorter duration (r=-0.10, p=0.452 and r=-0.02, p=0.779, respectively). After including smoking and time duration since onset of chest pain in the model hsCRP >2mg/L (OR= 1.40 (1.00-1.96)) and fibrinogen (p for trend = 0.035) remain associated with MI over UA while SAA was no longer significantly

associated with MI (Table 3). Time duration since onset of symptoms as such did not reach a statistically significant association with MI over UA (OR 1.41 (0.98-2.03), Table 3).

The strongest associations with MI over UA were found when haematological variables (blood cells) were analysed (Table 2 and 3). Of circulating inflammatory blood cells, higher counts of neutrophils and monocytes, and lower counts of eosinophils were associated with a worse outcome of an ACS (Table 2). These associations were not affected when adjusting for smoking and duration of symptoms (Table 3) or in trend tests where the associations were highly significant ($p < 0.001$). In contrast, lymphocyte and basophil counts showed no association with outcome. Also, we found that higher thrombocyte count was significantly associated with MI (Table 2 and 3). Interestingly, a smaller thrombocyte mean volume was significantly associated with MI when compared to UA. We found no significant interaction between sex and inflammatory response in relation to the outcome of ACS.

Discussion

In the current study we showed that levels of inflammatory biomarkers at time of admission are associated with a more severe outcome in the case of ACS (i.e. predisposition towards MI, rather than UA). We found significant differences in blood cell profiles between a MI or UA outcome, with elevated neutrophils, monocytes and platelets counts in MI, together with a reduced eosinophil count and a lower mean platelet volume. Plasma biomarkers for inflammation (hsCRP, fibrinogen and SAA) showed weaker associations.

The strength and novelty of the Carlscrona Heart Attack Prognosis Study (CHAPS) is due to the unique nature of the patient cohort. The patients were recruited before the introduction of PCI, CABG, and modern antithrombotic drugs in the standard management of ACS. These interventions would otherwise influence the thrombotic processes involved in ACS. The absence of them at that time made it possible for us to identify progression to MI or UA as distinct outcome groups within the cohort. Furthermore, the study was based in one centre with the same two cardiologists assessing and categorising all patients, using consistent criteria. There are limitations of the study that should be acknowledged. Analyses of hsCRP and SAA were performed using frozen samples stored at -80 C for 15 years, however biochemical analyses of fibrinogen and blood cells were performed over a period of four years, although the hospital routine diagnostic laboratory used accredited standardised methods, providing consistency over time. Furthermore, not all patients have complete data for laboratory analyses. As refined criteria and more sensitive and specific biomarkers are implemented the definition of MI continues to evolve. It is likely that some of the UA cases in our study would now been diagnosed as NSTEMI, using recent criteria required for MI diagnosis [7]. As CHAPS is a single centre study, and treatments and risk factor profiles have partly developed since the study was performed, the results would therefore not necessarily be generalised to a broader modern population.

Previously, we have shown, using the CHAPS material, that genetic variations of thrombotic factors are associated with ACS outcome [8], and furthermore that acquired risk factors, smoking and impaired glucose homeoestasis together with male sex, predispose to MI over UA[6]. Here we showed that a more pronounced state of

inflammation conferred an increased risk towards MI, rather than UA, in ACS. It is well established that a low grade inflammation has a pathogenic role for the progression of atherosclerotic coronary artery lesions[1] however it is less known to what extent a pre-existing inflammation can influence the outcome of ACS [2]. An elevation of inflammatory biomarkers in patients with ACS may reflect myocardial injury. Fibrinogen, CRP and SAA are induced by cytokine signalling, e.g. by IL1, TNF and IL6 [1 9 10]. However, due to a period of *de novo* synthesis and secretion of these proteins there is a time lag before a rise in plasma concentration becomes detectable during the acute phase of inflammation, with an average response time of 8 hours [9]. Furthermore, in patients with MI there is a known latency of 6-12 hours from onset of chest pain to a rise in CRP plasma concentrations [11]. Also we observed a correlation between hsCRP and time duration only in MI patients who had more than 4 hours since debut of symptoms before blood sampling. Thus, the associations with MI over UA that we observe in patients with duration of chest pain of less than 4 hours indicate that in ACS a higher pre-existing inflammation predisposes to a more severe outcome. In CHAPS we have previously found current smoking to be strongly associated with MI, but not UA [6]. We considered the possibility that these results could be explained by the known inflammatory effect of smoking [12 13 14]. However, the significant associations with MI for hsCRP and fibrinogen were still observed when adjusting for smoking. The strongest associations with MI over UA were observed when analysing circulating inflammatory blood cells, associations that were independent of smoking and time duration of symptoms to blood sampling. In contrast to plasma protein biomarkers that require synthesis before there is a detectable increase in levels, preformed blood cells can be quickly mobilised into circulation by demargination from the vessel wall and egress from the bone marrow [15]. Pro-inflammatory cytokines stimulate neutrophil and monocyte production in the

bone marrow. Stress induced release of endogenous catecholamine and glucocorticoids can mobilise these stores shortly after onset of chest pain. Thus, the magnitude of rise in cell count can reflect the size of the preformed cell pool that has been increased by a pre-existing low grade inflammation [15]. Thus, the difference we observed in neutrophil and monocyte count between MI and UA indicated a pre-existing inflammation preceding the ACS, consistence with our observations regarding hsCRP and fibrinogen levels. In a recent population-based cohort study Adamsson Eryd *et al.* found an association between increased neutrophil counts and incidence of coronary events and increased case fatality rate during follow-up [16], in line with a previous meta-analysis of several prospective population studies [17]. A possible explanation behind our observation that neutrophilia was associated with MI over UA is a hypercoagulable or thromboresistant state, as previously indicated by reduced efficiency of thrombolytic therapy or primary percutaneus coronary interventions in MI patients with elevated WBC [18-20]. In this context, it is interesting that a reduced efficiency of primary percutaneus coronary interventions in MI patients has recently been found to be associated with an increased amount of neutrophil extracellular traps (NETs) in aspirated coronary thrombi [21], adding support for an important role for neutrophils in the ACS thrombotic process. An association of an increased monocyte count and coronary events has previously been reported from population studies [22 23]. A possible mechanism relates to the heavy infiltration of monocytes/macrophages that is a characteristic of a thin fibrous cap of a vulnerable plaque [24]. Thus, a pre-existing monocytosis in MI might lead to a greater monocytoid infiltration, compared to UA, and predispose to a more extensive thrombotic process following plaque rupture [2 5]. Interestingly, we found significantly lower eosinophil counts in MI patients, compared to UA ones, consistent with recent reports [25]. Eosinophils have been detected in aspirates from thrombi in MI patients [25,26],

suggesting a possible role for this cell type in the progression of the thrombotic process in ACS. Our observation could indicate an active consumption of eosinophils in MI, or reflect a pre-existing condition of elevated eosinophil count and hypersensitivity inflammation that could predispose to UA. Indeed, Erdogan *et al.* reported a significant higher eosinophil count in UA patients, but not MI patients, when compared to controls [27]. Thrombocytes are key effector cells in an inflammatory process [28 29], and an increase of the thrombocyte count is part of an inflammatory condition [30]. Recently the role of thrombocytes in both vascular inflammation and the thrombotic process in CAD has been highlighted [5 31 32], with an increased mean platelet volume (MPV) reported to be associated with acute cardiovascular events [33 34]. In a systematic review and meta-analysis using pooled results from 16 cross-sectional studies involving 2809 patients, MPV was found to be significantly higher in patients with ACS than in patients with stable CAD or healthy individuals [33]. No significant difference in MPV was found between subjects with MI and those with UA. Individual studies have shown both higher and lower MPV in MI over UA patients [35 36]. In ACS thrombocytes are involved in a dynamic thrombotic process with consumption of preferentially more reactive large-sized thrombocytes [34]. This is extensive and permanent in MI, in contrast to the recurrent episodes of (temporary) coronary platelet aggregation and consumption in UA [37 38], tending to result in a lower MPV in MI than UA, as in our study and the study of Mathur et al[36]. This is however in most studies probably counterbalanced by the effects on thrombocytes of a more intense pre-existing inflammation in MI, leading to a similar MPV in MI and UA [33].

In conclusion, while inflammation is well established as a major risk factor for development of CAD and risk of future events, our study indicate the further role of

inflammation in a more severe outcome in the case of ACS. Our data suggests that neutrophil levels can have a prognostic value in patients with ACS, as previously suggested [16]. The observed differences in ACS outcome associated with inflammation and blood cell profiles raise several hypotheses that warrant further investigation. Establishing the mechanisms for this at the cellular level could lead to optimisation of pharmacological treatment for CAD and ACS.

Footnotes:

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Contributors statement:

HO, LR, and MF designed and initiated the original CHAPS cohort study on which the current study is based. MF conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. JO, HF, IV, HO, AH, LR, UL conceived and designed the current study. IV and MP collected and compiled the laboratory data. HF and UL performed the statistical analyses and compiled the results. JO, MF, HF, IV, HO, AH, LR, UL interpreted the results. JO, HO, UL drafted the paper. MF, HF, IV, LR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JO is the guarantor.

Conflict of interest

No conflict of interests to declare.

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for

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Data sharing: No additional data available

The lead author, Jacob Odeberg, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Table 1. Characteristics of the study population.

Risk factors	Men n=644 m	(SD)	Women n=264 m	(SD)
Age (years)	63.0	(8.6)	65.5	(8.0)
Serum cholesterol (mmol L^{-1})	6.0	(1.3)	6.6	(1.4)
Plasma glucose (mmol L^{-1})	6.8	(3.4)	7.3	(3.8)
HbA1c (%)	5.2	(1.3)	5.4	(1.7)
Hs-CRP (mmol L^{-1})	9.3	(21.9)	9.0	(21.4)
Duration (minutes)	287	(418)	360	(421)
	n	(%)	n	(%)
Hypertension	169	(27.2)	71	(28.1)
Diabetes	92	(14.8)	50	(19.7)
Smoking (current)	148	(24.1)	43	(17.1)
Duration ≥240 minutes	153	(31.1)	74	(38.5)
HsCRP >2 mg/L	341	(59.3)	141	(63.5)

Risk factors		Range	Men n=644 n (%)	Women n=264 n (%)
S-amyloid (mg/L)	Tert 1	0.111-3.25	209 (36.3)	57 (25.7)
	Tert 2	3.26-7.44	191 (33.2)	75 (33.8)
	Tert 3	7.45-1570	175 (30.4)	90 (40.5)
Fibrino (g/L)	Tert 1	1.5-3.3	233 (40.5)	77 (33.8)
	Tert 2	3.4-4.0	159 (27.7)	73 (32.0)
	Tert 3	4.1-10.0	183 (31.8)	78 (34.2)
Leuko (10^9/L)	Tert 1	2.49-7.39	196 (32.6)	85 (35.4)
	Tert 2	7.4-9.8	198 (32.9)	84 (35.0)
	Tert 3	9.82-80.9	207 (34.4)	71 (29.6)
Neutro (10^9/L)	Tert 1	0.14-4.79	191 (32.3)	84 (36.5)
	Tert 2	4.81-7.04	196 (33.1)	78 (33.9)
	Tert 3	7.05-20.06	205 (34.6)	68 (29.6)
Eosino (10^9/L)	Tert 1	0-0.06	155 (27.2)	84 (36.8)
	Tert 2	0.07-0.14	186 (32.6)	77 (33.8)
	Tert 3	0.15-9.12	229 (40.2)	67 (29.4)
Baso (10^9/L)	Tert 1	0-0.039	229 (40.7)	95 (43.0)
	Tert 2	0.04-0.059	174 (30.9)	60 (27.1)
	Tert 3	0.06-0.33	160 (28.4)	66 (29.9)
Lympho (10^9/L)	Tert 1	0.16-1.32	206 (34.8)	71 (30.9)
	Tert 2	1.33-1.88	191 (32.3)	80 (34.8)

	Tert 3	1.89-75.33	195 (32.9)	79 (34.3)
Mono (10^9/L)	Tert 1	0.04-0.4	167 (28.4)	116 (50.7)
	Tert 2	0.41-0.56	212 (36.0)	59 (25.8)
	Tert 3	0.57-1.60	210 (35.7)	54 (23.6)
T-cyt (10^9/L)	Tert 1	85-198	228 (38.1)	54 (23.6)
	Tert 2	199-247	182 (30.4)	94 (39.3)
	Tert 3	248-680	188 (31.4)	91 (38.1)
T-mcv (fL)	Tert 1	6.5-8.8	221 (39.4)	69 (31.4)
	Tert 2	8.9-9.4	167 (29.8)	78 (35.5)
	Tert 3	9.5-46.0	173 (30.8)	73 (33.2)

Data are means (m) and standard deviations (SD), or numbers (n) and proportions (%). HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Baso-Basophil cell count; Lympho-lymphocyte cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume. Missing data age (n=0), serum cholesterol (n=102), plasma glucose (n=82), HbA1c (n=108), Hs-CRP (n=111), duration (n=224), hypertension (n=34), diabetes (n=34), smoking (n=43), s-amyloid (n=111), fibrinogen (n=105), leukocytes (n=67), neutrophils (n=neutrophils (n=86), eosinophils (n=110), basophils (n=124), lymphocytes (n=86), monocytes (n=90), thrombocyte cell count (n=71), T-mcv (n=127).

Table 2 . Risk factors for a MI as outcome of an ACS (adjusted for differences in age and sex).

Risk factors		OR	95% CI	
Sex (male vs female)		1.59	1.19-2.13	
Age-group (by 10 years)		1.01	1.00-1.02	
HsCRP >2 mg/L		1.75	1.31-2.35	
Sex (male vs female)		1.72	1.25-2.37	
Age-group (by 10 years)		1.00	0.98-1.02	
S-amyloid	Tert 1	1.0		*p for trend 0.005*
	Tert 2	1.40	0.99-1.98	
	Tert 3	1.66	1.16-2.36	
Fibrino	Tert 1	1.00		*p for trend 0.010*
	Tert 2	1.37	0.96-1.95	
	Tert 3	1.57	1.11-2.20	
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.75	1.95-3.88	
	Tert 3	9.43	6.29-14.1	
Neutro	Tert 1	1.00		*p for trend <0.001*
	Tert 2	2.94	2.07-4.17	
	Tert 3	8.83	5.92-13.17	

Eosino	Tert 1	1.00		*p for trend 0.002*
	Tert 2	0.65	0.45-0.94	
	Tert 3	0.56	0.39-0.80	
Baso	Tert 1	1.00		*p for trend 0.099*
	Tert 2	1.04	0.74-1.46	
	Tert 3	1.36	0.96-1.93	
Lympho	Tert 1	1.00		*p for trend 0.572*
	Tert 2	0.86	0.61-1.21	
	Tert 3	0.91	0.64-1.28	
Mono	Tert 1	1.00		*p for trend* <0.001
	Tert 2	1.29	0.92-1.82	
	Tert 3	3.18	2.20-4.60	
T-cyt	Tert 1	1.00		*p for trend 0.027*
	Tert 2	1.14	0.81-1.60	
	Tert 3	1.48	1.05-2.08	
T-mcv	Tert 1	1.00		*p for trend* <0.001
	Tert 2	0.45	0.32-0.65	
	Tert 3	0.50	0.35-0.72	

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95% CI). Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The tertiles were then entered into the regression as a linear variable to test for trend. HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count;

Eosino-eosinophil cell count; Baso-Basophil cell count; Lympho-lymphocyte cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

Table 3. Risk factors for a MI as outcome of an ACS. Multivariate analysis, adjusted for differences in age, sex, smoking and duration of symptoms.

Risk factors		OR	95% CI	
Covariates in model: sex, age_group, smoking, duration				
Hscrp >2 mg/L		1.40	1.00-1.96	
Sex (male vs female)		1.50	1.04-2.17	
Age-group (by 10 years)		1.01	0.99-1.03	
Smoking (yes/no)		2.15	1.39-3.32	
Duration (≥4h vs <4h)		1.41	0.98-2.03	
S-amyloid	Tert 1	1.0		*p for trend 0.225*
	Tert 2	1.43	0.96-2.13	
	Tert 3	1.27	0.84-1.92	
Sex (male vs female)		1.51	1.04-2.18	
Age-group (by 10 years)		1.01	0.99-1.03	
Smoking (yes/no)		2.24	1.45-3.45	
Duration (≥4h vs <4h)		1.43	0.99-2.05	
Fibrino	Tert 1	1.00		*p for trend 0.035*
	Tert 2	1.26	0.84-1.87	
	Tert 3	1.55	1.03-2.34	
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.56	1.73-3.80	
	Tert 3	7.32	4.65-11.5	

Neutro	Tert 1	1.00	*p for trend <0.001*
	Tert 2	2.54	1.71-3.78
	Tert 3	7.33	4.65-11.6
Eosino	Tert 1	1.00	*p for trend 0.003*
	Tert 2	0.69	0.45-1.07
	Tert 3	0.53	0.35-0.81
Baso	Tert 1	1.00	*p for trend 0.130*
	Tert 2	1.14	0.77-1.70
	Tert 3	1.37	0.91-2.06
Lympho	Tert 1	1.00	*p for trend 0.855*
	Tert 2	0.77	0.52-1.14
	Tert 3	0.97	0.64-1.46
Mono	Tert 1	1.00	*p for trend <0.001*
	Tert 2	1.00	0.67-1.48
	Tert 3	2.36	1.54-3.63
T-cyt	Tert 1	1.00	*p for trend 0.021*
	Tert 2	1.11	0.75-1.65
	Tert 3	1.60	1.07-2.38
T-mcv	Tert 1	1.00	*p for trend <0.001*
	Tert 2	0.41	0.27-0.62
	Tert 3	0.45	0.30-0.69

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals

(95% CI). All models included sex, age-group, smoking, and duration of chest pain as covariates beside the specified risk factor itself. Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The tertiles were then entered into the regression as a linear variable to test for trend. HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Baso-Basophil cell count; Lympho-lymphocyte cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ***Page 1, 3***
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ***Page 3***
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ***Page 7***
Objectives	3	State specific objectives, including any prespecified hypotheses ***Page 7***
Methods		
Study design	4	Present key elements of study design early in the paper ***Page 8, page 9***
Setting	5	Describe the setting , locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ***Page 8, page 9***
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants ***Page 8, page 9***
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ***Page 8, page 9***
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ***Page 8, page 9***
Bias	9	Describe any efforts to address potential sources of bias ***No potential sources of bias identified***
Study size	10	Explain how the study size was arrived at ***Page 8, page 9***
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ***Page 10, page 11***
( ) Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ***Page 10, page 11***
		(*b*) Describe any methods used to examine subgroups and interactions ***Page 11***
		(*c*) Explain how missing data were addressed ***Page 11***
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy ***N.A***
		(*e*) Describe any sensitivity analyses ***N.A***

Results		
(□)Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ***Page 11***
		(b) Give reasons for non-participation at each stage ***Page 11***
		(c) Consider use of a flow diagram (a flow diagram was included in the previous BMJ article describing the CHAPS cohort)
□ Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ***Table 1***
		(b) Indicate number of participants with missing data for each variable of interest ***Table 1 footnotes***
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
□Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures ***Table 1***
□ Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ***Page 11-12 and Table 2, Table 3 footnotes***
		(*b*) Report category boundaries when continuous variables were categorized ***Table 1, footnotes Table 2 and Table 3***
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ***N.A***
□ Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ***Page 12***
Discussion		
□ Key results	18	Summarise key results with reference to study objectives ***Page 12***
□Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ***Page 13***
□Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ***Page 13-16***
□Generalisability	21	Discuss the generalisability (external validity) of the study results ***Page 13***
Other information		
(□) Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ***Page 18***

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The influence of pre-existing inflammation on the outcome of acute coronary syndrome: a cross sectional study

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The influence of pre-existing inflammation on the outcome of acute coronary syndrome: a cross sectional study

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Abstract

Objectives

Inflammation is a well-established risk factor for the development of coronary artery disease (CAD) and acute coronary syndrome (ACS). However, less is known about its influence on the outcome of ACS. The aim of this study was to determine if blood biomarkers of inflammation were associated specifically with acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Design

Cross sectional study

Setting

Patients admitted to the coronary care unit, via the emergency room, at a central county hospital over a four-year period (1992-96).

Participants

In a sub-study of Carlscrona Heart Attack Prognosis Study (CHAPS) of 5292 patients admitted to the coronary care unit, we identified 908 patients aged 30-74 years, who at discharge had received the diagnosis of either MI (527) or UA (381).

Main outcome measures

MI or UA, based on the diagnosis set at discharge from hospital.

Results

When adjusted for smoking, age, sex, and duration of chest pain, concentrations of plasma biomarkers of inflammation (hsCRP>2mg/L (OR=1.40 (1.00-1.96) and

fibrinogen (p for trend=0.035)) analysed at admission were found to be associated with MI over UA, in an event of an ACS. A strong significant association with MI over UA was found for blood cell markers of inflammation, i.e. counts of neutrophils (p for trend<0.001), monocytes (p for trend<0.001), and thrombocytes (p for trend=0.021), while lymphocyte count showed no association. Interestingly, eosinophil count (p for trend=0.003) was found to be significantly lower in patients with MI compared to UA.

Conclusions

Our results show that in patients with an ACS the blood cell profile and degree of inflammation at admission was associated with the outcome. Furthermore, our data suggest that a pre-existing low-grade inflammation may dispose towards MI over UA.

Keywords: Inflammation, acute coronary syndrome, unstable angina, myocardial infarction

Abbreviations:

ACS; Acute Coronary Syndrome

MI; Myocardial Infarction

UA; Unstable Angina

CAD; Coronary Artery Disease

PCI; Percutaneous Coronary Intervention

CABG; Coronary Arterial Bypass Graft Surgery

hsCRP; High sensitivity C-Reactive Protein

SAA; Serum Amyloid A

OR; Odds Ratio

CI; Confidence Interval

What is already known on this subject:

- Inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions
- The role of inflammation for the outcome of an acute coronary syndrome (ACS) to either myocardial infarction or unstable angina is less established

What this study adds:

- A pre-existing inflammation is a risk factor for a more severe ACS outcome.
- The early inflammatory response predicts the outcome of an ACS.
- A distinct difference in blood cell profile is associated with ACS outcome.

Strengths and limitations of this study

Strengths:

- The patients were recruited before the introduction of PCI, CABG and modern antithrombotic drugs in the standard management of ACS. Thus, it was possible to identify progression to UA or MI as distinct outcome groups within the cohort, in the absence of interventions that would otherwise influence the thrombotic processes involved in ACS.
- The study was based in a single centre with the same two cardiologists evaluating and categorising all 5292 patients, using consistent criteria.

Limitations:

- Some of the UA cases would likely have been diagnosed as NSTEMI using the most recent criteria of MI.
- Treatments and risk factor profiles have partly evolved since the study was performed.

Introduction

The acute coronary syndrome (ACS) is usually initiated by an atherosclerotic plaque rupture or disruption of the overlying endothelial surface. Subsequent thrombosis formation can permanently occlude the lumen of a coronary artery, causing myocardial cell death and the induction of myocardial infarction (MI). However, in other cases it can be transient, or only partially occlude the vessel, resulting in unstable angina (UA) [1 2]. It is not known why some patients progress to the former, rather than the latter outcome. It is well established that a low-grade inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions [1 2]. A role for inflammatory mediators during the evolution of an ACS is indicated by the widespread coronary inflammation found during UA, throughout the entire coronary artery bed, not only the artery containing the culprit lesion [3 4]. To what extent ACS outcome is related to a concurrent inflammatory response or to the degree of pre-existing inflammation is less established [2 5].

The Carlscrona Heart Attack Prognosis Study (CHAPS) constitutes a patient cohort recruited before the introduction of percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) surgery and modern antithrombotic drugs in the management of patients with ACS. Thus, to our knowledge, this study is unique in that MI and UA could be identified as distinct groups within an ACS population. In a previous CHAPS report we demonstrated that smoking, or impaired glucose homeostasis, were acquired risk factors for a severe ACS outcome [6]. In the current study the aim was to determine if blood biomarkers of inflammation, e.g. high sensitivity CRP (hsCRP), serum amyloid protein A (SAA), plasma fibrinogen, and blood cell counts and indices are associated specifically with either acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Materials and Methods

Study design:

We performed a sub-study of the Carlscrona Heart Attack Prognosis Study (CHAPS) of patients with suspected acute coronary syndrome. In this sub-study we included patients diagnosed with myocardial infarction or with unstable angina.

Patient recruitment

The patient material has previously been described in detail [6]. In brief, in CHAPS we recruited 5292 consecutive patients admitted to the coronary intensive care unit with acute chest pain (indicative of a possible ACS) at Blekinge Hospital, Karlskrona, between January 26, 1992 and January 25, 1996. Of the total number of admittances, 2992 were between 30-74 years of age at admittance. In patients with multiple admittances, only the first classifying admittance was included as 'event' (UA or MI) in the analysis. Informed consent was obtained from all included patients and the study complies with the Declaration of Helsinki.

Acute coronary syndrome patients

As previously described a diagnosis of ACS was confirmed in 908 of the eligible patients aged 30-74 years of age (644 men and 264 women) [6]. Two groups were identified: (i) patients experiencing at least one acute MI during the study (527) or (ii) patients experiencing no acute MI, but having at least one episode of UA during the study (381). Data on environmental and lifestyle factors, and blood samples, were collected on first admittance under the classifying diagnosis. The classifying diagnosis was set at discharge by one of two experienced cardiologists.

A diagnosis of acute MI was made when patients fulfilled at least two of the following criteria: (i) A history of chest pain of at least 15 min duration, (ii) an increase in activity of cardiac enzymes to at least twice the upper limit of normality, or (iii) characteristic ECG changes for MI (typical sequence change of ST segment and/or of T-waves and/or appearance of new Q-waves). These criteria included both patients with ST-elevation MI (STEMI) and non-ST elevation MI (NSTEMI).

A diagnosis of UA was made when patients fulfilled all of the following criteria: (i) no evidence of MI, (ii) acute chest pain of increased/modified character to any previously experienced, during the preceding 48 h and (iii) angina pectoris diagnosed and medically treated before admission, or alternatively, angina pectoris ascertained by clinical evaluation, including a bicycle exercise test prior to discharge from the hospital[6]. Post-infarction angina and patients with secondary angina were not included.

Patients admitted to the coronary intensive care unit were initially treated with aspirin, and in case of on-going chest pain, also nitrates and morphine. In cases of clear diagnosis of ST elevation MI, thrombolysis with streptokinase was given (194 of 527 patients with MI). If the diagnosis of MI was based on cardiac markers only, thrombolysis was not given. Acute coronary artery intervention was not available at this hospital at the time of the study.

Ethical approval

Carlscrona Heart Attack Prognosis Study (CHAPS) was approved by the Regional Ethical Review Board, Lund, Sweden (EPN 2009/762 and LU 298-91).

Risk factors

Information on risk factors and medical history were recorded at admission from patient history and/or extracted from earlier medical files, and the diagnosis and information were also verified at discharge from the hospital [6]. Smoking status was defined as current- or non-smoker. Patients who had quit smoking >1 month prior to admission were classified as non-smokers.

Laboratory analyses

Samples for laboratory analysis were collected at hospital admission. A standardised protocol for obtaining data for selected laboratory parameters was used. The procedures for blood sampling and laboratory analyses followed the routines of the Department of Clinical Chemistry at Blekinge County Hospital and analyses were performed in the certified hospital laboratory. Haematological variables (blood cell count and indices) and plasma fibrinogen were analysed using routine diagnostic methods in fresh samples at time of admission. Blood cell count was analysed in EDTA whole blood by ADVIA 2120 (Siemens, Germany) and plasma fibrinogen in Sodium Citrate blood samples on a Trombotrack instrument (Nycomed, Norway). Results were extracted from the computerised hospital laboratory records and entered into the study database. High sensitivity CRP (hsCRP) and Serum Amyloid A protein (SAA) were analysed in samples that had been stored at -80 and thawed. Both proteins were analysed by BN ProSpec (Siemens, Germany). These results were entered directly into the study database. The service provided by the laboratory is subject to regular internal precision and accuracy checks and external quality control measures in accordance with the guidelines of the Association of Clinical Chemists in Sweden. The external control system used is EQUALIS, Sweden and

Bio Rad UKNEQAS, England. The instruments from Roche and Siemens are validated according to the IVD directive. The verification performed by the laboratory includes intra-assay precision, correctness of measure intervals, minimal detectable concentration, interferences, pre-analytical factors and blood collection and handling. All laboratory results reported from the laboratory and included in the study are within determined intra assay range for each assay method.

Statistical methods

STATA and IBM SPSS Statistics (version 21) were used for data analyses. Standard methods were used for descriptive statistics. Associations were estimated by binary logistic regression and presented by odds ratios (OR) with 95% confidence intervals (CI) and p-values. Test for trends were performed using the continuous format of the variables, and the results are presented as p values. However, for concentrations of fibrinogen, eosinophil cell count, and thrombocyte median cell volumes the tertiles was entered as a linear variable to test for trend due to skewed distributions of these variables. Two-way interaction terms were used to explore the association of sex and the major risk factors with ACS outcome.

Age was entered into the regressions as continuous variable. Duration of chest pain from onset to blood sampling upon admission to the Emergency Room (ER) was divided in ≥ 4 hours or < 4 hours. Plasma levels of hs-CRP were dichotomized at 2 mg/L to categorise individuals into high and low risk groups. This cut off is based on the JUPITER study, which selected individuals at high vascular risk because of an enhanced inflammatory response as indicated by hsCRP levels ≥2 mg/L [7]. For other biomarkers, to categorise into risk groups we divided these into tertiles, using tertile 1 as reference to obtain measures of relative risks. The tertiles were then entered into the regression as a linear variable to test for trend. Confounding was considered by

stratification and by multivariate regression models forcing age, sex, current smoking, and duration ≥240 minutes into the same model. Individuals with a missing variable were automatically excluded in the respective analysis, thus each multivariate analysis includes only those with full data for every variable included. E.g., for analyses of neutrophils 86 patients were excluded when adjusted for age and sex only, but 268 when also adjusting for smoking and duration of chest pain. Numbers remaining in the regression were accordingly 822 (90%) and 640 (70%), respectively.

Results

We included 908 patients with ACS (527 MI, 381 UA). In table 1 patient characteristics are shown. Outcome was similar in men and women, with no significant interaction between sex and markers of inflammation associated with the outcome of ACS. Results for men and women are thus presented together. When analysing the plasma protein inflammatory biomarkers, adjusted for differences in age and gender, we found that high sensitivity CRP (hsCRP)>2 mg/L at hospital admission was significantly associated with MI over UA (OR=1.75 (1.30-2.34)). MI was significantly associated with higher fibrinogen (p for trend = 0.01), and also with Serum Amyloid A (SAA) in the highest tertile (OR=1.66 (1.16-2.36) but not in trend test (p for trend = 0.216) (Table 2).

To separate an inflammatory response to myocardial tissue necrosis in patients with MI from that of a possible pre-existing inflammation, we analysed hsCRP levels in relation to duration from onset of chest pain until blood sampling. Controlling for differences in age and sex we found a significant correlation of hsCRP with duration only in the MI patients that had ≥ 240 minutes duration since

onset of symptoms (r=0.19, p=0.033) but not in MI patients with a shorter duration (r=0.02, p=0.777), or in UA patients with ≥ 240 minutes duration or shorter duration (r=-0.10, p=0.452 and r=-0.02, p=0.779, respectively). After including smoking and time duration since onset of chest pain in the model hsCRP >2mg/L (OR= 1.40 (1.00-1.96)) and fibrinogen (p for trend = 0.031) remain associated with MI over UA (Table 3). Time duration since onset of symptoms as such did not reach a statistically significant association with MI over UA (OR 1.41 (0.98-2.03), Table 3*).*

The strongest associations with MI over UA were found when haematological variables (blood cells) were analysed (Table 2 and 3). Of circulating inflammatory blood cells, higher counts of neutrophils and monocytes, and lower counts of eosinophils were associated with a worse outcome of an ACS (Table 2). These associations were not affected when adjusting for smoking and duration of symptoms (Table 3) or in trend tests where the associations were highly significant (p=0.003). In contrast, lymphocyte and basophil counts showed no association with outcome (data not shown). Also, we found that higher thrombocyte count was associated with MI (Table 2 and 3). Interestingly, a smaller thrombocyte mean volume was significantly associated with MI when compared to UA (p for trend <0.001).

Discussion

Principal findings

In the current study we showed that levels of inflammatory biomarkers at time of admission are associated with a more severe outcome in the case of ACS (i.e. predisposition towards MI, rather than UA). We found significant differences in blood

cell profiles between a MI or UA outcome, with elevated neutrophils, monocytes and platelets counts in MI, together with a reduced eosinophil count and a lower mean platelet volume. Plasma biomarkers for inflammation (hsCRP, fibrinogen and SAA) showed weaker associations. Our results indicate that a pre-existing inflammation predispose to a more severe outcome in ACS.

Strengths and limitations.

The strength and novelty of the Carlscrona Heart Attack Prognosis Study (CHAPS) is due to the unique nature of the patient cohort. The patients were recruited before the introduction of PCI, CABG, and modern antithrombotic drugs in the standard management of ACS. These interventions would otherwise influence the thrombotic processes involved in ACS. The absence of them at that time made it possible for us to identify progression to MI or UA as distinct outcome groups within the cohort. Furthermore, the study was based in one centre with the same two cardiologists assessing and categorising all patients, using consistent criteria. There are limitations of the study that should be acknowledged. Biochemical analyses of fibrinogen and blood cells were performed over a period of four years. The hospital routine laboratory used standardised and certified methods, providing consistency over time. Analyses of hsCRP and SAA were performed using frozen samples stored at -80 C for 15 years; quality assurance work at the laboratory has shown that storage of samples at -80 C did not influence determined hsCRP and SAA levels. Furthermore, not all patients have complete data for laboratory analyses. In the different multivariate analyses performed, subjects with missing data for any included marker were automatically excluded, leaving about 70% left in the regression for the full model. Still, outcomes are strong and consistent with the age and sex adjusted model leaving 90% left in the regression. Furthermore, the overall patterns show a

high internal consistency. As refined criteria and more sensitive and specific biomarkers are implemented the definition of MI continues to evolve. It is likely that some of the UA cases in our study would now been diagnosed as NSTEMI, using recent criteria required for MI diagnosis [8]. As CHAPS is a single centre study, and treatments and risk factor profiles have partly developed since the study was performed, the results would therefore not necessarily be generalised to a broader modern population.

Plasma biomarkers of inflammation and the outcome of ACS

Previously, we have used the CHAPS material to show that genetic variations of thrombotic factors are associated with ACS outcome [9] and furthermore, that acquired risk factors, smoking and impaired glucose homeostasis together with male sex, predispose to MI over UA[6]. Here we showed that a more pronounced state of inflammation conferred an increased risk towards MI, rather than UA, in ACS. It is well established that a low grade inflammation has a pathogenic role for the progression of atherosclerotic coronary artery lesions[1] however, it is less known to what extent a pre-existing inflammation can influence the outcome of ACS [2]. It could be argued that elevation of inflammatory biomarkers in patients with ACS may reflect myocardial injury rather than underlying inflammation. However, fibrinogen, CRP and SAA are induced by cytokine signalling, e.g. by IL1, TNF and IL6 [1 10 11], and due to a period of *de novo* synthesis and secretion of these proteins there is a time lag before a rise in plasma concentration becomes detectable during the acute phase of inflammation. The average lag time of this response is 8 hours [10], and furthermore, in patients with MI there is a known latency of 6-12 hours from onset of chest pain to a rise in CRP plasma concentrations [12]. In line with this, we observed a correlation between hsCRP and time only in MI patients where the duration between symptom

onset and blood sampling exceeded 4 hours, indicating that the inflammatory response to myocardial injury had a lag time of several hours. Thus, the associations with MI over UA that we observe in patients with duration of chest pain of less than 4 hours indicate that in ACS a higher pre-existing inflammation predisposes to a more severe outcome. In CHAPS we have previously found current smoking to be strongly associated with MI, but not UA [6]. We considered the possibility that these results could be explained by the known inflammatory effect of smoking [13 14 15]. However, the significant associations between MI and hsCRP and fibrinogen were still observed when adjusting for smoking.

Circulating inflammatory blood cells and the outcome of ACS

The strongest associations with MI over UA were observed when analysing circulating inflammatory blood cells - independent of smoking and time duration of symptoms to blood sampling. In contrast to plasma protein biomarkers that require synthesis before there is a detectable increase in levels, preformed blood cells can be quickly mobilised into circulation by demargination from the vessel wall and egress from the bone marrow [16]. Pro-inflammatory cytokines stimulate neutrophil and monocyte production in the bone marrow. Stress induced release of endogenous catecholamine and glucocorticoids can mobilise these stores shortly after onset of chest pain. Thus, the magnitude of rise in cell count can reflect the size of the preformed cell pool that has been increased by a pre-existing low grade inflammation [16]. Thus, the difference we observed in neutrophil and monocyte count between MI and UA indicated a pre-existing inflammation preceding the ACS, consistent with our observations regarding hsCRP and fibrinogen levels. In a recent population-based cohort study Adamsson Eryd *et al.* found an association between increased neutrophil count and incidence of coronary events and increased case fatality rate

during follow-up [17], in line with a previous meta-analysis of several prospective population studies [18]. A possible explanation behind our observation that neutrophilia was associated with MI over UA is a hypercoagulable or thrombo-resistant state, as previously indicated by reduced efficiency of thrombolytic therapy or primary percutaneus coronary interventions in MI patients with elevated WBC [19-21]. In this context, it is interesting that a reduced efficiency of primary percutaneus coronary interventions in MI patients has recently been found to be associated with an increased amount of neutrophil extracellular traps (NETs) in aspirated coronary thrombi [22], adding support for an important role for neutrophils in the ACS thrombotic process. An association of an increased monocyte count and coronary events has previously been reported from population studies [23 24]. A possible mechanism relates to the heavy infiltration of monocytes/macrophages that is a characteristic of a thin fibrous cap of a vulnerable plaque [25]. Thus, a pre-existing monocytosis in MI might lead to a greater monocytoid infiltration, compared to UA, and predispose to a more extensive thrombotic process following plaque rupture [2 5]. Interestingly, we found significantly lower eosinophil counts in MI patients, compared to UA ones, consistent with recent reports [26]. Eosinophils have been detected in aspirates from thrombi in MI patients [26,27], suggesting a possible role for this cell type in the progression of the thrombotic process in ACS. Our observation could indicate an active consumption of eosinophils in MI, or reflect a pre-existing condition of elevated eosinophil count and hypersensitivity inflammation that could predispose to UA. Indeed, Erdogan *et al.* reported a significant higher eosinophil count in UA patients, but not MI patients, when compared to controls [28]. Thrombocytes are key effector cells in an inflammatory process [29 30] and an increase of the thrombocyte count is part of an inflammatory state [31]. Recently the role of thrombocytes in both vascular inflammation and the thrombotic process in CAD has been highlighted [5 32 33], with an

increased mean platelet volume (MPV) reported to be associated with acute cardiovascular events [34 35]. In a systematic review and meta-analysis using pooled results from 16 cross-sectional studies involving 2809 patients, MPV was found to be significantly higher in patients with ACS than in patients with stable CAD or healthy individuals [34]. No significant difference in MPV was found between subjects with MI and those with UA. Individual studies have shown both higher and lower MPV in MI over UA patients [36 37]. In ACS thrombocytes are involved in a dynamic thrombotic process with consumption of preferentially more reactive large-sized thrombocytes [35]. This is extensive and permanent in MI, in contrast to the recurrent episodes of (temporary) coronary platelet aggregation and consumption in UA [38 39], tending to result in a lower MPV in MI than UA, as in our study and the study of Mathur *et al.*[37]. This is however, in most studies, probably counterbalanced by the effects on thrombocytes of a more intense pre-existing inflammation in MI, leading to a similar MPV in MI and UA [34].

Conclusions and possible clinical implications

In conclusion, while inflammation is well established as a major risk factor for development of CAD and risk of future events, our study indicate the further role of inflammation in a more severe outcome in the case of ACS. Our data suggests that neutrophil levels can have a prognostic value in patients with ACS, as previously suggested [17]. The observed differences in ACS outcome associated with inflammation and blood cell profiles raise several hypotheses that warrant further investigation. Establishing the mechanisms for this at the cellular level could lead to optimisation of pharmacological treatment for CAD and ACS.

Footnotes:

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Contributors statement:

HO, LR, and MF designed and initiated the original CHAPS cohort study on which the current study is based. MF conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. JO, HF, IV, HO, AH, LR, UL conceived and designed the current study. IV and MP collected and compiled the laboratory data. HF and UL performed the statistical analyses and compiled the results. JO, MF, HF, IV, HO, AH, LR, UL interpreted the results. JO, HO, UL drafted the paper. MF, HF, IV, LR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JO is the guarantor.

Conflict of interest

No conflict of interests to declare.

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Data sharing: No additional data available

The lead author, Jacob Odeberg, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Table 1. Characteristics of the study population.

Risk factors	Men n=644 m	(SD)	Women n=264 m	(SD)
Age (years)	63.0	(8.6)	65.5	(8.0)
Serum cholesterol (mmol L^{-1})	6.0	(1.3)	6.6	(1.4)
Plasma glucose (mmol L^{-1})	6.8	(3.4)	7.3	(3.8)
HbA1c (%)	5.2	(1.3)	5.4	(1.7)
Hs-CRP (mmol L^{-1})	9.3	(21.9)	9.0	(21.4)
Duration (minutes)	287	(418)	360	(421)
	n	(%)	n	(%)
Hypertension	169	(27.2)	71	(28.1)
Diabetes	92	(14.8)	50	(19.7)
Smoking (current)	148	(24.1)	43	(17.1)
Duration ≥240 minutes	153	(31.1)	74	(38.5)
HsCRP >2 mg/L	341	(59.3)	141	(63.5)

Risk factors		Range	Men n=644 n (%)	Women n=264 n (%)
S-amyloid (mg/L)	Tert 1	0.111-3.25	209 (36.3)	57 (25.7)
	Tert 2	3.26-7.44	191 (33.2)	75 (33.8)
	Tert 3	7.45-1570	175 (30.4)	90 (40.5)
Fibrino (g/L)	Tert 1	1.5-3.3	233 (40.5)	77 (33.8)
	Tert 2	3.4-4.0	159 (27.7)	73 (32.0)
	Tert 3	4.1-10.0	183 (31.8)	78 (34.2)
Leuko (10^9/L)	Tert 1	2.49-7.39	196 (32.6)	85 (35.4)
	Tert 2	7.4-9.8	198 (32.9)	84 (35.0)
	Tert 3	9.82-80.9	207 (34.4)	71 (29.6)
Neutro (10^9/L)	Tert 1	0.14-4.79	191 (32.3)	84 (36.5)
	Tert 2	4.81-7.04	196 (33.1)	78 (33.9)
	Tert 3	7.05-20.06	205 (34.6)	68 (29.6)
Eosino (10^9/L)	Tert 1	0-0.06	155 (27.2)	84 (36.8)
	Tert 2	0.07-0.14	186 (32.6)	77 (33.8)
	Tert 3	0.15-9.12	229 (40.2)	67 (29.4)
Baso (10^9/L)	Tert 1	0-0.039	229 (40.7)	95 (43.0)
	Tert 2	0.04-0.059	174 (30.9)	60 (27.1)
	Tert 3	0.06-0.33	160 (28.4)	66 (29.9)
Lympho (10^9/L)	Tert 1	0.16-1.32	206 (34.8)	71 (30.9)
	Tert 2	1.33-1.88	191 (32.3)	80 (34.8)

	Tert 3	1.89-75.33	195 (32.9)	79 (34.3)
Mono (10^9/L)	Tert 1	0.04-0.4	167 (28.4)	116 (50.7)
	Tert 2	0.41-0.56	212 (36.0)	59 (25.8)
	Tert 3	0.57-1.60	210 (35.7)	54 (23.6)
T-cyt (10^9/L)	Tert 1	85-198	228 (38.1)	54 (23.6)
	Tert 2	199-247	182 (30.4)	94 (39.3)
	Tert 3	248-680	188 (31.4)	91 (38.1)
T-mcv (fL)	Tert 1	6.5-8.8	221 (39.4)	69 (31.4)
	Tert 2	8.9-9.4	167 (29.8)	78 (35.5)
	Tert 3	9.5-46.0	173 (30.8)	73 (33.2)

Data are means (m) and standard deviations (SD), or numbers (n) and proportions (%). HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Baso-Basophil cell count; Lympho-lymphocyte cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume. Missing data age (n=0), serum cholesterol (n=102), plasma glucose (n=82), HbA1c (n=108), Hs-CRP (n=111), duration (n=224), hypertension (n=34), diabetes (n=34), smoking (n=43), s-amyloid (n=111), fibrinogen (n=105), leukocytes (n=67), neutrophils (n=neutrophils (n=86), eosinophils (n=110), basophils (n=124), lymphocytes (n=86), monocytes (n=90), thrombocyte cell count (n=71), T-mcv (n=127).

Table 2 . Risk factors for a MI as outcome of an ACS (adjusted for differences in age and sex).

Risk factors		OR	95% CI	p
Sex (male vs female)		1.59	1.19-2.13	0.002
Age (years)		1.01	1.00-1.02	0.178
HsCRP >2 mg/L		1.75	1.30-2.34	*p for trend 0.037*
Sex (male vs female)		1.73	1.26-2.34	<0.001
Age (years)		1.00	0.98-1.02	0.872
S-amyloid	Tert 1	1.0		*p for trend 0.216*
	Tert 2	1.39	0.98-1.97	0.063
	Tert 3	1.66	1.16-2.36	0.006
Fibrino	Tert 1	1.00		*p for trend 0.010*
	Tert 2	1.26	0.90-1.94	0.174
	Tert 3	1.62	1.12-2.35	0.011
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.78	1.97-3.92	<0.001
	Tert 3	9.64	6.42-14.5	<0.001
Neutro	Tert 1	1.00		*p for trend <0.001*
	Tert 2	2.96	2.09-4.20	<0.001
	Tert 3	8.91	5.97-13.3	<0.001

Eosino	Tert 1	1.00		*p for trend 0.002*
	Tert 2	0.65	0.45-0.94	0.021
	Tert 3	0.56	0.39-0.80	0.001
Mono	Tert 1	1.00		*p for trend* <0.001
	Tert 2	1.29	0.92-1.82	0.140
	Tert 3	3.18	2.20-4.61	<0.001
T-cyt	Tert 1	1.00		*p for trend 0.016*
	Tert 2	1.14	0.81-1.61	0.445
	Tert 3	1.48	1.05-2.09	0.025
T-mcv	Tert 1	1.00		*p for trend* <0.001
	Tert 2	0.46	0.32-0.65	<0.001
	Tert 3	0.51	0.35-0.72	<0.001

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95% CI). Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The continuous format of the variables were used to test for trend, however, due to skewed distributions the tertiles were used as a linear variable for trend test of concentration of fibrinogen, eosinophil cell count and thrombocyte median cell volumes. HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

Table 3. Risk factors for a MI as outcome of an ACS. Multivariate analysis, adjusted for differences in age, sex, smoking and duration of symptoms.

Risk factors		OR	95% CI	p
Covariates in model: sex, age, smoking, duration				
				p for trend 0.225
Hscrp >2 mg/L		1.40	1.00-1.96	0.049
Sex (male vs female)		1.50	1.04-2.17	0.031
Age (years)		1.01	0.99-1.03	0.566
Smoking (yes/no)		2.15	1.39-3.32	0.001
Duration (≥4h vs <4h)		1.41	0.98-2.03	0.061
S-amyloid	Tert 1	1.0		*p for trend 0.679*
	Tert 2	1.43	0.96-2.13	0.078
	Tert 3	1.28	0.84-1.93	0.248
Sex (male vs female)		1.51	1.04-2.18	0.030
Age (years)		1.01	0.99-1.03	0.478
Smoking (yes/no)		2.24	1.45-3.45	<0.001
Duration (≥4h vs <4h)		1.43	0.99-2.06	0.055
Fibrino	Tert 1	1.00		*p for trend 0.031*
	Tert 2	1.19	0.82-1.74	0.349
	Tert 3	1.62	1.03-2.55	0.039
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.58	1.74-3.83	<0.001
	Tert 3	7.39	4.69-11.6	<0.001

Neutro	Tert 1	1.00		*p for trend <0.001*
	Tert 2	2.58	1.74-3.83	<0.001
	Tert 3	7.39	4.69-11.6	<0.001
Eosino	Tert 1	1.00		*p for trend 0.003*
	Tert 2	0.69	0.45-1.07	0.069
	Tert 3	0.54	0.35-0.81	0.003
Mono	Tert 1	1.00		*p for trend <0.001*
	Tert 2	0.99	0.67-1.47	0.978
	Tert 3	2.36	1.54-3.62	<0.001
T-cyt	Tert 1	1.00		*p for trend 0.052*
	Tert 2	1.12	0.75-1.66	0.584
	Tert 3	1.61	1.08-2.39	0.020
T-mcv	Tert 1	1.00		*p for trend <0.001*
	Tert 2	0.41	0.27-0.61	<0.001
	Tert 3	0.45	0.30-0.68	<0.001

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95% CI). All models included sex, age-group, smoking, and duration of chest pain as covariates beside the specified risk factor itself. Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The continuous format of the variables were used to test for trend, however, due to skewed distributions the tertiles were used as a linear variable for trend test of concentration of fibrinogen, eosinophil cell count and thrombocyte median cell volumes.

HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ***Page 1, 3***
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ***Page 3***
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ***Page 7***
Objectives	3	State specific objectives, including any prespecified hypotheses ***Page 7***
Methods		
Study design	4	Present key elements of study design early in the paper ***Page 8, page 9***
Setting	5	Describe the setting , locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ***Page 8, page 9***
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants ***Page 8, page 9***
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ***Page 8, page 9***
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ***Page 8, page 9***
Bias	9	Describe any efforts to address potential sources of bias ***No potential sources of bias identified***
Study size	10	Explain how the study size was arrived at ***Page 8, page 9***
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ***Page 10, page 11***
( ) Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ***Page 10, page 11***
		(*b*) Describe any methods used to examine subgroups and interactions ***Page 11***
		(*c*) Explain how missing data were addressed ***Page 11***
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy ***N.A***
		(*e*) Describe any sensitivity analyses ***N.A***

Results		
( )Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ***Page 11***
		(b) Give reasons for non-participation at each stage ***Page 11***
		(c) Consider use of a flow diagram (a flow diagram was included in the previous BMJ article describing the CHAPS cohort)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ***Table 1***
		(b) Indicate number of participants with missing data for each variable of interest ***Table 1 footnotes***
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures ***Table 1***
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ***Page 11-12 and Table 2, Table 3 footnotes***
		(*b*) Report category boundaries when continuous variables were categorized ***Table 1, footnotes Table 2 and Table 3***
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ***N.A***
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ***Page 12***
Discussion		
Key results	18	Summarise key results with reference to study objectives ***Page 12***
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ***Page 13***
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ***Page 13-16***
Generalisability	21	Discuss the generalisability (external validity) of the study results ***Page 13***
Other information		
( ) Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ***Page 18***

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The influence of pre-existing inflammation on the outcome of acute coronary syndrome: a cross sectional study

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The influence of pre-existing inflammation on the outcome of acute coronary syndrome: a cross sectional study

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Abstract

Objectives

Inflammation is a well-established risk factor for the development of coronary artery disease (CAD) and acute coronary syndrome (ACS). However, less is known about its influence on the outcome of ACS. The aim of this study was to determine if blood biomarkers of inflammation were associated specifically with acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Design

Cross sectional study

Setting

Patients admitted to the coronary care unit, via the emergency room, at a central county hospital over a four-year period (1992-96).

Participants

In a sub-study of Carlscrona Heart Attack Prognosis Study (CHAPS) of 5292 patients admitted to the coronary care unit, we identified 908 patients aged 30-74 years, who at discharge had received the diagnosis of either MI (527) or UA (381).

Main outcome measures

MI or UA, based on the diagnosis set at discharge from hospital.

Results

When adjusted for smoking, age, sex, and duration of chest pain, concentrations of plasma biomarkers of inflammation (hsCRP>2mg/L (OR=1.40 (1.00-1.96) and

fibrinogen (p for trend=0.035)) analysed at admission were found to be associated with MI over UA, in an event of an ACS. A strong significant association with MI over UA was found for blood cell markers of inflammation, i.e. counts of neutrophils (p for trend<0.001), monocytes (p for trend<0.001), and thrombocytes (p for trend=0.021), while lymphocyte count showed no association. Interestingly, eosinophil count (p for trend=0.003) was found to be significantly lower in patients with MI compared to UA.

Conclusions

Our results show that in patients with an ACS the blood cell profile and degree of inflammation at admission was associated with the outcome. Furthermore, our data suggest that a pre-existing low-grade inflammation may dispose towards MI over UA.

Keywords: Inflammation, acute coronary syndrome, unstable angina, myocardial infarction

Abbreviations:

ACS; Acute Coronary Syndrome

MI; Myocardial Infarction

UA; Unstable Angina

CAD; Coronary Artery Disease

PCI; Percutaneous Coronary Intervention

CABG; Coronary Arterial Bypass Graft Surgery

hsCRP; High sensitivity C-Reactive Protein

SAA; Serum Amyloid A

OR; Odds Ratio

CI; Confidence Interval

Strengths and limitations of this study

Strengths:

- The patients were recruited before the introduction of PCI, CABG and modern antithrombotic drugs in the standard management of ACS. Thus, it was possible to identify progression to UA or MI as distinct outcome groups within the cohort, in the absence of interventions that would otherwise influence the thrombotic processes involved in ACS.
- The study was based in a single centre with the same two cardiologists evaluating and categorising all 5292 patients, using consistent criteria.

Limitations:

- Some of the UA cases would likely have been diagnosed as NSTEMI using the most recent criteria of MI.
- Treatments and risk factor profiles have partly evolved since the study was performed.

Introduction

The acute coronary syndrome (ACS) is usually initiated by an atherosclerotic plaque rupture or disruption of the overlying endothelial surface. Subsequent thrombosis formation can permanently occlude the lumen of a coronary artery, causing myocardial cell death and the induction of myocardial infarction (MI). However, in other cases it can be transient, or only partially occlude the vessel, resulting in unstable angina (UA) [1 2]. It is not known why some patients progress to the former, rather than the latter outcome. It is well established that a low-grade inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions [1 2]. A role for inflammatory mediators during the evolution of an ACS is indicated by the widespread coronary inflammation found during UA, throughout the entire coronary artery bed, not only the artery containing the culprit lesion [3 4]. To what extent ACS outcome is related to a concurrent inflammatory response or to the degree of pre-existing inflammation is less established [2 5].

The Carlscrona Heart Attack Prognosis Study (CHAPS) constitutes a patient cohort recruited before the introduction of percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) surgery and modern antithrombotic drugs in the management of patients with ACS. Thus, to our knowledge, this study is unique in that MI and UA could be identified as distinct groups within an ACS population. In a previous CHAPS report we demonstrated that smoking, or impaired glucose homeostasis, were acquired risk factors for a severe ACS outcome [6]. In the current study the aim was to determine if blood biomarkers of inflammation, e.g. high sensitivity CRP (hsCRP), serum amyloid protein A (SAA), plasma fibrinogen, and blood cell counts and indices are associated specifically with either acute myocardial infarction (MI) or unstable angina (UA) in patients with ACS.

Materials and Methods

Study design:

We performed a sub-study of the Carlscrona Heart Attack Prognosis Study (CHAPS) of patients with suspected acute coronary syndrome. In this observational cohort sub-study we included patients diagnosed with myocardial infarction or with unstable angina.

Patient recruitment

The patient material has previously been described in detail [6]. In brief, in CHAPS we recruited 5292 consecutive patients admitted to the coronary intensive care unit with acute chest pain (indicative of a possible ACS) at Blekinge Hospital, Karlskrona, between January 26, 1992 and January 25, 1996. Of the total number of admittances, 2992 were between 30-74 years of age at admittance. In patients with multiple admittances, only the first classifying admittance was included as 'event' (UA or MI) in the analysis. Informed consent was obtained from all included patients and the study complies with the Declaration of Helsinki.

Outcome measures

Unstable angina or myocardial infarction as diagnosed at discharge from hospital.

Acute coronary syndrome patients

As previously described a diagnosis of ACS was confirmed in 908 of the eligible patients aged 30-74 years of age (644 men and 264 women) [6]. Two groups were

identified: (i) patients experiencing at least one acute MI during the study (527) or (ii) patients experiencing no acute MI, but having at least one episode of UA during the study (381). Data on environmental and lifestyle factors, and blood samples, were collected on first admittance under the classifying diagnosis. The classifying diagnosis was set at discharge by one of two experienced cardiologists.

A diagnosis of acute MI was made when patients fulfilled at least two of the following criteria: (i) A history of chest pain of at least 15 min duration, (ii) an increase in activity of cardiac enzymes to at least twice the upper limit of normality, or (iii) characteristic ECG changes for MI (typical sequence change of ST segment and/or of T-waves and/or appearance of new Q-waves). These criteria included both patients with ST-elevation MI (STEMI) and non-ST elevation MI (NSTEMI).

A diagnosis of UA was made when patients fulfilled all of the following criteria: (i) no evidence of MI, (ii) acute chest pain of increased/modified character to any previously experienced, during the preceding 48 h and (iii) angina pectoris diagnosed and medically treated before admission, or alternatively, angina pectoris ascertained by clinical evaluation, including a bicycle exercise test prior to discharge from the hospital[6]. Post-infarction angina and patients with secondary angina were not included.

Patients admitted to the coronary intensive care unit were initially treated with aspirin, and in case of on-going chest pain, also nitrates and morphine. In cases of clear diagnosis of ST elevation MI, thrombolysis with streptokinase was given (194 of 527 patients with MI). If the diagnosis of MI was based on cardiac markers only, thrombolysis was not given. Acute coronary artery intervention was not available at

this hospital at the time of the study.

Ethical approval

Carlscrona Heart Attack Prognosis Study (CHAPS) was approved by the Regional Ethical Review Board, Lund, Sweden (EPN 2009/762 and LU 298-91).

Risk factors

Information on risk factors and medical history were recorded at admission from patient history and/or extracted from earlier medical files, and the diagnosis and information were also verified at discharge from the hospital [6]. Smoking status was defined as current- or non-smoker. Patients who had quit smoking >1 month prior to admission were classified as non-smokers.

Laboratory analyses

Samples for laboratory analysis were collected at hospital admission. A standardised protocol for obtaining data for selected laboratory parameters was used. The procedures for blood sampling and laboratory analyses followed the routines of the Department of Clinical Chemistry at Blekinge County Hospital and analyses were performed in the certified hospital laboratory. Haematological variables (blood cell count and indices) and plasma fibrinogen were analysed using routine diagnostic methods in fresh samples at time of admission. Blood cell count was analysed in EDTA whole blood by ADVIA 2120 (Siemens, Germany) and plasma fibrinogen in Sodium Citrate blood samples on a Trombotrack instrument (Nycomed, Norway). Results were extracted from the computerised hospital laboratory records and entered into the study database. High sensitivity CRP (hsCRP) and Serum Amyloid A protein (SAA) were analysed in samples that had been stored at -80 and thawed.

Both proteins were analysed by BN ProSpec (Siemens, Germany). These results were entered directly into the study database. The service provided by the laboratory is subject to regular internal precision and accuracy checks and external quality control measures in accordance with the guidelines of the Association of Clinical Chemists in Sweden. The external control system used is EQUALIS, Sweden and Bio Rad UKNEQAS, England. The instruments from Roche and Siemens are validated according to the IVD directive. The verification performed by the laboratory includes intra-assay precision, correctness of measure intervals, minimal detectable concentration, interferences, pre-analytical factors and blood collection and handling. All laboratory results reported from the laboratory and included in the study are within determined intra assay range for each assay method.

Statistical methods

STATA and IBM SPSS Statistics (version 21) were used for data analyses. Standard methods were used for descriptive statistics. Associations were estimated by binary logistic regression and presented by odds ratios (OR) with 95% confidence intervals (CI) and p-values using females as reference group. Test for trends were performed using the continuous format of the variables, and the results are presented as p values. However, for concentrations of fibrinogen, eosinophil cell count, and thrombocyte median cell volumes the tertiles was entered as a linear variable to test for trend due to skewed distributions of these variables. Two-way interaction terms were used to explore the association of sex and the major risk factors with ACS outcome.

Age was entered into the regressions as continuous variable. Duration of chest pain from onset to blood sampling upon admission to the Emergency Room (ER) was divided in ≥ 4 hours or < 4 hours. Plasma levels of hs-CRP were dichotomized at 2

mg/L to categorise individuals into high and low risk groups. This cut off is based on the JUPITER study, which selected individuals at high vascular risk because of an enhanced inflammatory response as indicated by hsCRP levels ≥2 mg/L [7]. For other biomarkers, to categorise into risk groups we divided these into tertiles, using tertile 1 as reference to obtain measures of relative risks. The tertiles were then entered into the regression as a linear variable to test for trend. Confounding was considered by stratification and by multivariate regression models forcing age, sex, current smoking, and duration ≥240 minutes into the same model. Individuals with a missing variable were automatically excluded in the respective analysis, thus each multivariate analysis includes only those with full data for every variable included. E.g., for analyses of neutrophils 86 patients were excluded when adjusted for age and sex only, but 268 when also adjusting for smoking and duration of chest pain. Numbers remaining in the regression were accordingly 822 (90%) and 640 (70%), respectively.

Results

We included 908 patients with ACS (527 MI, 381 UA). In tables 1a and 1b patient characteristics are shown. Outcome was similar in men and women, with no significant interaction between sex and markers of inflammation associated with the outcome of ACS. Results for men and women are thus presented together. When analysing the plasma protein inflammatory biomarkers, adjusted for differences in age and sex we found that high sensitivity CRP (hsCRP)>2 mg/L at hospital admission was significantly associated with MI over UA (OR=1.75 (1.30-2.34)). MI was significantly associated with higher fibrinogen (p for trend = 0.01), and also with Serum Amyloid A (SAA) in the highest tertile (OR=1.66 (1.16-2.36) but not in trend test (p for trend = 0.216) (Table 2).

To separate an inflammatory response to myocardial tissue necrosis in patients with MI from that of a possible pre-existing inflammation, we analysed hsCRP levels in relation to duration from onset of chest pain until blood sampling. Controlling for differences in age and sex we found a significant correlation of hsCRP with duration only in the MI patients that had ≥ 240 minutes duration since onset of symptoms (r=0.19, p=0.033) but not in MI patients with a shorter duration (r=0.02, p=0.777), or in UA patients with ≥ 240 minutes duration or shorter duration (r=-0.10, p=0.452 and r=-0.02, p=0.779, respectively). After including smoking and time duration since onset of chest pain in the model hsCRP >2mg/L (OR= 1.40 (1.00-1.96)) and fibrinogen (p for trend = 0.031) remain associated with MI over UA (Table 3). Time duration since onset of symptoms as such did not reach a statistically significant association with MI over UA (OR 1.41 (0.98-2.03), Table 3*)*.

The strongest associations with MI over UA were found when haematological variables (blood cells) were analysed (Table 2 and 3). Of circulating inflammatory blood cells, higher counts of neutrophils and monocytes, and lower counts of eosinophils were associated with a worse outcome of an ACS (Table 2). These associations were not affected when adjusting for smoking and duration of symptoms (Table 3) or in trend tests where the associations were highly significant (p=0.003). In the multivariate models the outcome for smoking (highly significant) and duration of symptoms (borderline significant), were generally the same with all inflammatory biomarkers and is thus shown only in the first model with hsCRP. In contrast, lymphocyte and basophil counts showed no association with outcome (data not shown). Also, we found that higher thrombocyte count

was associated with MI (Table 2 and 3). Interestingly, a smaller thrombocyte mean volume was significantly associated with MI when compared to UA (p for trend <0.001).

Discussion

Principal findings

In the current study we showed that levels of inflammatory biomarkers at time of admission are associated with a more severe outcome in the case of ACS (i.e. predisposition towards MI, rather than UA). We found significant differences in blood cell profiles between a MI or UA outcome, with elevated neutrophils, monocytes and platelets counts in MI, together with a reduced eosinophil count and a lower mean platelet volume. Plasma biomarkers for inflammation (hsCRP, fibrinogen and SAA) showed weaker associations. Our results indicate that a pre-existing inflammation predispose to a more severe outcome in ACS.

8

Plasma biomarkers of inflammation and the outcome of ACS

Previously, we have used the CHAPS material to show that genetic variations of thrombotic factors are associated with ACS outcome [9] and furthermore, that acquired risk factors, smoking and impaired glucose homeostasis together with male sex, predispose to MI over UA[6]. Here we showed that a more pronounced state of inflammation conferred an increased risk towards MI, rather than UA, in ACS. It is well established that a low grade inflammation has a pathogenic role for the progression of atherosclerotic coronary artery lesions[1] however, it is less known to what extent a pre-existing inflammation can influence the outcome of ACS [2]. It could be argued that elevation of inflammatory biomarkers in patients with ACS may reflect myocardial injury rather than underlying inflammation. However, fibrinogen, CRP

and SAA are induced by cytokine signalling, e.g. by IL1, TNF and IL6 [1 10 11], and due to a period of *de novo* synthesis and secretion of these proteins there is a time lag before a rise in plasma concentration becomes detectable during the acute phase of inflammation. The average lag time of this response is 8 hours [10], and furthermore, in patients with MI there is a known latency of 6-12 hours from onset of chest pain to a rise in CRP plasma concentrations [12]. In line with this, we observed a correlation between hsCRP and time only in MI patients where the duration between symptom onset and blood sampling exceeded 4 hours, indicating that the inflammatory response to myocardial injury had a lag time of several hours. Thus, the associations with MI over UA that we observe in patients with duration of chest pain of less than 4 hours indicate that in ACS a higher pre-existing inflammation predisposes to a more severe outcome. In CHAPS we have previously found current smoking to be strongly associated with MI, but not UA [6]. We considered the possibility that these results could be explained by the known inflammatory effect of smoking [13 14 15]. However, the significant associations between MI and hsCRP and fibrinogen were still observed when adjusting for smoking.

Circulating inflammatory blood cells and the outcome of ACS

The strongest associations with MI over UA were observed when analysing circulating inflammatory blood cells - independent of smoking and time duration of symptoms to blood sampling. In contrast to plasma protein biomarkers that require synthesis before there is a detectable increase in levels, preformed blood cells can be quickly mobilised into circulation by demargination from the vessel wall and egress from the bone marrow [16]. Pro-inflammatory cytokines stimulate neutrophil and monocyte production in the bone marrow. Stress induced release of endogenous catecholamine and glucocorticoids can mobilise these stores shortly after onset of

chest pain. Thus, the magnitude of rise in cell count can reflect the size of the preformed cell pool that has been increased by a pre-existing low grade inflammation [16]. Thus, the difference we observed in neutrophil and monocyte count between MI and UA indicated a pre-existing inflammation preceding the ACS, consistent with our observations regarding hsCRP and fibrinogen levels. In a recent population-based cohort study Adamsson Eryd *et al.* found an association between increased neutrophil count and incidence of coronary events and increased case fatality rate during follow-up [17], in line with a previous meta-analysis of several prospective population studies [18]. A possible explanation behind our observation that neutrophilia was associated with MI over UA is a hypercoagulable or thrombo-resistant state, as previously indicated by reduced efficiency of thrombolytic therapy or primary percutaneus coronary interventions in MI patients with elevated WBC [19-21]. In this context, it is interesting that a reduced efficiency of primary percutaneus coronary interventions in MI patients has recently been found to be associated with an increased amount of neutrophil extracellular traps (NETs) in aspirated coronary thrombi [22], adding support for an important role for neutrophils in the ACS thrombotic process. An association of an increased monocyte count and coronary events has previously been reported from population studies [23 24]. A possible mechanism relates to the heavy infiltration of monocytes/macrophages that is a characteristic of a thin fibrous cap of a vulnerable plaque [25]. Thus, a pre-existing monocytosis in MI might lead to a greater monocytoid infiltration, compared to UA, and predispose to a more extensive thrombotic process following plaque rupture [2 5]. Interestingly, we found significantly lower eosinophil counts in MI patients, compared to UA ones, consistent with recent reports [26]. Eosinophils have been detected in aspirates from thrombi in MI patients [26,27], suggesting a possible role for this cell type in the progression of the thrombotic process in ACS. Our observation could indicate an active consumption of

eosinophils in MI, or reflect a pre-existing condition of elevated eosinophil count and hypersensitivity inflammation that could predispose to UA. Indeed, Erdogan *et al.* reported a significant higher eosinophil count in UA patients, but not MI patients, when compared to controls [28]. Thrombocytes are key effector cells in an inflammatory process [29 30] and an increase of the thrombocyte count is part of an inflammatory state [31]. Recently the role of thrombocytes in both vascular inflammation and the thrombotic process in CAD has been highlighted [5 32 33], with an increased mean platelet volume (MPV) reported to be associated with acute cardiovascular events [34 35]. In a systematic review and meta-analysis using pooled results from 16 cross-sectional studies involving 2809 patients, MPV was found to be significantly higher in patients with ACS than in patients with stable CAD or healthy individuals [34]. No significant difference in MPV was found between subjects with MI and those with UA. Individual studies have shown both higher and lower MPV in MI over UA patients [36 37]. In ACS thrombocytes are involved in a dynamic thrombotic process with consumption of preferentially more reactive large-sized thrombocytes [35]. This is extensive and permanent in MI, in contrast to the recurrent episodes of (temporary) coronary platelet aggregation and consumption in UA [38 39], tending to result in a lower MPV in MI than UA, as in our study and the study of Mathur *et al.*[37]. This is however, in most studies, probably counterbalanced by the effects on thrombocytes of a more intense pre-existing inflammation in MI, leading to a similar MPV in MI and UA [34].

Strengths and limitations.

The strength and novelty of the Carlscrona Heart Attack Prognosis Study (CHAPS) is due to the unique nature of the patient cohort. The patients were recruited before the introduction of PCI, CABG, and modern antithrombotic drugs in the standard

management of ACS. These interventions would otherwise influence the thrombotic processes involved in ACS. The absence of them at that time made it possible for us to identify progression to MI or UA as distinct outcome groups within the cohort. Furthermore, the study was based in one centre with the same two cardiologists assessing and categorising all patients, using consistent criteria. There are limitations of the study that should be acknowledged. Smoking was defined as current smoker or non-smoker, and thus ex-smokers (cessation >1 month ago) were classified into the non-smoker group, however previous studies indicate that the increased risk for cardiovascular events associated with smoking decreases rapidly after smoking cessation [40]. Furthermore, duration was based on time of onset as reported by patients at admission, which may confer a misclassification in some cases. Biochemical analyses of fibrinogen and blood cells were performed over a period of four years. The hospital routine laboratory used standardised and certified methods, providing consistency over time. Analyses of hsCRP and SAA were performed using frozen samples stored at -80 C for 15 years; quality assurance work at the laboratory has shown that storage of samples at -80 C did not influence determined hsCRP and SAA levels. Furthermore, not all patients have complete data for laboratory analyses. In the different multivariate analyses performed, subjects with missing data for any included marker were automatically excluded, leaving about 70% left in the regression for the full model. Still, outcomes are strong and consistent with the age and sex adjusted model leaving 90% left in the regression. Furthermore, the overall patterns show a high internal consistency. As refined criteria and more sensitive and specific biomarkers are implemented the definition of MI continues to evolve. It is likely that some of the UA cases in our study would now been diagnosed as NSTEMI, using recent criteria required for MI diagnosis [8]. Also, as CHAPS is a single centre study, and treatments and risk factor profiles have partly developed since the study

was performed, the results would therefore not necessarily be generalised to a broader modern population.

Conclusions and possible clinical implications

In conclusion, while inflammation is well established as a major risk factor for development of CAD and risk of future events, our study indicate the further role of inflammation in a more severe outcome in the case of ACS. Our data suggests that neutrophil levels can have a prognostic value in patients with ACS, as previously suggested [17]. The observed differences in ACS outcome associated with inflammation and blood cell profiles raise several hypotheses that warrant further investigation. It is possible that UA and MI represent different entities of ACS that involve different pathological mechanisms. Establishing such mechanisms at the cellular level could lead to optimisation of pharmacological treatment for CAD and ACS.

Footnotes:

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Contributors statement:

HO, LR, and MF designed and initiated the original CHAPS cohort study on which the current study is based. MF conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. JO, HF, IV, HO, AH, LR, UL conceived and designed the current study. IV and MP collected and compiled the laboratory data. HF and UL performed the statistical analyses and compiled the

results. JO, MF, HF, IV, HO, AH, LR, UL interpreted the results. JO, HO, UL drafted the paper. MF, HF, IV, LR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JO is the guarantor.

Conflict of interest

No conflict of interests to declare.

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Data sharing: No additional data available

The lead author, Jacob Odeberg, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

I, Jacob Odeberg the Corresponding Author of this article contained within the original manuscript which includes any diagrams & photographs within and any related or stand alone film submitted (the 'Contribution') has the right to grant on behalf of all authors and does grant on behalf of all authors, a licence to the BMJ Publishing Group Ltd and its licencees, to permit this Contribution (if accepted) to be published in the BMJ and any other BMJ Group products and to exploit all subsidiary rights, as set out in our licence set out at: http://www.bmj.com/about-bmj/resources-authors/forms-policies-and-checklists/copyright-open-access-and-permission-reuse."

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Table 1a. Clinical characteristics of the study population.

	Total n=908		Men n=644		Women n=264	
Risk factors	m	(SD)	m	(SD)	m	(SD)
Age (years)	63.7	8.5	63.0	(8.6)	65.5	(8.0)
Serum cholesterol (mmol L-1)	6.2	1.3	6.0	(1.3)	6.6	(1.4)
Plasma glucose (mmol L-1)	6.9	3.5	6.8	(3.4)	7.3	(3.8)
HbA1c (%)	5.3	1.4	5.2	(1.3)	5.4	(1.7)
Hs-CRP (mmol L-1)	9.2	21.8	9.3	(21.9)	9.0	(21.4)
Duration (minutes)	307	420	287	(418)	360	(421)

	n	(%)	n	(%)	n	(%)
Hypertension	240	(27.5)	169	(27.2)	71	(28.1)
Diabetes	142	(16.2)	92	(14.8)	50	(19.7)
Smoking (current)	191	(22.1)	148	(24.1)	43	(17.1)
Duration ≥240 minutes	227	(33.2)	153	(31.1)	74	(38.5)
HsCRP >2 mg/L	482	(60.5)	341	(59.3)	141	(63.5)

Table 1b. Characteristics of plasma protein inflammatory biomarkers categorised by tertiles in men and women, respectively.

Risk factors		Range	Men n=644 n (%)	Women n=264 n (%)
S-amyloid (mg/L)	Tert 1	0.111-3.25	209 (36.3)	57 (25.7)
	Tert 2	3.26-7.44	191 (33.2)	75 (33.8)
	Tert 3	7.45-1570	175 (30.4)	90 (40.5)
Fibrino (g/L)	Tert 1	1.5-3.3	233 (40.5)	77 (33.8)
	Tert 2	3.4-4.0	159 (27.7)	73 (32.0)
	Tert 3	4.1-10.0	183 (31.8)	78 (34.2)
Leuko (10^9/L)	Tert 1	2.49-7.39	196 (32.6)	85 (35.4)
	Tert 2	7.4-9.8	198 (32.9)	84 (35.0)
	Tert 3	9.82-80.9	207 (34.4)	71 (29.6)
Neutro (10^9/L)	Tert 1	0.14-4.79	191 (32.3)	84 (36.5)
	Tert 2	4.81-7.04	196 (33.1)	78 (33.9)
	Tert 3	7.05-20.06	205 (34.6)	68 (29.6)
Eosino (10^9/L)	Tert 1	0-0.06	155 (27.2)	84 (36.8)
	Tert 2	0.07-0.14	186 (32.6)	77 (33.8)
	Tert 3	0.15-9.12	229 (40.2)	67 (29.4)
Baso (10^9/L)	Tert 1	0-0.039	229 (40.7)	95 (43.0)

	Tert 2	0.04-0.059	174 (30.9)	60 (27.1)
	Tert 3	0.06-0.33	160 (28.4)	66 (29.9)
Lympho (10^9/L)	Tert 1	0.16-1.32	206 (34.8)	71 (30.9)
	Tert 2	1.33-1.88	191 (32.3)	80 (34.8)
	Tert 3	1.89-75.33	195 (32.9)	79 (34.3)
Mono (10^9/L)	Tert 1	0.04-0.4	167 (28.4)	116 (50.7)
	Tert 2	0.41-0.56	212 (36.0)	59 (25.8)
	Tert 3	0.57-1.60	210 (35.7)	54 (23.6)
T-cyt (10^9/L)	Tert 1	85-198	228 (38.1)	54 (23.6)
	Tert 2	199-247	182 (30.4)	94 (39.3)
	Tert 3	248-680	188 (31.4)	91 (38.1)
T-mcv (fL)	Tert 1	6.5-8.8	221 (39.4)	69 (31.4)
	Tert 2	8.9-9.4	167 (29.8)	78 (35.5)
	Tert 3	9.5-46.0	173 (30.8)	73 (33.2)

Data are means (m) and standard deviations (SD), or numbers (n) and proportions (%). HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Baso-Basophil cell count; Lympho-lymphocyte cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume. Missing data age (n=0), serum cholesterol (n=102), plasma glucose (n=82), HbA1c (n=108), Hs-CRP (n=111), duration (n=224), hypertension (n=34), diabetes (n=34), smoking (n=43), s-amyloid (n=111), fibrinogen (n=105), leukocytes (n=67), neutrophils (n=neutrophils (n=86), eosinophils (n=110), basophils (n=124), lymphocytes (n=86), monocytes (n=90), thrombocyte cell count (n=71), T-mcv (n=127).

Table 2 . Risk factors for a MI as outcome of an ACS (adjusted for differences in age and sex).

Risk factors		OR	95% CI	p
Male sex		1.59	1.19-2.13	0.002
Age (years)		1.01	1.00-1.02	0.178
HsCRP >2 mg/L		1.75	1.30-2.34	*p for trend 0.037*
Sex (male vs female)		1.73	1.26-2.34	<0.001
Age (years)		1.00	0.98-1.02	0.872
S-amyloid	Tert 1	1.0		*p for trend 0.216*
	Tert 2	1.39	0.98-1.97	0.063
	Tert 3	1.66	1.16-2.36	0.006
Fibrino	Tert 1	1.00		*p for trend 0.010*
	Tert 2	1.26	0.90-1.94	0.174
	Tert 3	1.62	1.12-2.35	0.011
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.78	1.97-3.92	<0.001
	Tert 3	9.64	6.42-14.5	<0.001
Neutro	Tert 1	1.00		*p for trend <0.001*
	Tert 2	2.96	2.09-4.20	<0.001
	Tert 3	8.91	5.97-13.3	<0.001

Eosino	Tert 1	1.00		*p for trend 0.002*
	Tert 2	0.65	0.45-0.94	0.021
	Tert 3	0.56	0.39-0.80	0.001
Mono	Tert 1	1.00		*p for trend* <0.001
	Tert 2	1.29	0.92-1.82	0.140
	Tert 3	3.18	2.20-4.61	<0.001
T-cyt	Tert 1	1.00		*p for trend 0.016*
	Tert 2	1.14	0.81-1.61	0.445
	Tert 3	1.48	1.05-2.09	0.025
T-mcv	Tert 1	1.00		*p for trend* <0.001
	Tert 2	0.46	0.32-0.65	<0.001
	Tert 3	0.51	0.35-0.72	<0.001

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95% CI) adjusting for differences in age and sex. Plasma levels of hs-CRP were dichotomized at 2 mg/L, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The continuous format of the variables were used to test for trend, however, due to skewed distributions the tertiles were used as a linear variable for trend test of concentration of fibrinogen, eosinophil cell count and thrombocyte median cell volumes. HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

Table 3. Risk factors for a MI as outcome of an ACS. Multivariate analysis adjusted for differences in age, sex, smoking and duration of symptoms.

Risk factors		OR	95% CI	p
Covariates in model: sex, age, smoking, duration of symptoms				
				p for trend 0.225
Hscrp >2 mg/L		1.40	1.00-1.96	0.049
Male sex		1.50	1.04-2.17	0.031
Age (years)		1.01	0.99-1.03	0.566
Smoking (yes/no)		2.15	1.39-3.32	0.001
Duration (≥4h vs <4h)		1.41	0.98-2.03	0.061
S-amyloid	Tert 1	1.0		*p for trend 0.679*
	Tert 2	1.43	0.96-2.13	0.078
	Tert 3	1.28	0.84-1.93	0.248
Fibrino	Tert 1	1.00		*p for trend 0.031*
	Tert 2	1.19	0.82-1.74	0.349
	Tert 3	1.62	1.03-2.55	0.039
Leuko	Tert 1	1.00		*p for trend* <0.001
	Tert 2	2.58	1.74-3.83	<0.001
	Tert 3	7.39	4.69-11.6	<0.001
Neutro	Tert 1	1.00		*p for trend <0.001*
	Tert 2	2.58	1.74-3.83	<0.001
	Tert 3	7.39	4.69-11.6	<0.001

Eosino	Tert 1	1.00		*p for trend 0.003*
	Tert 2	0.69	0.45-1.07	0.069
	Tert 3	0.54	0.35-0.81	0.003
Mono	Tert 1	1.00		*p for trend <0.001*
	Tert 2	0.99	0.67-1.47	0.978
	Tert 3	2.36	1.54-3.62	<0.001
T-cyt	Tert 1	1.00		*p for trend 0.052*
	Tert 2	1.12	0.75-1.66	0.584
	Tert 3	1.61	1.08-2.39	0.020
T-mcv	Tert 1	1.00		*p for trend <0.001*
	Tert 2	0.41	0.27-0.61	<0.001
	Tert 3	0.45	0.30-0.68	<0.001

Associations between risk factors and an adverse outcome of the ACS were estimated using binary logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95% CI). All models included sex, age, smoking, and duration of chest pain as covariates beside the specified risk factor itself. Plasma levels of hs-CRP ≥ 2 mg/L were compared to those below, while other biomarkers were divided in tertiles for categorical comparisons using tertile 1 as reference. The continuous format of the variables were used to test for trend, however, due to skewed distributions the tertiles were used as a linear variable for trend test of concentration of fibrinogen, eosinophil cell count and thrombocyte median cell volumes. HsCRP - high sensitivity CRP; Fibrino-fibrinogen; Leuko-leukocyte cell count; Neutro-neutrophil cell count; Eosino-eosinophil cell count; Mono-monocyte cell count; T-cyt thrombocyte cell count; T-mcv thrombocyte median cell volume

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ***Page 1, 3***
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ***Page 3***
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ***Page 7***
Objectives	3	State specific objectives, including any prespecified hypotheses ***Page 7***
Methods		
Study design	4	Present key elements of study design early in the paper ***Page 8, page 9***
Setting	5	Describe the setting , locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ***Page 8, page 9***
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants ***Page 8, page 9***
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ***Page 8, page 9***
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ***Page 8, page 9***
Bias	9	Describe any efforts to address potential sources of bias ***No potential sources of bias identified***
Study size	10	Explain how the study size was arrived at ***Page 8, page 9***
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ***Page 10, page 11***
( ) Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ***Page 10, page 11***
		(*b*) Describe any methods used to examine subgroups and interactions ***Page 11***
		(*c*) Explain how missing data were addressed ***Page 11***
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy ***N.A***
		(*e*) Describe any sensitivity analyses ***N.A***

Results		
(□)Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ***Page 11***
		(b) Give reasons for non-participation at each stage ***Page 11***
		(c) Consider use of a flow diagram (a flow diagram was included in the previous BMJ article describing the CHAPS cohort)
□ Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ***Table 1***
		(b) Indicate number of participants with missing data for each variable of interest ***Table 1 footnotes***
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
□Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures ***Table 1***
□ Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ***Page 11-12 and Table 2, Table 3 footnotes***
		(*b*) Report category boundaries when continuous variables were categorized ***Table 1, footnotes Table 2 and Table 3***
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ***N.A***
□ Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ***Page 12***
Discussion		
□ Key results	18	Summarise key results with reference to study objectives ***Page 12***
□Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ***Page 13***
□Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ***Page 13-16***
□Generalisability	21	Discuss the generalisability (external validity) of the study results ***Page 13***
Other information		
(□) Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ***Page 18***

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.