International Journal of Cardiology 197 (2015) 101–104

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Reduced CD31 expression on $CD14^+CD16^+$ monocyte subset in acute coronary syndromes

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ARTICLE INFO

Article history:
Received 18 May 2015
Accepted 18 June 2015
Available online 19 June 2015

Keywords:
Acute coronary syndromes
Immune system
Monocyte
CD31

Atherosclerosis is an inflammatory disease that involves both innate and adaptive immunity [1]. Critical impairment of immune response has been observed in a subset of patients with acute coronary syndromes (ACS) [2–4], however only few studies have highlighted the importance of the monocyte compartments in ACS [5,6].

Monocytes play an important role in the immune system linking innate and adaptive immunity and they are critical drivers in many inflammatory diseases. Genetic evidences showed the existence of three human monocyte subpopulations with distinct roles in the immune response, based on their surface CD14/CD16 expression: $CD14^{++}CD16^-$, $CD14^{++}CD16^+$, and $CD14^+CD16^+$ monocytes [7,8]. A recent study indicates that increased levels of $CD14^{++}CD16^-$ predict cardiovascular events [5]. Monocytes express CD31, a member of the immunoglobulin (Ig)-superfamily that plays critical roles in immune-modulation both in innate and adaptive immunity [9,10]. Recently, we showed that during the acute phase of the disease, CD31 expression is reduced on T-cell surface in ACS patients, leading to lymphocyte hyper-reactivity [3].

We performed multicolor flow-cytometry to assesses CD31 expression on different monocyte subsets in ACS patients ($n = 30$) as compared with stable angina patients (SA) ($n = 30$) and individuals without overt cardiovascular diseases (controls) ($n = 30$). 10 ACS and 10 SA were re-analyzed at one year of follow-up. Characteristics of study population are reported in Table 1. Methods are described in the Supplementary material. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The Ethics Committee of the Catholic University of Rome approved the study. The authors of this article have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

ACS patients showed reduced $CD14^+CD16^+$ and increased $CD14^{++}CD16^-$ frequency with respect to SA and controls ($P < 0.001$ for all comparison) (Fig. 1A). ACS showed a different expression of CD31 on monocyte subsets (Fig. 1B). Indeed, both SA and controls showed increased CD31 expression on $CD14^+CD16^+$ monocytes as compared with $CD14^{++}CD16^+$ and $CD14^{++}CD16^-$ (SA: $P < 0.001$ for $CD14^+CD16^+$ versus $CD14^{++}CD16^+$ and $CD14^{++}CD16^-$; controls: $P = 0.015$ and $P < 0.001$ for $CD14^+CD16^+$ versus $CD14^{++}CD16^+$ and $CD14^{++}CD16^-$, respectively), whereas in ACS patients no differences were observed in CD31 expression among the different monocyte subsets. We analyzed the ratio of CD31 MFI among the three subpopulations to quantify CD31 expression in ACS as compared with SA and controls. ACS patients showed reduced CD31 MFI $CD14^+CD16^+$/$CD14^{++}CD16^+$ ratio ($P < 0.001$ versus SA and $P = 0.005$ versus controls) and reduced CD31 MFI $CD14^+CD16^+/CD14^{++}CD16^-$ ratio ($P = 0.008$ versus SA and $P = 0.013$ versus controls). No differences were observed in CD31 MFI $CD14^{++}CD16^+/CD14^{++}CD16^-$ ratio (Fig. 1B).

Fig. 2 shows monocyte phenotypes and CD31 expression on monocyte subsets of ACS and SA patients at baseline and at 1-year follow-up. ACS patients showed a significant increase of $CD14^+CD16^+$ and a reduction of $CD14^{++}CD16^-$ frequency at 1-year follow-up as compared with baseline ($P = 0.006$ and $P < 0.001$ respectively) (Fig. 2A). All monocyte subsets showed increased CD31 expression in ACS patients after one year follow-up (follow-up vs baseline: $P = 0.022$ for $CD14^+CD16^+$; $P = 0.041$ for $CD14^{++}CD16^+$ and $P = 0.037$ for $CD14^{++}CD16^-$).

Although the outcome of ACS is considerably improved in the last decade, ACS represents the main cause of morbidity and mortality worldwide. The investigation of the mechanisms underlying the immune dysregulation is pivotal to identify new potential therapeutic targets and clinical biomarkers in a subset of ACS patients.

In our study, we observed reduced $CD14^+CD16^+$ and increased $CD14^{++}CD16^-$ frequencies during the acute phase of ACS and a reduced expression of the immunomodulatory molecule CD31 on $CD14^+CD16^+$ with respect to the other monocyte subpopulations. Notably, one year after the hospitalization, in a stable phase of the disease, the phenotype of monocyte subsets become similar to

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http://dx.doi.org/10.1016/j.ijcard.2015.06.039

Table 1
Clinical characteristics of study population.

	Controls	SA	ACS	P-value
Number of patients	30	30	30	
Sex (M/F)	20/10	21/9	20/10	0.95
Age (mean ± SD)	62 ± 11	65 ± 11	63 ± 12	0.62
Risk factors				
– Hypercholesterolemia, n (%)	16 (53)	18 (60)	13 (43)	0.44
– Hypertension, n (%)	15 (50)	26 (87)	19 (63)	0.01*
– Smoke, n (%)	13 (43)	14 (47)	22 (73)	0.04**
– Obesity, n (%)	4 (13)	8 (27)	6 (20)	0.44
– Family history of IHD, n (%)	5 (17)	5 (17)	13 (43)	0.02**
– Diabetes, n (%)	3 (10)	7 (23)	6 (20)	0.38
Previous history				
– ACS, n (%)	NA	NA	4 (16)	NA
– Previous PCI/CABG, n (%)	NA	NA	0	NA
Medications (at the time of blood sampling)				
– Aspirin, n (%)	11 (37)	19 (63)	22 (73)	0.011*,***
– Ticlopidine/clopidogrel, n (%)	0	5 (17)	6 (20)	0.04*,**
– Low molecular weight heparin, n (%)	0	1 (3)	2 (7)	0.35
– β-Blockers, n (%)	7 (23)	10 (33)	7 (23)	0.54
– ACE-inhibitors/ARBs, n (%)	13 (43)	16 (53)	16 (53)	0.68
– Statins, n (%)	11 (37)	18 (60)	14 (47)	0.18
– Insulin, n (%)	0	1 (3)	0	0.36
– Oral antidiabetic drugs, n (%)	0	3 (10)	2 (7)	0.22
In-hospital management				
– cTnT > 0.01 ng/mL, n (%)	0	0	30 (100)	NA
– LVEF (mean ± SD)	53 ± 8	51 ± 8	50 ± 7	0.78
– Multi-vessel disease, n (%)	NA	7 (28)	10 (40)	0.37
– PCI/CABG for the index event, n (%)	NA	19 (76)	21 (84)	0.48
Laboratory assay (mean ± SD)				
– Total cholesterol (mg/dL)	190 ± 53	171 ± 43	173 ± 38	0.16
– LDL (mg/dL)	114 ± 38	86 ± 30	93 ± 20	0.41
– HDL (mg/dL)	49 ± 14	50 ± 14	44 ± 7	0.12
– Triglycerides (mg/dL)	150 ± 101	118 ± 57	141 ± 75	0.31
– Lymphocyte count (10^9/L)	2 ± 0.5	2.2 ± 0.7	2.3 ± 1.2	0.76
– hs-CRP (mg/L), median (range)	1.0 (0.2–4.5)	0.7 (0.4–10.9)	5.8 (0.2–53.5)	0.02**

* P < 0.05 SA vs controls.
** P < 0.05 ACS vs SA and controls.
*** P < 0.001 ACS vs SA and controls.

that observed in SA patients. Indeed, there was an increase in $CD14^+CD16^+$ and a reduction in $CD14^{++}CD16^-$ frequency as compared with the time of hospitalization. Most importantly, at one year of follow-up, ACS showed increased CD31 expression on all monocyte subpopulations, in agreement with our previous observation in T-cells [3].

Monocyte subsets are believed to play a differential role in intra-plaque angiogenesis and tissue repair [7]. The reduction of $CD14^+CD16^+$ during the acute phase of the disease suggests a role of this subpopulation in the immune response of ACS patients. Furthermore, during the acute phase, ACS patients showed reduced expression of CD31, in particular on the surface of the $CD14^+CD16^+$ subpopulation. The simultaneous down-regulation of the anti-inflammatory $CD14^+CD16^+$ population and of the immunomodulatory molecule CD31, during the acute setting of ACS, might lead to an imbalance of self-tolerance contributing to plaque instability.

Our study is an observational prospective analysis, including a limited number of patients, we cannot exclude that these alterations might be part of the general stress response in the acute setting of ACS. Nevertheless, our study further examines the role of immune system alteration in coronary artery disease, highlighting the relevance of CD31 as potential therapeutic target and clinical biomarker in the subset of ACS patients in whom inflammation seems to play a role in the pathogenesis.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.06.039.

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