DIAGNOSTICA DI LABORATORIO IN EMOSTASI E TROMBOSI

FACTORS AFFECTING SPONTANEOUS PLATELET AGGREGATION IN HEALTHY SUBJECTS AND PATIENTS WITH STABLE CORONARY ARTERY DISEASE

M. Scavone[1], A. Fioretti[1], C. Ghali[1], B. Clerici[1], E. Popov[2], G.M. Podda[1], M. Cattaneo[3]

[1]Laboratorio di emostasi e trombosi, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Divisione di Medicina Generale II, ASST Santi Paolo e Carlo, Italy; [2]National Medical Research Center of Cardiology, Ministry of Health of the Russian Federation, Moscow, Russia; [3]Fondazione Arianna Anticoagulazione, Bologna, Italy

Background and Aims. Platelets of some subjects aggregate in light transmission aggregometer (LTA) in absence of exogenous agonists ("spontaneous platelet aggregation", SPA), reflecting "platelet hyper-reactivity". Two prospective studies showed that SPA predicts the risk of cardiovascular events in healthy subjects and recurrent coronary events and mortality patients with myocardial infarction. However, its clinical use has been limited by concerns that it represents an *in vitro* artifact, especially in consideration of the fact that it is detectable in platelet-rich plasma (PRP) anticoagulated in citrate but not in hirudin. We aimed to compare SPA in patients with stable coronary artery disease (CAD, cases) and control subjects, using different anticoagulants and aggregometers: LTA and the more sensitive Optical Density Fluctuations Aggregometer (ODFA), which can detect small aggregates of 2-3 platelets.

Methods. SPA was measured in citrate-PRP and hirudin-PRP from age- and sex-matched 139 controls and 102 cases, using both LTA and ODFA. Citrate-PRP was also tested after aspirin (100 µM) addition to inhibit potential thromboxane-A2 synthesis in a low-Ca^{2+} medium. Reproducibility of SPA was assessed in a second session, after at least two weeks, in a subset of subjects. All procedures adhered to international recommendations to minimize in vitro platelet activation.

Results. Citrate-PRP. SPA was higher in cases than controls with both LTA and ODFA, in presence and absence of added aspirin (Fig. 1). Using tertile stratification of data obtained in controls, the frequency of cases was lower in the first tertile and higher in the third tertile, compared to controls: LTA, 33% controls *vs* 21% cases in first tertile, X^2=4.58, P=0.032, 33% controls *vs* 45% cases in third tertile, X^2=3.59, P=0.058; ODFA 33% controls *vs* 22% cases in first tertile, X^2=3.52, P= 0.061, 33% controls *vs* 50%, cases in third tertile, X^2=6.72, P=0.009. The SPA phenotype was reproducible in 73% of subjects in the first tertile and 88% in the third tertile (LTA) and 58% in the first tertile and 83% in the third tertile (ODFA). Hirudin-PRP. A very low extent of SPA was detected by LTA only in 4 subjects in each group, while it was measurable in most subjects by ODFA, although its extent was much lower than in citrate-PRP (Fig. 1). At variance with citrate-PRP, there was no difference in the extent of SPA between cases and controls, suggesting that citrate-sensitive variables account for the observed difference between cases and controls. It is known that platelet aggregation in citrate-PRP is inversely related to hematocrit levels. However, hematocrit was not responsible for the observed difference in SPA, because it was similar in controls and cases (39.3% *vs* 40,2%, P=0.548). We found that agonists that induce platelet aggregation by binding to Gi-coupled receptors (ADP in presence of P2Y1 antagonist, epinephrine and sulprostone, a PGE2 analogue) induced platelet aggregation (LTA) in citrate-PRP, but not in hirudin-PRP, thus perfectly mimicking the results obtained with SPA and raising the hypothesis that a hyper-functional platelet Gi pathway regulates SPA in CAD patients.

Conclusions. SPA is more frequent and pronounced in CAD patients than in controls when assessed in citrate-PRP, but not in hirudin-PRP. Citrate likely unmasks platelet Gi signalling that is responsible for SPA and may be hyper-functional in CAD patients.

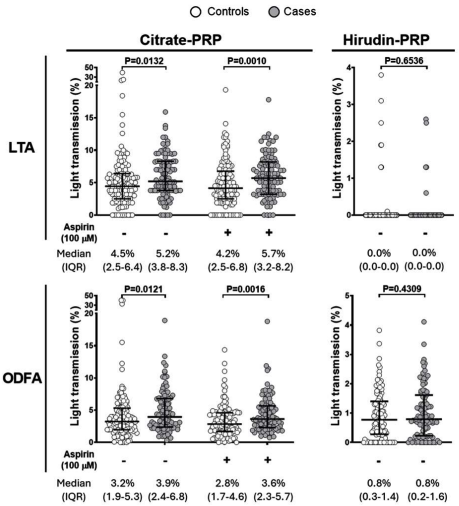


Figure 1. Comparison of SPA measurements by LTA and ODFA in citrate-PRP and hirudin-PRP
Citrate-PRP was analysed in absence (139 controls and 102 cases for LTA; 137 controls and 101 cases for ODFA) and presence of aspirin (100 µM) (132 controls and 102 cases for LTA; 129 controls and 100 cases for ODFA). Hirudin-PRP (128 controls and 97 cases for LTA; 123 controls and 98 cases for ODFA) was analysed without aspirin only. In all experiments, PRP was stirred at 1,000 rpm at 37°C for 6 minutes and analysed by LTA or ODFA without addition of exogenous platelet agonists. Changes in light transmission were interpreted as due to SPA. Data are presented as medians with interquartile ranges (IQR). Statistical comparisons were performed using the Wilcoxon test.