



PLATELET ACTIVATION AND THROMBOGENICITY IN ANTI-PHOSPHOLIPID ANTIBODY-POSITIVE SUBJECTS: AN INTEGRATED TGA AND T-TAS ANALYSIS

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Background and Aims. Antiphospholipid syndrome (APS) is characterized by the presence of antiphospholipid antibodies (aPL) and is associated with an increased risk of thrombotic events and pregnancy morbidity. However, risk stratification in aPL-positive subjects, particularly in those without prior clinical manifestations, remains challenging. This study aimed to evaluate platelet phenotype and thrombogenicity as potentially valuable tools for the identification and thrombotic risk stratification of patients with aPL.

Methods. Thirty-two patients (16 males, age: 50 ± 17 , $n=11$ on warfarin, $n=8$ on aspirin) with aPL positivity ($n=17$ APS, $n=10$ with thrombocytopenia) was compared with age- and sex-matched healthy controls ($n=40$). Platelet aggregation was assessed by light transmission aggregometry and expressed as percentage of maximal aggregation (%MA). Platelet activation markers (CD62P and PAC-1) were evaluated by flow cytometry as mean fluorescence intensity (MFI). Global hemostatic potential was assessed using thrombin generation assay (TGA) and total thrombus-formation analysis system (T-TAS), a microchip-based flow chamber evaluating platelet thrombus formation (PL chip) and whole-blood thrombogenicity (AR chip).

Results. Platelets from patients showed: i) reduced aggregability to $5 \mu\text{mol/l}$ ADP (57 ± 6 vs 72 ± 4 , $p=0.045$), 4 mg/l collagen (46 ± 6 vs 85 ± 4 , $p<0.01$), $5 \mu\text{mol/l}$ epinephrine (35 ± 6 vs

66 ± 7 , $p=0.02$), arachidonic acid (35 ± 7 vs 69 ± 5 , $p<0.001$); ii) increased basal CD62 expression (14 ± 2 vs 6 ± 0.4 , $p<0.001$) and reduced binding to PAC-1 in response to $5 \mu\text{mol/l}$ ADP (65 ± 4 vs 80 ± 2 , $p=0.02$) and $1 \mu\text{mol/l}$ TRAP-6 (0.74 ± 0.15 vs 1.3 ± 0.25 , $p=0.05$). Patients also showed altered TGA profiles characterized by prolonged lag time (tLAG: 9.6 ± 0.7 vs 6.2 ± 0.5 min, $p=0.005$.) and reduced thrombin production (PEAK: 273 ± 23 vs 3872 ± 636 nm/l, $p<0.03$) more evident in patients with the tetra positivity to aPL, and by altered T-TAS profiles: AUC-PL: 186 ± 34 vs 362 ± 11 ($p<0.001$) and AUC-AR: 944 ± 138 vs 1362 ± 25 ($p=0.01$). These alterations were found independently of anti-aggregant or anti-coagulant therapy. Strong correlations between T-TAS and TGA parameters were also found.

Conclusions. APS and aPL-positive subjects exhibit significant alterations in platelet activation and coagulation profiles. Platelets show reduced aggregability, increased expression of the activation marker CD62, and reduced PAC-1 binding, likely reflecting a shift toward a procoagulant phenotype. The positive correlation between thrombin generation parameters and T-TAS suggests that these two assays capture, at least in part, the same pathophysiological axis: the overall capacity to generate thrombin and to support thrombus formation and stability. For this reason, T-TAS may be a valuable tool to identify different aPL profiles can underlie different prothrombotic states.