



Correlation of thrombocyte size and total thrombocyte mass

In healthy people, the thrombocyte counts are between 150 and 400 G/l. There is an inverse relation between the mean platelet volume (MPV) and the number of thrombocytes. The lower the platelet count, the larger are the individual thrombocytes; accordingly, the greater the number, the smaller are the individual platelets. This leads to an almost constant thrombocyte mass, which implies that the body primarily strives to obtain the total mass and not a specific platelet count.

Giant thrombocytes

Giant thrombocytes refer to platelets having a diameter of 7 or more micrometers (normal erythrocyte size). The cause of this may be congenital diseases, such as «Bernhard-Soulier syndrome», or less commonly, the «May-Hegglin anomaly». Giant platelets are also found within the scope of myelodysplastic and myeloproliferative syndromes. If there are more giant thrombocytes in a sample, the MPV value (mean platelet volume) increases. Interferences may occur if the thrombocytes are measured with the impedance method on a device based solely on their volume. The giant platelets are allocated to microcytic erythrocytes and an inaccurately low platelet count is determined.

Reticular thrombocytes

Reticular thrombocytes are larger, one-to-two-day-old thrombocytes with a high RNA content (m-RNA from the megakaryocytes). Increased values refer to increased bone marrow activity with increased formation of new thrombocytes.

Introduction

Thrombocytes (platelets, PLT) are a part of corpuscular blood components and play important roles in blood coagulation. Thrombocytes (Tc) are strangulations of cytoplasm of the megakaryocytes. Thrombocytopoiesis is regulated by the hormone, thrombopoietin, formed in the kidneys and the liver. Each megakaryocyte produces 1000-3000 platelets, which circulate in the periphery for 7-10 days and are then removed. The initially inactive platelets circulate at about 70% in the peripheral blood. The remaining 30% are in the splenic pool, from where they can be mobilized at any time.

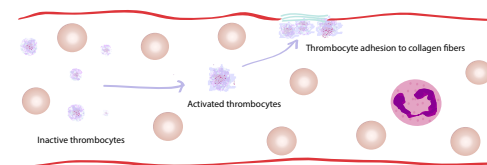
Morphology and function of thrombocytes

Thrombocytes have no nucleus, though they contain mRNA of megakaryocytes. They have a diameter in a blood smear of around 1-3 µm and their volume in the haemogram is 2-20 fl. Younger thrombocytes are larger than older thrombocytes and contain more mRNA. Upon activation, the thrombocytes change their shape.

The previously discoid plates develop pseudopodia (cytoplasm offshoots). When contacting exposed collagen fibers of vessels, the thrombocytes reversibly adhere to the vascular wall. In the process, the developed pseudopodia facilitate an optimal seal. Then irreversible adhesion (aggregation) occurs due to the fibrin strands cross-linking with the thrombocytes.

Platelet aggregation in blood samples may occur with faulty pre-analytics (e.g. unfavorable capillary blood sampling, insufficient mixing or overfilling EDTA tubes). In approximately 0.1% of samples, an EDTA-induced pseudo-thrombocytopenia is observed (in the process, the EDTA in the tube causes an agglutination of the thrombocytes, which is why we have to rely on a different coagulant, such as heparin or citrate).

Thrombocyte activation with vascular lesion



Methods to determine the platelet count

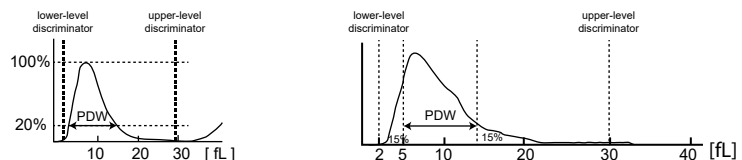
The only method to count platelets in the counting chamber long-term has now been replaced by more precise automated methods with hematology systems.

3-part diff systems

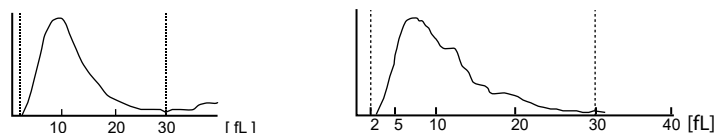
Measurement of thrombocytes and erythrocytes in the same measurement channel. Delimitation with discriminators due to cell volume.

A proper Tc curve starts with the lower-level discriminator (Sysmex: 3 fl, Microsemi 2 fl) on the baseline and ends once again at the variable upper-level discriminator (approx. 30 fl) on the baseline.

If this is not the case, a flag will appear for the PLT (the correctness of the value must be questioned and all subsequent PLT parameters are not reported). Possible causes are overlapping on the upper-level discriminator due to macro or giant thrombocytes, platelet aggregates, which are erroneously included in the erythrocytes or due to microcytes, erythrocyte fragments, non-lysed erythrocytes, very seldom bacteria in the event of sepsis, which lead to an erroneously high platelet count.



Left: thrombocyte diagram (PLT) of Sysmex XP-300. The PDW value corresponds with the width of the curve at 20% height. Right: thrombocyte diagram (PLT) of the Microsemi. The PDW value in percentage is calculated from the curve width of the middle 70% of thrombocytes.



Thrombocyte histograms from sample MQ 2018-4 H3B (chronic myeloid leukemia) with increased PDW. Left: the Sysmex XO-300 histogram with a PDW of 15.2 fL. Right: the Microsemi histogram with a PDW of 16.4 %.



Spotlight on hematology

IPF - «Immature platelet fraction»

The measurement of this «immature platelet fraction» determines the percentage of reticular (immature) thrombocytes in the total thrombocyte count based on the demonstrated mRNA content. The significance of the IPF has been and is the subject of various studies. Clinical relevance was able to be determined, for example, for the following questions:

Diagnostics and monitoring of thrombocytopenia

Thrombocytopenia with a low IPF speaks of a decreased formation of thrombocytes due to insufficiency of the bone marrow, e.g. during chemo and radiation therapy, medication side effects.

In contrast, an increased IPF occurs with increased thrombocyte consumption and simultaneously intact thrombopoietic output, e.g. due to bleeding, thrombocytopenia due to antibodies (ITP, AITP). This differential diagnostic statement was so far only possible through bone marrow puncture.

MPV, P-LCR and PDW

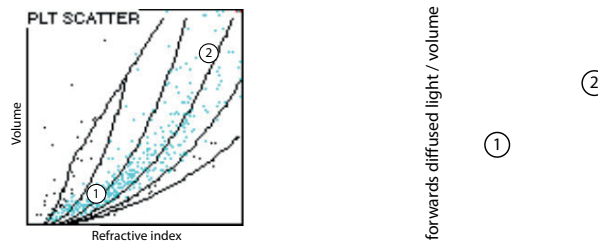
Increased MPV, P-LCR, and PDW values, which can be measured on 3-part diff systems, may require further evaluation of the IPF value. However, they can also be an indication of technical interferences of the thrombocyte/erythrocyte measurement.

5-part diff systems

These systems measure the platelet counts in conjunction with additional parameters, which largely precludes the interferences outlined above.

The Sysmex devices measure the platelet count by means of volume determination (impedance), diffused light, and fluorescence marking of platelet RNA. ADVIA 2120 uses laser technology for the measurement by measuring diffused light in two different angles, which on the one hand reflect the cell volume and on the other hand the refractive index (RI, correlates with platelet content).

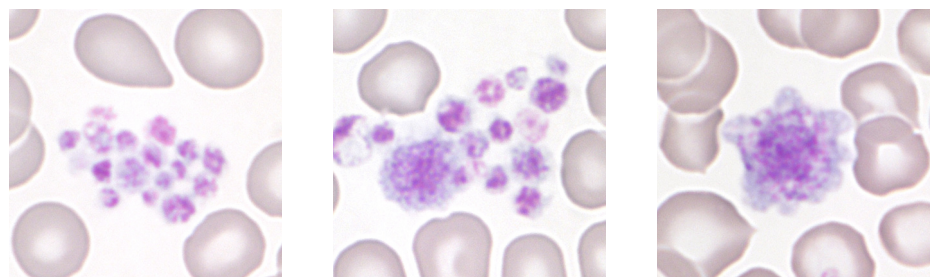
An extremely accurate, but time-consuming and costly method is fluorescence marking of the surface antigens CD61/CD41 with monoclonal antibodies and the subsequent flow cytometric measurement (this method was defined by the ISLH International Society of Laboratory Hematology as a reference method for determining thrombocytes).



Left: diffused light measurement with ADVIA 2120. The normal thrombocytes are in 1; the large thrombocytes are in 2. Right: Visual measurement with fluorescence coloration on the Sysmex XE5000. 1 are the normal thrombocytes; 2 are the immature thrombocytes.

Thrombocyte indices

Parameter	description	unit/ reference range	Interpretation changed values
MPV mean platelet Volume	mean thrombocyte volume	8-12 fl	increased: increased macro and giant thrombocytes or immature reticular thrombocytes
P-LCR Platelet large cell ratio	Relationship between total Tc and Tc with volume >12 fl	15-35 %	increased: increased macro and giant thrombocytes or immature reticular thrombocytes
PCT plateletcrit	percentage of thrombocyte volume in total blood volume	0.12-0.48%	decreased: decreased thrombocyte mass
PDW-SD platelet distribution width	Distribution width, size variability	9-14 fl	increased: Thrombocyte anisocytosis
IPF immature platelet fraction	Percentage of immature, reticular thrombocytes.	0.5-6.0 %	increased: increased percentage of immature thrombocytes. Increased thrombopoiesis. Reduced: lower percentage of immature thrombocytes. Reduced thrombopoiesis.



Thrombocytes in microscopic blood count 2018-4 H3B (chronic myeloid leukemia).

About

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