

## SEED HAEMATOLOGY



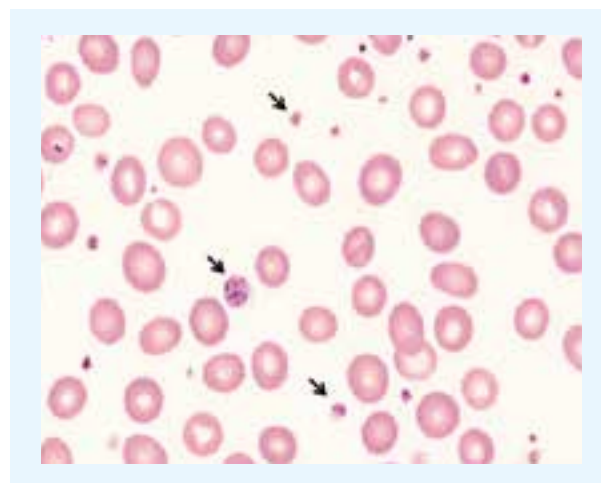
### The importance of thrombocytopenia and its causes

#### What is thrombocytopenia?

Thrombocytopenia is a disorder in which there is an abnormally low amount of platelets (thrombocytes) in peripheral blood. The adult reference intervals for the platelet count (PLT) are  $166 - 308 \times 10^9 / L$  for men and  $173 - 390 \times 10^9 / L$  for women [1]. Values outside this range do not necessarily indicate disease. Usually, a patient is considered thrombocytopenic when  $PLT < 150 \times 10^9 / L$ . Latest guidelines recommend doing a smear when an adult presents  $PLT < 100 \times 10^9 / L$  in order to check for cell abnormalities. With children, a threshold of  $150 \times 10^9 / L$  is preferable for smear review [2]. However, we highly recommend 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. The threshold for this depends on the clinic/ward, but commonly it is around  $20 \times 10^9 / L$ . Nevertheless, it is important to deliver 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.



**Fig. 1** Blood smear with RBC and PLT (indicated by arrows)

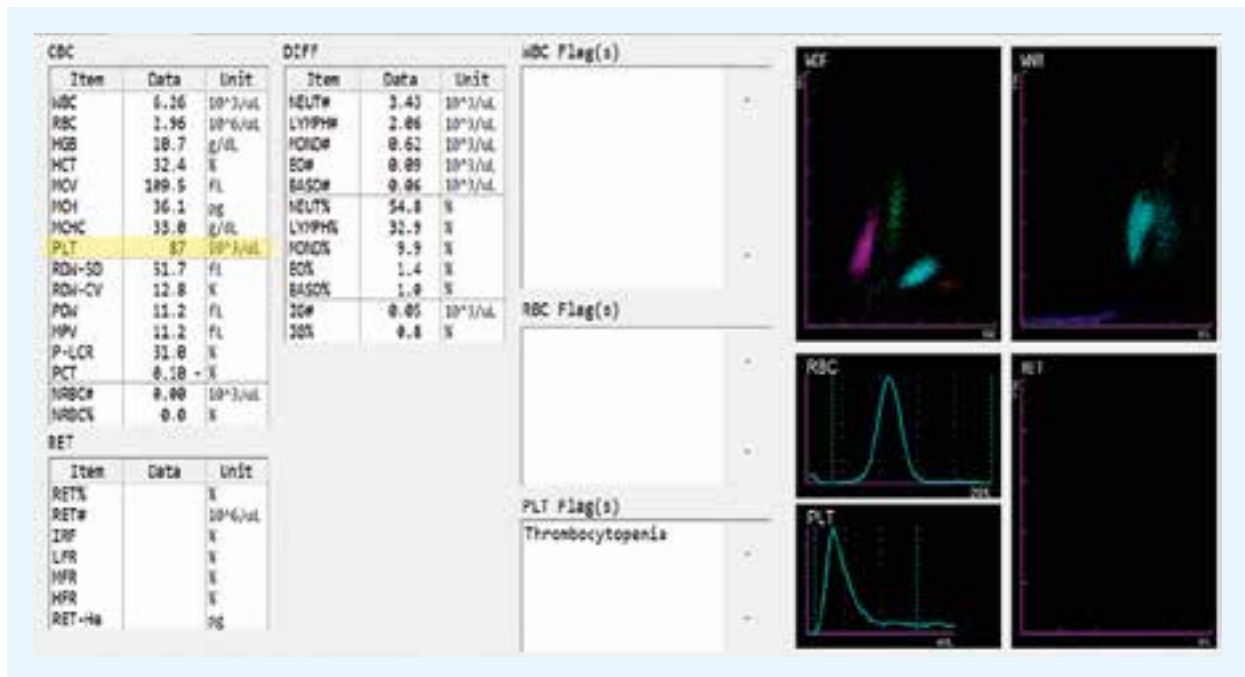


Fig. 2 Analysis result from an XN-Series analyser of a patient with thrombocytopenia

### Clinical manifestations of thrombocytopenia

Normally, especially with mild thrombocytopenia, patients have no symptoms so this disorder is usually discovered during a routine complete blood count (CBC). 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  $50 \times 10^9/L$ , 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.

### Causes of thrombocytopenia

The causes of thrombocytopenia can be generally classified as inherited or acquired [4], but it is also interesting to know if it is caused by a decreased production (also called productive thrombocytopenia) or by an abnormally high destruction of platelets in the blood (known also as consumptive thrombocytopenia).\*

### 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).

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

### 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.

#### 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, resulting in a high IPF.

#### 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.

#### 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.

#### Congenital amegakaryocytic thrombocytopenia

A rare inherited disorder resulting in the absence of megakaryocytes in the bone marrow, and therefore, no platelets are produced.

### Diagnosis of thrombocytopenia

There are different tests that can be done in the laboratory, like a CBC, analysis of liver enzymes, folic acid levels and vitamin B12 levels, or a blood smear. If the cause for the low platelet count remains unclear, a bone marrow biopsy is usually recommended to differentiate whether the low platelet count is due to decreased production or peripheral destruction [5], since analysis of the bone marrow can determine the number, size and maturity of the megakaryocytes. Nowadays, this information can also be obtained by looking at the immature platelet fraction (IPF), which informs about the activity of the bone marrow without the need of performing a bone marrow biopsy. This information will help the diagnosis and prompt subsequent treatment of the disease.

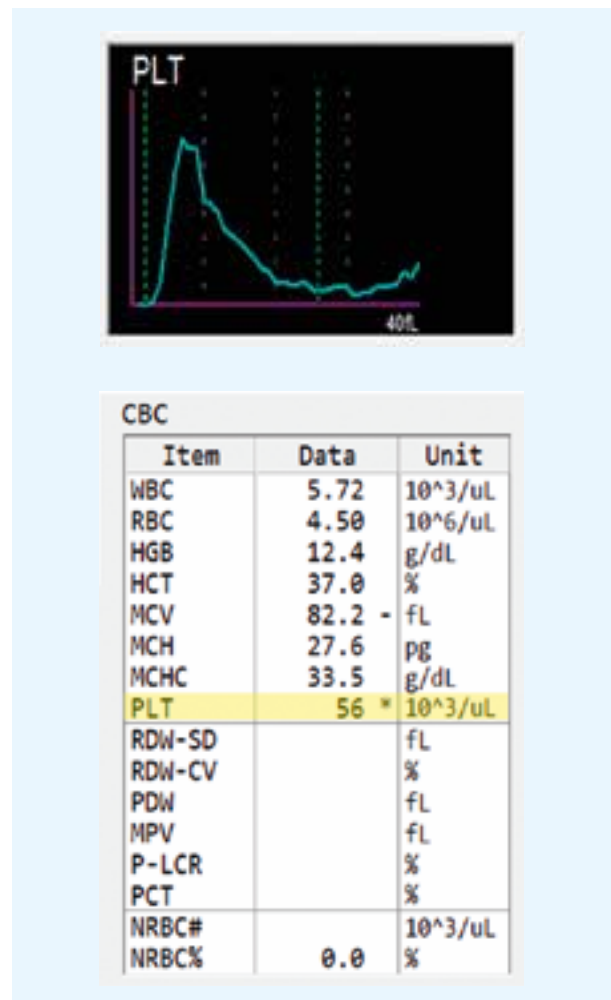


Fig. 3 PLT histogram with abnormal distribution and low PLT count in the CBC

### The importance of IPF

IPF refers to the immature platelet fraction in peripheral blood. Immature platelets were first described as reticulated platelets in 1969 [6], when RNA condensations in platelets were observed by microscopy.

In bone marrow, megakaryocytes pinch off immature, reticulated platelets, which develop into mature platelets within one or two days. The amount of immature platelets found in peripheral blood is an indication for the rate of thrombopoiesis in bone marrow. An active bone marrow will result in an increased IPF value.

This information enables differentiation between bone marrow failure or increased destruction or loss of platelets in peripheral blood as the cause of an observed thrombocytopenia. The analysis of IPF makes clinical information available that may reduce the necessity and number of bone marrow examinations.

Bone marrow biopsy has 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.

Using the IPF parameter, a clear differentiation between the causes of thrombocytopenia – whether there is platelet destruction or an aplastic bone marrow – can be easily

achieved. Increased IPF levels indicate a responsive bone marrow and thrombocytopenic states must therefore be related to excessive platelet consumption. 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.

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 [7–8]. 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.

Figure 4 shows how the immature platelets (IPF) can be clearly separated from the mature ones using an XN-Series haematology analyser with PLT-F channel. In this specialist channel the sample is treated with a specific reagent that exclusively labels the RNA inside platelets. 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 IPF fraction (green) has a higher fluorescence signal (SfL axis) as well as a bigger size (FSC axis) than the mature platelets.

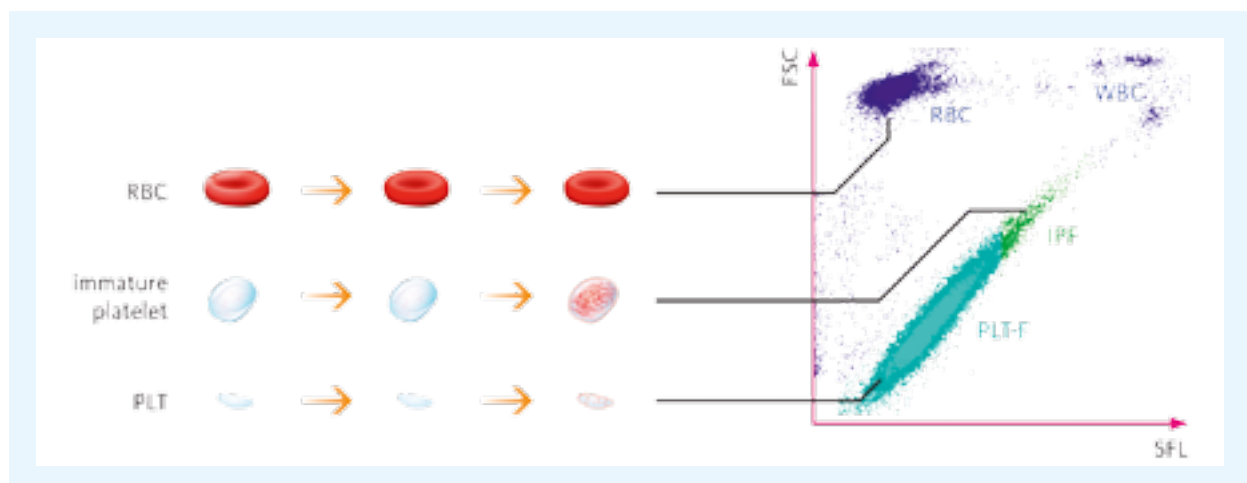


Fig. 4 PLT-F scattergram obtained from an XN-Series analyser

## 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. This means that its measurement provides an assessment of bone marrow platelet production from a peripheral blood sample. As has been explained in this document, there is a high clinical utility of the %IPF as a laboratory test for diagnosis and treatment of 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. The 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.

## References

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