

Diagnostica di laboratorio in emostasi e trombosi

ASSESSMENT OF PLATELET AGGREGATION FOR THE DIAGNOSIS OF INHERITED PLATELET DISORDERS USING A FULLY AUTOMATED COAGULATION ANALYSER: COMPARISON WITH LTA AND MEA

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Background and Aims. Light-transmission aggregometry (LTA) is the gold standard for the diagnosis of inherited platelet-function disorders (IPFD) but it requires skilled personnel and large sample volumes. Recently attempts have been made toward LTA automation to overcome some limitations of manual LTA, allowing the automated selection of agonists and their concentrations. Sysmex CN-6500 is a new haemostasis analyser that performs platelet aggregation with small volume platelet-rich plasma (PRP) (140µl) and automates reagent dilution and aggregation readouts. Aim of our study was to test the agreement between platelet aggregation results obtained with the automated Sysmex CN-6500, manual LTA and multiple electrode aggregometry (MEA) for the study of IPFD.

Methods. Patients aged >18 years and with an IPFD confirmed according to well-defined laboratory and/or molecular genetic criteria were enrolled. A group of 20 healthy subjects with no personal or family history of hemorrhage, with normal platelet aggregation and secretion, and not receiving drug known to interfere with platelet function during the previous 10 days, were used as controls during the same study period to establish the aggregation cut-off. Platelet aggregation was tested in PRP by LTA with the APACT 4 aggregometer (Helena) or with the CN-6500 analyser (Sysmex) and in whole blood with the Multiplate® (Roche) impedance aggregometer using different concentrations of ADP, arachidonic acid, collagen and epinephrine. Correlation between methods was assessed using the Pearson R coefficient and agreement using the Passing-Bablok regression test.

Results. 50 patients (28 females) with a median age of 43 years (95% IC 26-61 years) with various IPFD, including Glanzmann thrombasthenia (10), PT-VWD (2), Filamin A defect (2), TAR (1), FPD/AML (5), TxA2 receptor defect (2), HPS (7), δ-SPD (1), COX-1 like defect (1) and 19 with primary secretion defects were enrolled. The median platelet count in PRP was 260x109/L (95%CI: 176 to 369×109/L). No significant differences in maximal amplitude aggregation were found between manual and automated LTA using all agonists. Passing-Bablok regression analysis showed good agreement between Sysmex CN-6500 and LTA and low agreement with Multiplate (Table 1). Defective aggregation was detected in all the IPFD patients with both manual and automated LTA while MEA failed to identify aggregation abnormalities in 7 patients (6 HPS and 1TxA2 receptor defect). Platelet aggregation testing required 1.1 mL of PRP with the Sysmex CN-6500, 2 mL with manual LTA and 2.4 mL of whole blood with the Multiplate. The turnaround time was approximately 20 minutes for automated LTA, 10 minutes for Multiplate and about 30 minutes for manual LTA.

Conclusions. Automated LTA with the Sysmex CN-6500 provides comparable results to manual LTA in the identification of patients with IPFD, with the advantage of automated serial reagent dilution, smaller total sample volume and shorter turnaround time. Both methods have greater sensibility for IPFD compared with MEA.

Table 1. Passing Bablok regression analysis between Sysmex CN-6500 and the two reference methods. *R= Spearman correlation coefficient; n.d.= not done*

Agonist	LTA (%)				MEA (AUC)			
	slope	*intercept*	*R*	*p*	*slope*	*intercept*	*R*	*p*
ADP 5µM	0.91	12.8	0.76	<0.0001	385.56	-1299.78	0.66	<0.0001
Collagen 2µg/ml	1.14	-7.51	0.69	=0.0006	232.56	-3347.78	0.76	<0.0001
AA 1mM	11.13	-1.15	0.79	<0.0001	294.38	-5094.71	0.68	<0.0001