

# BMJ Open 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 4-year period (1992–1996).

**Participants:** In a substudy of Carlsrona 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 (high-sensitivity C reactive protein >2 mg/L (OR=1.40 (1.00 to 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 ACS. A strong significant association with MI over UA was found for blood cell markers of inflammation, that is, 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 those with UA.

**Conclusions:** Our results show that, in patients with 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.

## INTRODUCTION

Acute coronary syndrome (ACS) is usually initiated by an atherosclerotic plaque rupture or disruption of the overlying endothelial

## Strengths and limitations of this study

- The patients were recruited before the introduction of percutaneous coronary intervention, coronary artery bypass graft and modern antithrombotic drugs in the standard management of acute coronary syndrome (ACS). Thus, it was possible to identify progression to unstable angina (UA) or myocardial infarction (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.
- Some of the UA cases would likely have been diagnosed as non-ST elevation MI, using the most recent criteria of MI.
- Treatments and risk factor profiles have partly evolved since the study was performed.

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).<sup>1 2</sup> It is not known why some patients progress to the former, rather than the latter, outcome. It is well established that low-grade inflammation has a major pathogenic role for the progression of atherosclerotic coronary artery lesions.<sup>1 2</sup> A role for inflammatory mediators during the evolution of ACS is indicated by the widespread coronary inflammation found during UA, throughout the entire coronary artery bed, and not only in the artery containing the culprit lesion.<sup>3 4</sup> To what extent ACS outcome is related to a concurrent inflammatory response or to the degree of pre-existing inflammation is less established.<sup>2 5</sup>

The Carlsrona 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 the best of 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.<sup>6</sup> In the current study, the aim was to determine if blood biomarkers of inflammation, for example, high-sensitivity C reactive protein (hsCRP), serum amyloid protein A (SAA), plasma fibrinogen, and blood cell counts and indices, are associated specifically with either acute MI or UA in patients with ACS.

## MATERIALS AND METHODS

### Study design

We performed a substudy of CHAPS of patients with suspected ACS. In this observational cohort substudy, we included patients diagnosed with either MI or with UA.

### Patient recruitment

The patient data have been previously described in detail.<sup>6</sup> 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 26 January 1992 and 25 January 1996. Of the total number of admittances, 2992 were between 30 and 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

UA or MI as diagnosed at discharge from hospital.

### Patients with ACS

As previously described, a diagnosis of ACS was confirmed in 908 of the eligible patients 30–74 years of age (644 men and 264 women).<sup>6</sup> Two groups were identified: (1) patients experiencing at least one acute MI during the study (527) or (2) 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: (1) a history of chest pain of at least 15 min duration, (2) an increase in activity of cardiac enzymes to at least twice the upper limit of normality, or (3) 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 those with non-STEMI (NSTEMI).

A diagnosis of UA was made when patients fulfilled all of the following criteria: (1) no evidence of MI, (2) acute chest pain of increased/modified character to any previously experienced, during the preceding 48 h and (3) 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.<sup>6</sup> 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 also—in case of ongoing chest pain—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.

### 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.<sup>6</sup> Smoking status was defined as current smoker 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 routine 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 the 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. hsCRP and SAA were analysed in samples that had been stored at –80°C, after thawing. 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, as well as 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 (V.21) were used for data analyses. Standard methods were used for descriptive statistics. Associations were estimated by binary logistic regression and presented by ORs with 95% CIs and p values. Tests 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 were entered as linear variables 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 on admission to the emergency room (ER) was divided in  $\geq 4$  h or  $< 4$  h. Plasma levels of hsCRP were dichotomised at 2 mg/L to categorise individuals into high-risk 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  $\geq 2$  mg/L.<sup>7</sup> 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  $\geq 240$  min 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. As an example, for analyses of neutrophils, 86 patients were excluded when adjusted for age and sex only, but 268 were excluded 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). Patient characteristics are shown in tables 1 and 2. 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 hsCRP  $> 2$  mg/L at hospital admission was significantly associated with MI over UA (OR=1.75 (1.30 to 2.34)). MI was significantly associated with higher fibrinogen (p for trend=0.01), and also with SAA in the highest tertile (OR=1.66 (1.16 to 2.36), but not in the trend test (p for trend=0.216) (table 3).

To separate an inflammatory response to myocardial tissue necrosis in patients with MI from that from a possible pre-existing inflammation, we analysed hsCRP

**Table 1** Clinical characteristics of the study population

Risk factors	Total n=908		Men n=644		Women n=264	
	m	(SD)	m	(SD)	m	(SD)
Age (years)	63.7	8.5	63.0	(8.6)	65.5	(8.0)
Serum cholesterol (mmol/L)	6.2	1.3	6.0	(1.3)	6.6	(1.4)
Plasma glucose (mmol/L)	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)
hsCRP (mmol/L)	9.2	21.8	9.3	(21.9)	9.0	(21.4)
Duration (min)	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 $\geq 240$ min	227	(33.2)	153	(31.1)	74	(38.5)
hsCRP $> 2$ mg/L	482	(60.5)	341	(59.3)	141	(63.5)

Data are means (m) and SD, or numbers (n) and proportions (%). Missing data age (0), serum cholesterol (n=102), plasma glucose (n=82), HbA1c (n=108), hsCRP (n=111), duration (n=224), hypertension (n=34), diabetes (n=34), smoking (n=43). HbA1c, glycated haemoglobin; hsCRP, high sensitivity C reactive protein.

**Table 2** Characteristics of plasma protein inflammatory biomarkers categorised by tertiles in men and women

Risk factors	Range	Men n=644 n (%)	Women n=264 n (%)
Serum 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)
Leuco (10 <sup>9</sup> /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 <sup>9</sup> /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 <sup>9</sup> /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 <sup>9</sup> /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 <sup>9</sup> /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 <sup>9</sup> /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 <sup>9</sup> /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)

Missing data Serum amyloid (n=111), fibrinogen (n=106), leucocytes (n=67), neutrophils (n=68), eosinophils (n=110), basophils (n=124), lymphocytes (n=86), monocytes (n=90), thrombocyte cell count (n=71), T-mcv (N=127). Baso, basophil cell count; eosino, eosinophil cell count; fibrino, fibrinogen; leuco, leucocyte cell count; lympho, lymphocyte cell count; mono, monocyte cell count; neutro, neutrophil cell count; T-cyt, thrombocyte cell count; T-mcv, thrombocyte median cell volume; tert, tertile.

**Table 3** Risk factors for an MI as outcome of ACS (adjusted for differences in age and sex)

Risk factors	OR	95% CI	p Value
Male sex	1.59	1.19 to 2.13	0.002
Age (years)	1.01	1.00 to 1.02	0.178
hsCRP >2 mg/L	1.75	1.30 to 2.34	<i>p for trend 0.037</i>
Sex (male vs female)	1.73	1.26 to 2.34	<0.001
Age (years)	1.00	0.98 to 1.02	0.872
Serum amyloid			
Tert 1	1.0		<i>p for trend 0.216</i>
Tert 2	1.39	0.98 to 1.97	0.063
Tert 3	1.66	1.16 to 2.36	0.006
Fibrino			
Tert 1	1.00		<i>p for trend 0.010</i>
Tert 2	1.26	0.90 to 1.94	0.174
Tert 3	1.62	1.12 to 2.35	0.011
Leuco			
Tert 1	1.00		<i>p for trend &lt;0.001</i>
Tert 2	2.78	1.97 to 3.92	<0.001
Tert 3	9.64	6.42 to 14.5	<0.001
Neutro			
Tert 1	1.00		<i>p for trend &lt;0.001</i>
Tert 2	2.96	2.09 to 4.20	<0.001
Tert 3	8.91	5.97 to 13.3	<0.001
Eosino			
Tert 1	1.00		<i>p for trend 0.002</i>
Tert 2	0.65	0.45 to 0.94	0.021
Tert 3	0.56	0.39 to 0.80	0.001
Mono			
Tert 1	1.00		<i>p for trend &lt;0.001</i>
Tert 2	1.29	0.92 to 1.82	0.140
Tert 3	3.18	2.20 to 4.61	<0.001
T-cyt			
Tert 1	1.00		<i>p for trend 0.016</i>
Tert 2	1.14	0.81 to 1.61	0.445
Tert 3	1.48	1.05 to 2.09	0.025
T-mcv			
Tert 1	1.00		<i>p for trend &lt;0.001</i>
Tert 2	0.46	0.32 to 0.65	<0.001
Tert 3	0.51	0.35 to 0.72	<0.001

Associations between risk factors and an adverse outcome of ACS were estimated using binary logistic regression and expressed as ORs with 95% CIs adjusting for differences in age and sex. Plasma levels of hsCRP were dichotomised 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 was 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 T-mcv.

ACS, acute coronary syndrome; eosino, eosinophil cell count; fibrino, fibrinogen; hsCRP, high-sensitivity C reactive protein; leuco, leucocyte cell count; MI, myocardial infarction; mono, monocyte cell count; neutro, neutrophil cell count; T-cyt, thrombocyte cell count; T-mcv, thrombocyte median cell volume; tert, tertile.

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 those patients with MI who had  $\geq 240$  min duration since onset of symptoms ( $r=0.19$ ,  $p=0.033$ ) but not in patients with MI with a shorter

duration ( $r=0.02$ ,  $p=0.777$ ), or in patients with UA with  $\geq 240$  min 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



**Table 4** Risk factors for an MI as outcome of ACS

Risk factors	OR	95% CI	p Value
<i>Covariates in model: sex, age, smoking, duration of symptoms</i>			
			<i>p for trend 0.225</i>
hsCRP >2 mg/L	1.40	1.00 to 1.96	0.049
Male sex	1.50	1.04 to 2.17	0.031
Age (years)	1.01	0.99 to 1.03	0.566
Smoking (yes/no)	2.15	1.39 to 3.32	0.001
Duration (≥4 vs <4 h)	1.41	0.98 to 2.03	0.061
<i>Serum amyloid</i>			
			<i>p for trend 0.679</i>
Tert 1	1.0		
Tert 2	1.43	0.96 to 2.13	0.078
Tert 3	1.28	0.84 to 1.93	0.248
<i>Fibrino</i>			
			<i>p for trend 0.031</i>
Tert 1	1.00		
Tert 2	1.19	0.82 to 1.74	0.349
Tert 3	1.62	1.03 to 2.55	0.039
<i>Leuco</i>			
			<i>p for trend &lt;0.001</i>
Tert 1	1.00		
Tert 2	2.58	1.74 to 3.83	<0.001
Tert 3	7.39	4.69 to 11.6	<0.001
<i>Neutro</i>			
			<i>p for trend &lt;0.001</i>
Tert 1	1.00		
Tert 2	2.58	1.74 to 3.83	<0.001
Tert 3	7.39	4.69 to 11.6	<0.001
<i>Eosino</i>			
			<i>p for trend 0.003</i>
Tert 1	1.00		
Tert 2	0.69	0.45 to 1.07	0.069
Tert 3	0.54	0.35 to 0.81	0.003
<i>Mono</i>			
			<i>p for trend &lt;0.001</i>
Tert 1	1.00		
Tert 2	0.99	0.67 to 1.47	0.978
Tert 3	2.36	1.54 to 3.62	<0.001
<i>T-cyt</i>			
			<i>p for trend 0.052</i>
Tert 1	1.00		
Tert 2	1.12	0.75 to 1.66	0.584
Tert 3	1.61	1.08 to 2.39	0.020
<i>T-mcv</i>			
			<i>p for trend &lt;0.001</i>
Tert 1	1.00		
Tert 2	0.41	0.27 to 0.61	<0.001
Tert 3	0.45	0.30 to 0.68	<0.001

Multivariate analysis adjusted for differences in age, sex, smoking and duration of symptoms.

Associations between risk factors and an adverse outcome of ACS were estimated using binary logistic regression and expressed as ORs with 95% CIs. All models included sex, age, smoking and duration of chest pain as covariates beside the specified risk factor itself. Plasma levels of hsCRP ≥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 was 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 T-mcv.

ACS, acute coronary syndrome; eosino, eosinophil cell count; fibrino, fibrinogen; hsCRP, high-sensitivity C reactive protein; leuco, leucocyte cell count; MI, myocardial infarction; mono, monocyte cell count; neutro, neutrophil cell count; T-cyt, thrombocyte cell count; T-mcv, thrombocyte median cell volume; tert, tertile.

chest pain in the model, hsCRP >2 mg/L (OR=1.40 (1.00 to 1.96)) and fibrinogen (p for trend=0.031) remain associated with MI over UA (table 4). Time duration since onset of symptoms, as such, did not reach a statistically significant association with MI over UA (OR 1.41 (0.98 to 2.03), table 4).

The strongest associations with MI over UA were found when haematological variables (blood cells) were analysed (tables 3 and 4). Of circulating inflammatory blood cells, higher counts of neutrophils and monocytes, and lower counts of eosinophils, were associated with a worse outcome of ACS (table 3). These associations were not affected when adjusting for smoking and duration of symptoms (table 4) 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 (tables 3 and 4). 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 the time of admission are associated with a more severe outcome in the case of ACS (ie, predisposition towards MI, rather than UA). We found significant differences in blood cell profiles between an MI or UA outcome, with elevated neutrophils, monocytes and platelet counts in MI, together with a reduced eosinophil count and lower mean platelet volume (MPV). Plasma biomarkers for inflammation (hsCRP, fibrinogen and SAA) showed weaker associations. Our results indicate that a pre-existing inflammation predisposes to a more severe outcome in ACS.<sup>8</sup>

### Plasma biomarkers of inflammation and the outcome of ACS

Previously, we used the CHAPS material to show that genetic variations of thrombotic factors are associated with ACS outcome<sup>9</sup> and, furthermore, that acquired risk factors, smoking and impaired glucose homeostasis together with male sex, predispose to MI over UA.<sup>6</sup> We have here shown 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,<sup>1</sup> however, less known is to what extent a pre-existing inflammation can influence the outcome of ACS.<sup>2</sup> 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—for example, by interleukin (IL) 1, tumour necrosis factor and IL-6<sup>1 10 11</sup>—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 h<sup>10</sup> and, furthermore, in patients with MI, there is a known latency of 6–12 h from onset of chest pain to a rise in CRP plasma concentrations.<sup>12</sup> In line with this, we observed a correlation between hsCRP and time only in patients with MI where the duration between symptom onset and blood sampling exceeded 4 h, 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 <4 h indicate that in ACS a higher pre-existing inflammation predisposes to a more severe outcome. In CHAPS, we previously found current smoking to be strongly associated with MI, but not UA.<sup>6</sup> We considered the possibility that these results could be explained by the known inflammatory effect of smoking.<sup>13–15</sup> 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.<sup>16</sup> 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 the 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.<sup>16</sup> 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.*<sup>17</sup> found an association between increased neutrophil count, and incidence of coronary events and increased case fatality rate during follow-up, in line with a previous meta-analysis of several prospective population studies.<sup>18</sup> A possible explanation for our observation—that neutrophilia was associated with MI when compared to UA—is a hypercoagulable or thrombo-resistant state, as previously indicated by reduced efficiency of thrombolytic therapy or primary percutaneous coronary interventions in patients with MI

with elevated white cell count.<sup>19–21</sup> In this context, it is interesting that a reduced efficiency of primary PCIs in patients with MI has recently been found to be associated with an increased amount of neutrophil extracellular traps (NETs) in aspirated coronary thrombi,<sup>22</sup> adding support to the possibility of 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.<sup>23 24</sup> A possible mechanism relates to the heavy infiltration of monocytes/macrophages that is characteristic of a thin fibrous cap on a vulnerable plaque.<sup>25</sup> Thus, a pre-existing monocytosis in MI, compared to UA, might lead to greater monocytoid infiltration, and predispose to a more extensive thrombotic process following plaque rupture.<sup>2 5</sup> Interestingly, we found significantly lower eosinophil counts in patients with MI, compared to those with UA, consistent with recent reports.<sup>26</sup> Eosinophils have been detected in aspirates from thrombi in patients with MI,<sup>26 27</sup> 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.*<sup>28</sup> reported a significantly higher eosinophil count in patients with UA, but not in patients with MI, when compared to controls. Thrombocytes are key effector cells in an inflammatory process<sup>29 30</sup> and an increase of the thrombocyte count is part of an inflammatory state.<sup>31</sup> Recently, the role of thrombocytes in vascular inflammation and the thrombotic process in coronary artery disease (CAD) has been highlighted,<sup>5 32 33</sup> with an increased MPV reported to be associated with acute cardiovascular events.<sup>34 35</sup> 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 in healthy individuals.<sup>34</sup> No significant difference in MPV was found between patients with MI and those with UA. Individual studies have shown both higher and lower MPV in patients with MI over those with UA.<sup>36 37</sup> In ACS, thrombocytes are involved in a dynamic thrombotic process, with consumption of preferentially more reactive large-sized thrombocytes.<sup>35</sup> This is extensive and permanent in MI, in contrast to the recurrent episodes of (temporary) coronary platelet aggregation and consumption in UA,<sup>38 39</sup> tending to result in a lower MPV in MI than in UA, as in our study and the study of Mathur *et al.*<sup>37</sup> 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.<sup>34</sup>

### Strengths and limitations

The strength and novelty of 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 either 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.<sup>40</sup> 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 4 years. The hospital laboratory used standardised and certified methods, providing consistency over time. Analyses of hsCRP and SAA were performed using frozen samples stored at  $-80^{\circ}\text{C}$  for 15 years; quality assurance work at the laboratory has shown that storage of samples at  $-80^{\circ}\text{C}$  did not influence determined hsCRP and SAA levels. Furthermore, not all patients have complete data for laboratory analyses. In the different multivariate analyses performed, participants with missing data for any included marker were automatically excluded, leaving about 70% of patients in the regression for the full model. Still, outcomes are strong and consistent with the age and sex adjusted model, leaving 90% of patients 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 be diagnosed as NSTEMI, using recent criteria required for MI diagnosis.<sup>8</sup> 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 indicates the further role of inflammation in a more severe outcome in the case of ACS. Our data suggest that neutrophil levels can have a prognostic value in patients with ACS, as previously proposed.<sup>17</sup> 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.

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