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SYSMEX EDUCATIONAL ENHANCEMENT AND DEVELOPMENT | November 2023*

SEED Haematology

The importance of thrombocytopenia and its causes

What is thrombocytopenia?

Thrombocytopenia is a condition in which there is an abnormally low number of platelets (thrombocytes) in peripheral blood. The adult reference intervals for the platelet count (PLT) are 164 – 369 × 10^9/L for men and women [1]. Values outside this range do not necessarily indicate disease. Usually, a patient is considered thrombocytopenic when PLT < 150 × 10^9/L. The French-speaking cellular haematology group (GFHC) recommends performing a smear when an adult presents with PLT < 100 × 10^9/L in order to check for cell abnormalities (Figure 1). In children, a threshold of 150 × 10^9/L is preferable for smear review [2]. However, Sysmex highly recommends that the reference ranges are always examined for suitability in a given patient population according to the method recommended by the International Federation of Clinical Chemistry and Laboratory Medicine [3].

The function of platelets is to initially stop bleedings by clumping and clotting blood vessel injuries and starting the coagulation cascade. That is why it is so important that thrombocytopenia is diagnosed and treated rapidly. When PLT levels decrease, even minor injuries could become life threatening for the patient. When the level is extremely low, a platelet transfusion may be required in order to avoid spontaneous bleeding. The threshold for this depends on the clinic/ward, and can range from 10 to 50 × 10^9/L [4]. Nevertheless, it is important to transfuse platelet concentrates only when they are necessary, since platelet transfusions are expensive and can have serious side effects including febrile reactions, transmission of viral infections, haemolytic transfusion reactions and graft versus host disease [5].

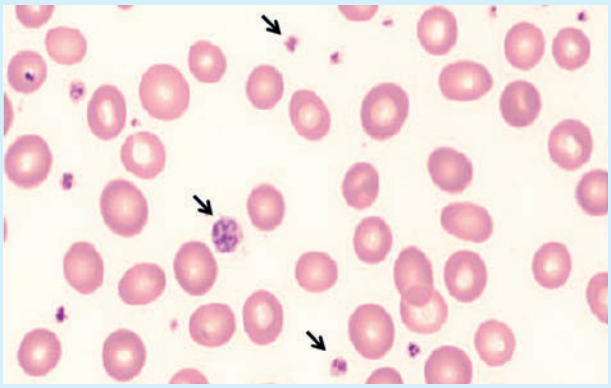
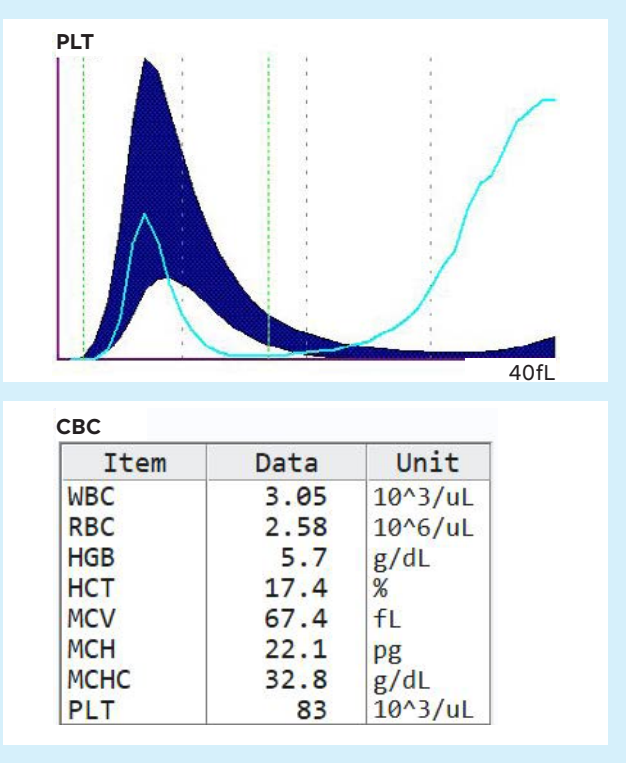


Fig. 1 Peripheral blood smear with red blood cells and platelets (indicated by arrows).

* Revision of the original article published in August 2016.

Clinical manifestations of thrombocytopenia

Patients with mild thrombocytopenia usually have no symptoms, so this condition is discovered during a routine complete blood count (CBC) (Figure 2). General symptoms are bleeding in the mouth and of the gums, easy bruising, nosebleeds and rashes. In severe thrombocytopenia, i.e., when PLT counts are below 50 × 10^9/L, excessive bleeding can occur if the person is cut or injured. Spontaneous bleeding can also happen when platelet numbers are severely diminished. Some women may have heavier or longer menstruation. A person with thrombocytopenia may also complain of malaise, fatigue and general weakness [6].



Item	Data	Unit
WBC	3.05	10^3/uL
RBC	2.58	10^6/uL
HGB	5.7	g/dL
HCT	17.4	%
MCV	67.4	fL
MCH	22.1	pg
MCHC	32.8	g/dL
PLT	83	10^3/uL

Fig. 2 PLT histogram and CBC profile of a thrombocytopenic sample. The solid turquoise line represents the PLT distribution of the sample, and the dark blue area the normal range of PLT.

Causes of thrombocytopenia

The causes of thrombocytopenia can be generally classified as inherited or acquired, but it is also interesting to know if it is caused by a decreased production in the bone marrow (also called productive thrombocytopenia) or by an abnormally high destruction of platelets in the peripheral blood (known also as consumptive thrombocytopenia) [7].* Usually, the underlying cause of thrombocytopenia is identified with blood tests such as analysis of liver enzymes, folic acid levels and vitamin B12 levels, a peripheral blood smear or a bone marrow biopsy.

Decreased production
In this case, insufficient numbers of platelets are produced in the bone marrow. There are different conditions in this group, such as aplastic anaemia, cancer infiltration in the bone marrow, cirrhosis, folate deficiency, myelodysplastic syndrome, or vitamin B12 deficiency. Also, the use of certain drugs may lead to a low production of platelets in the bone marrow. The most common example is chemotherapy treatment.

Increased destruction
This type of thrombocytopenia is due to an increased destruction of platelets in the bloodstream, spleen or liver. Examples are disseminated intravascular coagulation (DIC), drug-induced, hypersplenism, immune thrombocytopenic purpura (ITP) or thrombotic thrombocytopenic purpura (TTP).

Most common types of thrombocytopenia

Aplastic anaemia
Aplastic anaemia is caused by decreased numbers of pluripotent haematopoietic stem cells resulting in reduced haematopoiesis. The outcome of this is pancytopenia, which is the reduction of all types of blood cells: white blood cells, red blood cells and platelets [8].

Immune thrombocytopenic purpura (ITP)
ITP is an autoimmune haematological disorder in which accelerated platelet destruction leads to a reduction in peripheral blood platelets. It causes a characteristic purpuric rash and a tendency to bleed. The diagnosis of ITP is a process of exclusion. Megakaryopoietic activity of the bone marrow may be enhanced [9].

Thrombotic thrombocytopenic purpura (TTP)
TTP is usually caused by a lack or deficiency of the enzyme ADAMTS13, which cleaves multimers of von Willebrand factor in the peripheral vasculature. Accumulation of the uncleaved multimers leads to spontaneous aggregation of platelets, activation of coagulation and clot formation [9].

Heparin-induced thrombocytopenia (HIT)
Patients that are under heparin treatment (an anticoagulant) may develop thrombocytopenia because of abnormal blood clot formation inside their blood vessels. Like in TTP, the patient becomes thrombocytopenic because platelets are consumed in the clot formation and their count decreases [9].

Congenital amegakaryocytic thrombocytopenia
A rare inherited disorder resulting in the absence of megakaryocytes in the bone marrow, and therefore, no platelets are produced [9].

* For deeper information about the aetiology of thrombocytopenia, please check the white paper called 'Differential diagnosis of thrombocytopenia'.

The importance of IPF

IPF refers to the immature platelet fraction in peripheral blood. Immature platelets were first described as reticulated platelets in 1969, when RNA condensations in platelets were observed by microscopy [11].

In bone marrow, megakaryocytes pinch off immature, reticulated platelets, which develop into mature platelets within one or two days. A bone marrow biopsy can directly assess the megakaryocyte population, and thus the thrombopoietic activity, but this procedure comes with several disadvantages: It is an invasive procedure and patients experience pain when the needle is inserted for taking the sample. General anaesthesia is typically not given and some patients experience side effects like fever, chills and swelling in the area of the biopsy [12].

The number of immature platelets found in peripheral blood is another indication for the rate of thrombopoiesis in bone marrow, with a more active bone marrow releasing more immature platelets in the peripheral blood. Benlachgar N *et al.* summarised the studies showing that the IPF parameter correlates with bone marrow activity without the need for a bone marrow biopsy. Thus, IPF could aid in the rapid diagnosis and prompt subsequent treatment of thrombocytopenia [10]. The analysis of IPF makes clinical information available that may reduce the necessity and number of bone marrow examinations.

Figure 3 shows how the immature platelets (IPF) can be clearly separated from the mature ones using a Sysmex haematology analyser with the PLT-F channel that uses a combination of reagents that label the RNA inside platelets.

The younger platelets have a bigger size and a higher amount of RNA than the mature ones and this is represented in the scattergram, where the immature platelet fraction (green) has a higher fluorescence signal (SFL axis) as well as a bigger size (FSC axis) than the mature platelets. The staining in the PLT-F channel is highly specific, as demonstrated by Wada A *et al.* in a pool of platelets, red blood cells and fragmented red blood cells, only the platelets were stained with the PLF-F reagents, and these stained cells were also positive for the platelet-specific markers CD61 and CD41 [13].

Studies have shown that by using the IPF parameter from peripheral blood, a clear differentiation between the causes of thrombocytopenia – whether there is platelet destruction or an aplastic bone marrow – can be easily achieved [14–17]. Increased IPF levels indicate a responsive bone marrow, so thrombocytopenic states must therefore be related to excessive platelet consumption [14, 15]. On the other hand, normal or low IPF values with thrombocytopenic patients indicate a non-responsive bone marrow, indicating in turn that the observed thrombocytopenia may be a result of impaired or failing thrombopoiesis [16, 17].

Since the IPF count increases before the overall PLT count does, IPF could be used to predict e.g., bone marrow recovery after chemotherapy or the effect of treatment on thrombocytopenic patients [14, 18]. IPF represents the young fraction of the platelets, and the possibility to detect it without having to wait for the mature ones to appear in peripheral blood allows faster and better monitoring of response to therapy, which would finally provide a better treatment for the patient [14, 16].

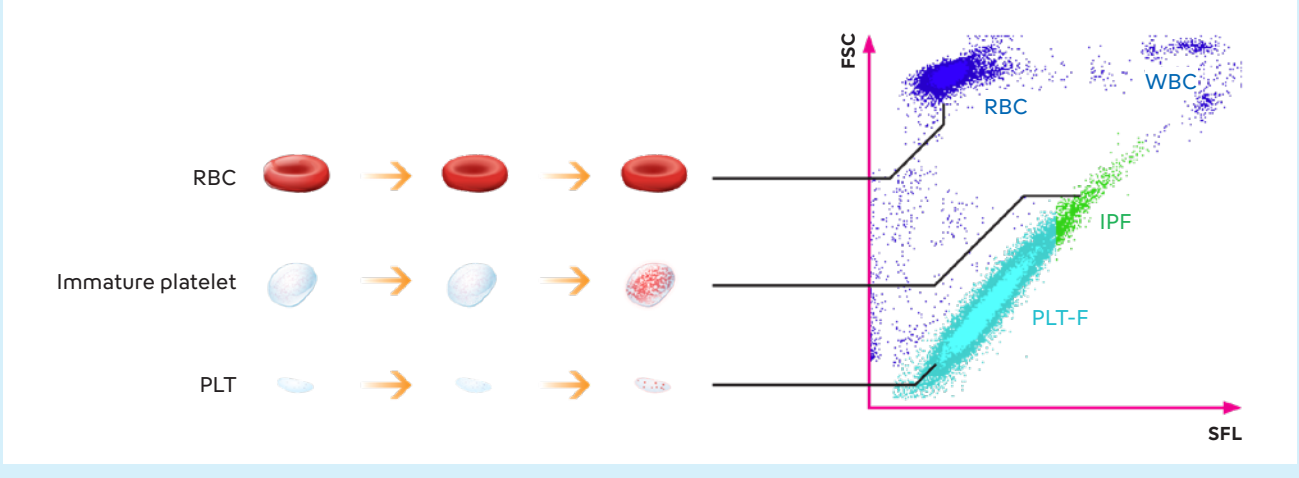


Fig. 3 Distribution of cells in the PLT-F measurement channel of Sysmex haematology analysers.

Conclusion

The immature platelet fraction (IPF) is a PLT-related parameter that measures young, reticulated platelets in peripheral blood. IPF levels rise as bone marrow production of platelets increases [14]. This means that measurement of IPF supports the assessment of the bone marrow activity from a peripheral blood sample. As has been explained in this document, the IPF has a high clinical utility as a laboratory test to help diagnose and manage thrombocytopenia, due to the ability to relate raised %IPF levels to increased peripheral platelet destruction. It is particularly useful for supporting the diagnosis of autoimmune thrombocytopenic purpura and thrombotic thrombocytopenic purpura, and for distinguishing these from bone marrow suppression or failure. IPF can also be a sensitive measure for evaluating thrombopoietic recovery during aplastic chemotherapy. Transfusions may only be considered if the %IPF values are not rising as this would indicate poor intrinsic thrombopoietic activity [19].

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