Acute Monocyte Subset Counts in Patients Undergoing Coronary Surgery

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Abstract

Elevated proinflammatory monocyte levels may predict cardiovascular events in several diseases. The present paper reports on changes in Classical (CD14++CD16-), intermediate (CD14++CD16+) and particularly non-classical (CD14++CD16+) monocyte counts in patients undergoing coronary bypass surgery with respect both to baseline levels in healthy subjects. In an observational descriptive study, baseline counts in 31 patients scheduled for coronary revascularization surgery were compared with 25 healthy controls. In a subsequent longitudinal study in patients undergoing surgery were monitored at 5 time points up until 48 hours after surgery; biochemical markers for ischemia and acute inflammation were measured, and monocyte subset percentages were quantified by flow cytometry. Baseline and endpoint classical monocyte counts in coronary patients were significantly lower than those of healthy controls (p<0.05). Intermediate monocytes populations were significantly higher at all time points surgery group than in controls, though progressively declining over time to approach control levels. Baseline counts for the non-classical subset, widely linked to cardiovascular risk, were significantly higher in the coronary group than in controls (p<0.001) and, though falling over time, did not attain normal levels over the study period (p<0.001).

Given the modulatory effect of extracorporeal circulation on biochemical parameters, a secondary analysis was performed to ascertain the potential effect of ECC on monocyte subset counts: no significant inter-group difference in counts was observed.

Keywords: Coronary disease; Monocytes; Revascularization

Introduction

Atherosclerotic coronary heart disease is the most common type of cardiovascular condition, and is among the main causes of death in industrialized countries [1]. Coronary artery bypass graft (CABG) is one of the treatments of choice in patients with severe coronary disease. CABG has a number of major benefits, enhancing quality of life and improving life expectancy [2]. Inflammation is a major contributor to coronary artery disease, playing a key role in atherosclerosis and disease progression [3]. Elevated levels of proinflammatory immune cells are reported in patients with atherosclerosis or acute myocardial infarction, reflecting not only the acute inflammatory response to ischemia/reperfusion injury but also the adaptive immunological mechanisms associated with chronic ischemic disease that lead to increased vulnerability during acute events [4]. Elevated levels of blood enzymes such as Creatine Phosphokinase (CPK), Creatine Kinase Isoenzyme MB (CPK MB) and troponin I are currently considered markers of acute-phase myocardial injury Monocytes play a key role in atherogenesis, their accumulation in the vessel wall marking a major step in the onset and progression of atherosclerosis [5]. The findings of in vitro and animal studies suggest that the various monocyte subsets are selectively involved at various stages in the development of atherosclerotic plaques [6], while research in patients with chronic renal failure and with coronary artery disease indicates that increased monocyte-subset counts may be predictive of cardiovascular...
Infectious diseases than in healthy subjects [5]. CD14++CD16+ are characterized by greater cell-membrane expression of the CD14 molecule. Elevated levels are associated with a higher incidence of cardiovascular events and greater CV mortality in patients with chronic kidney disease not undergoing dialysis [13]. Recent research has shown that CD14++CD16+ monocytes independently predicted cardiovascular events in subjects referred for elective coronary angiography [14].

Cardiac surgery with Extracorporeal Circulation (ECC) is associated with a systemic inflammatory response, characterized by vasodilation and diminished systemic vascular resistance, triggered by the contact of blood with the extracorporeal circuit and influenced by the development of ischemia and reperfusion injury with subsequent endotoxin release [15]. The extent and duration of the inflammatory response are governed by a number of factors, including the drugs used, ischemia time and extracorporeal circulation time, as well as mechanical and physical processes inherent in the perfusion process. In general, however, ECC is widely reported to be beneficial for patients [16], and is the procedure of choice for most heart surgery. This study sought to determine the influence of coronary surgery on peripheral blood CD14++CD16-, CD14+CD16+ and CD14++CD16+ monocyte counts in patients with diagnosed revascularizable coronary artery disease (CAD), comparing baseline counts in these patients with those of patients undergoing simple valve replacement and those of healthy controls; a secondary aim was to determine whether use of Extracorporeal Circulation (ECC) influenced changes in monocyte subset counts.

**Patients and Methods**

**Study design and patient selection**

This was a two-part study. The first part was a descriptive transversal study to compare counts for 3 monocyte subsets (CD14++CD16-, CD14+CD16+ and CD14++CD16+) in healthy controls with baseline (pre-surgery) and endpoint (48 hours post-surgery) counts in patients undergoing coronary bypass surgery. In a subsequent prospective longitudinal study, monocyte subset counts were measured in patient group at five different timepoints, from induction of anesthesia to 48 hours post-surgery. Two groups were thus involved in the study: patients with diagnosed isolated Coronary Artery Disease (CAD) and healthy controls with no cardiovascular risk factors.

Forty patients admitted for coronary revascularization surgery were recruited. A 31 patients completed follow-up; 2 died and 7 withdrew consent originally given at admission. Diagnosis was by cardiac catheterization jointly managed by the Hospital’s cardiology and cardiovascular surgery units. Patients requiring valve or aorta surgery during the same operative procedure were excluded from the study. Surgery, performed at the Hospital by 4 experienced heart surgeons, was aimed at achieving complete revascularization using the left or right mammary artery, radial artery or internal saphenous vein depending on the requirements of each patient. All patients received standard anesthesia, heparinization (3 mg/kg) and subsequent total reversion with protamine. Extracorporeal circulation was established in patient group as follows: standard aortic cannulation and single vena cava, followed by aortic clamp, antegrade and retrograde cold blood cardioplegia with reperusions every 20 minutes; proximal anastomosis of each graft was followed by moderate hypothermia (34°C). The Stöckert SS Heart Lung Machine

![Figure 1: Results for the three human monocyte subsets by flow cytometry.](image1)

![Figure 2: Values for CD14++CD16+ monocytes in coronary patients in 5 Time points.](image2)
was used for extracorporeal perfusion, with a centrifugal pump and oxygenator depending on patient theoretical flow. The decision to use extracorporeal circulation for CAD patients was taken by the surgeon on a case-by-case basis.

On the day of surgery, informed consent was obtained from patient group and the following data were collected: a) age, sex, weight, and height for calculating body mass index (BMI); b) preoperative risk factors including history of hypertension, diabetes, smoking, dyslipidemia, peripheral vascular disease, previous acute myocardial infarction, diagnosed chronic kidney failure; c) medical treatment with ACE inhibitors, aspirin, isosorbide dinitrate.

The first sample collection took place in the operating theatre after orotracheal intubation and induction of anesthesia; the final sample was collected 48 post-surgery. Samples were also collected at this stage from age- and sex-matched healthy controls with no symptoms, no cardiovascular risks and normal ECG.

At a total of 5 time points, peripheral blood samples (10 ml) were collected under standard conditions and placed in two 5 ml tubes containing lithium heparin anticoagulant: T1 (after orotracheal intubation and induction of anesthesia); T2 (15 minutes after aortic declamping or, in patients not undergoing ischemia/reperfusion, 15 minutes after completion of the anterior descending artery bypass procedure); T3 (4 hours post-ischemia); T4 (24 hours post-ischemia); and T5 (48 hours post-ischemia). One tube was sent to the hospital’s experimental unit for processing, isolation of polymorphonuclear cells (PMNs) and measurement of monocyte subset counts. The

Table 1: Patient demographic data.

<table>
<thead>
<tr>
<th></th>
<th>CORONARY PATIENTS</th>
<th>VALVE PATIENTS</th>
<th>HEALTHY SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n:31</td>
<td>n:10</td>
<td>n:25</td>
</tr>
<tr>
<td>Age</td>
<td>64.1 ± 9.1</td>
<td>59.1 ± 11.7</td>
<td>55.9 ± 4.9</td>
</tr>
<tr>
<td>Male sex (n, n%)</td>
<td>25 (84.4%)</td>
<td>4 (40%)</td>
<td>19 (76%)</td>
</tr>
<tr>
<td>Body Mass Index(Kg/m²)</td>
<td>28.5 ± 5.16</td>
<td>28.4 ± 3.11</td>
<td>28.03 ± 1.9</td>
</tr>
<tr>
<td>PREOPERATIVE RISK FACTORS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EUROSORE</td>
<td>3.5 ± 1.7</td>
<td>2.8 ± 1.3</td>
<td>n/a</td>
</tr>
<tr>
<td>Previous acute myocardial infarction</td>
<td>21 (67.7%) **</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic kidney failure</td>
<td>2 (6.4%) **</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>5 (16.1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DIABETES</td>
<td>16 (51.6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>21 (67.7%) **</td>
<td>6(60%)</td>
<td>0</td>
</tr>
<tr>
<td>Arterial Hypertensión</td>
<td>25 (80.6%) **</td>
<td>6 (60%)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>5 (16.1%) **</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>INTRAOPERATIVE FACTORS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracorporeal circulation</td>
<td>22 (70.9%) **</td>
<td>10 (100%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Mean ECC time</td>
<td>109 min</td>
<td>104 min</td>
<td>n/a</td>
</tr>
<tr>
<td>Mean ischemia time</td>
<td>83.4 min</td>
<td>78 min</td>
<td>n/a</td>
</tr>
<tr>
<td>Mean bypass</td>
<td>2.39</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Use of left mammary artery</td>
<td>96.4%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>POSTOPERATIVE FACTORS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean days in Intensive care</td>
<td>2.09</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>MORTALITY</td>
<td>2 (6.45%) **</td>
<td>0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*Level of statistical significance by Student’s t and Chi-squared tests.

**p<0.05 Coronary vs. Healthy
other tube was referred to the hospital’s central laboratory for measurement of the following blood biochemical parameters: creatine phosphokinase (CPK, normal: 30-200U/L), troponin I (0.00-0.50 ng/ml), procalcitonin (0.05-0.50 ng/ml) and C-reactive protein (CRP, 0.0-5.0mg/L). Samples from healthy controls (5 ml) were collected in standard conditions, placed in one tube, and referred for isolation of PMNs and monocyte subset counts; since these subjects had no CV risks and were asymptomatic, acute-phase reactants and myocardial damage markers were not measured.

**Peripheral-blood monocyte subset counts**

Peripheral-blood monocyte subset counts were performed by flow cytometry, using the laboratory’s standard protocol [17,18]. Analysis (FSC/SSC) was performed in a region including all monocytes, from which subsets were separated using anti-CD14 and anti-CD16 antibodies (Becton Dickinson, San Jose, CA). Briefly, blood was incubated for 30 minutes at 4°C with peridinin chlorophyll protein (PerCP)-conjugated mAb to CD14 (M5E2) and fluorescein isothiocyanate (FITC)-conjugated mAb to CD16 (3G8). Flow cytometric analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson*). Mean fluorescence intensity (MFI) ranges for CD14 and CD16 were as follows: CD14+: 200-700; CD14++: 700-1000; CD16-: 0-300; and CD16+: 300-1000. Results for the three human monocyte subsets are shown in Figure 1. (A) CD14++CD16-; (B) CD14++CD16+ and (C) CD14+CD16++.

**Statistical analysis**

Statistical analysis of study data was performed using the PASW Statistics 19 (SPSS19) software package. Data are expressed as mean ± Standard Deviation (SD) for normally-distributed data or median and Interquartile Range (IQR) for data not normally distributed. For comparison of means between healthy subjects and patients, Student’s t test or the Mann-Whitney U test were used depending on whether data fitted a normal distribution. Data for the five timepoints in the patient groups (T1, T2, T3, T4 and T5) were subjected to repeated measures ANOVA or the Friedman test where appropriate. Values of p < 0.05 were considered statistically significant.

**Results**

Demographic and clinical data for the subjects enrolled in the study (31 coronary patients and 25 healthy subjects) are shown in Table 1. Significant differences were recorded for mean age between healthy controls and coronary patients (55.9±4.9 vs. 64.1±9.1, respectively; p < 0.05). No significant inter-group differences were recorded for gender or for BMI (28 kg/m2 in all groups). Two coronary patients (6.25%) died on postoperative day 3, one due to postoperative myocardial infarction and the other due to sepsis. All patients underwent diagnostic coronary angiography prior to surgery, and all patients had previously received treatment with ACE inhibitors, aspirin and isosorbide dinitrate. Half the coronary patients were diabetic. ECC (ONCAB) was required in 71.9% of coronary patients. 3.1 Peripheral-blood monocyte subset counts following surgery.

Monocyte subset counts in the patient group are shown in Figure 2, and quantitative and statistical details are provided in Table 2. In coronary patients, the percentage of CD14++CD16- monocytes fell at T2, but increased significantly at T3, thereafter remaining elevated until T5 (p<0.013). CD14+CD16+ monocyte counts did not decrease significantly until T5 (p=0.017). Values for CD14++CD16+ monocytes remained stable until T3, thereafter declining significantly until T5 (p<0.001). Baseline CD14++CD16+ counts were significantly higher in patient group. As Table 3 shows, baseline and endpoint CD14++CD16+ counts were significantly lower than those of healthy subjects (p<0.05). Baseline and endpoint CD14+CD16+ counts were significantly higher in coronary patients than in healthy controls, although endpoint values were lower than baseline values and approached those of controls. Baseline CD14++CD16+ monocyte counts in patients were significantly higher than those of healthy subjects (18.1±1.1 vs 3.1±; p<0.001), and although they declined over time, they remained elevated with respect to controls (12.4±1.7, p<0.001). No significant differences in monocyte subset percentages were observed between ECC and non-ECC coronary patients (Figure 3).

**Discussion**

This is the first paper to address the influence of coronary revascularization surgery on the relative proportions of the three monocyte subsets. In patients undergoing surgery, low baseline peripheral-blood CD14++CD16- monocyte counts increased postoperatively, while CD14+CD16+ and, particularly, CD14++CD16+ counts diminished; by contrast, high CD14++CD16- counts and low CD14+CD16+ and CD14++CD16+ counts were recorded in healthy controls. A number of studies have highlighted the "plasticity" of monocytes, i.e. their ability to differentiate into various cell phenotypes in response to their environment [19]. In healthy subjects, the monocyte population generally expresses high levels of CD 14 molecules and does not express CD 16. CD14+CD16- is a marker for monocytes resident in the bone marrow; CD14++CD16- is a marker for monocytes in the peripheral blood. CD14+CD16+ and CD14++CD16+ are markers for monocytes that have recently migrated to the blood. The percentage of CD14++CD16- monocytes in patients increased postoperatively, while CD14+CD16+ and, particularly, CD14++CD16+ counts diminished; by contrast, high CD14++CD16- counts and low CD14+CD16+ and CD14++CD16+ counts were recorded in healthy controls. A number of studies have highlighted the "plasticity" of monocytes, i.e. their ability to differentiate into various cell phenotypes in response to their environment [19].

**Table 2: Mean monocyte subset counts in coronary patients (5 time points).**

<table>
<thead>
<tr>
<th>Subset</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14++CD16-</td>
<td>46.7 ± 1.8</td>
<td>44.5 ± 2</td>
<td>47.3 ± 1.8 * T2</td>
<td>46.2 ± 1.9</td>
<td>46.2 ± 1.6</td>
<td>0.013</td>
</tr>
<tr>
<td>CD14+CD16+</td>
<td>5.3 ± 0.7</td>
<td>5.4 ± 0.5</td>
<td>4.2 ± 0.3</td>
<td>3.7 ± 0.4</td>
<td>2.8 ± 0.3 * T2,T3</td>
<td>0.017</td>
</tr>
<tr>
<td>CD14++CD16+</td>
<td>18.1 ± 1.1 * T5</td>
<td>18 ± 0.8</td>
<td>18 ± 1.2 * T5</td>
<td>14.9 ± 1.3</td>
<td>12.4 ± 1.7 * T2,T3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Level of statistical significance by repeated-measures ANOVA.
†: p<0.05 after post-hoc comparisons of time factors (T1, T2, T3, T4 and T5).

**Table 3: Mean monocyte subset counts in CAD patients and healthy controls.**

<table>
<thead>
<tr>
<th>Subset</th>
<th>Controls</th>
<th>CAD patients (endpoint)</th>
<th>CAD patients (baseline)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14++CD16-</td>
<td>45.7</td>
<td>46.1</td>
<td>45.3</td>
<td>†: 0.002</td>
</tr>
<tr>
<td>CD14+CD16+</td>
<td>3.1</td>
<td>2.8</td>
<td>5.5</td>
<td>†: 0.002</td>
</tr>
<tr>
<td>CD14++CD16+</td>
<td>3.8</td>
<td>12.6</td>
<td>18.5</td>
<td>†: 0.001</td>
</tr>
</tbody>
</table>

* Level of statistical significance by comparison of mean values (controls vs. CAD patients).
†: baseline levels (†), endpoint levels (‡)
Atherosclerosis is a chronic inflammatory state involving a number of different inflammatory pathways [26]. In atherosclerotic patients, monocytes accumulate continuously during atheroma formation, and accumulation reportedly increases in proportion to lesion size [27]. Migration of monocytes into the vessel wall contributes to the onset and progression of atherosclerosis [28], and research has confirmed a link between levels of soluble inflammatory markers such as PCR and proinflammatory cytokines and the incidence of cardiovascular events [29]. The present study unsurprisingly revealed in coronary patients a statistically-significant correlation between baseline levels of cardiac troponin I, a highly sensitive and specific marker of myocardial damage [30], and levels of CD14+CD16+ monocytes, associated with cardiovascular events (r= -0.357; p= 0.057). During the postoperative period, a significant inverse correlation was observed between anti-inflammatory CD14++CD16- monocyte counts and levels of the soluble inflammatory marker PCR (r= -0.322; p= 0.083).

Few studies to date have focused on the effect of surgery and ECC on monocyte subset counts in patients with coronary artery disease, and none report a direct effect on monocyte subsets as a function of CD16 surface expression. However, high levels of TLR2, TLR4 and TREM-1 surface expression have been observed in CD14++CD16- monocytes, confirming their proinflammatory profile [31,32]. Research in vitro using simulated ECC models [33,34], suggests that ECC may stimulate polymorphonuclear leukocyte (PMN) and monocyte production through mechanisms yet to be ascertained. However, such models cannot fully reproduce the situation of a patient undergoing heart surgery, particularly where there is concurrent lung ischemia–reperfusion injury, as in the present study.

Monitoring of monocyte subset counts showed that CD16+ monocytes were, as expected, the dominant subset in healthy controls. CD16+ monocyte counts were significantly lower in coronary patients at baseline; however, a progressive and significant increase was observed over the postoperative period. These monocytes are involved in new vessel formation and in the production of anti-inflammatory molecules. Levels of proinflammatory CD16+ monocytes—both the CD14+CD16+ subset linked to chronic inflammation and the non-classical CD14++CD16+ linked to cardiovascular disease—were significantly lower in controls than in patients prior to surgery and nearly all patients had established atherosclerosis and coronary artery disease; 67% had at least one prior episode of infarction, and almost 17% had additional diagnosed peripheral vascular disease. In a 200-patient study reported in 2003 by Schlitt et al. [35], patients with chronic CAD also had higher CD14+ CD16+ monocyte counts than healthy controls. Nahrendorf et al. [36] in mice and Tsujioka in humans [37] observed a two-phase monocyte response to myocardial infarction: over the first 2-3 days post-injury, i.e. during left ventricular remodeling, there was a predominance of Ly-6C+ monocytes in mice and of CD16- monocytes in humans, along with marked phagocytic and proteolytic activity. Between 4 and 7 days post-injury, the accumulation of Ly-6C+ monocytes in mice and CD16+ monocytes in humans initially promoted reparative processes such as angiogenesis and extracellular matrix deposition, the classical features of granulation tissue formation; levels remained high throughout chronic inflammation. In the present study, patients exhibited high baseline counts for CD16+ monocytes (CD14+CD16+ and CD14+++CD16+), whilst a two-phase process was observed during the postoperative period. In the first phase, i.e. during and immediately after surgery, the habitual systemic inflammatory response [38] prompted by surgical trauma, contact of the blood with the ECC circuit, myocardial ischemia during bypass [39], subsequent reperfusion the lesion may be temporarily exacerbated when circulation is restored to ischemic tissue [40] and endotoxin release, triggered the inflammatory cascade, leading to a transient increase in inflammatory factors, including monocytes. In the later phase, there was a marked decline in CD16+ monocyte counts, which finally approached those of healthy subjects. However, this study ended 48 hours post-surgery, and further research is required in order for reliable patterns to be discerned. Contrary to expectations, ECC appeared to have no effect on relative monocyte subset counts: no significant difference was observed between non-ECC coronary patients and either valve-replacement patients or ECC coronary patients. Although many of the present findings could not be compared with the results of earlier research, since there have been no previous studies of changes in monocyte levels in patients undergoing coronary surgery, it would appear that surgical coronary revascularization may play a major role in modulating the innate immune system, and specifically the behaviour of CD14+CD16+ and CD14+++CD16+ monocyte subsets closely involved in the onset and progression of atherosclerosis and cardiovascular disease. This may help to explain why the prognosis for coronary patients undergoing surgery is better than that of non-surgically revascularized patients.

This study was affected by two major constraints: the relatively small number of patients, and the failure to chart patient progress and monocyte subset counts over a longer follow-up period.

**Conclusion**

In conclusion, our data provide evidence of an association of coronary artery disease with increased levels of intermediate monocytes and non-classical monocytes and this levels decrease after coronary artery bypass graft (CABG).

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