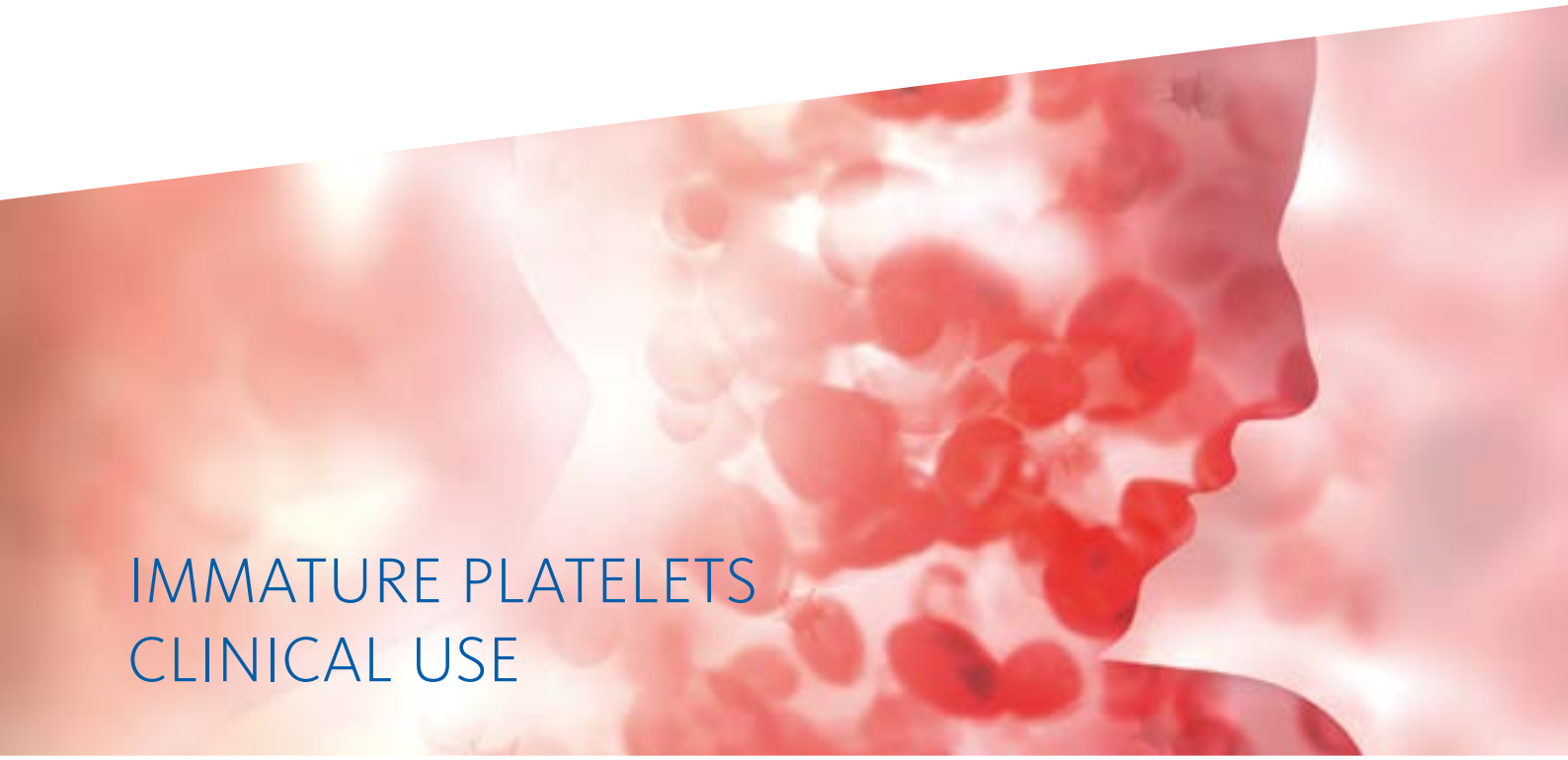
WHITE PAPER



IMMATURE PLATELETS CLINICAL USE

Differential diagnosis of thrombocytopenia

Thrombocytopenia and automated platelet measurement

Thrombocytopenia is a condition characterised by an abnormally low platelet count – lower than the normal platelet count in adults that ranges from 150 x 10^9/L to 450 x 10^9/L. Severe thrombocytopenia, with platelet counts lower than 20 x 10^9/L, is associated with spontaneous bleeding (not caused by injury). Overlooking a severe thrombocytopenia can have serious consequences for the patient, so obtaining reliable platelet counts is essential for making clinically important decisions with confidence.

Yet obtaining accurate platelet counts, particularly from samples with thrombocytopenic conditions, is not always easy and a challenging task for the laboratory. Sysmex offers a readily available platelet counting solution with PLT-F. You will find more information on this topic in the section 'Challenges in determining an accurate and precise platelet count'.

Aetiology of thrombocytopenia

Although thrombocytopenia is defined by low platelet concentrations, PLT counts alone do not reveal the underlying causes, which can be inherited or acquired. The causes can be divided into two main categories: decreased bone marrow production and increased destruction/consumption of platelets in peripheral blood. Often, the clinical question is whether thrombocytopenia is due to bone marrow failure as observed in conditions such as aplastic anaemia (AA) or myelodysplastic syndromes (MDS), or due to increased peripheral destruction/consumption such as in immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura (TTP) or disseminated intravascular coagulation (DIC). Invasive bone marrow biopsies are usually recommended to investigate the underlying aetiology.

Differential diagnosis of thrombocytopenia

Differential diagnosis of thrombocytopenia is complex and requires an investigation of the patient's medical history, an evaluation of clinical symptoms, functional platelet tests and an assessment of blood-derived platelet parameters. Historically, some clinicians have used the mean platelet volume (MPV) as a surrogate marker for platelet production because immature platelets tend to be larger than mature platelets. However, the presence of schistocytes, microcytes or other particles with a volume similar to platelets can make the MPV unreliable. Additionally, MPV values are imprecise or impossible to determine, particularly in samples with very low PLT counts, for which information about platelet production is needed most.

The immature platelet fraction (IPF) is a better marker for platelet production and indicates the percentage of immature platelets in relation to the total PLT count. It was described in 1992 by Ault *et al.*, who coined the term 'reticulated platelets' to describe newly released platelets with an elevated RNA content, whose numbers correlated with megakaryocytic activity [1]. IPF is a reproducible parameter that correlates well with the reticulated platelet count obtained from CD61 flow cytometry [2]. Although there is only a partial correlation with the MPV, immature platelets tend to be larger than mature platelets: one study found that 61 % of reticulated platelets were in the tertile containing the largest platelets, while 32 % and 7 % were in the middle and small tertiles, respectively [3]. In addition, immature platelets are more reactive than mature platelets. They contain higher amounts of RNA and are able to produce various proteins typical for active platelets (e. g. GPIIb/IIIa, P-selectin) [3].

IPF reference ranges

Several studies have established reference ranges for IPF on the Sysmex XE- and XN-Series [4 – 12]. Generally, there is good consistency among these studies with a reference range of approximately 1.1 – 6.1 %. One study established an IPF reference range for neonates: 0.7 – 7.9 % [13]. However, reference ranges should be always examined for suitability in a given patient population according to the method recommended by the International Federation of Clinical Chemistry and Laboratory Medicine [14].

Thrombocytopenia with an increased IPF may indicate increased destruction in peripheral blood, loss of platelets or a hereditary macrothrombocytopenia.

Thrombocytopenia with a normal or decreased IPF may indicate decreased platelet production in bone marrow.

Several publications reported that IPF obtained from the Sysmex XE- and XN-Series analysers is higher in patients with thrombocytopenia caused by excessive platelet destruction/consumption than in patients with thrombocytopenia caused by decreased platelet production in bone marrow [2, 5, 10 – 12, 15 – 19]. For example:

- Briggs *et al.* (2004) followed patients with ITP and TTP, which are both caused by excessive platelet consumption: IPF values were markedly elevated in these two patient groups, while patients under chemotherapy (causing decreased bone marrow production of platelets), and ITP and TTP patients in remission had normal IPF values (Fig. 1) [10].
- Kickler *et al.* (2006) reported high IPF values in thrombocytopenic patients with increased platelet destruction, while normal to slightly increased values were observed in patients with decreased platelet production (Table 1) [11].
- Abe *et al.* (2006) used a cut-off of 7.7 %, resulting in a sensitivity of 86.8 % and a specificity of 92.6 % for the differential diagnosis of ITP and AA. In addition, IPF was significantly more useful than the mean platelet volume [15].
- Jung *et al.* (2010) found that IPF is higher in ITP patients than in AA patients and that a cut-off of 7.3 % could be used to distinguish ITP from AA, resulting in a sensitivity of 54.0 % and a specificity of 92.2 % [12]. (The lower sensitivity compared to the study of Abe *et al.* could be due to different patient cohorts: Patients with acute ITP typically have a high IPF, while patients in remission can also have a normal IPF.)
- Strauss *et al.* (2011) studied children with thrombocytopenia and observed that IPF was low in children with platelet production defects, while it was markedly increased in ITP patients, indicating accelerated platelet turnover [17].
- Sakuragi *et al.* (2015) found that IPF from the XN-Series had a higher precision and was affected by fewer interferences than IPF from the XE-series. Using a cut-off of 5.8 % resulted in a sensitivity of 85.1 % and a specificity of 89.3 % to distinguish ITP from aplastic thrombocytopenia [19].

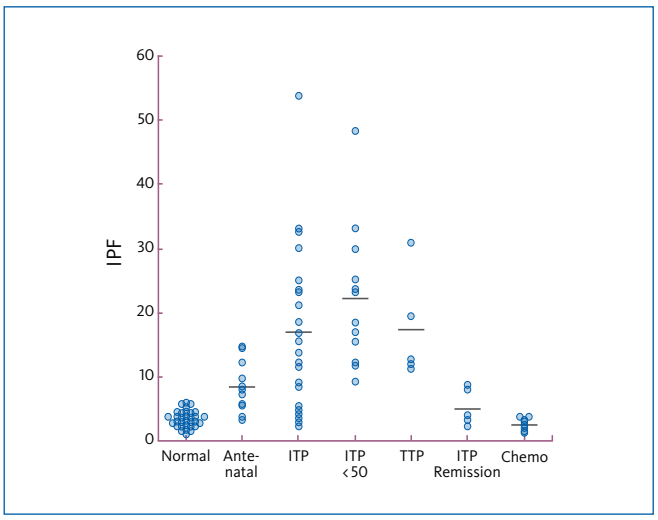


Fig. 1 *IPF values in different patient groups. ITP: immune thrombocytopenia; ITP < 50: ITP patients with a PLT count below 50 x 10⁹/L; TTP: thrombotic thrombocytopenic purpura; Chemo: patients under chemotherapy. Adapted from Briggs et al. [10].*

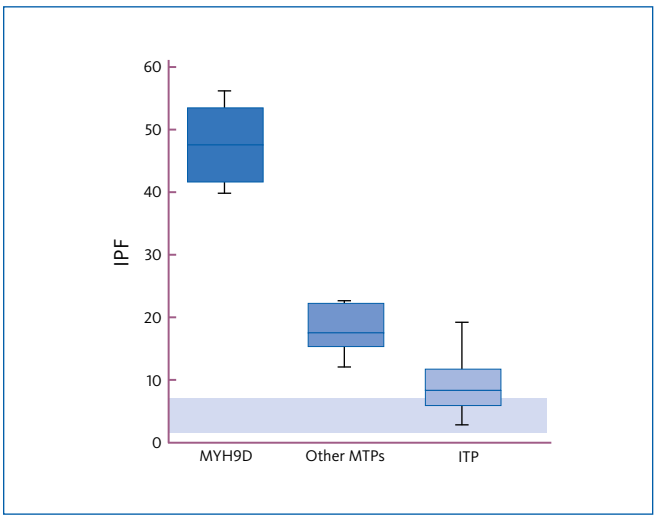


Fig. 2 *IPF values in different patient groups. MYH9D: MYH9 disorders; MTPs: macrothrombocytopenias; ITP: immune thrombocytopenia. Adapted from Miyazaki et al. [21].*

Table 1 *IPF values in different patient groups. ITP: immune thrombocytopenia; DIC: disseminated intravascular coagulation; AA: aplastic anaemia; PNH: paroxysmal nocturnal haemoglobinuria; *: statistical p-value compared to healthy controls; NS: not statistically significant compared to control. Adapted from Kickler et al. [11].*

Subject	Sample size	Mean	*p**
Healthy	80	3.1	–
Destruction			
ITP	37	15.0	<.0001
DIC	25	9.5	<.0001
All destruction	62	12.8	<.0001
Suppression			
AA/PNH	3	6.1	.019
Cancer	16	3.8	NS
All suppression	19	4.1	.05

In summary: the IPF parameter provides an assessment of bone marrow platelet production and aids differentiation between thrombocytopenia caused by decreased bone marrow production and thrombocytopenia caused by increased destruction/consumption. It delivers additional information, which might help avoid an invasive bone marrow biopsy.

The impact of IPF on the differential diagnosis of congenital thrombocytopenia

The IPF can also contribute to the differential diagnosis of suspected congenital thrombocytopenia. Congenital thrombocytopenia is usually suspected in the case of neonatal thrombocytopenia, the onset of bleeding symptoms in childhood, a family history of thrombocytopenia, or when the PLT count is unresponsive to ITP treatment. The MPV is often used for the differential diagnosis of hereditary thrombocytopenia [20] but, as mentioned before, the MPV is affected by interferences and its value is often imprecise or impossible to determine in samples with very low PLT counts.

Several recent publications describe how IPF can contribute to the differential diagnosis of congenital thrombocytopenia. For example, Miyazaki *et al.* (2015) revealed that the IPF was about five times higher in May-Hegglin MYH9 disorders (48.6 % ± 1.9) and approximately double in other macrothrombocytopenia conditions (18.4 % ± 2.1) compared to ITP patients with similar PLT counts (9.2 % ± 0.3) (Fig. 2) [21]. In contrast, patients with Wiskott-Aldrich congenital microthrombocytopenia (WAS) had a lower IPF than would be expected for their level of thrombocytopenia, and the IPF in these patients was lower than in ITP patients [22]. Similar findings were reported in seven children with WAS, who had a lower absolute IPF count than age-matched chronic ITP patients [23].

Challenges in determining an accurate and precise platelet count

Automated haematology analysers generally deliver an accurate and precise measurement of platelet counts based on the impedance method (PLT-I). However, interfering particles can result in falsely high counts while precision may be limited when patients suffer from severe thrombocytopenia (PLT ≤ 20 x 10^9/L) as the low platelet count limits the number of analysed cells. To resolve this, XN-Series analysers can perform reflex measurements with alternative flow cytometry methods (PLT-O or PLT-F) in case interfering particles or severe thrombocytopenia are suspected (Table 2).

In XN analysers equipped with PLT-I and PLT-O, a switching algorithm automatically determines whether a PLT-O reflex measurement is required for reporting an accurate PLT count; in analysers equipped with PLT-I and PLT-F, a PLT-F reflex measurement may be required and is performed likewise. Especially if a severe thrombocytopenia is suspected, a more precise measurement is needed to obtain reliable results for confidently making clinically important decisions. Here, PLT-F would be the method of choice.

Table 2 *Comparison of different platelet counting methods available on the Sysmex XN-Series*

	Impedance count (PLT-I)	Optical count (PLT-O)	Fluorescence count (PLT-F)
Workflow	Default and routine automated method	Often a reflex method	Often a reflex method
Analysis	Part of complete blood count	Part of reticulocyte count	Dedicated PLT count
Precision, accuracy and interferences	■ Low precision if PLT < 20 x 10^9/L ■ Low accuracy for samples with interferences: containing particles with a volume similar to platelets (reagent crystals, air bubbles, microcytes, RBC fragments)	■ Low precision if PLT < 20 x 10^9/L ■ High accuracy when RBC abnormalities are present ■ Low accuracy for samples with WBC fragments (apoptosis/ necrosis)	■ High precision down to PLT = 3 x 10^9/L due to five-fold counting volume ■ Virtually no interferences ■ Comparable with reference method (CD41/CD61) [24, 25]
Diagnostic parameters beyond the PLT count	PDW, MPV, PCT, P-LCR	None	IPF, IPF#

Conclusion and clinical interpretation

PLT counts alone do not reveal the underlying aetiology of thrombocytopenia. The causes of thrombocytopenia can be due to decreased platelet production in bone marrow or an increased destruction/consumption of platelets in peripheral blood. The IPF is an established parameter that provides guidance for treating physicians when determining the cause of thrombocytopenia based on the aetiology of various congenital and acquired thrombocytopenic states, as described in this white paper.

A high IPF suggests consumptive thrombocytopenic disorders or congenital macrothrombocytopenia, and may also indicate an appropriate bone marrow response to thrombocytopenia. In contrast, a low or normal IPF is seen in aplastic states (Table 3). IPF measurements can be ordered for certain patient populations or measured as a reflex test for unknown patients with unclear thrombocytopenia.

Table 3 *Aetiology of thrombocytopenia and associated IPF values. The ranges in the table are provided for guidance only; interpretation of IPF should always occur within the complete clinical context, including clinical symptoms and other laboratory tests.*

Acquired		**Hereditary**
Ineffective platelet production	Increased platelet destruction/consumption	Congenital macrothrombocytopenia
IPF 1.1 – 6.1 %	IPF > 6.1 %	IPF > 12 %
Bone marrow damage ■ Myelodysplastic syndromes ■ Neoplastic bone marrow infiltration ■ Aplastic anaemia caused by chemicals, drugs or infections ■ Chronic ITP with apoptotic megakaryocytes	**Immune causes** ■ Immune thrombocytopenia (ITP) ■ Heparin-induced thrombocytopenia (HIT) type II	**IPF > 12 %** ■ Bernard-Soulier syndrome ■ ACTN1-related thrombocytopenia ■ αδ-storage pool disease ■ Variant form of Glanzmann thrombasthenia
Ineffective production ■ Megaloblastic anaemia	**Non-immune causes** ■ Thrombotic thrombocytopenic purpura (TTP) ■ Haemolytic uraemic syndrome (HUS) ■ Disseminated intravascular coagulation (DIC) ■ HIT type I ■ Bleeding	**IPF > 40 %** ■ May-Hegglin MYH9 disorders

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