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Diagnosis of Inherited Platelet Function Disorders using two different Diagnostic Approaches

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ABSTRACT

Objective: To compare the effectiveness of immunophenotyping by flow cytometry (FC) and light transmission aggregometry (LTA) in diagnosing inherited platelet function disorders (IPFDs) i.e., Glanzmann thrombasthenia (GLT) and Bernard-Soulier syndrome (BSS).

Study Design: Prospective longitudinal study.

Place and Duration of Study: Armed Forces Institute of Pathology Rawalpindi, Pakistan from Jul 2020 to Jun 2022.

Methodology: A total of 76 patients were included in the study based on their clinical presentation and elevated bleeding time, as assessed using the ISTH-SSC Bleeding Assessment Tool severity score. Flow cytometry was performed on blood samples collected in EDTA anticoagulant to detect platelet membrane glycoproteins (CD41, CD61, CD42a, and CD42b). In contrast, LTA was used to record platelet responses to collagen, epinephrine, ADP, and Ristocetin, with platelet-rich plasma prepared from blood specimens collected in citrate anticoagulant.

Results: A total of 76 patients were included in the study based on their clinical presentation and increased bleeding time. Amongst them 42(58.0%) were males, while the mean age was 10.61±8.74 years. Chronic history of epistaxis was the presenting symptom in majority of the patients; 26(32%). Flow cytometry revealed a total of 16 patients suffering from IPFDs (GLT=10; BSS=06) whereas LTA confirmed the diagnosis of platelet dysfunction among 14 cases (GLT=10; BSS=04). Both showed concurrent positive results in 76.5% subjects (κ=0.84) while flow cytometry showed a relatively higher value of sensitivity, specifically in patients with low platelet count like BSS.

Conclusion: Both LTA and flow cytometry show a significant level of mutual diagnostic agreement.

Keywords: Flow cytometry; Functional and molecular analysis; Inherited platelet disorder; Light transmission aggregometry; Platelet aggregation.

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INTRODUCTION

Platelets are involved in the primary phase of haemostasis and their activation and subsequent aggregation play a pivotal role in the ultimate formation of a stable thrombus. Platelet function is derived from a well-executed coordination between a multitude of intrinsic and extrinsic ligands and a number of membrane receptors which bind these ligands.[1] Any genetic aberration in the overall process of platelet function can subsequently provoke the pathogenesis of inherited platelet function disorders (IPFDs), a group of rare haematological conditions which contribute to a lifelong mild-to-moderate risk of abnormal bleeding.[2] The commonly encountered IPFDs are the bleeding dyscrasias such as Glanzmann thrombasthenia (GLT) and Bernard-Soulier Syndrome (BSS) where a plethora of diagnostic modalities have been established to diagnose these disorders, in contrast less recognized disorders still require a complex set of molecular tests. These multidimensional techniques range from routine clinical assessment and blood counts or microscopic examination to advanced diagnostic approaches in the form of flow cytometry (FC) and light transmission aggregometry (LTA).

The clinical suspects of IPFDs should be monitored by platelet function assays (PFAs) where both LTA and FC can differentiate between the subtypes of IPFDs. Often considered as the gold standard test for PFA, the LTA process involves transmission of light through platelet-rich plasma (PRP), the light transmission increases as platelets aggregate. The amount and rate of light transmission are dependent on platelet reactivity following addition of a agonists (ADP, Collagen, Epinephrine and Ristocetin) provided other variables like temperature, mixing speed are controlled.[5] Concurrently, any abnormal changes in platelet aggregation are analysed. Variable reactivity of the PRP towards different aggregating agonists forms the characteristic diagnostic point to help differentiate between GLT

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and BSS.[6] On the other hand, fluorescent antibody-labelled platelets are identified by means of a laser beam in Flow Cytometry. This analysis can broadly depict if there is any underlying defect of platelet surface markers including CD41 or CD61 (GLT) and CD42a or CD42b (BSS). Regardless of these complex PFAs, a restricted access to platelet studies and a relative scarcity of highly specialized laboratories for molecular analysis are obstacles in diagnosing IPFDs. Nonetheless, evidence has indicated that an accurate estimation of IPFDs primarily depends upon the overall efficacy of PFAs.[7]

LTA can be of limited use sometimes in IPFDs where PRP is difficult to obtain like BSS which may present with thrombocytopenia. This could limit the diagnostic potential of LTA and alternate techniques like Flow Cytometry would enhance the sensitivity of identifying IPFDs.

This study aimed at diagnosing the potential cases of IPFDs i.e. Glanzmann thrombasthenia and Bernard-Soulier syndrome by means of Flow Cytometry and Light Transmission Aggregometry and to compare the relative effectiveness of the two PFA techniques.

METHODOLOGY

This prospective longitudinal study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan over a period of 2 years from July 2020 to June 2022. Ethical approval for the study was obtained from the ethical committee of the institution (vide FC-HEM20-20/READ-IRB/22/957). The sample size was calculated using a WHO calculator with prevalence of IPFDs (GTT and BSS) at 12.1%, which came out be 64. However, to increase the statistical power of evaluation all 76 patients presenting with clinical suspicion of IPFD to our institute during the duration of our study were included.

Inclusion Criteria: All patients presenting with mucocutaneous bleeding in absence of any known pathology, of all ages, both gender and normal coagulation profile (except bleeding time) were included in the study.

Exclusion Criteria: Patients with known coagulation factor deficiency, vascular disorders, thrombocytopenia due to other causes, liver disease and those who consumed an antiplatelet medication in the past 2 weeks, were excluded from the study population.

Prior to initiating our study, a written consent was obtained from each patient. In the paediatrics group, the written consent was provided by the patient's parent/guardian. Patients, their families, and the control group were fully informed about the aim of the research, and research was carried out in line with the Helsinki protocol. The bleeding score (BS) questionnaire was used for analysing the bleeding tendency of patients, the most frequent bleeding symptoms encountered were recorded following which a numerical assessment for bleeding severity was carried out.[8] Patients with bleeding severity range ≥ 3 in male patients, ≥ 5 in females, and ≥ 2 within the paediatric population were included in the final analysis. Hence, a total of 76 patients were finalized as the study population. Before any additional testing, it was assured that all these patients had undergone biochemical testing of their hepatic function so as to assess their clotting profile. Ristocetin cofactor activity and Von Willebrand Factor (vWF) antigen (to rule out Von willibrand disease as D/D of BSS) and Fibrinogen assay (to rule out disorders of fibrinogen as D/D of GTT) were done for all patients. All the medical record analyses and laboratory examinations were performed by two senior physicians at our institution. All the blood samples were collected into Potassium EDTA tubes for complete blood counts and Flow Cytometry. For LTA 10 ml blood was collected in Trisodium Citrate.

CBC was performed using SYSMEX XP-100 and Leishman stained peripheral film was examined under microscopy to exclude other causes of thrombocytopenia.

For LTA, platelet-rich plasma (PRP) from whole blood was prepared by centrifugation at 150g for 10 min in cryocentrifuge at a temperature of 20-25°C. Control platelet poor plasma (PPP) was prepared with centrifugation of the same tube at around 1000 g for 20 min. PRP formed was run on chronolog 700 for assessment of platelet function with following agonists collagen (2 µg/mL), Epinephrine (100 µmol/L), ADP (10 µmol/L) , Ristocetin (1.4 mg/mL) and the percentage of light transmission of PRP over baseline (PPP) changes for a maximum of 300 sec was recorded. The specimens of LTA were preincubated at 37°C for 3min. In our study, an abnormal platelet aggregation was defined as the one with an amplitude lower than 40% of normal. Variables that can cause Pre analytic/ analytic errors including lipemic sample, high haematocrit, centrifugation speed, initial

refractory period of 30 mins after PRP formation and temperature were kept in mind.

Expression of major platelet membrane glycoproteins were assessed by Flow Cytometry using standard Lyse Wash procedure. Fluorochrome labelled monoclonal antibodies against CD41, CD61,CD42a and CD42b were used and resultant suspension was analysed using BD FACS CANTO II.

The statistical calculations were performed using the Statistical Package for the Social Sciences version 23.0. Mean and standard deviation (SD) values were calculated for age while percentage was calculated for type of clinical presentation of bleeding. Relative effectiveness of both the diagnostic measures was expressed in the form of sensitivity and specificity. Meanwhile, their positive and negative predictive values were also calculated. Moreover, Cohen's kappa (κ) constant was determined to reflect the degree of agreement between the results obtained from LTA and FC.

RESULTS

Among the 76 patients (n = 76) 58.0% patients (n=44) were males and 42.0% (n=32) were females while the mean age of patient cohort was 10.61±8.74 years. A majority of patients (32%) had a chronic history of epistaxis whereas cutaneous petechiae (15.8%) and severe intraoperative bleeding (14.5%) were the next commonest bleeding manifestations (Table-I).

Table-I. Baseline Parameters of Study Participants (n = 76)

Mean Age		**10.61±8.74 years**
Gender	Males	44(58.0%)
	Females	32(42.0%)
Bleeding Manifestations	Epistaxis	26(32.0%)
	Skin petechiae	12(15.8%)
	Intraoperative bleeding	11(14.5%)
	Menorrhagia	13(17.1%)
	Gastrointestinal bleeding	7(9.2%)
	Oral bleed after dental extraction	6(7.9%)
	Haemarthrosis	1(1.3%)

LTA and FC were utilized to predict the diagnosis of Glanzmann thrombasthenia or Bernard-Soulier syndrome. FC revealed a total of 16 patients suffering from IPFDs (GLT = 10; BSS = 06) while LTA confirmed the diagnosis of platelet function abnormalities among 14 cases (GLT= 10; BSS = 04). Both showed concurrent positive results in case of 76.5% of subjects. Both the techniques were compared with each other in terms of their sensitivity, specificity, and positive and negative predictive values. While evaluating sensitivity or specificity for IPT, LTA was considered as the gold standard test and vice versa (Table-II).

Both the diagnostic modalities under investigation showed a substantial level of mutual agreement where Cohen's constant (κ) was calculated to be 0.88 which shows almost perfect agreement. Genetic defect by IPT/Flow cytometry and Defect by LTA shown in Table-III.

Table-II. Sensitivity, Specificity and Positive/Negative Predictive Values for Immunophenotyping (IPT) vs Light transmission aggregometry (LTA) (n=76)

Test Parameters	**Diagnostic Methods for IPFDs**	
	Immunophenotyping (IPT)	**Light Transmission Aggregometry (LTA)**
Sensitivity	92.9%	81.3%
Specificity	95.2%	98.3%
Positive Predictive Value	81.3%	92.9%
Negative Predictive Value	98.3%	95.2%

Table-III. Genetic defect by Immunophenotyping/Flow cytometry and Defect by Light Transmission Aggregometry. Crosstabulation (n=76)

	Defect by Light Transmission Aggregometry			Total
	Non	**Glanzmann**	**BSS**	
Defect by IPT/ None	59(96.7%)	01(9.1%)	0(0%)	60
Glanzmann	0(0%)	10(90.9%)	0(0%)	10
BSS	02(3.3%)	0(0%)	04(100.0%)	06
Total	61	11	04	76

BSS: Bernard-Soulier Syndrome; IPT: Immunophenotyping

DISCUSSION

Our study found a higher incidence of GLT as compared to BSS. Both flow cytometry (62.5%) and LTA (71.4%) showed a significantly greater number of cases of GLT among patients presenting with bleeding dyscrasias. Recurrent epistaxis was the most prevalent manifestation of bleeding disorders. Although both techniques had a substantial correlation, IPT had a relatively higher level of sensitivity, whereas both revealed a comparable degree of specificity.

Due to their complex and heterogeneous pathophysiology, diagnosing the platelet function disorders can be highly intriguing, and consequently, there is no single method which can be qualified as

highly sensitive and specific for diagnosing all the IPFDs. In one study, majority of the patients had a significantly raised bleeding assessment tool score even without being labelled as positive on either LTA or FC.[9] Similarly, up to 79% individuals in the contemporary study were not diagnosed with either GLT or BSS despite their presentation with chronic bleeding abnormalities. A successful implementation of flow cytometry has reasonably improved the overall diagnostic accuracy in case of IPFDs. In their study, Sharma *et al*., noted a substantial level of inter-rater agreement between flow cytometry and LTA (κ=0.792), which further strengthens the validity of the former method in detecting platelet cell dysfunction (10). A moderate level of agreement (κ=0.57) was also determined by Navred *et al*., who used LTA as a standard technique to evaluate the positive and negative predictive values for IPT.[9] These were estimated to be 70% and 87% respectively in contrast to 81.3% and 98.3%, as estimated by our study. Moreover, flow cytometry had a lower degree of sensitivity and specificity as compared to the current study.

The present study indicated a substantial inter-technique concurrence (~76.5%). Similarly, other authors have also conducted a comparative analysis where Huskens *et al*., showed that up to 62% of their cases revealed a comparable diagnostic accuracy between the LTA and flow cytometry (κ=0.23), which was substantially less as estimated in the current study. Nonetheless, the latter methodology was found to be more accurate in detection of platelet dysfunction.[11] A fair agreement was also described by Asten *et al*., who found an inter-rater reliability equivalent to 0.32 between FC and LTA.[12] In addition, a combination of these two techniques has also been considered to be highly effective in improving the overall diagnostic potential.

Light transmission aggregometry is apparently the gold standard for clinical diagnosis of disorders pertinent to platelet function if there is no severe thrombocytopenia.[13] However, it is worth noting that besides having a lower sensitivity as compared to flow cytometry, laboratory evidence has also suggested that LTA requires a significantly greater volume of blood sample which downplays its efficient use among children with IPFDs.[14-15] On the other hand, flow cytometry has been mainly targeted as a potential screening tool for IPFDs, but the current evidence suggests a much broader role for FC.[16-17] Therefore, it is advisable to design a comprehensive approach by combining these two diagnostic techniques where both can play a complementary role to potentiate the diagnosis of IPFDs specifically in patients with lower platelet count.[18]

LIMITATION OF STUDY

The study consisted of a relatively restricted sample size where only up to 21% of subjects with elevated bleeding scores were diagnosed with an IPFD. Moreover, the authors did not correlate the patient's bleeding scores with the end results of either LTA or flow cytometry. It is also noteworthy that the relatively higher values of sensitivity and specificity in the study could have been a possible outcome of a lack of a neutral procedure serving as the gold-standard for diagnosing inherited platelet dysfunction.

CONCLUSION

The Light Transmission Aggregometry and Flow Cytometry show a significant level of diagnostic agreement, However, Flow Cytometry is a more accurate choice for the detection of inherited platelet function defects specifically in BSS.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MWA & SAKK: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

SZ & SZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MU & NT: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Wu J, Heemskerk JWM, Baaten CCFMJ. Platelet membrane receptor proteolysis: Implications for platelet function. Front Cardiovasc Med 2021;7:608391 https://doi.org/10.3389/fcvm.2020.608391
2. Boeckelmann D, Glonnegger H, Sandrock-Lang K, Zieger B. Pathogenic Aspects of Inherited Platelet Disorders. Hamostaseologie 2021;41(6):460-468. https;//doi: 10.1055/a-1665-6249
3. Le Blanc J, Mullier F, Vayne C, Lordkipanidzé M. Advances in platelet function testing—light transmission aggregometry and beyond. J Clin Med 2020 ;9(8):2636 https://doi.org/10.3390/jcm9082636

4. Podda G, Femia EA, Cattaneo M. Current and emerging approaches for evaluating platelet disorders. Int J Lab Hematol 2016;38(S1):50–58. http://doi.org/10.1111/ijlh.12539
5. Rubak P, Nissen PH, Kristensen SD, Hvas A-M. Investigation of platelet function and platelet disorders using flow cytometry. Platelets 2016;27(1):66-74. https://doi.org/10.3109/09537104.2015.1032919
6. Hvas AM, Grove EL. Platelet Function Tests: Preanalytical Variables, Clinical Utility, Advantages, and Disadvantages. Methods Mol Biol 2017;1646:305-320. https://doi.org/10.1007/978-1-4939-7196-1_24
7. Lambert MP. Updates in diagnosis of the inherited platelet disorders. Curr Opin Hematol 2020;27(5):333–340.
8. Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, et al. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. J Thromb Haemost 2010;8(9):2063–2065. https://doi.org/10.1111/j.1538-7836.2010.03975.x
9. Navred K, Martin M, Ekdahl L, Zetterberg E, Andersson NG, Strandberg K, et al. A simplified flow cytometric method for detection of inherited platelet disorders—A comparison to the gold standard light transmission aggregometry. PLoS One 2019;14(1):e0211130. https://doi.org/10.1371/journal.pone.0211130
10. Sharma P, Sachdeva MUS, Kumar N, Bose S, Bose P, Uppal V, et al. A comparative study between light transmission aggregometry and flow cytometric platelet aggregation test for the identification of platelet function defects in patients with bleeding. Blood Res 2021;56(2):109–118. https://doi.org/10.5045/br.2021.2020232
11. Huskens D, Li L, Florin L, de Kesel P, de Laat B, Roest M, et al. Flow cytometric analysis of platelet function to improve the recognition of thrombocytopathy. Thromb Res 2020 ;194:183–189. https://doi.org/10.1016/j.thromres.2020.06.037
12. Van Asten I, Schutgens REG, Baaij M, Zandstra J, Roest M, Pasterkamp G, et al. Validation of flow cytometric analysis of platelet function in patients with a suspected platelet function defect. J Thromb Haemost 2018;16(4):689–698. https://doi.org/10.1111/jth.13952
13. Sun P, McMillan-Ward E, Mian R, Israels SJ. Comparison of light transmission aggregometry and multiple electrode aggregometry for the evaluation of patients with mucocutaneous bleeding. Int J Lab Hematol 2019;41(1):133–140.https://doi.org/10.1111/ijlh.12937
14. Lambert MP. Inherited platelet disorders. Hematol Oncol Clin North Am 2019;33(3):471–87. https://doi.org/10.1016/j.hoc.2019.01.008
15. Naz A, Jamal MY, Amanat S, Din ujjan I, Najmuddin A, Patel H, et al. Autosomal recessive inherited bleeding disorders in Pakistan: a cross-sectional study from selected regions. Orphanet J Rare Dis 2017;12(66):06206 http://doi.org/10.1186/s13023-017-0620-6
16. Irum S, Robert HM, Mahmood A, Mahmood R, Khurshid A, Ishaq M. Frequency inherited platele function disorders–Armed Forces Institute of Pathology experience. Pak Armed Force Med J 2020;70(1):22–26.
17. Kim B. Diagnostic workup of inherited platelet disorders. Blood Res 2022;57(S1):S11–19. http://doi.org/10.5045/br.2022.2021223
18. Palma-Barqueros V, Revilla N, Sánchez A, Zamora Cánovas A, Rodriguez-Alén A, Marín-Quílez A, et al. Inherited platelet disorders: An updated overview. Int J Mol Sci 2021;22(9):4521. http://doi.org/10.3390/ijms22094521