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Hematology

Importance of the Immature Platelet Fraction in the Etiological Diagnosis of Thrombocytopenia

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Abstract **Original Research Article**

Thrombocytopenia is defined as a platelet count of less than 150 G/L in the blood. It can be of central or peripheral origin. Newly-formed immature platelets are called reticulated platelets (RP) and are expressed as the immature platelet fraction or IPF. This automated hematological parameter enables us to distinguish between peripheral and central thrombocytopenia in a simple, quick, easily reproducible and non-invasive way. In the present study, our aim was to evaluate the clinical utility of the IPF and its ability to differentiate between central and peripheral thrombocytopenia. In this prospective study, we collected 50 cases of thrombocytopenia in our series. The IPF and all other CBC parameters were measured using the Sysmex XN-1500 analyser. Based on the clinical context and myelogram findings, the subjects in our study were separated into a central thrombocytopenia group and a peripheral thrombocytopenia group. We then assessed variations in IPF between the two groups. The mean IPF in the peripheral thrombocytopenia group was significantly higher than that in the central thrombocytopenia group (15.71 ± 12.02% vs. 5.51 ± 3.04%; $p<0.001$) and this difference persisted regardless of platelet count. We also established a sensitivity and specificity ROC curve, which showed that the IPF had excellent diagnostic value for differentiating between central and peripheral thrombocytopenia, with an area under the curve of 0.914. We also defined a discriminative cut-off value of 8.5% with a sensitivity of 77.8% and a specificity of 86.4% for defining the origin of thrombocytopenia. Thus, an IPF value above 8.5% points to peripheral thrombocytopenia with increased platelet regeneration. In conclusion, the results of our study have enabled us to formulate recommendations for improving the diagnostic strategy for thrombocytopenia using the immature platelet fraction or IPF.

Keywords: Immatures Platelets, Peripheral Thrombocytopenia, Central Thrombocytopenia, Reticulated Platelets, Fluoro-Flowcytometry.

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1. INTRODUCTION

Thrombocytopenia represents a common anomaly in the complete blood count, it is defined by a platelet count below 150 G/L of blood. Etiologies are varied and sometimes challenging to confirm.

The primary question that clinicians face with any thrombocytopenia is determining its central or peripheral origin. For a long time, only the study of bone marrow could answer this question, it is easy to do and it is often providing a reliable diagnosis in a short time. However, it is an invasive and painful procedure which is closely related to the quality of the sample, that is also time consuming. Sternal aspiration may result in severe complications such as pneumothorax or hemopericardial effusion, while posterior iliac crest aspiration can result in allergic reactions to local anesthesia, excessive bleeding, infection or long-lasting discomfort [1]. Due to these constraints, hemogram analyzer manufacturers have developed a new parameter called “IPF” or “Immature Platelet Fraction”, which could be a promising tool to discriminate central from peripheral thrombocytopenia, then avoiding bone marrow aspiration to assess megakaryocyte density [1].

The immature platelet fraction (IPF) is the percentage of immature platelets compared to the total number of platelets. They are newly released platelets. They are supposed to reflect bone marrow capacity to produce platelets, and could be a surrogate marker for megakaryocytic activity. They can be distinguished from mature platelets by their RNA content and their larger

size. They are suggested to be the platelet analogue of the red cell reticulocytes [1].

A new automated method to reliably quantify reticulated platelets, expressed as the immature platelet fraction (IPF), is available on new generation hemocytometers. Results are expressed as a percentage of total platelet count (IPF%), or as an absolute number [1].

The aim of this study was to evaluate how IPF could be used in management and diagnosis of thrombocytopenia of unknown origin. Our objective was to determine a cut-off value to differentiate central from peripherl thrombocytopenia in a prospective study.

2. MATERIALS AND METHODS

This is a prospective descriptive study on the value of the new hematological parameter "IPF" or "Immature Platelet Fraction", and its role in the etiological diagnosis of thrombocytopenia, spread over 6 months from 01/01/2023 to 01/07/2023.

In the first phase, we established normal values for this parameter in a group of control patients. Subsequently, we evaluated and compared the IPF (Immature Platelet Fraction) in two groups: the first group comprised patients with central origin thrombocytopenia (CT), and the other group had peripheral origin thrombocytopenia (PT).

2.1. Patients

2.1.1. Control Group

We analyzed prospectively, IPF% values among 50 normal subjects with normal hematological findings at the Avicenna Military Hospital (HMA) in Marrakech. We excluded from our study any subject with an abnormal blood count, as well as patients with known thrombopathy.

2.1.2. Thrombocytopenic Patients

We analyzed prospectively, IPF% values among consecutive 50 thrombocytopenic patients with platelet counts below 100 G/L who underwent bone marrow aspiration, collected at the Hematology Laboratory of Avicenna Military Hospital (HMA) in Marrakech. Decision to realize bone marrow aspiration was indicated by the clinical physician, and validated by a hematologist. Only patients with an identified etiology of thrombocytopenia were included. Exclusion criteria were thrombocytopenia with poor quality BM smears (hemodilution for example), as well as patients with incomplete medical records.

2.2. METHODS

2.2.1. Data Collection

The collection of clinical data was done using the medical records of patients referred to the hematology laboratory. All data were processed using an exploitation form that included sociodemographic, clinical, and biological aspects of each patient, with a focus on hematological data.

Text and tables were entered in Word, and graphs in Excel XP. Statistical analysis, ROC curve generation and interpretation were carried out using SPSS software. The Student's T-test was used to compare two means. The comparison is considered significant when the p-value is less than 0.05.

2.2.2. Diagnostic Resources

Peripheral venous blood samples were collected in a fasting state, using K2-EDTA (ethylene diamine tetra-acetic acid) anticoagulant bottles, immediately identified, placed in plastic bags, transported to the laboratory at room temperature and analyzed within 6h after collection.

We conducted a complete blood count for all patients, including a blood cell count and a blood smear, allowing for a quantitative and qualitative analysis of various blood components. The obtained results were confronted with clinical data as well as the results of the bone marrow examination for each patient.

In our study, the complete blood count was performed using the Sysmex XN-1500 analyzer (figure 1) on blood samples. It’s a fully-automated hematology analyzer that is part of the new reference range from Sysmex Japan, which includes a fully integrated spreader-stainer with a small footprint. In our structure, the hematology Sysmex XN-1500 analyzer was calibrated and then quality controlled every 24 hours using XN CHECK (Sysmex Corporation).

Figure 1: Sysmex XN-1500 in the HMA hematology laboratory

The first generation of the fully automated analyzers has a reticulocyte/platelet channel that measures by flow cytometry the fraction of immature platelet to their total number [2]. However, the Sysmex XN-1500 uses a specific channel (PLT-F) for platelet count and IPF measurement (IPF% and IPF#) using a fluoro-flowcytometry method which consists, on the one

hand, by searching for platelet membrane glycoproteins using specific antibodies (anti-CD41, anti-CD61), and on the other hand by measurement of cell size and flurorescence.

The platelet membranes are perforated by the lysis reagent, then fluorescents dyes (polymethine and ozazine) enter into the cells, staining the RNA content in the platelets. The stained cells are passed through a semiconductor diode laser beam and resulting forward scatter light (cell volume) and fluorescence intensity (RNA content) measured. Two-dimensional scattergrams were plotted, with the X-axis representing the intensity of the side fluorescent light (SFL) reflecting RNA content, and the Y-axis indicating the intensity of the forward scattered light (FSC) reflecting cell size.

IPF is characterized as the platelet with large size and high fluorescence intensity, relative to the total number of platelets (figure 3). Data analysis is done by specially designed algorithm software (IPF-software). This fully automated technique allows for obtaining a standardized and quick result in less than 1 minute.

3. RESULTS

3.1. Control Group

Median age was 59 (18-90), 52% were men (n = 26). Median platelet count was 254 G/L. Median IPF% was 3.1% (0.7-6.8).

3.2. Thrombocytopenic Group

We included 50 patients in this study (table 1), median age was 54, (18–92), 58% were males (n=29). Thrombocytopenia was the sole abnormality in 21 patients (42%), or was associated with anaemia (n = 18, 36%), neutropenia (n = 2, 4%), or both (n = 9, 20%).

Thrombocytopenia was peripheral (PT) in 28 patients (56%) and central (CT) in 22 (44%). Median platelet count was 57,2 G/L, range (1-100); median PLT was 54 G/L (1-100) in the PT group and 59 G/L (5-84) in the CT group (p=0,002).

In the PT group, mechanisms for thrombocytopenia were platelet consumption (n = 8, 16%), destruction (n = 19, 38%) or hypersplenism (n = 1, 2%). In the CT group, diagnoses were acute leukaemia (n = 11, 22%), myelodysplastic syndrome (n = 5, 10%), bone metastasis (n = 3, 6%), macrophage activation syndrome (n = 1, 2%), primary myelofibrosis (n = 1, 2%) or drug toxicity (n=1, 2%).

Table 1: Population characteristics

	Patients
Nombre de patients	50 (100%)
Age	54 (18-90)
Males	30 (60%)
Cytopenias	
Thrombocytopenia alone	21 (42%)
+ Anemia	18 (36%)
+ Neutropenia	2 (4%)
Pancytopenia	9 (18%)
Mechanism	
Peripheral thrombocytopenia	28 (56%)
Consumption	8 (16%)
Hemorrhage/thrombosis	2 (4%)
Thrombotic micro-angiopathy	4 (8%)
Inflammation	2 (4%)
Hypersplenism	1 (2%)
Destruction	19 (38%)
Immune thrombocytopenia	18 (36%)
Evans syndrome	1 (2%)
Central thrombocytopenia	22 (44%)
Acute Leukaemia	11 (22%)
Myelodysplastic syndrome	5 (10%)
Bone metastasis	3 (6%)
Macrophage activation syndrome	1 (1%)
Primary myelofibrosis	1 (2%)
Drug toxicity (heparin treatment)	1 (2%)
Platelet count, G/L	
Overall	57,2 [1–100]
PT	54 [1–100]
CT	59 [5–84]
IPF, %	
Overall	11,77 [1,1–67.3]
PT	15.71 [5.9–67.3]
CT	5.51 [1,1–18.5]

Median IPF was 11.22 % (2.3-67.3) in this population (table 1). IPF% was significantly higher in the PT group compared to the CT group (figure 4), with median values of 15.71% (5.9–67.3) vs. 5.51% (1,1–18,5), respectively (p<0,001).

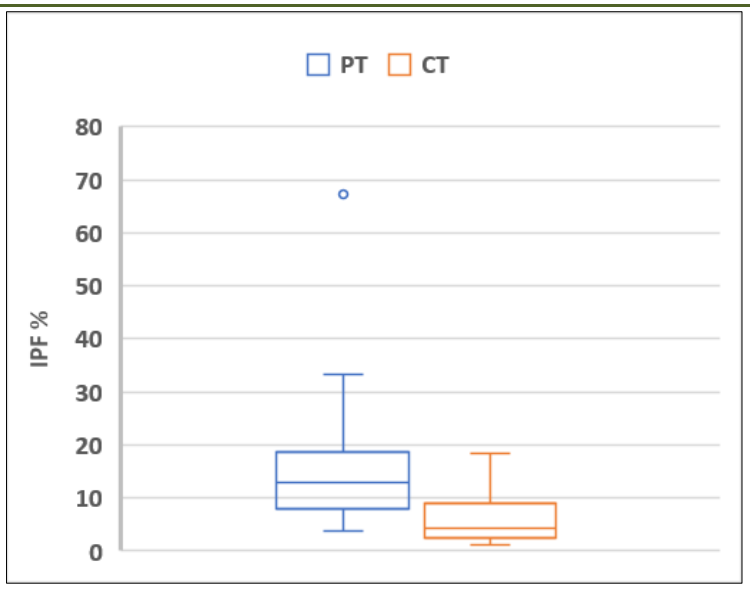

Figure 2: Box plot representing IPF in central thrombocytopenia (CT) and peripheral thrombocytopenia (PT) groups

To study the impact of platelet count on the IPF% of thrombocytopenic patients, we analyzed the groups (CT, PT) by separating them based on their platelet count.

Thus, when the platelet count was below 50 G/L., median IPF of the PT group was significantly higher than that of the CT group (21.63 ± 19.05% vs. 6.63 ± 4.45%; p=0.004). Similarly, when the platelets were above 50 G/L., median IPF of the PT group remained significantly higher than that of the CT group (12 ± 4% vs. 5 ± 2.5%; p=0.001).

The aim of the study was to determine an IPF% cut-off. Therefore, we used a Receiver Operating Characteristic (ROC) curve of sensitivity and specificity to analyze the ability of IPF to differentiate between peripheral and central thrombocytopenias (Figure 3).

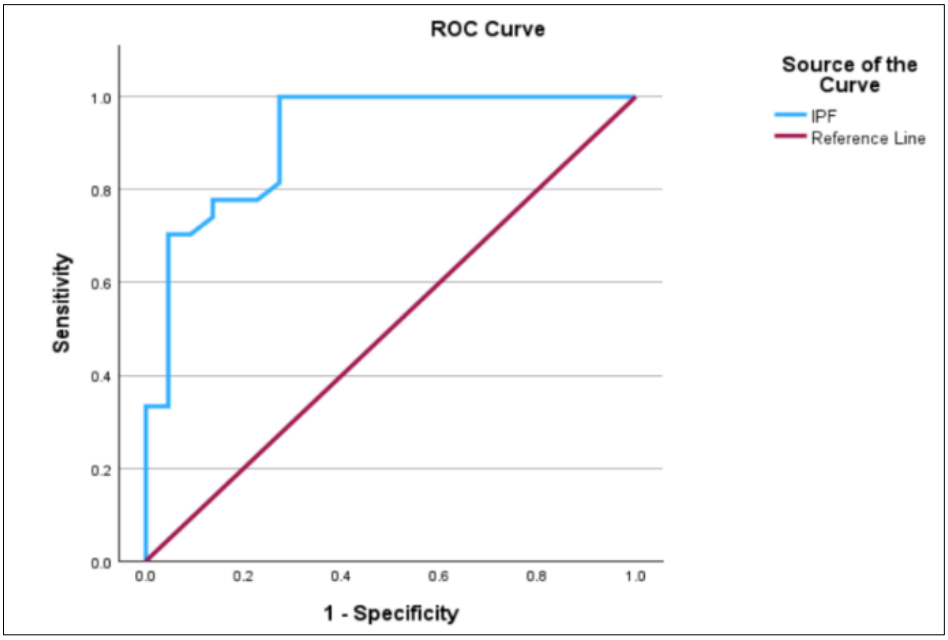

Figure 3: ROC curve showing the sensitivity and specificity of IPF in differentiating between central and peripheral thrombocytopenias

The analysis of the curve in this study demonstrated the ability of IPF to differentiate between peripheral and central thrombocytopenias with an area under the curve of 0.914. Consequently, we were able to define a discriminative cut-off of 8,5% for IPF through our study, allowing differentiation between central and peripheral thrombocytopenias with a sensitivity of 77.8% and a specificity of 86.4% (Table 2).

Table 2: IPF cut-off value found in our study, along with its sensitivity and specificity

Cut-off value	Sensitivity	Specificity	PPV	NPV	Youden's Index	ASC
8,5%	77,8%	86,4%	97,25%	77,14%	0,641	0.914

In our study, 23 patients had peripheral thrombocytopenia with IPF > 8,5%, bone marrow aspiration could have been avoided in those 23 patients (46%), particularly in patients with isolated thrombocytopenia.

4. DISCUSSION

In our study, all peripheral venous blood samples were collected in K2-EDTA (ethylene diamine tetra-acetic acid) tube. Most studies adopted the use of K2-EDTA tube [3]. Nishiyama *et al*., reported that for healthy individuals, the IPF values were almost stable at room temperature for K2-EDTA, acide-citrate dextrose solution (ACD) and cirate-theophylline-adenosine-dipyridamole (CTAD) anticoagulants for up to 4 day-storage [4].

Recently, reticulated platelets (now called as IPF) measurement has been developed to evaluate the pathophysiology of thrombocytopenia, but these are still not labeled IVD by scientific hemostasis societies [5, 6]. IPF value in our control population was 4.9% (0.7-9.6). The data obtained in our study significantly expand on those of similar studies wich have suggested reference range values. Table 3 shows 10 studies about the evaluation of the IPF by Sysmex instruments (XE/XN) in healthy population (Table 3). These results and the large variations between the studies could be attributed to the varying sample sizes, ethnic differences, as well as the various methods used for measuring IPF.

Table 3: IPF reference values in adult's healthy person with Sysmex XE and XN series

1st author	Year of publication	Population	N	Sysmex instrument	% IPF
Ali U [7]	2017	UK	2292	XN 1000	1.6–10.1
Yang B [8]	2017	China	2179	XN 9000	0.7–8.4
Ko YJ [9]	2014	Korea	2104	XE 2100/XN	0.3–7.4
Mogongoa LF [10]	2012	South Africa	60	XE 2100	0.7–5.5
Sachdev R [11]	2014	India	945	XE 2100	0.3–8.7
Jung H [12]	2012	Korea	2039	XE 2100	0.4–3.2
Joergensen MK [13]	2016	Denmark	1674	XE 5000	1.3–9
Naz A [14]	2016	Pakistan	94	XE 2100	1.1–17.8
Cybulska A [15]	2016	Germany	97	XE 2100	0.9–8.5
Morkis IVC [16]	2016	Brazil	132	XE 5000	0.8–6.1
Our study	2023	Morocco	100	XN 1500	0.7-9.6

Immature platelets or reticulated platelets are thrombocytes newly released into the peripheral blood. They can be identified by their large size and high concentration of RNA in their cytoplasm. IPF represents the percentage of immature platelets in circulation relative to the total number of platelets. This parameter is a direct reflection of megakaryopoiesis, and thus, the higher the bone marrow produces young platelets, the higher the IPF [2].

In the PT group, median IPF was 15.71% (5.9-67.3). This result is consistent with data from other studies. For instance, Cannavo [17], found a value of 15.8% in his series, and Abe [18], in turn, obtained 17.4% as IPF median in his ITP group.

For the CT group, median IPF in our study was 5.51% (1.1-18.5). This value is moderately low compared to the literature. For instance, median IPF was 11.9% in Cannavo's series [17], and a median of 6.4% was observed in Abe's series [18], for his group of marrow aplasias.

IPF was significantly increased in the PT group compared to the CT group in our study ($p < 0.001$). This is explained by the fact that in central thrombocytopenias, the marrow production of platelets is insufficient, either due to marrow infiltration, solid cancer metastases, vitamin deficiencies, or myelodysplastic syndrome, to name a few examples. Similarly, this has been described in several studies, especially those of Ferreira *et al*., [19], Jung H *et al*., [12], Cannavo [17], ($p < 0.029$), and Abe *et al*., [18], ($p < 0.01$), which included large populations. Furthermore, Adly *et al*., showed that the median IPF was significantly higher in patients with thrombocytopenia owing to increased peripheral platelet destruction than in those with thrombocytopenia owing to decreased platelet production, and that the IPF could be a marker for the diagnosis of ITP with high sensitivity and specificity [20].

We used receiver operating characteristic (ROC) curves to demonstrate the excellent discriminatory power of the IPF by determining his cut-off value at the best sensitivity and specificity. In this study, a cut-off of 8.5% would differentiate peripheral thrombocytopenias from central thrombocytopenias, this cut-off is interesting, because of its high sensitivity (77.8%), specificity (86.4%) and predictive positive

value (97,25%). Thus, an IPF greater than 8.5% helps guide us toward a peripheral origin of thrombocytopenia. This result is consistent with several studies (table 4). Abe *et al*., [18], who found that IPF was a better tool in managing thrombocytopenia compared to MPV, reported a cut-off of 7.7%, with a sensitivity and a specificity of 86.8% and 92.6%, respectively (figure 4). Min Ji Jeon [21], found a cut-off of 7% with a sensitivity and specificity of 61% and 70%, respectively (figure 5). Moreover, Jung H *et al*., [12], with the same device (XE) reported a cut-off of 7,3% with sensitivity and a specificity of 54% and 92.2%, respectively (table 4). This difference in the sensitivity could be explained by the difference between the comparative population (hematological malignancies, aplastic anemia …), and the severity of the thrombocytopenia in ITP patients.

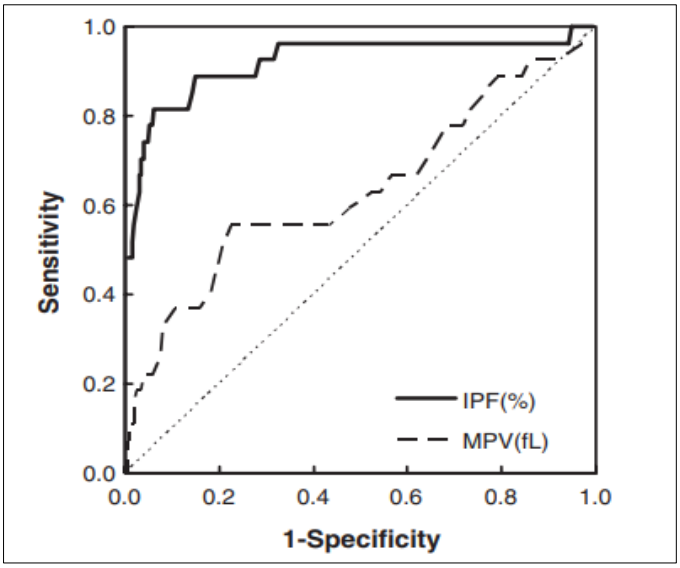

Figure 4: ROC curves in IPF and MPV. The cut-off value was 7,7% with a sensitivity of 86,8% and specificity of 92,6% [18]

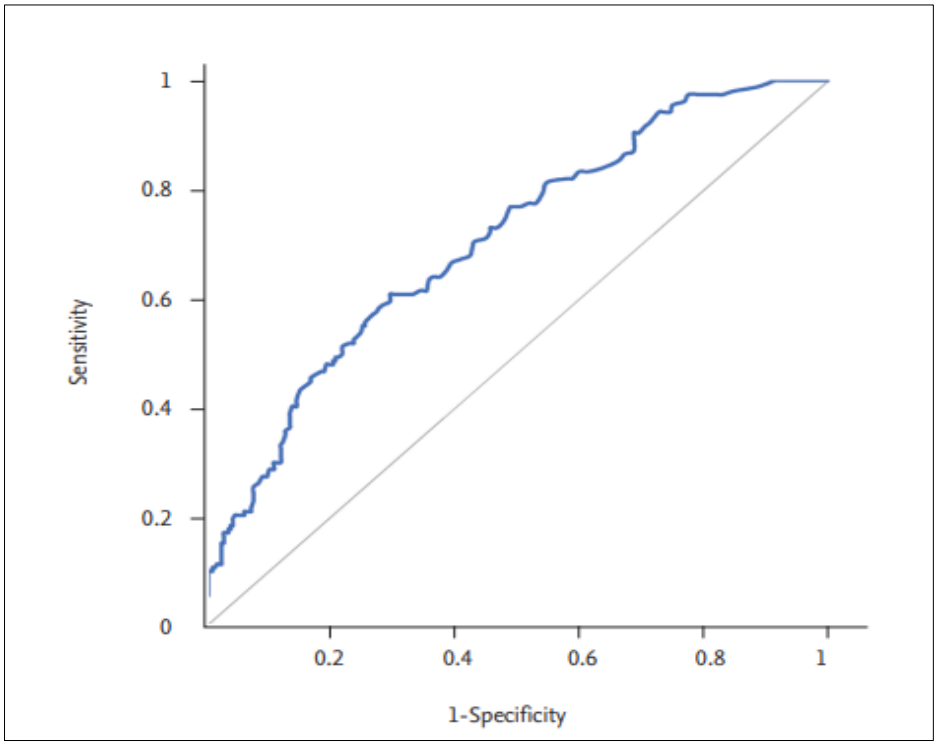

Figure 5: Receiver-operating characteristic (ROC) curve of immature platelet fraction (IPF) in the immune thrombocytopenic purpura (ITP) group and non-ITP group. The optimal cut-off value of IPF (%) for differentiating ITP was 7.0% with a sensitivity of 61% and specificity of 70% [21]

Table 4: Different cut-off values across series XN series

	IPF% cut-off	Sensibility (%)	Specificity (%)
Jung *et al*., 2010, Korea [12]	7,3	54	92,2
Abe *et al*., 2006, Japan [18]	7,7	86,8	92,6
Asghar *et al*., 2023, Pakistan [22]	7,95	92	86
Goel *et al*., 2021, India [23]	5,95	88	75,9
Cho YG [24]	6,1	92,9	82,9
Min Ji Jeon [21]	7	61	70
Our study	8,5	77,8	86,4

Several methodological limitations were encountered during the completion of this work, including the small size of our sample due to the novelty of the IPF and its infrequent prescription by clinicians, the limitation of the national and international documents related to our topic, making it challenging to compare with information available in the literature, as well as the lack of clinical and paraclinical information in the files of the subjects in our study, which made effective analysis difficult.

In spite of all, the present study provides solid evidence that the IPF can allow the separation of peripheral and central thrombocytopenia with sensitivity and specificity. Currently, our hematology laboratory is collaborating with other laboratories from various university hospitals in the kingdom, with the aim of establishing a national cut-off in the hope of having the IPF parameter labeled as an IVD (In Vitro Diagnostic) by hemostasis scientific societies.

5. CONCLUSIONS

Thrombocytopenia is among the most common hematological disorder. It can be either central or peripheral. The complete blood count (CBC) is the initial step in establishing the etiological diagnosis of any thrombocytopenia, followed by the role of the bone marrow aspiration (myelogram), the gold standard examination to determine its origin, but which remains an invasive and operator-dependent procedure. However, the "fraction of immature platelets or IPF" provides a faster, easier, non-invasive, and more cost-effective alternative to bone marrow aspiration in diagnosing thrombocytopenias. This parameter is fully automated, available as a routine test, and facilitates the management of thrombocytopenias. This study allowed us to establish a discriminative threshold of 8.5% with a sensitivity of 77.8% and a specificity of 86.4%, from which thrombocytopenias can be classified based on their origin.

Conflicts of Interest: "The authors declare no conflicts of interest."

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