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Blood 142 (2023) 1197–1198

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Multimodality Platelet Evaluation By Mass Cytometry and Genetic Analysis in Patients with Bleeding Disorders

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Introduction: Bleeding disorders are a diverse group of conditions characterized by abnormal bleeding tendencies. Platelet dysfunction is a common underlying cause and although traditional platelet aggregometry is important in the clinical work-up, platelet functional assays often provide limited diagnostic insights, prompting the need for more advanced techniques and approaches. This study aims to explore the utility of mass cytometry combined with genetic analysis in assessing platelet function in patients with otherwise unspecified bleeding tendencies.
Methods: In a single-center prospective study, enrolled patients with clinical and laboratory suspicion of platelet-related bleeding disorders were subjected to multimodality evaluation. Mass cytometry (CyTOF) allows for simultaneous evaluation of multiple platelet markers on single cells, including those involved in platelet activation, aggregation, and adhesion, through a panel of heavy metal-conjugated antibodies. Whole genome sequencing with targeted analysis was utilized to identify potential genetic variants associated with quantitative and qualitative platelet disorders and correlated with CyTOF findings.
Results: CyTOF revealed altered platelet markers associated with activation, aggregation, and adhesion in 40% (n=14) of total participants (n=35) with suspected platelet-related bleeding disorders (**Table 1**). CyTOF showed significant concordance with platelet aggregometry in 74% of participants (data not shown). Alterations in specific CyTOF markers (e.g., PAC-1, CD40L) correlated with bleeding risk by univariate analysis of patients stratified to high vs low risk groups based on ISTH bleeding assessment tool (ISTH-BAT) (**Table 1**). Multivariate analysis using a random forest machine learning algorithm identified variables most predictive of bleeding risk, including CD40L, PAC-1, P-selectin, and mean platelet volume. Genetic analysis identified one or more variants in genes implicated in qualitative and quantitative platelet disorders in 61% (n=19) of total patients analyzed (**Figure 1**). Abnormal CyTOF findings were associated with greater proportion of variants detected (79% with abnormal CyTOF vs 53% with normal CyTOF) (**Figure 1**). Direct associations between specific mass cytometry findings and genetic variants (i.e., GPIIb, GPIIIa, GPIb, GPIX, etc.) were discovered for several patients, providing important information in evaluating variant pathogenicity and diagnostic validation.
Conclusion: This study highlights the potential of integrating mass cytometry and genetic analysis to assist in the diagnosis of otherwise unspecified bleeding tendencies, bleeding risk stratification, and patient management. Our findings enhance the understanding of platelet function in patients with bleeding disorders and provide valuable insights into the complex relationship between platelet phenotypes and genetic determinants. Further investigations with a larger patient cohort are warranted to validate and expand upon these initial correlations and explore their clinical implications in diagnosis and management of bleeding disorders.

Disclosures Neparidze: *Janssen:* Research Funding; *GSK:* Research Funding.

Table 1. Demographics and Clinical / Laboratory Characteristics of Bleeding Patients

Characteristic[a]	Total Patients (N=35)	Low Bleeding Risk (N=16)	High Bleeding Risk (N=19)	*P*-value[b]
N (%)	35 (100)	16 (46.7)	19 (54.3)	
Age, y	39.4 ± 21.5	34.1 ± 23.1	43.8 ± 19.5	0.19
Male sex	9 (25.7)	4 (25.0)	5 (26.3)	0.93
Race or ethnic group				
White	30 (85.7)	15 (93.8)	15 (79.0)	0.21
Hispanic	5 (14.3)	1 (6.3)	4 (21.1)	0.21
Bleeding score (ISTH-BAT)	5.4 ± 3.8	2.1 ± 1.5	8.2 ± 2.7	<0.0001
PT (sec)	10.42 ± 0.67	10.34 ± 0.47	10.49 ± 0.80	0.58
aPTT (sec)	25.29 ± 2.05	25.25 ± 2.46	25.33 ± 1.74	0.93
Platelet count (x 10^9/L)	243.3 ± 148.2	266.6 ± 82.4	223.7 ± 186.9	0.40
Thrombocytopenia	6 (17.1)	2 (12.5)	5 (26.3)	0.31
Mean Platelet Volume (fL)	10.72 ± 1.69	10.11 ± 1.59	11.24 ± 1.63	0.05
Elevated MPV	5 (14.3)	2 (25.0)	3 (26.3)	0.93
Platelet aggregometry abnormal	20 (57.1)	8 (50.0)	12 (63.2)	0.43
Platelet CyTOF abnormal	14 (40.0)	5 (31.3)	9 (47.4)	0.33
CyTOF: Constituitive markers				
CD41 / GPIIb (MMI)	293.4 ± 73.4	309.6 ± 76.6	279.8 ± 70.24	0.24
CD61 / GPIIIa (MMI)	44.0 ± 13.6	42.3 ± 11.2	45.4 ± 15.5	0.51
CD42b / GPIb (MMI)	106.2 ± 33.1	112.0 ± 31.7	101.3 ± 34.5	0.35
GPVI (MMI)	128.2 ± 37.7	132.8 ± 37.4	124.3 ± 38.6	0.49
CD36 (MMI)	16.5 ± 8.5	18.9 ± 6.4	14.4 ± 9.7	0.12
CyTOF: Activation markers				
PAC-1 (MMI)	106.0 ± 50.8	126.2 ± 31.9	88.9 ± 57.8	0.03
CD62P / P-selectin (MMI)	35.3 ± 13.3	39.4 ± 8.3	31.8 ± 15.7	0.09
CD63 / LAMP-3 (MMI)	19.5 ± 8.4	20.1 ± 3.6	18.9 ± 11.0	0.70
CD107a / LAMP-1 (MMI)	1.99 ± 0.81	1.93 ± 0.74	2.05 ± 0.89	0.66
CD154 / CD40L (MMI)	9.3 ± 3.9	8.0 ± 1.4	10.4 ± 4.9	0.07
Genetic Variants				
One or more detected	19 (61.2)	7 (50.0)	12 (70.5)	0.26
Variant(s) per patient	0.97 ± 0.88	0.79 ± 0.89	1.12 ± 0.86	0.30

[a]Nominal variables presented as N (%) and continuous variables as mean ± SD

[b]*P*- value comparing high vs low bleeding risk

MMI = median metal intensity

Figure 1

https://doi.org/10.1182/blood-2023-177946