PLATELET ANALYSIS ON FLOW CYTOMETRY : MEDIAN FLUORESCENCE INTENSITY

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Introduction: Flow cytometry stands as an advanced analytical method for screening and diagnosis of inherited platelet disorders(IPD). Therefore the optimization of prenanalytical conditins , sample processing and analysis strategies is crucial to ensure the reproducibility and accuracy of results . One of the most importnat considerations is the choice of anticoagulant as it may affect platelet function and while anticoagulation with citrate was the preferred approach, experts were divided on the acceptability of employing EDTA.

Aim : The aim of this study is to compare the median fluorescence intensity (MFI) of platelet surface glycoproteins when using citrate versus EDTA as anticoagulants.

Methods: We obtained Platelet-Rich Plasma samples using both EDTA and Citrate tubes from two distinct patient groups. Group 1: 8 patients with Glanzman thrombasthenemia and 1 patient with Bernard Soulier Syndrom and Group 2:21 healthy donors. Then, we assessed the MFI of CD61 and CD41a(GPIIbIIIa glycoproteins),CD42a and CD42b(GPIb_IX proteins) and CD62P (Platelet activation marker) for each group using both EDTA and Citrate samples. The analysis was conducted using the BD FACSLyricTMflow cytometer. Data were analysed by SPSS version 29, a p-value <0.05 was considered as significant statistical difference

Results: The results of our study are illustrated in the tables below

Group 1 : Patients

MFI	FSC	SSC	CD61	CD41a	CD42a	CD42b	CD 62P
EDTA sample	9196	8309	42	193	10077	3054	853
Citrate sample	9810	8763	32	304	11325	3871	445
p	0.757	0.27	0.331	0.895	0.508	0.627	**0.047**

Group 2 : 21 Healthy donors

MFI	FSC	SSC	CD61	CD41a	CD42a	CD42b	CD 62P
EDTA sample	5590	7289	4373	3517	4396	1755	780
Citrate sample	5561	7659	3675	4806	5255	2177	328
p	0.876	0.195	0.058	0.054	**0.002**	**0.017**	**0.005**

Conclusions: CD62P, is expressed on the surface of activated platelets,and mainly used for assessing activation of non-stimulated platelets.In our study , CD62P had a higher expression intensity in EDTA samples which reflects platelet activation, such a spontaneous activation with EDTA may interfere with the results of flow cytometry analysis

Our results suggest that CD62P evaluation is best conducted with Citrate as the anticoagulant in the blood sample, as several studies indicate an elevation in its expression on platelet surface in EDTA samples .

CD41a expression was reported to have a time-dependent decrease in EDTA samples if incubated at 37°, however platelet surface glycoproteins are assessed immdiately after blood collection without any invubation , which supports the absence of significant difference found in our study .

Our study showed a significant difference in CD42a and CD42b expression among the group of heathy donors but no similar results were illustrated in other studies .

Our results show that the choice of anticoagulant depends on the parameters we intend to measure. According to our preliminary results we believe we should assess platelet glycoproteins with both samples and validate study results on larger samples .

Our results assessed only spontaneous platelet expression of glycoproteins , an analysis conducted on platelets before and after activation would be interesting to assess qualitative platelets defects .