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P-Selectin as a new gender associated biomarker in patients with metabolic syndrome

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Metabolic syndrome (MetS) is a cluster of risk factors for atherosclerosis, including abdominal obesity, hypertension, insulin resistance, dyslipidemia with high triglycerides and low high-density lipoprotein cholesterol [1]. Affected patients have a significantly increased risk of developing cardiovascular disorders. This is probably due to a blood hypercoagulability as well as to endothelial cell activation [2]. Furthermore, several epidemiological studies, the Framingham in particular [3], have investigated into the evolution of cardiovascular disease (CVD) hypothesizing the presence of a gender difference in the pathogenetic and progression determinants detectable in men and women. For example, women were found to outlive men and to experience fewer atherosclerotic cardiovascular events, with an incidence lagging behind that in men by 10 to 20 years [4]. On these bases, and considering that several risk factors for vascular diseases have been associated with inflammation and platelet activation [5], a pilot study has been conducted in platelets from a low number of patients with MetS of both sexes. In particular, we focused our attention on P-Selectin protein expression. This is a transmembrane protein present in the alpha granules of platelets that, following activation, is rapidly translocated to the cell surface and then released in the blood flow.

The study population included 56 ambulatory subjects with MetS (31 men, and 25 women, aging 50–70 years) and 40 age-matched healthy donors (HD) (22 men and 18 women). MetS was diagnosed according to the amended National Cholesterol Education Program's Adult Treatment Panel III (ATP-III) guidelines [1]. Healthy donors were identified on the basis of the absence of CVD risk factors and a completely normal CVD screening. We included in the study patients with carotid IMT increase (>1 mm) but in the absence of known or suspected coronary artery disease (CAD) and only women in post-menopausal and without hormone-replacement therapy.

Patients with previous myocardial infarction, previous coronary artery by-pass graft, coronary angioplasty or positive exercise ECG testing, depression, inflammatory diseases and ACEI treatment were excluded from the study. The nature and purpose of the study were explained to all participants who gave their informed consent following the rules of good medical practice. This study was approved by the Institutional Review Board of "Sapienza" University of Rome (Italy). The investigation was conformed to the principles outlined in the Declaration of Helsinki.

Platelet-rich plasma was prepared by centrifugation of whole blood as previously described [6]. Samples were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, Milan, Italy) and stained with monoclonal antibody against P-Selectin (Chemicon International, Inc. Temecula, CA, USA) followed by an anti-mouse FITC-conjugated antibody (Sigma). Platelets were analyzed with an Olympus BX51 Microphot fluorescence microscope and with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA) for quantitative analyses. Plasmatic P-Selectin was measured using a commercially available immunoassay kit (Human sP-Selectin, R&D Systems). Cytofluorimetric results were statistically analyzed by using the non-parametric Kolmogorov–Smirnov test using Cell Quest Software. At least 20,000 events were acquired. The median values of fluorescence intensity histograms were used to provide a semi-quantitative analysis. Statistical analyses were performed by using Student's *t*-test.

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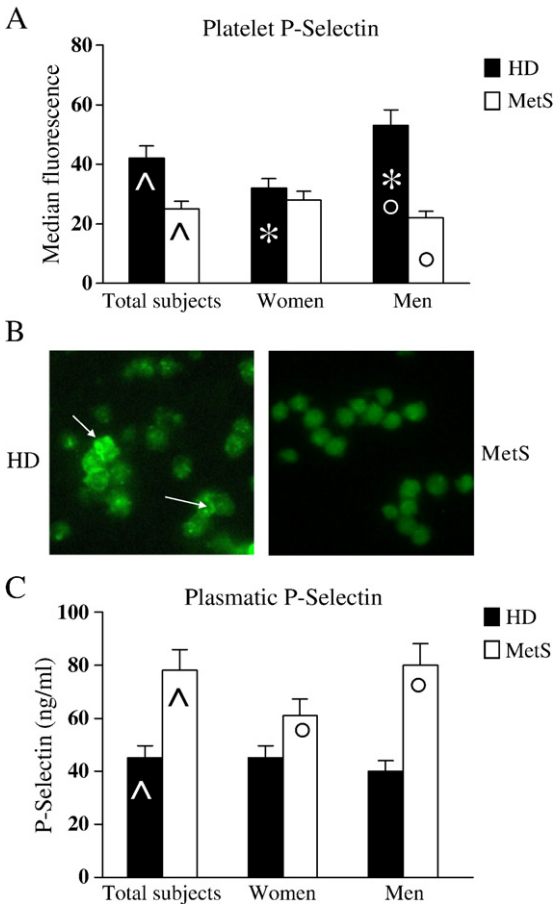

Fig. 1. (A): Histogram representing flow cytometry evaluation of P-Selectin in platelets from patients with MetS and from HD. P-Selectin expression decreased significantly only in men with MetS. Numbers represent median values of fluorescence intensity histograms ± SD. (B): Two representative micrographs obtained by immunofluorescence microscopy of P-Selectin distribution detected in platelets from a male HD (left panel) and with MetS (right panel). Note the presence of P-Selectin-positive clumps (arrows) in platelets from HD and the absence of positive clumps in platelets from one representative patient with MetS. (C): Histogram ± SD representing plasmatic levels of soluble P-Selectin. Significantly higher levels of P-Selectin were detectable in plasma from men with MetS with respect to women with MetS. (^) $P<0.01$, HD *vs* patients with MetS. (*) $P<0.01$, healthy men *vs* healthy women. (°) $P<0.01$, men with MetS *vs* healthy men.

As shown in Fig. 1A a decreased expression of P-Selectin was detected in platelets from MetS patients with respect to HD. More interestingly, a gender difference was also detected. In fact, in HD the P-Selectin was significantly more expressed in platelets from men with respect to those from women and this expression significantly decreased in platelets from men with MetS. Two representative micrographs obtained by immunofluorescence microscopy are reported in Fig. 1B. Regarding the soluble P-Selectin, increased levels have been found in patients with MetS (Fig. 1C). Importantly, when a gender-biased analysis was carried out in male and female patients, significantly higher levels of P-Selectin were detected in plasma from men with MetS in comparison to women with MetS.

Our experimental pilot analyses carried out in patients without any sign of coronary artery disease clearly display a gender disparity as concerns P-Selectin. It is known that increased plasmatic levels of this molecule are possibly due to an increased platelet shedding [5]. Fittingly, we found that increased plasmatic levels of P-Selectin corresponded to a significant decrease of this molecule on platelets. Furthermore, a significant gender disparity was found. In fact, P-Selectin major changes were found in the blood samples from men with MetS. Hence, the potential interest of P-Selectin as MetS biomarker in male subjects should be taken into account in future more extensive studies. In addition, the possible pathogenetic implication of this molecule in the progression of the disease, i.e. in plaque formation, should also be taken into account. These results clearly suggest a reappraisal of the role of P-Selectin as sex-associated progression determinant in metabolic syndrome.

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