Review

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The role of platelets in bleeding in patients with thrombocytopenia and hematological disease

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Abstract: This review evaluates the role of platelets in bleeding risk among patients with hematological disease and thrombocytopenia. Platelets are pivotal in primary hemostasis, and possess non-hemostatic properties involved in angiogenesis, tissue repair, inflammation and metastasis. Also, platelets safeguard vascular integrity in inflamed vessels. Overall, bleeding risk depends on the underlying disease, and patients with cancer and platelet count $<6-10 \times 10^9/L$ have a markedly increased bleeding risk, while the platelet count does not correlate with bleeding risk at higher platelet counts. Other factors might affect platelet properties and thus bleeding risk, for example, drugs, low hematocrit, coagulation system impairments or transfusion of dysfunctional donor platelets. For patients with leukemia and immune thrombocytopenia, reduced platelet activation, platelet aggregation, or thrombopoiesis, reflected by the reduced presence of reticulated platelets, are associated with bleeding phenotype. However, mechanistic insight into the cause of reduced platelet function in different thrombocytopenic conditions is sparse, except for some inherited platelet disorders. Promising tools for platelet function studies in thrombocytopenia are flow cytometry and biomarker studies on platelet constituents. An important message from this current paper is that bleeding risk assessment must be tailored to specific patient populations and cannot be applied broadly to all patients with thrombocytopenia.

Keywords: hemorrhage; neoplasms; platelet count; platelet function tests; thrombocytopenia.

Introduction

Platelets are pivotal in hemostasis [1]. Accordingly, patients with hematological disease and thrombocytopenia experience increased bleeding risk. To avoid bleeding, much effort has been put into prophylactic strategies, focusing mainly on the platelet count as a trigger for transfusion among patients with hematological cancers and thrombocytopenia [2]. However, the platelet count is not the only determinant of bleeding risk [3–5]. Thus, other factors contribute to the bleeding risk. In this regard, the platelet function might be relevant, as impaired platelet function would reduce the hemostatic capacity of platelets. The current literature suggests that impaired platelet function could be relevant for bleeding in thrombocytopenia; a relationship which has been particularly explored in hematological disease [6–10]. When interpreting the existing literature, it is, however, important to consider which methods have been used for the evaluation of platelet properties and platelet function, as some methods do not produce reliable results in samples with low platelet count.

The aim of the current review is to evaluate the role of platelet function in bleeding risk among patients with hematological disease.

Bleeding in hematological disease

In 1962, Gaydos et al. studied 92 patients with acute leukemia, and found an increasing bleeding risk with declining platelet count [11]. Others reported that bleeding was a contributing cause of death in 67% of cases with leukemia [12]. At that time, platelet transfusions were casuistically shown to treat bleeding in thrombocytopenia, as first reported by Duke in 1910 [13]. Platelet transfusions became widely available during the 1960s and 1970s and the platelet count became the main transfusion trigger.

While bleeding is frequent in hematological cancers with bone marrow failure [5], bleeding is thought to be rarer in thrombocytopenic conditions with high platelet turnover. Thus, bleeding was found in 14%, 6%, and 12% of admissions for thrombocytopenic purpura (TTP),

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heparin-induced thrombocytopenia (HIT) and immune thrombocytopenia (ITP), respectively [14]. In these conditions, the platelet count drops when platelets are consumed through accelerated coagulation processes; and platelet transfusions increase the thrombosis risk and the mortality [14].

It shall, however, be mentioned that the definition of bleeding differs in published studies, which could influence the reported incidences of bleeding in thrombocytopenia [15]. Also, bleeding is notoriously challenging to document correctly as some mucosal bleeds remain occult; further, there is a risk of bias because the patients' recollection and retelling of the episodes are subjective. Hence, standardization in methods for registration of bleeding is much needed [15].

The platelet count

The platelet count is used for monitoring thrombopoiesis; it exhibits large inter-individual variation, but is relatively stable in healthy individuals with an overall slight decline with age [16, 17]. Thrombocytopenia has been inconsistently defined in the literature as platelet count $<100 \times 10^9/L$ or $<150 \times 10^9/L$ [18, 19], and does not reflect the natural occurring age-, sex-, and ethnicity-dependent differences with a lower reference limit for platelet count around $125 \times 10^9/L$ for men and $150 \times 10^9/L$ for women with ethnical differences [17, 20]. Nevertheless, a consensus panel defined primary ITP as a platelet count $<100 \times 10^9/L$ in the absence of other causes or disorders that may be associated with thrombocytopenia [19]. Thereby a limit has been set for what should be regarded as a pathologically low platelet count and consequently should result in diagnostic considerations.

A reduced platelet count has in numerous studies been confirmed to be a bleeding risk factor [3, 4, 11, 21]. Spontaneous fecal blood loss was observed at platelet count $<10 \times 10^9/L$, and increased markedly when the platelet count was $<5 \times 10^9/L$ in a study among patients with aplastic anemia [22].

The platelet count is, however, not the only determinant for bleeding risk in thrombocytopenia. Two large studies in patients with hemato-oncological cancers congruently found that bleeding risk is increased at platelet counts $<80 \times 10^9/L$ with no clear pattern of decreasing risk with increasing platelet counts [3, 4]. Even severe thrombocytopenia does not *per se* equal bleeding, as major bleeding was observed in only 31% of days with a platelet count $<1 \times 10^9/L$ in patients with acute leukemia [11]. Hence, the current praxis of giving prophylactic transfusions at

a platelet count $<10 \times 10^9/L$ results in patient over-treatment. On the other hand, a no-prophylactic platelet transfusion strategy increases the overall risk of major bleeding [23, 24], but might be safe in patients undergoing autologous transplantations [23]. Thus, factors other than the platelet count contribute to bleeding risk in hematological disease.

Mechanistic insight into properties of platelets to prevent bleeding

Platelet plug formation and bleeding – classic hemostatic properties

Platelets are important in primary hemostasis as they adhere, spread, and aggregate at the site of vessel injury (Figure 1), which in the best case stops the bleed. In an injured vessel, platelets adhere to immobilized von Willebrand factor via platelet glycoprotein (GP)Iba [25, 26] and via collagen binding to $\alpha 2\beta 1$ and GPVI [27]. During this process, platelets become activated and release platelet granules, change shape, and activate the fibrinogen receptor GPIIb/IIIa [1]. Granule content, particularly ADP, rapidly activates a large numbers of platelets [1]. The shape change results in platelet spreading, which limits bleeding and provides a surface for platelet aggregation [25]. When platelets aggregate, they are linked through binding of ligands from plasma to the GPIIb/IIIa receptor. Fibrinogen is the primary ligand for the GPIIb/IIIa receptor, but several other ligands contribute to platelet aggregate formation particularly at high shear, including von Willebrand factor and fibronectin [28]. The major platelet activation pathways are via binding of collagen to the integrin $\alpha 2\beta 1$ and GPVI, thrombin via the protease-activated receptor (PAR)-1 and PAR-4 or ADP via the P2Y1 and P2Y12 receptors [25]. Vessel injury also engages the secondary hemostasis which leads to fibrin mesh formation that strengthens the clot [1, 25]. Via surface exposure of negatively charged phospholipid phosphatidylserine, platelets serve as an adhesion site for coagulation factors and enhance the efficiency of the coagulation reaction [1, 25].

Platelets and the vasculature – extended hemostatic properties

Evidence is mounting for several other platelet properties to be involved in angiogenesis, tissue repair, inflammation, and metastasis of cancer. However, these so-called

Figure 1: The platelet properties that prevent bleeding.

Platelets prevent bleeding via an effect on the vasculature and form plug to stop bleeding in case of injury (1) platelet plug formation – classic hemostasis: in an injured vessel, platelets adhere to immobilized von Willebrand factor via platelet glycoprotein (GP)Iba and via collagen binding to $\alpha 2\beta 1$ and GPIV. Platelets are activated and release their granules, change shape and activate the fibrinogen receptor GPIIb/IIIa. Granule content rapidly activates large numbers of platelets. The shape change results in platelets spreading, which not only limits bleeding but also provides a surface for platelet aggregation. When platelets aggregate, they are linked through binding of ligands from plasma to the GPIIb/IIIa receptor; (2) vascular integrity – extended hemostasis: platelets close gaps in the endothelium. Platelets secrete granules that contain proteins that regulate angiogenesis, wound healing and remodeling, immune cell recruitment and inflammation. These factors may regulate the vessel stability. The receptors glycoprotein VI and C-type lectin receptor 2 (CLEC-2) are critical in inflammatory hemostasis.

‘non-hemostatic’ properties appear to be relevant for the prevention of bleeding and a more fitting terminology would therefore be non-classic hemostatic properties or extended hemostatic properties. Circulating platelets support the barrier function of the resting endothelium by mechanically filling the gaps in the endothelial lining [29] (Figure 1). This is in accordance with the clinical observation that spontaneous blood loss in stool increases considerably with platelet counts $<5-10 \times 10^9/L$ in patients with aplastic anemia [22].

In mice, thrombocytopenic bleeding typically occurs in areas with pre-existing inflammation which points to the role of platelets in vascular integrity during inflammation [30, 31]. In a study with transgenic mice, the receptors glycoprotein VI and C-type lectin receptor 2 (CLEC-2) were critical in hemostasis in the inflamed skin and lung [32]. GPVI receptors have only a minor impact on hemostasis under normal circumstances in humans, as the lack of GPVI results in only a mild bleeding phenotype [33]. Further, a lack of platelet granules induced intracerebral bleeding in mice with ischemic brain

infarction, but did not result in bleeding in experimental inflamed skin or lung models [31]. Thus, the platelet involvement in inflammatory hemostasis appears to be tissue-specific.

Interestingly, platelet granule secretion also prevented thrombocytopenia-induced tumor bleeding [34]. This is related to the content of platelet granules, including regulators of tissue repair, immune responses, and vasoconstriction that are differentially and selectively secreted in an active manner [1, 35]. Platelets constituents either originate from the megakaryocytes, are synthesized *de novo*, or are taken up from the platelets’ surroundings. Albeit platelets are anucleate, they express RNAs and are able to translate mRNAs into proteins. Further, they are capable of intercellular transferring of microparticles and RNAs, which might regulate cellular functions [36]. Moreover, the processes by which platelets maintain vascular integrity at the site of inflammation appears to be independent from the ability to form clots [30, 32]. These important observations permit studies of individualized treatment based on the clinical disease spectrum.

Why do patients with hematological disease have impaired platelet function?

For some inherited platelet disorders there is a well-described association between the molecular defects and the phenotype. In inherited platelet disorders, specific genetic mutations result in changes in platelet composition; some of which are associated with increased bleeding risk [37]. For example, absence of platelet α -granules is observed in cases with mutations in *NBEAL2* and is characterized by a mild to moderate bleeding phenotype [38]. Other categories of patients with thrombocytopenia might experience impaired platelet function, which has primarily been investigated among patients with acute myeloid leukemia (AML) and ITP, but mechanistic insights into the nature of these acquired platelet function defects are sparser. A brief overview of platelet function in hematological disease is shown in Table 1.

Whether platelet function defects are mainly introduced by mechanisms related to the underlying disease, remains to be established. However, other blood constituents such as drugs, coagulation defects, changes in red blood cells, and platelet transfusions might also impair platelet function in thrombocytopenia. For example, up to 45% of admitted patients with thrombocytopenia receive antiplatelet or anticoagulant therapy [46].

Further, other factors influence the overall hemostatic capacity of platelets and contribute to bleeding risk. Impairments in the coagulation system could decrease the thrombin generation potential and thus reduce the

stimulus for platelet activation *in vivo*, while an induction of fibrinolysis would reduce clot strength [47]. It may be speculated that a compensatory rise in von Willebrand factor to some extent could compensate for the platelet functional defect. Red blood cells influence platelet aggregation capacity *in vitro* [48]. While studies are lacking, it is important to keep in mind that the matrix for platelets influences the test result when choosing a platelet function test.

The rheological properties of blood are important for limiting bleeding *in vivo*, but is not evaluated in any available method. To execute their hemostatic function, platelets must be placed close to the vessel wall. During flow, red blood cells cause marginalization of platelets and thus increase the platelet concentration at the site of action. Accordingly, low hematocrit is a risk factor for bleeding in thrombocytopenia [4, 46, 49].

Thrombocytopenic patients might receive platelet transfusion and it is evident that *in vitro* platelet function declines during storage of platelet concentrates for transfusion [50]. Thus, it is a concern whether variations in platelet function of platelet concentrates affect bleeding risk in recipients with thrombocytopenia. Unfortunately, no model accurately predicts the interplay between donor and patient platelets or the hemostatic effects of transfused platelets [50, 51]. A recent study found that transfused platelets are not cleared from the bloodstream dependent on the platelet function *in vitro* [52]. While this area remains to be fully studied due to methodological limitations of platelet aggregation tests during thrombocytopenia, it would be very relevant for the interpretation of recent studies of bleeding in thrombocytopenia.

Table 1: Platelet function in hematological disease.

Disease	Platelet function
Acute myeloid leukemia	<ul style="list-style-type: none">– Reduced platelet count and IPF– Reduced platelet activation determined as the percentage of platelets positive for or the expression of activated GPIIb/IIIa, CD63, and P-selectin before and after stimulation with TRAP, collagen-related peptide and ADP [6, 39, 40]– Reduced GPIb expression [6, 39, 40]– Reduced platelet aggregation with ADP, TRAP and collagen-related peptide, associated with bleeding [6, 10]
Immune thrombocytopenia (ITP)	<ul style="list-style-type: none">– Low platelet count and high IPF, reduced adhesion and clot strength with viscoelastic methods [41–43]– Reduced platelet activation as determined either as the percentage of positive platelets or the expression of platelet activated GPIIb/IIIa and P-selectin was found with TRAP, and convulxin while data on ADP conflicts [7, 8, 44]– Higher GPIb after stimulation with TRAP and ADP than normal [44] and high GPIb is associated with bleeding [8]– Reduced flow cytometric platelet aggregation with phorbol myristate acetate (PMA) in patients with severe bleeding symptoms [7]
Others hematological diseases	<ul style="list-style-type: none">– Lack of specific platelet receptors or properties is diagnostic for various inherited platelet disorders, e.g. lack of functional GPIIb/IIIa is seen in Glanzmann’s thrombasthenia [37, 45]– Specific knowledge is lacking in other hematological diseases

ADP, adenosine diphosphate; GP, glycoprotein; IPF, immature platelet fraction; TRAP, thrombin-receptor activating peptide.

Current methodology and clinical studies of platelet function in patients with thrombocytopenia and hematological disease

Platelet indices

Platelet size, such as the mean platelet volume (MPV), might reflect functional platelet properties as large

platelets exhibit higher platelet aggregation capacity *in vitro* than small platelets [53]. One study from 1982 proposed that low MPV identified bleeding in patients with hematological disease [54]. However, platelet size measurements have methodological limitations which restrict their use [9, 55] (Table 2) and newer studies were unable to show that MPV is a bleeding risk marker [7–9].

Immature platelets might be more hemostatically active than older platelets [69]. As immature, newly released platelets are rich in mRNA, they can be identified with fluorescence labeling [56]. Immature platelet fraction

Table 2: Methods for measuring platelet characteristics and function with focus on technical and clinical aspects.

Method	Technical aspects	Clinical aspects
Platelet count [3, 56]	Available on automated hematological equipment	Low platelet count is associated with bleeding. However, the platelet count does not correlate with bleeding risk
Platelet size, e.g. MPV [9, 55]	Available on automated hematological equipment. Size enlarges during sample storage, lack of standardization, cannot be obtained in up to 64% of samples, inaccurate if platelets are extraordinarily small or large	Not a suitable clinical marker due to technical limitations
IPF [41, 56, 57]	Limited availability and lack of standardization	Reflect thrombopoiesis and aids determining the cause of thrombocytopenia. Reduced IPF associated with bleeding as small platelets are less hemostatically active
Platelet content [58]	Isolated platelets can be obtained from centrifugation or gel filtration. Various secretion assays exist that determine the extent of granule release. Granule content can be evaluated after platelet activation. No common standards exists	A potential source of novel biomarkers, but studies are lacking. Platelet secretion assays can aid diagnosing inherited platelet disorders
Platelet adhesion, e.g. Cone-and-Plate[let] analyzer [43, 59]	Limited access to commercial tests	Reduced adhesion associated with bleeding risk as clot formation at site of vascular damage is reduced
Platelet aggregometry [60–62]	(1) LTA is the gold standard, but limited at a platelet count $<150 \times 10^9/L$, up-concentration is possible but requires a large blood volume and might affect platelet function. Time consuming and requires experienced operators (2) Whole blood impedance aggregometry, e.g. Multiplate is easy to operate. Applicable but results are influenced by low platelet counts. Large CV%	<i>In vivo</i> , reduced platelet aggregation is associated with a lack of clot formation and bleeding. A lack of studies on Multiplate and bleeding at low platelet counts. Large CV% makes Multiplate less likely to be useful for decision making. LTA is not recommended
Flow cytometry [6, 63–65]	With use of fluorescently labelled antibodies and fluorescent dyes, single cells are evaluated independent of the platelet count. Detailed phenotyping possible. Receptor expression, platelet activation and platelet aggregation can be evaluated. Technically difficult and not widely available. Lack of standardization	Lack of receptors might indicate functional problems and can be diagnostic for inherited platelet disorders. Reduced platelet activation and aggregation capacity is associated with bleeding
Platelet function analyzer (100/200) [66]	Global approach reflecting the interplay between platelets and other components in hemostasis – affected results might have multiple causes. Not applicable to samples with a platelet count $<50 \times 10^9/L$	Not recommended for testing platelet function in thrombocytopenia
Viscoelastic methods [41, 42, 67, 68]	Global approach reflecting the interplay between platelets and other components in hemostasis. Clot strength is dependent on the platelet count	Low clot strength associated with bleeding. The independent value for bleeding risk assessment unknown as it might reflect low platelet count

CV, coefficient of variation; IPF, immature platelet fraction; LTA, light transmission aggregometry; MPV, mean platelet volume.

(IPF, the percentage of immature platelets) is dependent on the cause of thrombocytopenia. A high IPF suggests increased thrombopoiesis, which is seen in conditions with peripheral platelet consumption or destruction, such as ITP or TTP [57]. In bone marrow failure, IPF is low and a rise may predict platelet recovery, for example, following hematopoietic progenitor cell transplantation [56]. Low IPF was associated with bleeding phenotype in ITP [8, 41, 57]; but only when platelet count was low. A recent study found that the absolute immature platelet number was lower in ITP patients with episodes of severe bleeding than in those without, despite similar platelet counts [57]. In patients with hematological cancer and a platelet count $\leq 50 \times 10^9/L$, a low absolute immature platelet number was associated with bleeding within the next day [9], while IPF did not identify bleeding risk in patients with cancer and thrombocytopenia in another study [46].

Global tests

The attempt of using global whole blood tests to mimic the *in vivo* hemostatic process is theoretically advantageous. In global assays, platelets are more or less indirectly activated dependent on the interplay of multiple hemostatic processes. Viscoelastic methods are global approaches which have gained ground in the monitoring of acute critical bleeding. It facilitates the timely and appropriate use of transfusions during acute critical bleeding [67]. Among other parameters, the clot strength is measured, a parameter that depends on the platelet count [67]. Low clot strength using viscoelastic methods identified the bleeding phenotype in severe thrombocytopenia of various causes [68] and in patients with ITP [41, 42]. Although these methods might be less sensitive for platelet function defects, Estcourt et al. found that results from viscoelastic methods predicted bleeding among patients with hematological cancer and thrombocytopenia, after adjustment for the platelet count [9]. Other currently available global tests are not useful for thrombocytopenic samples. The Platelet function Analyzer-100® (PFA-100®, Siemens Healthcare Diagnostics GmbH, Marburg, Germany) is not applicable to samples with a platelet count $< 50 \times 10^9/L$ as the low platelet count prolongs the blood clotting time, which often rises above the upper measurement range [66]. Bleeding time *ad modum* Ivy is no longer recommended [60].

Platelet adhesion

Platelet adhesion is pivotal for both platelet plug formation and relevant for the extended hemostatic effects of

platelets. Reduced platelet adhesion was associated with bleeding tendency in patients with thrombocytopenia [43, 59] and correlated with the severity of bleeding symptoms in ITP [59]. These studies used the Cone-and-Plate[let] analyzer (Impact-R™, DANED SA, Belgium) which is a commercially available platelet adhesion test. More clinical studies into platelet adhesion during thrombocytopenia would be relevant for insights into the disease mechanisms and could potentially reveal markers of bleeding as platelet adhesion is a critical process in hemostasis. However, the field is limited by the lack of commercially available easy operable tests that specifically reflect platelet adhesion.

Platelet aggregation

Platelet aggregation testing with light transmission aggregometry (LTA) remains the *gold standard* for platelet function testing and has been widely used [60, 61]. It is, however, important to be aware that the method requires a platelet count on at least $150 \times 10^9/L$ [61]. Nevertheless, LTA and PFA-100® identified bleeding phenotype in persons with ITP and normal platelet counts [70]. Lumiaggregometry, an extension of the classical light transmission aggregometer, might be more sensitive to reduced platelet function at low platelet counts than LTA [71], but studies of bleeding risk in thrombocytopenia are lacking. The Multiplate® Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) is an impedance aggregometer that evaluates platelet aggregation in whole blood. Technically, the method can be used with samples with severely reduced platelet counts [62, 72], but its results are significantly reduced at platelet counts $< 150 \times 10^9/L$ [72], which, however, does not exclude a relationship with the bleeding phenotype. Studies among hematological diseases are lacking.

Flow cytometry

Importantly, the evidence is mounting for the use of flow cytometry to study platelet function. By flow cytometry each cell is characterized separately, and flow cytometry is thus applicable regardless the patient's platelet count [63, 65, 73]. The method has been widely used to study expression levels of surface receptors and to provide information about the platelet activation level in unstimulated and stimulated samples [65, 73, 74]. The platelet activation marker P-selectin is an alpha-granule-membrane integrated receptor that is incorporated into the cell surface upon platelet activation [65, 73]. Platelet surface granulophysin (CD63)

expression represents dense granule release and the expression of the activated GPIIb/IIIa receptor or fibrinogen binding to the receptor is a measure of the capacity for platelet aggregation. Platelet reactivity or activation capacity is reported as the change in expression of these platelet activation markers when platelets are stimulated *in vitro* with agonists [65]. Evaluation of Annexin V has been used as a tool to study apoptosis [75]. Recently, it was further proposed that also platelet adhesion [64] and platelet aggregation, independent of the platelet count, could be measured with flow cytometry [63, 76].

Flow cytometry has been established as a part of the investigation for inherited platelet disorders, some of which are associated with bleeding risk [37]. Patients with ITP and a positive bleeding phenotype had higher levels of spontaneous platelet activation (platelet activation in unstimulated samples) [8], reduced platelet reactivity in terms of lower P-selectin expression [7, 8, 77], and low activated GPIIb/IIIa expression after platelet stimulation than ITP patients without bleeding [8, 77]. Platelet function in ITP was consistent over time and was associated with both concurrent and subsequent bleeding severity [78]. Congruently, ITP patients with bleeding phenotype were shown to have decreased flow cytometric platelet aggregation [7]. However, a recent study showed that eltrombopag, a drug used for raising the platelet count, only had a minor impact on platelet function in ITP [44]. Overall, platelet activation capacity is higher in thrombocytopenic patients with ITP than AML [77].

For thrombocytopenic patients with AML or myelodysplastic syndrome, platelet activation capacity was lower than in healthy individuals and there was an association between reduced platelet activation and bleeding [6, 39]. In one study, a preserved platelet aggregation response by flow cytometry identified all patients without bleeding phenotype [6]. In contrast, others found higher levels of activated fibrinogen receptor (GPIIb/IIIa) levels and P-selectin in stimulated samples in patients with AML with bleeding than without bleeding. Lower platelet activation capacity (low P-selectin expression in stimulated samples) was correlated with bleeding scores in patients with hematological cancers of whom the majority had thrombocytopenia [79]. Thus, flow cytometry is proving to be useful for studying the relationship between platelet function and bleeding in thrombocytopenia, as well as for achieving more detailed knowledge regarding the role of platelets in disease. It may also be valuable for studying extended hemostatic properties of platelets in vascular integrity. However, the method is not standardized, and the protocols are often laborious to perform, which limits their applicability, not least in urgent situations.

The protocols are also often specific for a certain condition, meaning that the test is not universally suitable as a biomarker.

Platelet content

Finally, it is noteworthy that platelets can be isolated from whole blood by centrifugation or gel-filtration. This permits evaluation of platelet constituents in platelet lysate [58]. It has been applied for studying granule release [58] and might be used to evaluate whether other platelet constituents, such as growth factors and immune mediators, influence bleeding risk in thrombocytopenia. While studies in animals, point to the relevance of platelet constituents in bleeding, human clinical studies in thrombocytopenia are lacking. Especially, information about granule constituents could be important for understanding bleeding at sites of inflammation.

Thus, several platelet function tests exist, however, most methods are limited in thrombocytopenia as the results are influenced by the low platelet count. Flow cytometry seems particularly promising for providing novel bleeding risk markers. Potentially, other relevant biomarkers can be established through studies on platelet composition and platelet adhesion.

Perspectives for future research

Future research should focus on establishing novel biomarkers of bleeding. In this regard the evaluation with flow cytometry and determination of platelet content could be helpful for research purposes. For clinical decision making, further development is, however, needed to make tests applicable for widespread use. That is, they should be affordable, easy to perform, and interpret. For currently available markers, several tests are promising, but standardization is required in order to make results from published studies comparable.

Conclusions

Platelet function varies in patients with thrombocytopenia and might identify bleeding risk. Mechanistic insight into the cause of reduced platelet function is sparse, except for some inherited platelet disorders. For the study of platelet function in thrombocytopenia, flow cytometry is a promising tool, while evaluation of platelet constituents and platelet adhesion could be relevant. An important

message from the current paper is that the determination of bleeding risk must be tailored to specific patient populations and cannot be applied broadly to all patients with thrombocytopenia. In this regard, for example, a reliable *in vitro* model for prediction of effect of platelet transfusion would be helpful.

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