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ABSTRACT N. PO52

COMPREHENSIVE ASSESSMENT OF VARIABLES AFFECTING SPONTANEOUS PLATELET AGGREGATION.

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Background and Aims: Platelets in citrate-plasma from some subjects aggregate in light transmission aggregometer (LTA) without stimulation by exogenous agonists ("spontaneous platelet aggregation", SPA). Although SPA has been associated with cardiovascular risk, its clinical use is hampered by concerns about potential artifacts stemming from the use of citrate anticoagulant or platelet activation during incorrect blood samples collection/processing. In this study, we aimed to comprehensively assess SPA in a large cohort of subjects without cardiovascular disease, exploring multiple variables including anticoagulants, platelet aggregation measurement methods, and individual factors such as age, sex, and platelet count, in order to clarify the nature and clinical relevance of SPA. To this end, we collected blood samples in different anticoagulants and measured SPA using both the traditional LTA and the more sensitive Optical Density Fluctuations Aggregometer (ODFA), capable of detecting small aggregates of 2-3 platelets. Importantly, all procedures were performed in strict adherence to international recommendations to minimize untoward *in vitro* platelet activation, thereby ensuring the reliability of the findings.

Methods: We measured SPA in platelet-rich plasma (PRP) in citrate, hirudin or citrate+hirudin from 100 subjects. Citrate-PRP was tested also in presence of aspirin (100 µM), to inhibit potential thromboxane-A_2 synthesis by aggregating platelets in low Ca^{2+} medium. SPA was evaluated using both LTA and ODFA, and platelet aggregate size was assessed by ODFA.

Results: SPA was detectable in most citrate-PRP samples using both LTA and ODFA. Median SPA values, expressed as percent increase in light transmission, were significantly higher when measured by LTA compared to ODFA [4.4% (3.0-6.0) vs 3.3% (2.1-5.0), P<0.0001]. In contrast, SPA was undetectable in hirudin-PRP samples by LTA, except in four subjects, while it was detectable in most subjects by ODFA [0.0% (0.0-0.0) vs 0.8% (0.1-1.3), P<0.0001]. The mean size of platelet aggregates, which is measurable only by ODFA, was significantly higher in citrate-PRP compared to hirudin-PRP [1.8 RU (1.3-2.2) vs 1.1 RU (1.0-1.2), P<0.0001]. SPA in citrate+hirudin-PRP were comparable to those in citrate alone [2.1% (1.5-4.3) vs 2.0% (1.1-4.1); P=0.0513], indicating that formation of trace amounts of thrombin is not responsible for the generation of SPA in citrate-PRP. The *in vitro* addition of aspirin to citrate-PRP samples marginally reduced the median extent of SPA both in LTA [4.4% (2.9-6.0) vs 4.0% (2.5-6.2), P=0.0004] and ODFA [3.3% (2.1-5.0) vs 2.8% (1.7-4.4), P<0.0001]. This effect was mainly attributable to the inhibition of secondary waves of platelet aggregation, while primary aggregation remained largely unaffected. Age, sex and platelet count affected SPA in citrate-PRP but not in hirudin-PRP.

Conclusions: SPA is not a mere artifact due to *in vitro* platelet activation by inappropriate sample processing, formation of trace-amounts of thrombin in citrate PRP or other effects of citrate, which only amplifies it, making it detectable also by the lower-sensitivity LTA. Our results demonstrate that SPA occurs to variable extents in most individuals without cardiovascular events and is influenced by individual variables. ODFA, a more sensitive detection method than LTA, allows the detection of SPA also in hirudin-PRP.

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