

ASSESSMENT OF THE IMMATURE PLATELET FRACTION'S RELEVANCE IN DISTINGUISHING BETWEEN CENTRAL AND PERIPHERAL THROMBOCYTOPENIA

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ABSTRACT :

The immature platelet fraction (IPF) is mainly used in the differential diagnosis between peripheral and central thrombocytopenias. The objectives of our study is to determine the reference intervals of the immature platelet fraction in our population, to evaluate its ability to differentiate between central and peripheral thrombocytopenias, and to define a threshold that discriminates between the two mechanisms. We worked on 127 samples, including 81 controls for determining reference intervals, and 46 thrombocytopenic patients. A platelet count and an immature platelet fraction measurement were performed using the complete blood count (CBC) MINDRAY BC6800 PLUS automated system. The comparative study was conducted using the Mann-Whitney test, and the discriminating threshold was defined using the ROC curve. The average of the immature platelet fraction in the controls was 7%, our reference values ranged between (1.6% and 14.76%), 56.52% of the thrombocytopenias were of central origin. Our study showed a highly significant difference in the results of the immature platelet fraction between patients with peripheral thrombocytopenia and those with central thrombocytopenia ($p=1.59 \times 10^{-08}$). The discriminatory threshold for this fraction between the two etiologies of thrombocytopenia was 11.75%, with a sensitivity of 100% and a specificity of 95.7%. Conclusion, the use of the immature platelet fraction effectively guides the etiological diagnosis of thrombocytopenia.

Key words: Central thrombocytopenia, Immature platelets, Peripheral thrombocytopenia.

RESUME : ANALYSE DE LA PERTINENCE DE LA FRACTION DES PLAQUETTES IMMATURES DANS LA DISTINCTION ENTRE LES THROMBOPENIES CENTRALES ET LES THROMBOPENIES PERIPHERIQUES.

La fraction immature des plaquettes (IPF) est principalement utilisée dans le diagnostic différentiel entre les thrombocytopénies périphériques et centrales. Les objectifs de notre étude sont de déterminer les intervalles de référence de la fraction des plaquettes immatures dans notre population, d'évaluer sa capacité à différencier les thrombocytopénies centrales et périphériques, et de définir un seuil permettant de distinguer les deux mécanismes. Nous avons travaillé sur 127 échantillons, dont 81 témoins pour déterminer les intervalles de référence et 46 patients thrombocytopéniques. Une numération plaquettaire et une mesure de la fraction des plaquettes immatures ont été réalisées à l'aide du système automatisé de numération globulaire complète (CBC) MINDRAY BC6800 PLUS. L'étude comparative a été réalisée à l'aide du test de Mann-Whitney, et le seuil de discrimination a été défini à l'aide de la courbe ROC. La moyenne de la fraction des plaquettes immatures chez les témoins était de 7 %, nos valeurs de référence variaient entre (1,6 % et 14,76 %), 56,52 % des thrombocytopénies étaient d'origine centrale. Notre étude a montré une différence très significative dans les résultats de la fraction des plaquettes immatures entre les patients atteints de thrombocytopénie périphérique et les patients atteints de thrombocytopénie centrale ($p = 1,59 \times 10^{-08}$). Le seuil discriminatif de cette fraction entre les deux étiologies de thrombocytopénie était de 11,75 %, avec une sensibilité de 100 % et une spécificité de 95,7 %. Conclusion, l'utilisation de la fraction des plaquettes immatures guide efficacement le diagnostic étiologique de la thrombocytopénie.

Mots clés : Thrombocytopénie centrale, Plaquettes immatures, Thrombocytopénie périphérique.

INTRODUCTION

Immature platelets constitute a subpopulation of platelets freshly released into the bloodstream by megakaryocytes in the bone marrow. They are distinguished from mature platelets by the presence of residual RNA, indicating their young age and high metabolic activity, and morphologically, immature platelets are slightly larger than mature platelets [1].

The immature platelet fraction (IPF) represents the percentage of circulating reticulated platelets relative to the total number of platelets. The advent of new automated measurement technologies has allowed for better standardization and more precise analysis of this hematological parameter, paving the way for in-depth investigations into its role in cases of thrombocytopenia [2].

The clinical implications of reticulated platelets are vast and mainly concern the evaluation and monitoring of hematological and cardiovascular pathologies [1,2].

In pathological contexts, their levels vary depending on the nature of the underlying disorder. An increase in young platelets is frequently observed in peripheral thrombocytopenias, characterized by destruction or excessive consumption of platelets, such as in immune thrombocytopenic purpura or hypersplenism. Conversely, a decrease in their number is observed in central-origin thrombocytopenias, associated with bone marrow insufficiency, particularly in myelodysplastic syndromes, bone marrow aplasia, or bone marrow suppression induced by chemotherapy [3,4].

The objectives of our study are to determine the reference intervals of the IPF in our population, taking into account the variations between ethnicities as well as the lack of harmonization between measurement systems, to evaluate its ability to differentiate central thrombocytopenias from peripheral thrombocytopenias, and to define a discriminative threshold allowing for differential diagnosis between the two mechanisms, enabling an initial diagnostic orientation with a simple blood count and limiting unnecessary complementary examinations, sometimes invasive like the myelogram.

PATIENTS AND METHODS

This is a descriptive cross-sectional study conducted in the hemobiology-blood transfusion department of the Constantine University Hospital in Algeria, on 127 samples including 81 control subjects and 46 thrombocytopenic patients. The control population consisted of healthy blood donors, with no known pathology or ongoing treatment, and they must present a normal platelet count (150 to 400 giga/l). They were included in our study after a rigorous medical selection, each subject is thoroughly examined clinically, tracing their medical and therapeutic history in search of a treatment or pathology that may influence the platelet count. For our study, we only included thrombocytopenic patients with a platelet count below 150 giga/l whose etiological diagnosis was definitively established, the myelogram being the key examination to confirm the peripheral origin of the thrombocytopenia when it is rich in megakaryocytes or its central origin when it is poor in megakaryocytes. We excluded all patients whose etiological diagnosis of thrombocytopenia was not precisely established. For each sample, the platelet count and IPF measurement were performed immediately after the collection of whole blood with EDTA anticoagulant within two hours of the collection. In our study, we used the MINDRAY BC6800 PLUS blood cell counter, which identifies immature platelets due to their richness in residual RNA, using a reticular fluorescent dye.

The reference intervals for IPF in our population were determined from the results of the control subjects using the non-parametric method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) EP28-A3C [5]. The comparative study of IPF values between the three populations studied, the control subjects, those with central thrombocytopenia, and those with peripheral thrombocytopenia, was conducted using the Mann-Whitney non-parametric test, the difference was considered significant when p-value was less than 0,05. The discriminative threshold of IPF to differentiate peripheral thrombocytopenias from central thrombocytopenias was defined using the ROC curve. These statistical tests were performed using SPSS V22 software.

RESULTS

Our control population was aged between 20 and 62 years with an average age of 32,84 years. Out of the 81 healthy subjects, 74 were male, which is 91,36%, with a male-to-female ratio of 10,57. The average platelet count was $272,47 \times 10^9/L$ ($179 - 398 \times 10^9/L$). Regarding the IPF, the average value found was 7.00%, the reference values for our population ranged between 1.6% and 14.76%.

Regarding the population of patients with thrombocytopenia, it included 27 women (58.70%) and 19 men (41.30%), with a male-to-female ratio of 0,70. The average age was 50 years (ranging from 15 to 80 years). Central thrombocytopenias accounted for 56.52% of thrombocytopenias, with the majority (70% of cases) linked to a mechanism of bone marrow infiltration, and 30% of cases linked to aplastic marrow, whereas peripheral thrombocytopenias, which represented 43.48% of thrombocytopenias, were caused in 65% of cases by immune thrombocytopenic purpura (ITP). 58.70% of thrombocytopenic subjects had a platelet count below $50 \times 10^9/L$ and 41.30% had a platelet count above $50 \times 10^9/L$. In 47.83% of cases, thrombocytopenia was isolated, whereas pancytopenia was present in 28.26% of cases, and bicytopenia was present in 23.91% of cases, of which 81.82% of the associations were with anemia. The comparative study (Table 1) showed a very significant difference in the IPF results of patients with peripheral thrombocytopenia when compared both to the control population ($p < 0,001$) and to patients with central thrombocytopenia ($p < 0,001$).

Table 1. Comparative study of IPF between different populations.

The mean IPF of the control population and subjects with central thrombocytopenia are very similar (7.00% and 6.67%, respectively), while the mean IPF of subjects with peripheral thrombocytopenia is significantly higher than the two previous populations (30.13%), with values reaching up to 69.6%.

Parameter (%)	Controls (n = 81)	Central Thrombo cytopenia (n = 26)	Peripheral Thrombo cytopenia (n = 20)
Minimum	0,80	0,90	11,90
Median	6,25	6,10	24,95
Maximum	17,70	14,70	69,60
Mean $\pm$ SD	7,00 $\pm$ 4,09	6,67 $\pm$ 3,21	30,13 $\pm$ 15,55
P val in comparison with peripheral thrombo- cytopenia	$p = 4,87 \times 10^{-7}$	$p = 1.59 \times 10^{-8}$	/

However, no significant difference was recorded between the controls and subjects with central thrombocytopenia ($p = 0.09$), whose IPF values were comparable but slightly lower for subjects with central thrombocytopenia compared to the control

population (Figure 1).

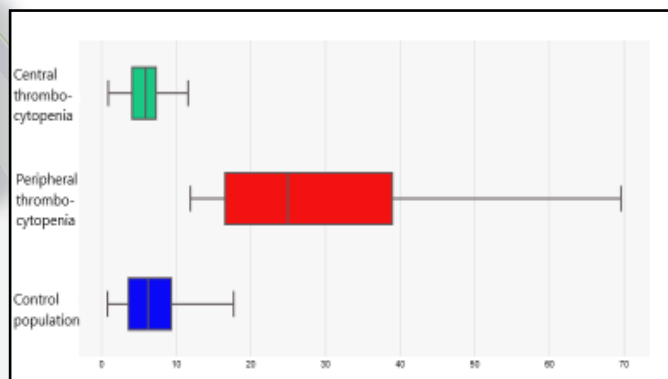


Figure 1. Comparison of IPF in different populations in boxplot form.

This figure shows that the distribution of IPF in the control population and subjects with central thrombocytopenia is very similar, with slightly lower values for subjects with central thrombocytopenia, while subjects with peripheral thrombocytopenia have significantly higher IPF values than the two previous populations.

Based on these results and using the ROC curve (Figure 2), the discriminative threshold of the IPF allowing for an initial orientation towards the central or peripheral etiology of thrombocytopenias in our study was 11.75% with a sensitivity of 100% and a specificity of 95.7%.

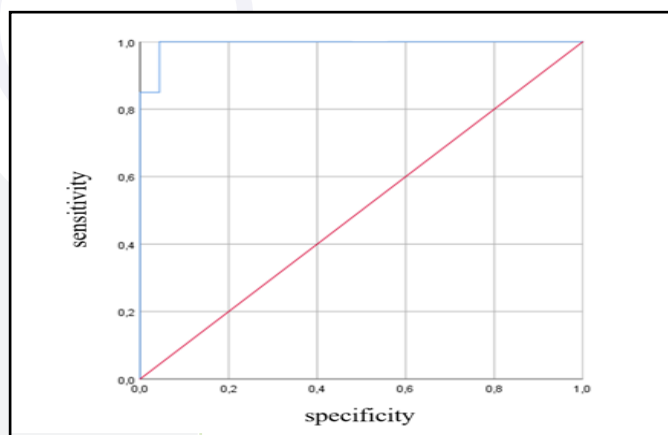


Figure 2. ROC curve. This curve gave us a threshold value for the IPF that allows initial differentiation between peripheral and central thrombocytopenia of 11.75% with a good area under the curve (0.993), a sensitivity of 100%, and a specificity of 95.7%.

DISCUSSION

Among the 81 healthy subjects constituting the control population, the age limits were between 20 and 62 years, which is related to the fact that the control population is made up of blood donors and the eligibility conditions for blood donation in Algeria regarding age limits are between 18 and 65 years. For the sex ratio, 91,36% of the subjects were male because men represent the vast majority of blood donors in our facility, but this distribution does not influence our results in any way as there is no clear evidence that the immature fraction of platelets varies physiologically with sex, its clinical relevance is more related to the evaluation of platelet production in various pathological conditions, regardless of sex [6,7]. The average platelet count in our control population was $272,47 \times 10^9/L$ with limits between $(179 - 398 \times 10^9/L)$, these results were comparable to those of the study by Garima et al in India [8]. Which found an

average platelet count of $254.7 \times 10^9/L$. Regarding the IPF, the average value found was 7.00% and the reference values of our population were between 1.6% and 14.76%. These results are not comparable to a study conducted on a Korean population by Kim et al [9]. Whose reference values were between (0.6% and 5.6%), nor to that of Goelet al [8]. Whose reference values were between (0.7% and 5.7%). It is essentially our upper limit that is significantly higher than the two cited studies, whereas these last two studies recorded reference ranges that are very comparable to each other [8,9]. This difference may be largely related to ethnic differences because these studies used the same analysis system, and it is useless to compare the reference intervals of IPF unless they were measured with the same method. In the absence of international standardization, it is not possible to compare IPF from different analyzers [10,11].

58.70% of thrombocytopenic subjects were female, with a male-to-female ratio of 0,70. This may be related to a more pronounced female predisposition to certain pathologies compared to men. The average age of our patients was 50 years, with extremes ranging from 15 to 80 years, with etiological pathologies mainly affecting the elderly, which meant that our patient population was older than that of healthy subjects.

In our study, we only included subjects whose etiological diagnosis had been established with certainty, so patients whose diagnosis was still ambiguous were excluded. We included more subjects with central thrombocytopenia (56.52%) in the comparative study, whose diagnostic criteria are well codified compared to peripheral thrombocytopenias (43.48%), some of whose etiologies have a diagnosis of exclusion such as immune thrombocytopenic purpura (ITP).

The comparative study (Table 1) showed a very significant difference in IPF results on one hand between patients with peripheral thrombocytopenia compared to the control population ($p < 0,001$) and on the other hand between patients with peripheral thrombocytopenia and those with central thrombocytopenia ($p < 0,001$).

Our results show that the IPF rate reflects platelet production by the bone marrow, and due to the strong medullary regeneration of platelets in response to peripheral thrombocytopenia, the rate of young platelets increases significantly. Unlike subjects with central thrombocytopenia, in whom the bone marrow is unable to respond in the same way. This gives this parameter the property of well differentiating the central origin from the peripheral origin of thrombocytopenia and predicting platelet recovery [12]. On the other hand, we did not notice a significant difference between the controls and subjects with central thrombocytopenia ($p=0.09$), which means that the bone marrow of a subject with central thrombocytopenia is unable to increase the production of their IPF in response to thrombocytopenia.

The use of the ROC curve (Figure 2) provided us with a discriminative threshold for the IPF of 11.75% with a sensitivity of 100% and a specificity of 95.7%. This means that in a thrombocytopenic patient, an IPF level above 11.75% suggests a peripheral origin of this thrombocytopenia, while an IPF below 11.75% suggests a central origin.

This threshold value is incomparable to the studies of Goel et al [8]. Who found a threshold value of 5.95%, this difference is very likely related to the difference in the ethnic origins of the two populations studied. This initial orientation of the etiological diagnosis conferred by the IPF threshold value will allow us to avoid unnecessary and even invasive additional examinations such as the myelogram, and to ensure early and effective management.

CONCLUSION

Thrombocytopenia represents a very common hemostatic disorder, with varied etiologies; it can indicate a malignant pathology, reflect the severity of a benign pathology, or even endanger the vital prognosis.

The use of new platelet parameters such as the IPF, currently available on several automated analyzers, allows for the differentiation between central and peripheral origins of thrombocytopenia through a simple blood test, and effectively guides its etiological diagnosis, enabling better early and effective management, while avoiding, in many cases, unnecessary and burdensome invasive examinations and procedures.

CONFLICTS OF INTEREST :

All authors declare that they have no conflicts of interest.

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