THE ROLE OF THE IMMATURE PLATELET FRACTION (IPF) IN THE DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOPENIA
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., 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 and superior to the measurement of reticulated platelets using traditional flow cytometry 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/μL) is vital for the maintenance of hemostasis. Platelet counts of less than 150,000/μL are defined as thrombocytopenic, and spontaneous bleeding not associated with injury has been linked to platelet counts of less than 20,000/μL. 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.

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).\(^6\)\(^7\)

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”\(^8\).

**IPF in the Clinical Setting**

**Normal Ranges**

In 2017, Sysmex determined reference range intervals according to 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 \(\times 10^3\)/\(\mu\)L, 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**

<table>
<thead>
<tr>
<th>Study performed By:</th>
<th>IPF% Normal Range</th>
<th>IPF# Normal Range</th>
<th>N Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sysmex America, Inc.</td>
<td>1.2 - 8.6%</td>
<td>3.6 – 20.0 (\times 10^3)/(\mu)L</td>
<td>247</td>
</tr>
<tr>
<td>U. Ali, et al(^9)</td>
<td>1.8 – 10.0% (male)</td>
<td>1.5 – 10.1% (female)</td>
<td>2366</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.37 – 23.21 (\times 10^3)/(\mu)L</td>
<td></td>
</tr>
</tbody>
</table>

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.\(^{10}\) followed AITP (Autoimmune Thrombocytopenic Purpura) and TTP (Thrombotic Thrombocytopenic Purpura) patients with platelet counts of less than 50,000/\(\mu\)L 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.\(^{11}\)

A study by Kickler\(^{12}\) 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.

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**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
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.13 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.

IPF in Bone Marrow Recovery

Several groups 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 and 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.16 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 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.

### Table 1: IPF% in different populations of thrombocytopenic patients

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

Figure 2: IPF% in different populations of thrombocytopenic patients

![Figure 3: Mean days to recovery for IPF, Immature Reticulocyte Fraction (IRF), platelet count (PLT) and Absolute Neutrophil Count (ANC) following stem cell transplantation.](image)
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. 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. showed that patients receiving TPO-A have a higher IPF.

What’s Out?
1. Inaccurate PLT measurements at the very low levels, as can be seen in severe thrombocytopenia.
2. Morphologic identification of large/young platelets using only MPV.
3. Waiting for absolute neutrophil count recovery to determine bone marrow recovery.

What’s In?
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.

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

Immature Platelet Fraction is a well-studied parameter that offers significant clinical information specific to 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 how it is applied, the use of the Immature Platelet Fraction and Count will support the delivery of high quality, cost effective patient care for a positive impact on patient outcomes.

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


Disclaimer

The uses or clinical applications described in these publications have not been approved or cleared by the FDA. It is the clinician's responsibility to validate any off-label applications for use in routine clinical practice.

Notice of Intended Use

The Immature Platelet Fraction (IPF) parameter on the XN- and XE-Series Automated Hematology Analyzers is intended for *in vitro* diagnostic use to enumerate the immature platelet fraction.