

PLATELET ACTIVATION PROFILE IN RELAPSING-REMITTING MULTIPLE SCLEROSIS AND ITS RELATIONSHIP WITH NEURONAL AND ASTROGLIAL BIOMARKERS

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Background and Aims. Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease involving complex immune-nervous system interactions. Beyond their role in hemostasis, platelets contribute to inflammation and immune responses. This study aimed to investigate platelet phenotype in relapsing-remitting MS (RRMS) patients and its association with biomarkers of neuronal damage and astroglial activation.

Methods. Citrated-blood was collected from 49 RRMS patients and 41 control subjects. ADP (2 µM)-induced platelet aggregation, ATP secretion and dense granule content (ATP and ADP) were assessed in platelet-rich plasma (PRP) using lumiaggregometry. Platelet activation markers, such as P-selectin expression, GPIIb/IIIa activation (PAC-1 binding), platelet-monocyte (PMAs), platelet-granulocyte (PGAs) and platelet-lymphocyte (PLAs) aggregates were evaluated by flow cytometry, along with platelet procoagulant markers (tissue factor expression and phosphatidylserine exposure) and reactive oxygen species (ROS) generation. Serum neurofilament light chain (sNfL) and glial fibrillary acidic protein (sGFAP) were measured by SIMOA technology as markers of neuronal damage and astroglial activation, respectively.

Results. ADP (2 µM)-induced platelet aggregation and ATP secretion did not differ between RRMS patients and CTRL [82% (61-89) *vs* 76% (52-90), P=0.5022; 0.2 nmol/10^8 platelets (0.0-0.4) *vs* 0.1 nmol/10^8 platelets (0.0-0.4), P=0.4702, respectively]. However, ADP content in platelet dense granule was reduced in RRMS patients, resulting in an increased ATP/ADP ratio compared with controls (Figure 1A-B). Markers of platelet activation, including P-selectin expression, PAC-1 binding, PMAs, PGAs and PLAs were all significantly increased in RRMS patients (Figure 1C-G). In contrast, tissue factor (TF) expression, phosphatidylserine exposure (lactadherin binding) and ROS generation were not increased in RRMS patients compared to controls [3.7% (1.9-5.3) *vs* 2.8% (1.8-3.8), P=0.1108; 0.4% (0.3-0.4) *vs* 0.6% (0.3-0.8), P=0.0424; 5.8% (4.0-11) *vs* 10% (4.8-15), P=0.1591, respectively]. Notably, both biomarkers, sNfL and sGFAP, positively correlated with PMAs (sNfL: r=0.342, P=0.026; sGFAP: r=0.311, P=0.045) and PGAs (sNfL: r=0.546, P<0.001; sGFAP: r=0.315, P=0.042), but not with P-selectin expression (sNfL: r=0.0127, P=0.423; sGFAP: r=0.225, P=0.151) or PAC-1 binding (sNfL: r=0.192, P=0.223; sGFAP: r=0.131, P=0.408).

Conclusions. Patelets from RRMS patients show preserved agonist-induced functional responses but a distinct activated phenotype, characterized by altered dense-granule nucleotide content and increased platelet–leukocyte aggregate formation, without evidence of enhanced platelet procoagulant activity. The association of sNfL and sGFAP with PMAs and PGAs suggests that platelet-immune cell interactions, rather than isolated platelet activation, may be linked to neuronal and astroglial injury in RRMS. These findings support platelet-leukocyte aggregates as potential biomarkers of thrombo-inflammatory disease activity and as possible targets for strategies aimed at modulating platelet-immune crosstalk in MS.

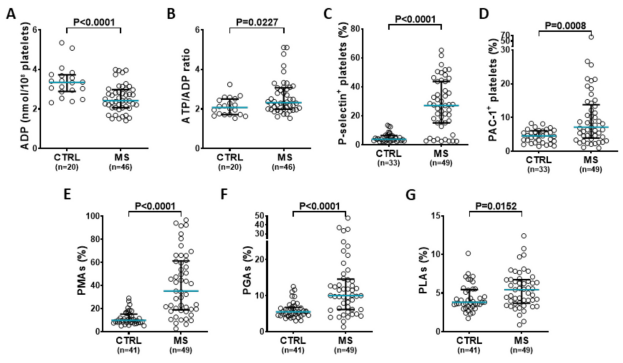


Figure 1. Platelet profile in relapsing-remitting multiple sclerosis patients RRMS) and control subjects (CTRL). Dense granule content was evaluated (**A**) ADP (nmol/10^8 platelets) and (**B**) ATP/ADP ratio. Flow cytometry analysis was performed to evaluate (**C**) P-selectin expression, (**D**) PAC-1 binding (GPIIb/IIIa activation), (**E**) platelet-monocyte aggregates (PMAs), (**F**) platelet-granulocyte aggregates (PGAs) and (**G**) platelet-lymphocyte aggregates (PLAs). Each dot represents an individual subject; horizontal blue lines indicate medians ± interquartile range. Statistical analysis was performed using unpaired t-test or Mann–Whitney test, as appropriate.