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ORIGINAL ARTICLE

Biomarker-associated Monocyte Inflammatory Signaling in Myocardial Infarction

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Abstract

Background: Monocytes are essential components in inflammatory signaling, and their recruitment is crucial in the signaling pathway, which directs and determines cell adhesion to the activated endothelium. A better understanding of the correlation between monocyte subsets and inflammatory signaling in patients with atherosclerotic disease in acute coronary syndrome (ACS) is essential for the development of more effective therapies for the prevention and treatment of cardiovascular diseases.

Objective: To analyze differences between biomarkers and monocyte activation in the setting of ischemic heart disease.

Methods: This was a case-control study comparing biomarkers and monocyte subsets between patients with ACS with and without ST-segment elevation and individuals without coronary stenosis. The nonparametric Kruskal-Wallis test was used to assess differences between groups, and Dunn's post hoc test was used to identify which groups were different. Cuzick's test for ordered group trends was used to assess falling or rising trends. Participants were classified into 3 groups: control (0); non-ST-elevation myocardial infarction (NSTEMI) (1); ST-elevation myocardial infarction (STEMI) D1 (2).

Results: Forty-seven patients with ACS and 19 controls with no obstructive lesions on coronary angiography were recruited. Monocyte profile assessment was statistically different regarding time of symptom onset and the presence or absence of atherosclerotic disease (Kruskal-Wallis, p = 0.0009). Dunn's post hoc test showed a significant difference between the control group and the STEMI D1 (p = 0.0014), STEMI D3 (p = 0.0036), and STEMI D7 (p = 0.0195) groups, corresponding to a 2-fold increase in classical (p = 0.0022) and nonclassical (p = 0.0031) monocytes compared with controls. For classical monocytes, there was a difference between the control group and all STEMI groups and between the NSTEMI group and the STEMI D1, D3, and D7 groups. For nonclassical monocytes, there was a difference between the control group and the STEMI D7 group (p = 0.0056) and between the NSTEMI group and the STEMI D7 group (p = 0.0166).

Conclusion: This study found that there was an increase in total and classical monocyte mobilization at the time of acute myocardial infarction in patients with ACS.

Keywords: Myocardial infarction; Monocytes; Atherosclerosis.

Introduction

Despite advances in technology and treatment, cardiovascular disease remains the leading cause of mortality in developed and developing countries (except during the pandemic, when COVID-19 was the leading cause).[1] Atherosclerosis is an inflammatory process associated with elevated levels of low-density lipoprotein cholesterol (LDL-C) involving the activation and infiltration of monocytes in arterial walls. This inflammatory process is responsible for the development of atherosclerosis, as well as for plaque rupture or erosion that leads

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to thrombosis, characterizing an episode of acute coronary syndrome (ACS).[2,3] Monocytes play a key role in inflammatory signaling, and their recruitment is essential in inflammatory signaling processes. Monocyte mobilization promotes cell adhesion to the activated endothelium and constitutes a key element in the mechanism of atherosclerosis. These findings suggest that interventions targeting these activation and mobilization pathways may become possible therapeutic targets in the management and prevention of cardiovascular diseases.[4] Monocytes are grouped into three subsets according to the surface markers CD14 and CD16: $CD14^{++}CD16^{-}$ are termed classical, $CD14^{++}CD16^{+}$ are termed intermediate, and $CD14^{+}CD16^{++}$ are termed nonclassical monocytes.[5] In noninflammatory states, classical monocytes are predominant and may account for up to 90% of monocytes. However, inflammatory states lead to an increased proportion of nonclassical monocytes compared with baseline.[6,7] Understanding the contribution of each monocyte subset in episodes of ACS and differences between biomarkers may improve our knowledge of the pathophysiology of atherosclerotic disease and its clinical manifestations in ACS.

Methods

Study

This was an observational, prospective, single-center study. The study was conducted in accordance with the Helsinki declaration and was approved by the Fundação Universitária de Cardiologia, Research Ethics Committee. All participants signed an informed consent form.

Enrollment

Using the fourth universal definition of myocardial infarction,[8] we evaluated patients undergoing cardiac catheterization at the catheterization laboratory of Instituto de Cardiologia do Rio Grande do Sul, in Porto Alegre, from April 2016 to April 2017. We consecutively recruited patients with a diagnosis of acute ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). The control group consisted of patients with no obstructive lesions on coronary angiography. Controls and cases were matched 2:1 by age and sex.

Inclusion criteria

(a) Adults > 18 years old with an (b) episode of ACS in the last 24 hours and (c) established coronary artery disease who (d) consented to participate in the study.

Exclusion criteria

Patients with (a) severe conditions and a life expectancy < 5 years, (b) a family history of blood dyscrasias, (c) severe anemia, (d) cancer, active infection, or inflammatory diseases, (e) renal failure on dialysis, (f) previous splenectomy, or (g) bipolar or mental disorders; patients who underwent (h) surgery in the last 7 days; patients (i) on immunosuppressants; and any patient (j) from whom we were not able to collect blood samples.

Initial procedures

Patient demographics and clinical characteristics were collected, as well as a blood sample for identifying monocyte subsets through flow cytometry. Additional blood samples were collected from patients in the STEMI group on days 3, 7, and 30. Peripheral venous blood was collected and processed by flow cytometry in 60 minutes. Plasma was stored at 70°C for analysis.

Flow cytometry

Flow cytometry analyses were performed at the Laboratory of Cellular and Molecular Cardiology of Fundação Universitária de Cardiologia using anti-CD14 (fluorescein isothiocyanate and phycoerythrin) and anti-CD16 (PE-CyTM5) antibodies. The protocol was performed according to Imanishi et al.[9]

From the total blood volume, 500 µl were incubated with 5 mL of lytic solution for 15 minutes, and 20 mL of FCS buffer was later added to the sample. The sample underwent centrifugation at 1,500 pm for 5 minutes. After centrifugation, an additional 2 mL of buffer was added to the solution, which was then separated into 10 200-µL tubes and 5 500-µL frozen vials. The samples were subsequently marked with 3 µL of CD14/CD16, and 400 µL of FCS was added to the solutions, which were incubated for 60 minutes. After incubation, the samples were washed with 1 mL of buffer and divided into 200-µL tubes. Finally, flow cytometry (FACSCanto™, BDBiosciences) was conducted using Cell Quest and FACS Diva software, version 6.1.3 (BD

Biosciences). Flow cytometry and biomarker analysis were performed at the Molecular Biology Laboratory of Instituto de Cardiologia.

Statistical analysis

Sample size calculation: with a power of 80% and a significance level of $p < 0.05$, 28 patients would be required to detect a difference of 30 cells/μL in $CD14^{++}CD16^{+}$ monocytes with a standard deviation (SD) of 39 cells/μL. To detect a difference of 250 cells/μL in $CD14^{+}CD16^{-}$ monocytes with a SD of 250 cells/μL, the sample would need to include 17 patients. The Shapiro-Wilk test was used to assess the normality of continuous variables. Variables with normal distribution were described as means and SDs, whereas variables without normal distribution were described as median and interquartile range. Categorical variables were expressed as absolute numbers and percentages. The nonparametric Kruskal-Wallis test was used to assess differences between groups, and Dunn's post hoc test was used to identify which groups were different. Monocyte measurements and groups were obtained on days 1, 3, 7, and 30 in STEMI patients. Friedman's test was used to evaluate repeated measures. Cuzick's test for ordered group trends was used to assess falling or rising trends.[10] Statistical analyses were performed using Stata software, version 13.0. The significance level was set at 5%.

Results

Baseline patient characteristics

We consecutively recruited 47 patients with ACS, of whom 34 had STEMI and 13 had NSTEMI. Mean patient age (mean ± SD) was 9.9 ± 7.3 years in the STEMI group and 60.2 ± 5.2 years in NSTEMI group. Men accounted for 71% of participants in the STEMI group and 58% in the NSTEMI group. The control group consisted of 19 patients, 37% of whom were men, with a mean age of 59.7 ± 6.4 years (Table 1).

Monocyte profile

The monocyte profile of each group was analyzed by flow cytometry. There was an average of 2,620 events per microliter in the control group, 2,539 events per microliter in the NSTEMI group, and 5,571 events per microliter in the STEMI group (Graph 1). The difference between groups was statistically significant, and the control group was different from the STEMI D1 group.

There was a difference between groups (Kruskal-Wallis, $p = 0.00301$) regarding classic monocyte count (Graph 2). Dunn's post hoc test showed that the control group was different from the STEMI D1 group ($p = 0.0058$). There was a significant rising trend between groups according to Cuzick's test ($p = 0.00112$). There was no difference between STEMI and NSTEMI groups according to Friedman's test.

There were no differences between groups when comparing intermediate monocytes (Kruskal-Wallis, $p = 0.3119$; and Friedman, $p = 0.1761$).

In nonclassical monocyte analysis, there was no difference between groups (Kruskal-Wallis, $p = 0.3658$) (Graph 03). In patients with STEMI, there was an increase in nonclassical monocyte count from the first collection to the seventh day, but the difference was not statistically significant according to Friedman's test ($p = 0.1519$).

Discussion

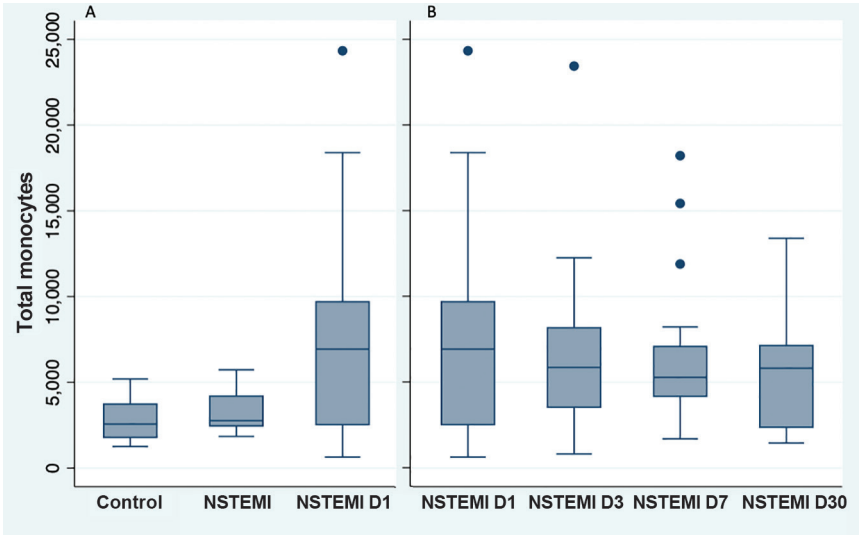
Monocyte count (nonclassical and intermediate) is associated with several characteristics of cardiovascular risk, including serum levels of tumor necrosis factor and onset of coronary atherosclerosis.[11] In patients with unstable angina, there were elevated counts of $CD14^{+}CD16^{+}$ monocytes (both intermediate and nonclassical), which are associated with coronary fibrous cap thickness in atherosclerotic lesions.[9] In patients with stable angina, $CD14^{+}CD16^{+}$ was shown to be associated with atherosclerotic plaque vulnerability.[12] $CD14^{+}CD16^{+}$ monocytes have greater ability to interact with endothelial cells, increased antigen-presenting capacity, and increased expression of inflammatory cytokines compared with the $CD14^{+}CD16^{+}$ subset.[9] These findings demonstrate that $CD14^{++}CD16^{+}$ monocytes are associated with cardiovascular diseases and atherosclerotic plaque progression and instability. The time course of circulating monocytes after an acute event differs according to each subset, as demonstrated in another study. Tsujioka et al.[13] reported a peak of $CD14^{+}CD16^{-}$ (classical) monocytes 2.6 days after AMI onset and a peak of $CD14^{+}CD16^{+}$ (nonclassical) 4.8 days after onset. Our study showed a statistically significant difference in total and classical monocyte increase in the STEMI D1 group. We observed a decrease in the number of classical monocytes and an increase in the number of nonclassical monocytes up to D7 similarly to Tsujioka et al.,[13] but with no statistical significance.

Multiple experimental and clinical evidence have associated inflammation with atherogenesis and its complications.[14-21] The inflammatory status, monitored by

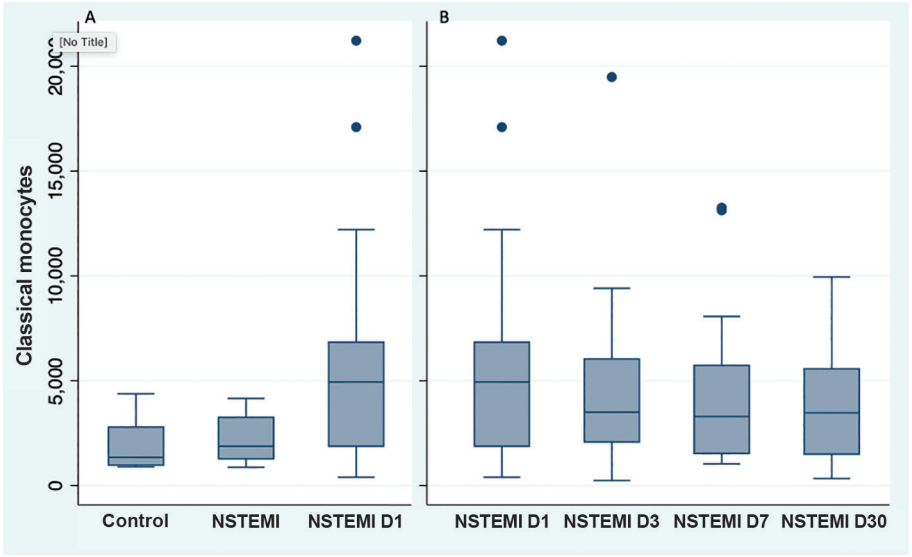
Table 1 – Baseline patient characteristics

Characteristics	Controls (n = 19)	NSTEMI (n = 13)	STEMI (n = 14)
Age	55 (45-64)	62 (56-71)	59 (53-71)
Male	7 (37%)	7 (58%)	24 (71%)
BMI*	23.6±2.3	24±1.7	25.6±11.2
Previous AMI	0	3 (23%)	11 (32%)
Hypertension	0	1 (8%)	11 (32%)
Smoking	2 (11%)	3 (23%)	20 (59%)
Diabetes	1 (5%)	3 (23%)	7 (21%)
Dyslipidemia	5 (26%)	3 (23%)	10 (29%)
Family history of CAD	0	0	5 (14%)
Peripheral vascular disease	0	0	2 (6%)
COPD	0	0	1 (3%)
Previous CABG	0	1 (8%)	1 (3%)
Previous PCI	0	3 (23%)	11 (32%)
Medications			
Aspirin	4 (21%)	11 (85%)	14 (41%)
Clopidogrel	0	2 (15%)	4 (12%)
Prasugrel	0	1 (8%)	0
Ticagrelor	0	0	0
Statins	5 (26%)	11 (85%)	12 (35%)
Beta-blocker	6 (32%)	9 (69%)	12 (35%)
ACE inhibition of angiotensine conversion enzyme	2 (11%)	7 (54%)	8 (24%)
Angiotensin receptor blocker	1 (5%)	1 (8%)	1 (3%)
Calcium-channel blocker	0	0	4 (12%)
Diuretic	1 (5%)	1 (8%)	3 (9%)
Laboratory tests			
Total cholesterol	175 (144-187)	176 (162-194)	170 (144-201)
LDL	95 (75-113)	98 (91-115)	96 (72-129)
HDL	47 (40-53)	42 (35-47)	38 (33-47)
Triglycerides	152 (98-173)	152 (134-174)	156 (94-189)
Creatinine	0.9 (0.8-1.1)	1.1 (0.9-1.2)	0.9 (0.8-1.1)
Echocardiogram			
Ejection fraction (%)	65 (59-73)	68 (47-70)	55 (49-66)
Ethnicity: Caucasian	8 (42%)	7 (54%)	20 (60%)
Ethnicity: Black	10 (53%)	5 (38%)	11 (32%)
Ethnicity: others	1 (5%)	1 (8%)	3 (8%)

** Normal distribution. Numbers in parentheses correspond to interquartile range in continuous variables and percentages in categorical variables. AMI: acute myocardial infarction; CABG: coronary artery bypass graft surgery; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; ACE: angiotension-converting enzyme inhibitor; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction; BMI: body mass index.*

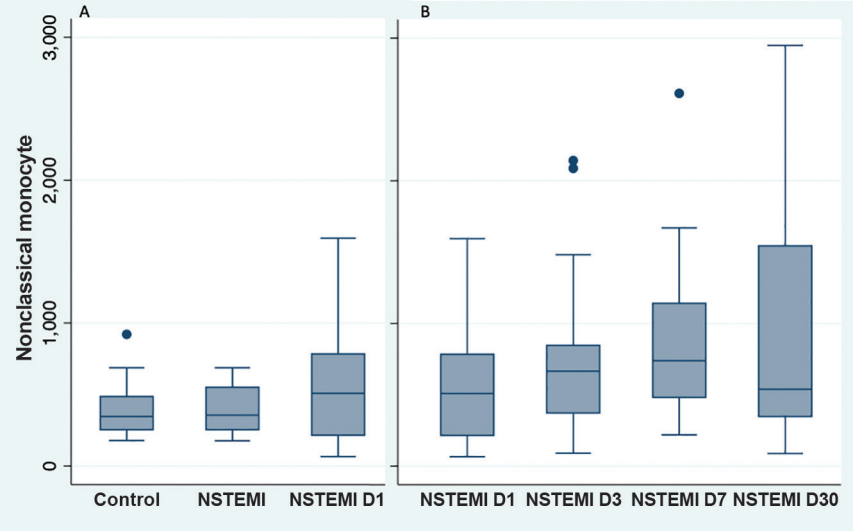


Graph 1 – (A) Total monocyte count according to each group. The difference between groups was statistically significant (Kruskal-Wallis, p = 0.002357). Dunn's post hoc test showed a difference between the control group and the STEMI D1 group (p = 0.0029). (B) Repeated total monocyte count in patients with STEMI. There was no difference between groups (Friedman, p = 0.2576).
STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction.



Graph 2 – (A) Classical $CD14^{++}CD16^{-}$ monocyte count according to each group. The difference between groups was statistically significant (Kruskal-Wallis p = 0.00301). Dunn's post hoc test showed a difference between the control group and the STEMI D1 group (p = 0.0058). (B) Repeated total monocyte count in patients with STEMI. There was no difference between groups (Friedman, p = 0.2576).
STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction.

ultrasensitive C-reactive protein concentrations measured with a high-sensitivity test and interleukins, may improve the prediction of early cardiovascular events.[20] Despite advances in therapeutic strategies for ACS, patients remain at risk of recurrent ischemic events.[21] The reduction in the number of events with statin use results in part from the reduction of circulating LDL-C levels, but also from a possible reduction in inflammation, which is one of



Graph 3 – (A) Nonclassical CD14⁺CD16⁺⁺ monocyte count according to each group. The difference between groups was not statistically significantly (Kruskal-Wallis, p = 0.3658). (B) Repeated total monocyte count in patients with STEMI. There was no difference between groups (Friedman, p = 0.1519).
STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction.

the pleiotropic effects of statin therapy.[22,23] The release of inflammatory cytokines contributes to the development of a number of inflammatory diseases, as well as to the development of atherosclerosis.[24] More recently, the use of low-dose colchicine in an attempt to reduce inflammatory cascade activation lead to a reduction in major cardiovascular events in patients with ACS.[25] Other studies also suggest that there may be a correlation between monocyte subset mobilization, high-density lipoprotein cholesterol levels, and the development of complications in treated patients with ACS, including stent thrombosis.[26]

A better understanding of the correlation between monocyte subsets and inflammatory signaling in patients with atherosclerotic disease is essential for the development of more effective therapies in the prevention and treatment of cardiovascular diseases.[27]

Limitations

This was an observational, single-center study with a limited number of participants. In addition, there was a predominance of male participants and a non-negligible heterogeneity regarding the use of statin and dual antiplatelet therapies between groups. This study is intended to raise hypotheses and should be complemented with new evidence in this clinical and laboratory setting.

Conclusions

This study demonstrated that, among patients with ACS, classical monocyte mobilization and count were increased on the day of AMI onset. We also identified a nonsignificant trend of late increase in nonclassical monocytes in peripheral blood when compared with controls. In severe ACS manifestations (STEMI), there was an increase in total and classical monocyte counts compared with patients with NSTEMI.

Author Contributions

Conception and design of the research and acquisition of data: Guimarães RB, Marchini J, Manica AL; analysis and interpretation of the data: Guimarães RB, Marchini J, Gomez LM, Manica AL; statistical analysis: Guimarães RB, Gomez LM; writing of the manuscript: Guimarães RB; critical revision of the manuscript for intellectual content: Guimarães RB, Leite RS, Dutra O, Castro I, Manica AL.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Fundação Universitaria de Cardiologia under the protocol number 1001.533. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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