Editorial

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Up-to date platelet testing

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Platelets are involved in numerous conditions such as in vasoconstriction of damaged blood vessels, in forming temporary platelet plugs in primary hemostasis, and to secrete procoagulants. They are pivotal for the local concentration of clotting factors in secondary hemostasis as well as in dissolving blood clots and modulating repair processes in vascular injury and vascular inflammation.

From the analytical perspective, testing for normal platelet function is still challenging. Indications for platelet testing are frequent including the exclusion of coagulopathies, bone marrow function testing such as under chemotherapy or irradiation or during excessive bone marrow activation, and increased turnover or increased reactive production of platelets.

While the laborious platelet function tests are requested in few medical conditions only, in most in- and outpatients the medical laboratory should deliver the attending physician reliable information whether the number and presumably the overall function of the platelets are within the expected ranges. About 10^{11} up to 10^{12} (in times of increased demand) platelets are produced per day but platelets have a short half-life and a high turnover which makes all platelet-dependent functions (which is highly related to the total platelet mass in a patient) vulnerable to the efficacy of megakaryopoiesis, to the diminished half-life of platelets as well as to changes in the mean platelet volume (MPV).

Any laboratory report indicating disturbances of platelet concentration and function should trigger rapid evaluation of the causes and of the possible therapeutic alternatives by the attending physicians as well as by the medical laboratory. This special issue of the Journal of Laboratory Medicine will give up-to date reviews on several aspects of platelets with a focus on a medical laboratory perspective.

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As counting platelets is one of the most frequent tests ordered in a medical laboratory, these test results are relevant for numerous patients and even preanalytical issues as well as clinical situations with diminished platelet count or function occurring in a very low percentage of all tests become very relevant from an economic perspective because of rather high absolute numbers. Normal platelet counting is a very economical and efficient method and if additional tests besides platelet counting become necessary for assessing platelet functions, they should consume little resources only. Of special interest are tests which can be obtained as a by-product in parallel to the regular blood count with a normal hematology analyzer such as the MPV or the quantitative analysis of reticulated thrombocytes (called “immature platelet fraction [IPF]”). With a lifespan of <1 day, reticulated platelets indicate the activity of megakaryopoiesis and measuring reticulated platelets can be of clinical use in the differential diagnosis of thrombocytopenia, in the recovery of thrombopoiesis after cytostatic therapy and transfusion management, in acute coronary artery disease and in ischemic stroke as well as in antiplatelet therapy and in infection. Meintker und Schulze [1] give a detailed overview about the current knowledge about the clinical application as well as the methodological background of measuring reticulated platelets (IPF).

Despite the first counting of platelets was performed about 180 years ago, there is still no best practice for the necessary anticoagulation of blood. Mannuß [2] presents in his paper an illustrative historical overview of the different anticoagulants used and describes in detail the ongoing challenges with selecting the adequate whole blood anticoagulant such as K_2EDTA , K_3EDTA , Na_2EDTA , citrate, $MgSO_4$, or hirudin. These anticoagulants have an enormous impact on the quality and reliability of platelet testing. Any time dependent changes of platelet morphology by the anticoagulant will affect MPV as well as the morphology and might necessitate a rapid sample transport and short storage until analysis. Since automated platelet counting often employs even a combination of different techniques (such as impedance method, scattered light optical method, and flow cytometry) together with different algorithms for the automatic analysis of the raw data, the effects of certain anticoagulants will be dependent even on the specific analyzer used. Selecting the best-suited anticoagulant gets even more challenging for

platelet function testing such as by light transmission aggregometry and for determining the activation state of platelets where subtle defects of platelet function should be confirmed or excluded with high confidence. Side effects of certain anticoagulants such as ethylenediaminetetraacetic acid (EDTA)-induced pseudothrombocytopenia (PTCP) occur rather frequently and unpredictably. Schuff-Werner et al. [3] summarize their own extensive experience as well the current knowledge of PTCP from the literature. PTCP due to anticoagulant-induced platelet aggregation is crucial, because a misinterpretation as a “true” thrombocytopenia may lead to serious diagnostic and therapeutic consequences such as bone marrow biopsy, initiation of corticosteroid therapy, platelet transfusion, delay of necessary surgical procedures, or even splenectomy.

While many analytical techniques focus on automated platelet analysis, there is still a continuous need for a precise morphological estimation of platelet morphology and platelet number. Abnormal erythrocytes as well as erythrocyte fragments, cryoglobulins, fragments of leucocytes or fungi can be mistaken for platelets and might falsely indicate a normal platelet count in the case of life-threatening thrombocytopenia or might falsely produce a result of thrombocytosis. When platelet dysfunction is assumed, light microscopy for the assessment of platelet size and morphology is an indispensable tool to narrow down the possible causes. Robier [4] presents an up-to-date overview including illustrative microscopic examples of preanalytical artefacts and typical clinical conditions. Light microscopy attained an even larger importance in the last years since for many genetic disorders of platelet function the causative genes have been identified and a detailed microscopic analysis including megakaryocytes, micromegakaryocytes and megakaryoblasts morphology will aid in the straightforward selection of the possible gene mutations for sequencing analysis.

Several standards for reporting of platelet morphology in clinical laboratory routine are currently used. However, differences between these standards can infringe patients’ safety in particular when physiological observations such as a few giant platelets or dysplastic platelets in an otherwise normal blood count are mistaken for a pathological condition. The article [4] describes in detail the caveats of these different reporting standards and gives recommendations for the uniform reporting of qualitative platelet abnormalities.

Causes of thrombocytopenia can be a reduced thrombocytopoiesis, an enhanced platelet destruction or consumption or an enhanced splenal pooling. Platelet antibodies can cause an increased turnover of endogenous

platelets and even of transfused platelets. Therefore, in-depth testing for platelet antibodies can be necessary when conditions such as immune thrombocytopenia (ITP), fetal or neonatal alloimmune thrombocytopenia (FNAIT) and post-transfusion purpura (PTP) are suspected or when platelet transfusions do not result in the expected increase due to alloimmunization. It is noteworthy, that these platelet antibodies may induce alterations of platelet function even in the absence of thrombocytopenia.

The presence of platelet antibodies can be dramatic as well for the survival of the patient as well as for the physicians selecting a suited immunotherapy and/or in need for transfusing selected donor platelets not affected by the patient’s antibodies. Rapid and reliable tests are needed for the correct detection of these antibodies: The increased knowledge about the antigens causing these antibodies as well as huge improvements in the available tests in the routine laboratory as well as in the reference laboratory occurred within the last decades: while the first tests available studied antibody binding to platelets and used as a readout the rather crude effects of these binding, the second generation of tests employed antibody binding on platelets in analogy to Coombs testing. The current generation of tests uses an array of different *monoclonal antibody-specific immobilization of platelet antigens* (MAIPA) tests as well as immunobead assays. Kiefel describes in depth the challenges of antibody testing such as low avidity binding and the advantages such as the much better standardization of the current tests compared to previous generation assays [5].

Therapy of thrombocytopenia by platelet transfusion

In case of thrombocytopenia, one therapeutic option is the transfusion of platelets and 480.000 units of platelet concentrates were transfused in 2019 in Germany. Klüter and Wuchter [6] review in detailed the current technologies available for platelet concentrate production and therapeutic application and in particular the novel methods, which allow storage of platelet concentrates over prolonged time, can achieve an inactivation of germs in the platelet concentrate and will allow a safe transfusion to the patient.

Platelet transfusions are rather challenging since the very short shelf life and the storage at room temperature together with the often-complicated selection of the best-suited platelet concentrate preparation has to consider the characteristics of the concentrate as well as the immunological response of the recipient. Recent technological

advances in platelet concentrate production have a marked impact on patient outcome. Since transfusions are regulated by the German transfusion act (Transfusionsgesetz [TFG]), the article also describes in detail the mandatory rules of the Hemotherapy Directives (*Richtlinie Hämotherapie*) of the German Medical Association (Bundesärztekammer).

Medical conditions: thrombocytopenia in pregnancy

Bleeding in pregnancy is a dramatic event and prevention is crucial for the benefit of mother and child. The platelet count in the mother during pregnancy is affected physiologically by dilution (increased maternal plasma volume), by sequestration of platelets in the spleen due to increased spleen size during pregnancy, and by pooling of platelets within the intervillous space of the placenta. Clinically more confusing is thrombocytopenia during pregnancy caused by the gestational thrombocytopenia (GT) which usually is benign and does not warrant further testing or special medical care. It can be concluded from clinical reports that almost all asymptomatic pregnancies with platelet counts between $100 \times 10^9/L$ and $150 \times 10^9/L$ are GT without increased risk of maternal or fetal bleeding complications. However, GT has to be differentiated from the clinically relevant immune thrombocytopenia, from preeclampsia and from the Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP) syndrome. GT with a platelet count less than $150 \times 10^9/L$ at time of delivery occurs frequently affecting 5–10% of uncomplicated pregnancies. A recent report by Reese et al. reported that platelet counts in all pregnant women including non-Hispanic white, non-Hispanic black, and Hispanic decrease from the first trimester until the time of delivery [7]. However, little is known about Asian and the article by Xu et al. [8] describes the time course of the platelet count in Chinese women during pregnancy.

MPV as a marker of hypercoagulable states

On the contrary, platelets are also involved in hypercoagulable states and platelet mass is regarded as a risk factor for certain thrombotic disorders. Different indices of platelets can be obtained in analogy to the red blood cell parameters such as the plateletcrit (PCT), the platelet distribution width (PCDW), and the MPV [9]. The MPV has received the widest application and seems to be able to give

a reliable estimate of the concentration resp. total volume of hyper-reactive, newly-formed platelets. Lippi et al. summarize the current knowledge about the role of the MPV in patients with different arterial and venous thrombotic disorders [10]. The MPV can be obtained easily and is a good surrogate marker for the platelet size: Since platelet number and volume/size may reflect a unfavorable hypercoagulable state, the observation that the MPV may be substantially increased in patients with acute episodes of coronary artery disease, with venous thromboembolism and with portal vein thrombosis, with stroke, with erectile dysfunction, and preeclampsia might lead to therapeutic pathways as well as modes of intervention for primary and secondary prevention. An increased MPV can also be an indicator of unfavorable outcome of thrombotic states. However, preanalytical issues such as the anticoagulant used, the timing of sample collection, and the sample storage conditions as well as differences within the analytics and calculations used and postanalytical issues such as the use of different reference range limits and diagnostic cutoff values obscure the comparability of studies using different techniques/analyzers currently.

Conclusions

In summary, in this special issue the articles scrutinize the challenges of testing for platelet diseases in a routine medical laboratory. The articles emphasize the need for highly standardized preanalytics to avoid artefacts as well as an unambiguous reporting of microscopic findings. In case of abnormal findings in the routine testing, the decision to submit the patient sample to a reference laboratory such as for in-depth platelet antibody testing has to be made in a timely manner and the selection of the therapy (i.e., immunomodulatory and/or by platelet transfusion) has to be made in very close connection with the local blood bank. In the last years, huge success has been made both in the diagnostics as well in the therapy of platelet disorders which allows to focus the medical efforts to the patients in need and to avoid collateral damage (overtreatment) in patients with physiological or self-limiting platelet disturbances.

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