

WHITE PAPER

THE ROLE OF THE IMMATURE PLATELET FRACTION (IPF) IN  
THE DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOPENIA



The value-driven  
laboratory

# The Value-Driven Laboratory

## Immature Platelet Fraction (IPF): An insight into assessment and treatment of platelet disorders

We define the value-driven laboratory as one that meets market demands by providing more and new types of diagnostic information to support the delivery of high quality and cost-effective patient care. This paper sets forth the value of the advanced platelet parameters, the Immature Platelet Fraction (IPF%) and the Immature Platelet Fraction Count (IPF#), to help physicians understand the pathophysiological mechanisms leading to low platelet count. The IPF can help physicians determine in a timelier fashion if a thrombocytopenia is due to decreased platelet production by the bone marrow or increased peripheral consumption/destruction of platelets.

### Introduction

The Immature Platelet Fraction (IPF) is a measure of thrombopoietic activity that was first described in 1992 by Ault et al.<sup>1</sup>, who coined the term “reticulated platelets” to describe large platelets with elevated ribonucleic acid (RNA) content. Reticulated platelets can be an analog to the reticulocytes in red cell populations. Ault’s study, and others have shown IPF to be a stable and reproducible parameter<sup>2</sup> and superior to the measurement of reticulated platelets using traditional flow cytometry<sup>3,4</sup> for providing information to the physician on the rate of thrombopoiesis. The immature platelet population rises as production and release of platelets increases in the bone marrow. Using a peripheral blood sample, the IPF provides an assessment of bone marrow platelet production similar to how a reticulocyte count provides a measure of red cell production. The IPF result is useful in monitoring patients with thrombocytopenia and helps physicians differentiate thrombocytopenia caused by platelet destruction versus compromised platelet production. Therefore, when using this parameter, physicians can reach decisions on appropriate care pathways in a timelier manner.

### An Overview of Platelet Parameters

#### Platelet Count (PLT)

A normal platelet count (150,000 – 450,000/ $\mu$ L) is vital for the maintenance of hemostasis. Platelet counts of less than 150,000/ $\mu$ L are defined as thrombocytopenic, and spontaneous bleeding not associated with injury has been linked to platelet counts of less than 20,000/ $\mu$ L. Current hematology instruments enable accurate measurement of low platelet counts; however, such a measurement only reflects the circulating platelet count at a specific point in time.

#### Mean Platelet Volume (MPV)

Historically, some clinicians have used an elevated MPV result to assist in the evaluation of thrombocytopenia, or as a surrogate marker for platelet production. The normal range for MPV is approximately 9 – 12 femtoliters (fL); platelets newly released from the bone marrow are generally larger and, as they age, become smaller. However, interference from similarly sized particles – such as schistocytes or microcytic RBCs – can make the MPV result unreliable. Additionally, the

MPV can show greater imprecision or be unmeasurable (suppressed by the analyzer) in samples with low platelet counts.<sup>5</sup>

#### Immature Platelet Fraction (IPF)

The Immature Platelet Fraction (IPF%) and Immature Platelet Fraction Count (IPF#) reflect the percentage and absolute number of immature platelets in a patient’s peripheral blood. These immature platelets, newly released from the bone marrow, contain increased amounts of nucleic acid that allow them to be differentiated from mature platelets using fluorescent flow cytometry. Select Sysmex XN- Series™ Automated Hematology Analyzers are capable of reporting the IPF% and IPF# results as a direct cellular measurement of thrombopoiesis, which can be used with other available clinical information to help physicians determine the pathophysiological mechanism of thrombocytopenia.

Clinicians have previously used immature platelet information as a measure of thrombopoietic activity in the bone marrow, which can be important in assessing the likelihood of bleeding. IPF# has been used in clinical trials to assess

the treatment effect of thrombopoietin receptor agonists, such as eltrombopag, and has also been studied in patients with Immune Thrombocytopenia (ITP).<sup>6,7</sup>

In studies assessing the effect of platelet transfusion on relative and absolute IPF, the IPF% was found to decrease after transfusion due to the increased circulating platelet count, but the IPF# result was not affected, thus “validating the assay as a reflection of ongoing platelet production by the marrow”.<sup>8</sup>

## IPF in the Clinical Setting

### Normal Ranges

In 2017, Sysmex determined reference range intervals according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The study of 129 females and 118 males showed the IPF% normal range to be 1.2 - 8.6% and the IPF# normal range to be 3.6 – 20.0 x 10<sup>3</sup>/μL, with no statistically significant difference between the two genders for either parameter. These findings are supported by an independent study published in 2017 based on 2366 samples; however, that study produced a statistically significant difference between the sexes for the IPF% parameter, but not for the absolute IPF. (Figure 1)

### IPF Normal Ranges by Source

Study performed By:	IPF% Normal Range	IPF# Normal Range	N Value
Sysmex America, Inc.	1.2 - 8.6%	3.6 – 20.0 x 10 <sup>3</sup> /μL	247
U. Ali, et al <sup>9</sup>	1.8 – 10.0% (male) 1.5 – 10.1% (female)	4.37 – 23.21 x 10 <sup>3</sup> /μL	2366

Figure 1: Correlation of reference ranges for IPF% and IPF#

### IPF in the Differentiation of Increased Platelet Destruction versus Decreased Platelet Production

Briggs et al.<sup>10</sup> followed AITP (autoimmune thrombocytopenic purpura) and TTP (thrombotic thrombocytopenic purpura) patients with platelet counts of less than 50,000/μL to determine if there is a difference in the IPF between peripheral thrombocytopenia due to bone marrow failure and increased peripheral destruction. The study showed that IPF% values were elevated in both patient groups due to excessive platelet consumption; the IPF% then decreased as the platelet count recovered. These findings were confirmed by Sakuragi et al. in a study that compared IPF% values in patients with primary immune thrombocytopenic purpura (ITP) and aplastic thrombocytopenic disorders.<sup>11</sup>

A study by Kickler<sup>12</sup> at Johns Hopkins looked at IPF values as differentiators in cases of thrombocytopenia (Figure 2). High IPF% values were found in patients with increased production, particularly if associated with platelet destruction, while normal values were seen in decreased platelet production.

## Causes of Thrombocytopenia

- Myeloablative therapy for hematologic malignancies
- ITP/TTP
- Hepatitis C
- Bone marrow transplant
- Acute myelogenous leukemia
- PNH/aplastic anemia
- DIC
- Splenomegaly
- Autoimmune Disease
- Bacteremia
- HIT
- Sepsis
- Pregnancy
- Drug Induced Thrombocytopenia (DIT). Medications may include quinine, quinidine, sulfa-containing medications, interferon

Subject	No. of Cases	Mean	SD	Range	95% Confidence Level	P*
Healthy	80	3.1	1.6	1.0 - 7.0	2.8 - 3.5	—
Destruction						
ATP	37	15.0	7.1	3.6 - 35.4	12.7 - 17.2	<.0001
DIC	25	9.5	5.2	2.3 - 18.6	7.5 - 11.5	<.0001
All destruction	62	12.8		2.3 - 35.4	11.0 - 14.5	<.0001
Recovery						
Chemotherapy	79	4.1	2.0	1.2 - 10.9	3.6 - 4.5	.001
BMT	8	8.0	4.2	4.3 - 15.3	5.1 - 10.8	<.0001
All recovery	87	4.4	2.5	1.2 - 15.3	3.9 - 4.9	<.0001
Suppression						
AA/PNH	3	6.1	2.5	3.9 - 8.8	3.2 - 8.9	.019
Cancer	16	3.8	2.0	0.8 - 7.1	2.8 - 4.7	NS
All suppression	19	4.1	2.2	0.8 - 8.8	3.1 - 5.1	.05

Figure 2: IPF% in different populations of thrombocytopenic patients<sup>2</sup>

### IPF in Hepatitis C/Liver Disease

An additional example of the clinical utility of IPF% is the evaluation of thrombocytopenia mechanisms in chronic hepatitis C patients as described by Zucker et al.<sup>13</sup> The authors concluded that the IPF% was elevated in thrombocytopenic patients with and without splenomegaly, supporting the idea that peripheral destruction and sequestration are the major causes of the thrombocytopenia in Hepatitis C and liver disease in general.

colleagues followed IPF% in 50 patients undergoing peripheral hematopoietic cell transplants and found that IPF recovered, on average, 3.1 days earlier than platelet count alone and 3.8 days earlier than absolute neutrophil counts (ANC) (Figure 3). Takami et al.<sup>15</sup> also reported the use of IPF as a predictor of platelet engraftment following allogenic stem cell transplantation, finding that IPF preceded engraftment.

Other uses for IPF have been described. Briggs<sup>3</sup> et al. have suggested that the IPF parameter may be able to alleviate the need for bone marrow examinations as well as to determine if prophylactic platelet transfusions are necessary for the HPC transplant patients.

### IPF in Bone Marrow Recovery

Several groups<sup>1,4,8,14</sup> have studied the clinical utility of the IPF in varying patient populations: following hematopoietic progenitor cell (HPC) transplantation; myeloablative chemotherapy for hematologic malignancy; disseminated Intravascular Coagulation (DIC); non-myeloablative therapy in cancer and aplastic anemia; and paroxysmal nocturnal hemoglobinuria (PNH).

Although it is customary to follow neutrophil recovery after hematologic transplantation procedures, IPF may be another reliable indicator of hematopoietic recovery. Zucker<sup>14</sup> and

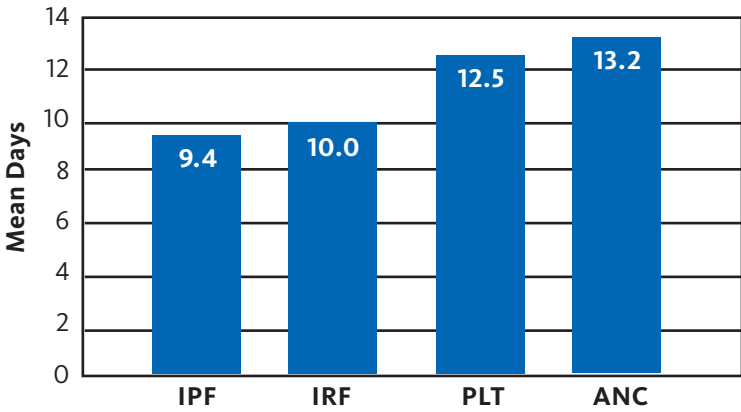


Figure 3: Mean days to recovery for IPF, Immature Reticulocyte Fraction (IRF), platelet count (PLT) and Absolute Neutrophil Count (ANC) following stem cell transplantation.<sup>14</sup>

## IPF in Acute Myeloid Leukemia and Myelodysplasia (AML/MDS) Versus Immune Thrombocytopenia (ITP)

It has been difficult to measure platelet function in the presence of severe thrombocytopenia, which is a finding in both AML/MDS and ITP patients. A study conducted by Psaila et al.<sup>16</sup> concluded that the difference in bleeding tendencies between these two groups of patients was explained by the quality of the platelets produced. Samples from ITP patients showed younger, larger platelets

with increased IPF values versus those from patients with AML/MDS who had lower IPF values and smaller platelets (Figure 4).

### IPF in the Assessment of Thrombopoietin Agonists

Another key role of IPF is in determining the efficacy of various treatment protocols for ITP, such as thrombopoietin receptor agonists (TPO-A). A study by Barsam et al.<sup>7</sup> showed that patients receiving TPO-A have a higher IPF.

### What may be possible with IPF?

1. A comprehensive platelet evaluation beginning with a fluorescent platelet count and simultaneous measurement of the IPF to evaluate the maturity of circulating platelets as an indicator of rate of PLT production (thrombopoiesis), i.e., using IPF to assist the physician in defining the best clinical pathway based on destruction/consumption vs. production issues.
2. Use of IPF to predict bone marrow recovery and rate of thrombopoiesis.
3. Evaluating TPO medication's effects on thrombopoiesis.
4. Evaluating the use of the IPF% parameter as a reflex test in the presence of thrombocytopenia for reporting to hematologists/ oncologists.

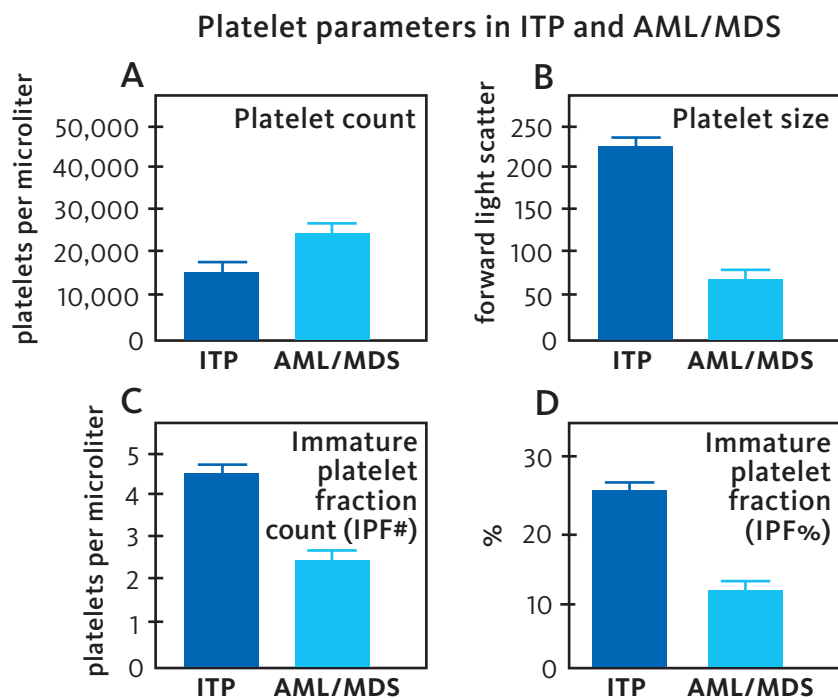


Figure 4: Platelet parameters in ITP and AML/MDS. (A) Platelet count; (B) Platelet size; (C) Immature Platelet Fraction Count; and (D) Immature Platelet Fraction.<sup>16</sup>

## IPF - A Unique Parameter

- Automated, easy to perform, standardized results
- Inexpensive – utilizes routine CBC reagents and controls
- Results available 24/7 for rapid response to clinicians
- May provide useful information for tailoring treatment regimens by allowing clinicians to link treatment options to mechanisms of low cell counts
- Could reduce time and cost of diagnosis of thrombocytopenia by the physician
- Provides the clinician with valuable information in the assessment of the mechanism of newly discovered thrombocytopenias and secondary thrombocytopenias (HIV, Hepatitis C, drugs etc.)

## Conclusion

The Immature Platelet Fraction is a well-studied parameter that offers significant cellular information useful for many patient populations. IPF can function as a routine value reported with every platelet count in certain patient populations or as a reflex test based on your laboratory's platelet count cut-off. It provides guidance to the physician on the etiology of various thrombocytopenic states, and may help anticipate bone marrow and platelet recovery. Regardless of the application the practitioner chooses, the use of the Immature Platelet Fraction and Count may assist in the delivery of high quality, cost effective patient care.

### Clarify the current strategy for thrombocytopenia management

1. How often are you unable to report a low platelet count because of linearity or result vote-out issues with your hematology analyzer?
2. What percentage of your pre-admission CBCs are identified as thrombocytopenic patients?

### Identify the clinical lab tests that currently trigger follow-up testing by physicians

3. What existing laboratory tests or clinical conditions trigger additional platelet studies in your institution?
4. What are the clinical conditions associated with thrombocytopenia at your organization?
5. What hospital service lines would need to know that a patient is thrombocytopenic?
6. What classes of pharmacologic agents have thrombocytopenia as an adverse event?

### Identify potential improvements that the clinical laboratory can support

7. Given that the IPF recovers prior to the recovery of the peripheral blood platelet count, are we offering enough information with just a platelet count?
8. How can the laboratory educate physicians and nurses, pharmacists, coding and other ancillary departments about the value of the IPF parameter in the differential diagnosis of thrombocytopenia?

## Bibliography

- <sup>1</sup> Ault KA, Rinder HM, Mitchell J, Carmody MB, Vary CP, Hillman RS. The significance of platelets with increased RNA content (reticulated platelets). A measure of the rate of thrombopoiesis. *Am J Clin Pathol* 1992; 98:637-46.
- <sup>2</sup> Jung H, Jeon HK, Kim HJ, Kim SH. Immature platelet fraction: establishment of a reference interval and diagnostic measure for thrombocytopenia. *Korean J Lab Med* 2010 OCT;30(5):451-9.
- <sup>3</sup> Briggs C, Hart D, Kunka S, Oguni S, Machin SJ. Immature platelet fraction measurement: a future guide to platelet transfusion requirement after haematopoietic stem cell transplantation. *Transfus Med* 2006 Apr;16(2):101-9
- <sup>4</sup> Pons I, Monteagudo M, Lucchetti G, Munoz L, Perea G, et al. Correlation between Immature Platelet Fraction and reticulated platelets. Usefulness in the etiology diagnosis of thrombocytopenia. *Eur J Haematol* 2010; 85(2): 158-63.
- <sup>5</sup> Abe Y, Wada H, Tomatsu H, Sakaguchi A, Nishioka J, Yabu Y, et al. A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). *Thromb Res* 2006;118(4):463-9.
- <sup>6</sup> Serrando M, Marull A, Ruiz M, Perez Del CD, Puig-Pey I, Munoz JM, et al. Clinical significance of IPF% measurement in diagnosing thrombocytopenic disorders: distinguishing primary immune thrombocytopenia from other disorders. *Int J Lab Hematol* 2016 Jun;38(3):E65-E68.
- <sup>7</sup> Barsam SJ, Psaila B, Forestier M, Page LK, Sloane PA, Geyer JT, et al. Platelet production and platelet destruction: assessing mechanisms of treatment effect in immune thrombocytopenia. *Blood* 2011 May 26;117(21):5723-32.
- <sup>8</sup> Bat T, Leitman SF, Calvo KR, Chauvet D, Dunbar CE. Measurement of the absolute immature platelet number reflects marrow production and is not impacted by platelet transfusion. *Transfusion* 2013 June;53(6):1201- 04.
- <sup>9</sup> Ali U, Knight G, Gibbs R, Tsitsikas D. Reference intervals for absolute and percentage immature platelet fraction using the Sysmex XN-10 automated haematology analyser in a UK population. *Scand J Clin Lab Invest* 2017 Dec;(77)8:658-664.
- <sup>10</sup> Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Br J Haematol* 2004 Jul;126(1):93-9.
- <sup>11</sup> Sakuragi, M., et al. Clinical significance of IPF% and RP% measurement in distinguishing primary immune thrombocytopenia from aplastic thrombocytopenic disorders. *Int J Hematology*. Jan. 2015.
- <sup>12</sup> Kickler TS, Oguni S, Borowitz MJ. A clinical evaluation of high fluorescent platelet fraction percentage in thrombocytopenia. *Am J Clin Pathol* 2006 Feb;25(2):282-7.
- <sup>13</sup> Zucker ML, Hagedorn CH, Murphy CA, Stanley S, Reid KJ, Skikne BS. Mechanism of thrombocytopenia in chronic hepatitis C as evaluated by the immature platelet fraction. *Int J Lab Hematol* 2012 Oct; 34(5):525-32.
- <sup>14</sup> Zucker ML, Murphy CA, Rachel JM, Martinez GA, Abhyankar S, McGuirk JP, et al. Immature platelet fraction as a predictor of platelet recovery following hematopoietic progenitor cell transplantation. *Lab Hematol* 2006;12(3):125-30.
- <sup>15</sup> Takami A, Shibayama M, Orito M, Omote M, Okumura H, Yamashita T, Shimadoi S, Yoshida T, Nakao S, Asakura H. Immature platelet fraction for prediction of platelet engraftment after allogeneic stem cell transplantation. *Bone Marrow Transpl* 2007;39:501-7.
- <sup>16</sup> Psaila B, Bussel JB, Frelinger AL, Babula B, Linden MD, Li Y, et al. Differences in platelet function in patients with acute myeloid leukemia and myelodysplasia compared to equally thrombocytopenic patients with immune thrombocytopenia. *J Thromb Haemost* 2011 Nov;9(11):2302-10.

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