# Flow Cytometry With Inflorescence - An Accessible Method in the Study of Qualitative and Quantitative Modifications of Blood Platelets in Immune Thrombocytopenic Purpura

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*The immune thrombocytopenic purpura (ITP) is a polymorphic and hematologic pathology, in terms of both clinical manifestations and etiopathogenic mechanisms, which bring on this disease. Establishing the type of thrombocytopenia, which can either be primary or secondary, peripheral or central, is essential for a further proper therapeutic conduct. The current means of diagnosis include a large variety of hematologic, immunologic and biochemical explorations, from the traditional ones to the latest methods, either genetical or molecular. The purpose of this paper is to emphasize the importance of a classical method of diagnosis, used in the primary triage of patients suffering from thrombocytopenia, highly valuable in the diagnosis process: the analysis of the mean platelet volume (MPV) and of the platelet distribution width (PDW), parameters revealed by the CBC (complete blood count). Evaluating the size of the platelets is a useful tool in the differential diagnosis between immune thrombocytopenic purpura and hereditary macrothrombocytopenia. The platelet distribution width and the mean platelet volume are read simultaneously, in order to differentiate the immune peripheral thrombocytopenia from the central one. The classical, viable and accessible method facilitates the measurement of MPV and PDW with the automatic analyzer, which functions according to the principle of flow cytometry with inflorescence, by using a LASER semiconductor and hydrodynamic focusing.*

*Keywords: immune thrombocytopenic purpura, platelet volume, platelet distribution width, flow cytometry.*

Flow cytometry with inflorescence is a regular technique, but of a high sensitivity and specificity, which facilitates the diagnosis of patients suffering from thrombocytopenia, who are also suspect of having an autoimmune disorder [1]. In addition, together with other tests which investigate the platelet morphology, the peripheral blood smear and bone marrow type, the flow cytometry offers precious data in differentiating between the peripheral and central, hereditary or acquired thrombocytopenia.

Flow citometry is a method of sorting the cells or of detecting the biomarkers. This method is based on sorting the cells according to the obtained florescence by exposing them to a laser ray, after marking it with specific antibodies, connected to a fluorochrome [2]. This method has a large scope, both in medical and surgical specialties: immunology, oncology, hematology, neonatology, in vitro fertilization, genetics. Some of the diseases for which this method has proved efficient, are the immunodeficiencies, leukemias, lymphomas, autoimmune thrombocytopenia purpura etc. Also, this method is useful in the diagnosis of genetic diseases, prenatal diagnosis, as well as in the in vitro fertilization techniques [3,4].

The immune thrombocytopenic purpura implies a decrease in the number of platelets below 100.000/mm$^3$ blood and the presence of antiplatelet antibodies [5]. The CBC is part of the evaluation screening tests, probably the most used one in the laboratory. It offers important information about the hematologic status of a patient and it also represents the first step in the diagnosis of several pathologies in the hematologic field and not only. Reading the parameters of the CBC orients the algorithm diagnosis and facilitates the recommendation of some targeted analytical tests, necessary in carrying out an accurate diagnosis, in a short notice. The CBC based on the flow cytometry principle is the first step in the diagnosis algorithm of immune thrombocytopenia purpura. The decrease in the number of platelets is revealed in this stage, along with the values of the mean platelet volume and platelet distribution width, valuable parameters in choosing the next diagnosis conduct [6].

## Experimental part

### *Materials and methods*

The hereby study is based on observations and it was carried out on 40 subjects hospitalized at the Sf. Spiridon University Hospital from Iasi, between 2013 and 2015. The patients are aged between 18 and 74 and come mainly from an urban environment. They agreed upon being included in a study, by signing an informed agreement. The study complies with the law no. 677/2001 pertaining to the collection, use and processing of personal data, and the protocol was approved by the Commission of Ethics of the Gr. T. Popa University of Medicine and Pharmacy from Iasi.

The initial stage of the study consists of an evaluation of the hematologic parameters by using a CBC. The platelet count was performed by using the automatic analyzer with laser semiconductor and hydrodynamic focusing. The used biological specimen is venous blood, collected with an EDTA anticoagulant. The collecting container is a vacutainer with a purple/pink cover - K3 EDTA. The tube was at least three quarters full, in order for the blood/anticoagulant report to be optimum (the EDTA recommended concentration is of 1.2 - 2.0 mg/mL blood) [7,8]. The content was mixed by tube inversion, approximately ten times.

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The values of MPV and PDW were obtained by means of a CBC. The automatic analyzer calculated the MVP by using the following formula:

$$MPV\ (fL) = \frac{PCT\ (Platelecrit)\ (\%)}{No.\ of\ platelets\ (x10^3/\mu L)} \quad x\ 10000$$

Reference values: MPV = 8.5-12 fL or $\mu m^3$ [9-11].

The platelet distribution width (PDW) is calculated by the automatic analyzer using the following formula:

$$PDW\ (CV\%) = \frac{Standard\ platelet\ size\ deviation\ x\ 100}{MTV}$$

Reference values: PDW = 10 – 18% coefficient of variation (CV) of the platelet size.

Interferences: The platelets tend to increase their volume in the first two hours after the contact with EDTA. The get smaller once with the prolongation of sample storage time. These oscillations hinder the levelling of measurements. Also, the MPV and PDW can record false values, if the number of platelets is below 10.000/μL [12, 13].

## Results and discussions

In the hereby study, the whole batch of patients suffer from thrombocytopenia, with a mean platelet (PLT) count of 45.93 x 20.10 x $10^3$/μL and a variance of 43.7%, which is within the range of 8 - 98 x $10^3$/μL since all the values are below the minimum reference limit. Thrombocytopenia is the common criterium for all the patients included in the batch of study.

The count of the platelets was performed by using the automatic analyzer when executing the CBC. The benchmark range for the values of the platelets is of 150-450.000 platelets/μL, and the reading of the pathological values in the sense of the decrease or increase in the number of platelets, was carried out based on this range of normal values. The analysis of the number of platelets is indicated in order to diagnose the quantitative platelet pathologies, such as thrombotic thrombocytopenic purpura, immune thrombocytopenic purpura, thrombocytosis.

With a variance of 31.9%, the series of MPV values varied between 5.70 and 20.90 fL, while 10% was below the minimum reference limit and 60% exceeded the maximum reference limit (8.5 - 12 fL). The average value of the study batch was of 13.52±4.31 fL, which is within the suggestive limits of immune thrombocytopenic purpura (ITP). The connection between PLT and MPV reveals the independency of these parameters, since only 4.2% of the increased values of PLT were under cover of the increased values of MVP, ($r = +0.042$; $R^2 = 0.0018$; $p = 0.797$) (fig.1). These results confirm the increase of MPV, associated with the peripheral decrease in the number of platelets in ITP, in the absence of other indices showing a possible central cause of thrombocytopenia.

The mean platelet volume is a size index of the platelets with normal values, comprised between 8.5 and 12 fL or $\mu m^3$. The reading of the MPV values reveals valuable information, which help the clinician establish the etiology of the thrombocytopenia and in further investigations, for a more accurate diagnosis. The MPV shows increased values in case of platelet damage through peripheral mechanism, while the normal or decreased values of MPV, are connected to the central etiology of thrombocytopenia: the decrease of platelet aggregation in the bone marrow by hindering the activity of megakaryocytes [14, 15].

Young and immature platelets can be differentiated from the mature ones through a different morphologic aspect. Young platelets tend to have an increased volume, which is emphasized by the increase of MPV values. The presence of new platelet generations and of an increased MPV, display a normal activity in the bone marrow, while maintaining its regenerative capacity.

In cases of hereditary thrombocytopenia (Bernard-Soulier syndrome and hereditary macrothrombocytopenia with an autosomal dominant transmission), the MPV has values specific to gigantic platelets (MPV = 16 - 30 fL) (114, 116). The increased values of MPV, specific to hereditary thrombocytopenia, have not been found in any patient from the batch of study.

Platelet distribution width (PDW) is analyzed together with the MPV and the number of platelets. The range of normal values of PDW is 10 -18% coefficient of variation (CV) of the platelet volume. Our results show a variation of the PDW values in the range of 5.60 to 23%, with a variation of 28.9%, while 10% of the samples are below the minimum reference limit, and 57.5% exceed the maximum reference limit (10 -18%). The mean values of the batch of study were of 16.93%±4.89.

The correlation between the number of platelets and PDW was direct, very weak intensity ($r = +0.058$; $R^2 = 0.0033$; $p = 0.724$), and this result cannot be extrapolated to the whole population (fig. 2). The PDW does not correlate directly with the number of platelets, since thrombocytopenia cannot exist without the alteration of platelet morphology, especially in the primary stages of the disease. Most of the times, the morphological alterations are caused by the action of the platelet antibodies over the platelet membrane glycoproteins in the case of peripheral thrombocytopenia, or they are represented by morphological anomalies caused by central aggregation disorders [15, 16].

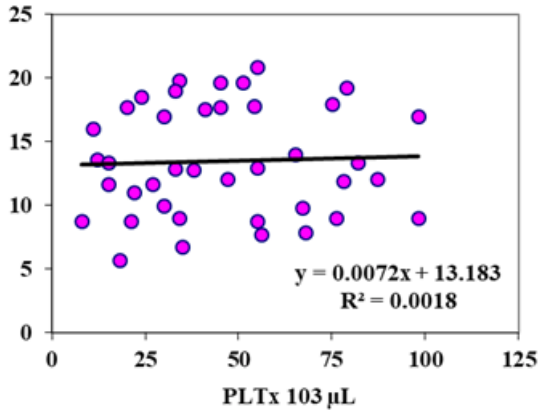


Fig 1. The correlation between MPV and PLT

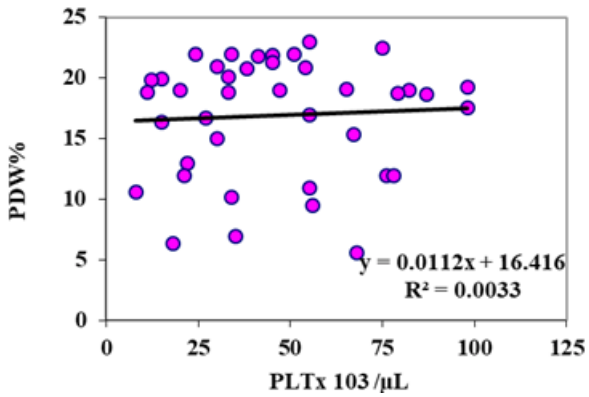


Fig. 2. The correlation between PLT and PDW

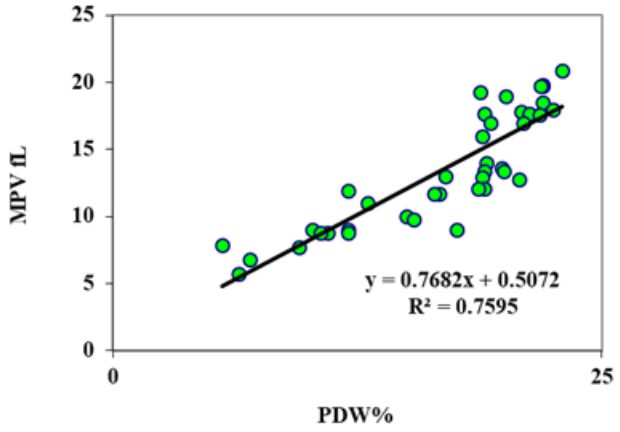

Fig. 3. The correlation between MPV and PDW

The correlation between MPV and PDW reveals a strong direct correlation ($r = +0.872$; $R^2 = 0.7595$; $p = 0.001$), since 87% of the increased values of PDW were under cover of the increased values of MPV; this result which can be extrapolated to the whole population (fig. 3).

The MPV is used together with the PDW, in order to distinguish between the conditions associated with a low production of platelets and the ones connected to an increased platelet damage. According to the results of our study, the MPV and PDW had slightly increased values, associated to a decreased number of platelets when compared to the normal range. All this data proves the importance of determining the MPV and PDW to the diagnosis of peripheral thrombocytopenia and its characterization.

## Conclusions

The flow cytometry with florescence, on the basis of which the automatic analyzer for the CBC works, is a traditional, but accessible method, with a high sensitivity and specificity in detecting primary stage thrombocytopenia in patients with purpura and morphology disorders in the blood platelets. We aim to stress out the importance of analyzing the number of platelets in correlation with the values of the MPV and PDW platelet indices, in order to confirm the peripheral or central nature of thrombocytopenia. In this study, the correlation between the number of platelets and MPV reveals their interdependency, while 95.8% of the decreased values of blood platelets are associated with increased values of MPV, which suggests a peripheral cause of thrombocytopenia in immune thrombocytopenic purpura. Also, the values of MPV and PDW are highly correlated; over 87% of the increased values of PDW were under cover of the increased values of MPV, and this result can be extrapolated to the whole population. The purpose of this paper is to emphasize the importance of using flow cytometry in detecting thrombocytopenia and in identifying platelet morphology disorders in the immune thrombocytopenic purpura, by analyzing the MPV and PDW values.

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