Platelet populations and priming in hematological diseases

Blood Reviews 31 (2017) 389–399

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

Review

Platelet populations and priming in hematological diseases

Constance C.F.M.J. Baaten[a], Hugo ten Cate[a,b], Paola E.J. van der Meijden[a,*,1], Johan W.M. Heemskerk[a,*,1]

[a] Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands
[b] Internal Medicine, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

ARTICLE INFO

Keywords:
Activation markers
Platelet heterogeneity
Positive priming
Thrombus

ABSTRACT

In healthy subjects and patients with hematological diseases, platelet populations can be distinguished with different response spectra in hemostatic and vascular processes. These populations partly overlap, and are less distinct than those of leukocytes. The platelet heterogeneity is linked to structural properties, and is enforced by inequalities in the environment. Contributing factors are variability between megakaryocytes, platelet ageing, and positive or negative priming of platelets during their time in circulation. Within a hemostatic plug or thrombus, platelet heterogeneity is enhanced by unequal exposure to agonists, with populations of contracted platelets in the thrombus core, discoid platelets at the thrombus surface, patches of ballooned and procoagulant platelets forming thrombin, and coated platelets binding fibrin. Several pathophysiological hematological conditions can positively or negatively prime the responsiveness of platelet populations. As a consequence, *in vivo* and *in vitro* markers of platelet activation can differ in thrombotic and hematological disorders.

1. Introduction

Individual platelets interact in numerous ways with the vessel wall or adherent blood cells. This versatility is fundamental to the role of platelets in a wide range of (patho)physiological processes, ranging from vascular repair, hemostasis and thrombosis, to inflammation progression, innate immunity and tumor metastasis. In the past years, evidence has been accumulating that circulating platelets are markedly heterogeneous in properties, which has led to the suggestion that identifiable populations of platelets with specialized response spectra are best suited for specific roles. In the present paper, we resume the current evidence for heterogeneity in terms of composition and functions of platelets during their formation from megakaryocytes, when circulating over time in the circulation, and once adhered to a vessel wall. We further describe how various physiological and pathophysiological conditions can change or prime the responsiveness of circulating platelets, and hence alter the distribution of platelet populations. We finally define how *in vitro* and *in vivo* markers of platelet activation phenotypes can be judged in relation to thrombotic and hematological disorders.

2. Intrinsic factors of platelet heterogeneity

Platelets from a given subject, healthy or diseased, greatly vary in receptor expression levels and markedly diverge in responsiveness once activated. Different populations of activated platelets can be distinguished, which can differently interact with the inflamed or injured vessel wall and differently support hematological processes. This heterogeneity is explained by several intrinsic factors, including variability of clonal megakaryocytes, unequal division of megakaryocyte-derived proplatelets, and modifications upon ageing of the newly formed platelets.

2.1. Heterogeneity between megakaryocytes and platelets

Heterogeneity between megakaryocytes, whether or not linked to a specific niche in the bone marrow or lungs, is a likely cause of inter-platelet variability, although there is only limited literature available on this subject. Several authors have described that polyploid megakaryocytes, either cultured from $CD34^+$ hematopoietic stem cells or derived from immortalized cell lines, considerably differ from cell to cell, in terms of levels of cytoplasmic and membrane proteins, as well as in agonist-induced Ca^{2+} transients [1–3]. Recently, this was demonstrated also for megakaryocytes derived from single-cell clones of

* Corresponding authors at: PO Box 616, 6200 MD Maastricht, The Netherlands.
E-mail addresses: c.baaten@maastrichtuniversity.nl (C.C.F.M.J. Baaten), h.tencate@maastrichtuniversity.nl (H. ten Cate), p.vandermeijden@maastrichtuniversity.nl (P.E.J. van der Meijden), jwm.heemskerk@maastrichtuniversity.nl (J.W.M. Heemskerk).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.blre.2017.07.004

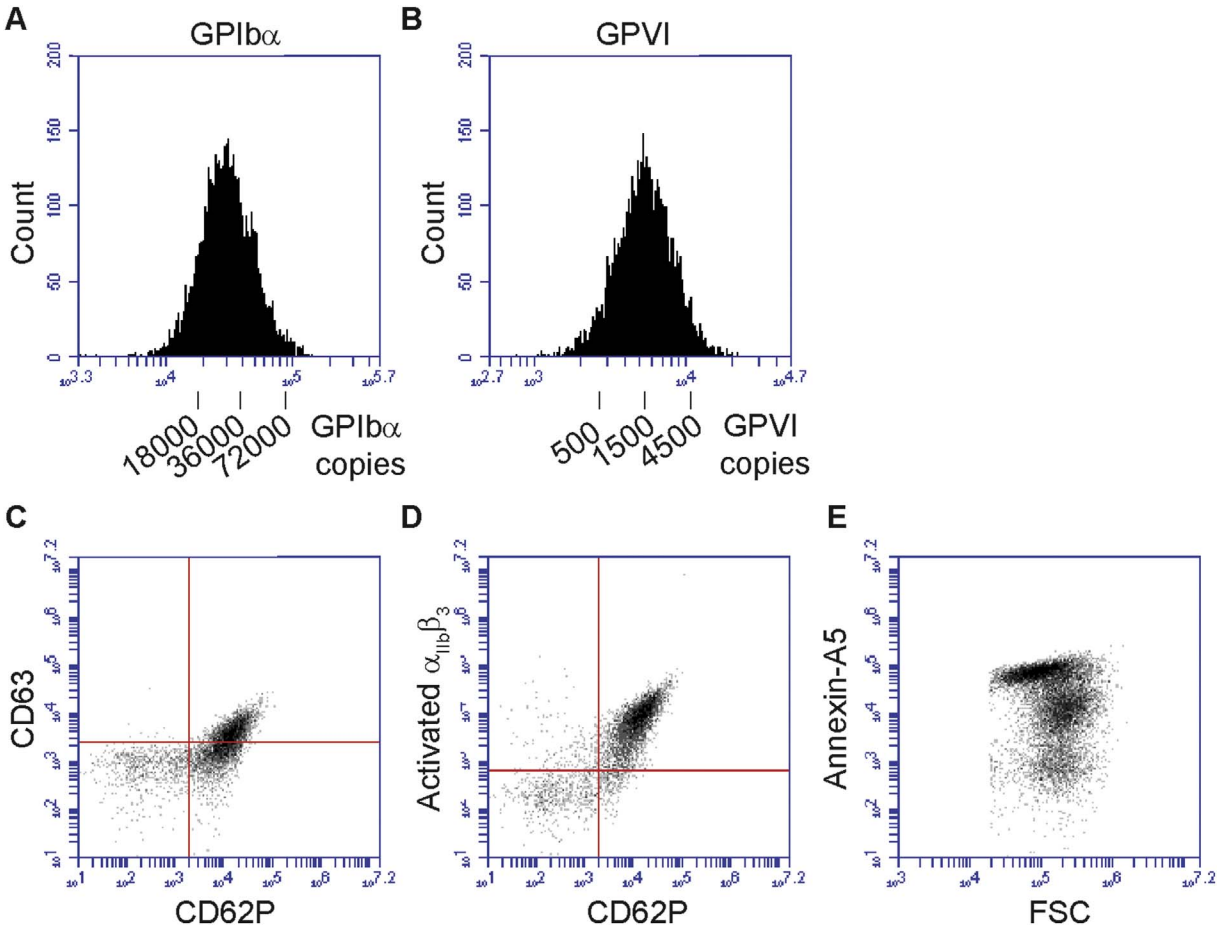

Fig. 1. Intrinsic heterogeneity in platelet composition and functions.
(A, B) Differential expression of key adhesive receptors in the total platelet population, immunologically stained for GPIbα or GPVI. Indicated in the flow cytometric histograms are the estimated copy numbers, based on proteome analysis [128]. (C, D) Platelets stimulated with a GPVI agonist immunologically stained for CD62P in combination with CD63; or CD62P in combination with a marker for activated integrin $\alpha_{IIb}\beta_3$. Shown is the presence of different platelet populations with alpha granule secretion ($CD62P^+$) with or without dense granule secretion or $\alpha_{IIb}\beta_3$ activation. (E) Two apoptotic platelet populations after stimulation with BH3 mimetic ABT-737, displaying low or high phosphatidylserine (PS) exposure.

forward-programmed human pluripotent stem cells. After re-programming, individual megakaryocytes of the same clone showed a 100-fold difference in expression levels of common receptor proteins, such as glycoprotein (GP)Ibα, GPVI and integrin $\alpha_{IIb}\beta_3$, as assessed by flow cytometry [4]. No studies are yet available to demonstrate whether distinct megakaryocytes (in the bone marrow) also yield different types of platelets. However, one can expect that single platelets 'inherit' at least in part their expression profiles of receptors and other signaling molecules from their precursor megakaryocyte. It has been shown that when the environment of megakaryocytes changes (*e.g.* upon inflammation or diabetes), the transcriptome of platelets is influenced as well [5,6]. For instance, patients diagnosed with the autoimmune disease systemic lupus erythematosus (SLE) may have an altered platelet transcriptome. The changes in platelet mRNA and protein levels were linked to increased procoagulant activity and platelet-monocyte interactions in SLE patients [7].

Proplatelets are formed from the demarcation compartment of megakaryocytes by pinching off the cytosol containing cell membrane compartment and organelles [8]. Although studies are lacking, it can be conceived that part of the heterogeneity between platelets may come from unequal retention or re-distribution of the cellular components from the mother cell, including surface receptors, actin and tubulin cytoskeletons, signalosomes, internal membrane vesicles, secretory granules, ribosomes, and mitochondria.

Clear evidence for heterogeneity in platelet size and volume comes from flow cytometric profiles and blood count histograms. Forward side scatter plots (flow cytometry) point to a considerable variation in size, which is in agreement with the relative large distribution width of the platelet volume (blood cell count). Sizable heterogeneity between platelets is also detectable by quantifying the abundantly expressed receptors. Thus, flow cytometric profiles of platelets that are stained with fluorescent antibodies against integrin β_3 (CD61) point to a large inter-platelet variation in expression levels of this integrin [9,10]. Similarly, staining for GPIbα or GPVI gives histograms, which show an about 10-fold difference in fluorescence intensity (indicative of expression level) of platelets in the lower and upper 10% percentiles (Fig. 1A–B). Evidence for signaling heterogeneity comes from recordings of the Ca^{2+} responses of single platelets from an arbitrary donor, which appear to vary greatly in the type of transient Ca^{2+} fluxes after stimulation *via* ADP, thrombin or collagen receptors [11].

2.2. Populations of activated platelets

Platelets, upon activation by agonists, are known to form populations with different surface properties [10]. For instance, platelets stimulated with a maximal dose of a GPVI agonist showing CD62P expression (marker of α-granule secretion) only display CD63 expression to a certain degree (marker of δ-granule and lysosome release), while the majority of platelets that express CD62P do have activated integrin $\alpha_{IIb}\beta_3$ (Fig. 1C–D). Inter-platelet heterogeneity in granule release has also been observed by electron microscopy [12]. Thus, secretion of granule content can occur in two different ways: single granule

secretion (both α and δ granules), or secretion of multiple granules fused into one large compartment (mainly α granules) [12]. Dependent on the strength of an activation trigger, secretion of the granule content appears to be regulated differently and can result in different secretion patterns (Fig. 1C–D) [12,13]. Another consistent observation is that, regardless of the agonist used, a fraction of 10–20% of the platelets fails to stain for CD62P [14], suggesting that some platelets are refractory to α-granule secretion.

A striking heterogeneity is observed when platelets in suspension are stimulated with combinations of collagen and thrombin receptor agonists. In addition to a population showing integrin $\alpha_{IIb}\beta_3$ activation and granule secretion, another population of swollen platelets appears with exposed phosphatidylserine at their outer membrane surface, inactivated/closed integrins, and capability of coagulation factor binding [15,16]. Depending on the activating conditions, the majority of the latter platelets also have a transglutaminase-dependent fibrin coat at their surface [17], which is in agreement with the early description of coated or COAT platelets [18]. As described below, the various platelet populations are considered to have different roles, in supporting platelet aggregation, procoagulant activity and fibrin formation. The formation of phosphatidylserine-exposing, procoagulant platelets is linked to a prolonged, high cytosolic Ca^{2+} level [19,20] *i.e.* a condition required for swelling and phospholipid scrambling *via* the Ca^{2+}-dependent anoctamin-6 channel (gene *ANO6* or *TMEM16F*) [21,22]. It can be speculated that, in single platelets, differences in both receptor expression levels and in activity of the Ca^{2+}-flux machinery determine the formation of the platelet populations.

Populations of platelets with different surface properties are also formed after stimulation with the proapoptotic BH3 mimetic ABT-737 [23]. Typically, platelets with low and high phosphatidylserine exposure are formed in this case (Fig. 1E).

2.3. Changes with platelet maturation, ageing and apoptosis

Early studies supposed that platelet activity correlates with platelet size and, hence, receptor and granule numbers [24,25]. Later papers have focused on platelet age, considering that newly formed platelets are larger in size and therefore more active than older platelets [26,27]. However, there is still not much support for the idea that platelet size is the only or main factor determining platelet responsiveness to agonists. Association studies do not suggest such a relation. For instance, in a cohort of healthy individuals, mean platelet volume negatively correlated with the amount of newly formed platelets, suggesting that young platelets are not necessarily the largest ones [28].

A consistent finding is that newly formed platelets are enriched in mRNA, as observed by specific mRNA-staining and cell counting analyses [29]. Young platelets with detectable mRNA in the cytoplasm have been termed as reticulated platelets [30,31] or immature platelet fraction [32]. The latter fraction, with a normal reference range of 1–5%, is considered to reflect the rate of platelet production in the bone marrow. Given that platelets have overall lost > 90% of the mRNA of their progenitor cells - while retaining the stable circular RNAs - [33], it is clear that the residual mRNA levels in reticulated platelets reflect incomplete mRNA maturation. Interestingly, subject age does not seem to be an important modifier of reticulated platelets, as in older subjects only ~2% of all > 6000 identified mRNA and miRNA species were found to be changed in expression levels [34]. On the other hand, a Spanish hemophilia study showed that the reticulated platelet fraction has a high heritability component [35]. Recent papers describe that certain combinations of mRNA and miRNA correlate with platelet phenotype, raising the possibility to use these RNA species as biomarkers of platelet function [36,37].

With a circulation time of 7–10 days and a remarkably constant platelet count over time, the daily turnover (production and removal) of platelets is extremely well regulated, being estimated at about 10^{11} platelets per day [8,38]. Platelet removal from the circulation occurs *via* both clearance and "consumption" (at the enormous vascular bed in the human body). Clearance can be triggered by prior platelet activation and an apoptotic process, *e.g.* resulting in the surface exposure of PS [39], or by other still unknown causes.

Evidence for a link between platelet ageing and apoptosis comes from experiments with mice, lacking the pro-apoptotic proteins Bax/Bak, in which platelet survival is greatly extended [40]. Because aged, Bak-deficient platelets showed a reduced capability of thrombin- and collagen-induced secretion, these were designated as exhausted platelets [41]. On the other hand, in mice lacking the anti-apoptotic protein Bcl-xL, platelet lifetime was shortened and platelet function was also reduced [40]. The interpretation was that a loss of Bcl-xL leads to activation of Bax/Bak to induce a pro-apoptotic condition. In patients with malignancies, who were treated with the Bcl-xL-degrading drug Navitoclax (a BH-3 mimetic also known as ABT-263), platelet lifetime was also shortened, thus resulting in mild thrombocytopenia [39]. Characteristics of apoptotic platelets are mitochondrial dysfunction, caspase proteolytic activity, refractoriness to integrin activation, and surface exposure of phosphatidylserine [42,43].

Another finding was that aged platelets gradually loose sialic acid residues from their surface membrane glycoproteins [38]. Desialylated platelets were found to be recognized by Ashwell-Morell receptors in the liver, mediating platelet clearance and, interestingly, also mediating release of thrombopoietin, which is the main cytokine regulating platelet formation from megakaryocytes [44]. These data are suggestive for a gradual, ongoing formation of the population of (pro)apoptotic platelets.

3. Environmental factors of platelet heterogeneity

Platelets can interact with a large variety of glycoproteins in the inflamed or damaged vessel wall. The adhesive interactions include: activated endothelial cells expressing von Willebrand factor (VWF); subendothelial matrix components such as laminins, collagens, thrombospondins; adhered plasma proteins, such as VWF, fibrin(ogen), fibronectin and vitronectin; leukocytes under inflammatory conditions; lymphatic wall components like podoplanin; tumor cells; and other platelets *via* fibrinogen bridges. Given that these adhesive ligands all induce different signaling pathways, the response of an adhered platelet will vary with the precise location and substrate of adhesion. Well studied is the environmental directed heterogeneity in responses of platelets adhering to collagen and assembling into a thrombus [10,16]. The formation of platelet populations in thrombus formation is further stimulated by the local generation of thrombin [45,46].

3.1. Platelet heterogeneity upon adhesion and activation

Platelets will respond differently, when interacting with specific vascular wall-derived proteins. This response heterogeneity is studied most extensively for platelets adhering to fibrillar collagen *via* the receptors GPVI and integrin $\alpha_2\beta_1$ [16,47]. Both receptors act in synergy to immobilize and thereby activate platelets *via* the GPVI signalosome, resulting in Ca^{2+} elevation, integrin activation and granular secretion [48,49]. Collagen fibrils allowing platelet adhesion to both receptors are most strongly activating, and form the largest platelet aggregates. A population of the collagen-adhered platelets – subjected to the highest Ca^{2+} fluxes –, responds by surface exposure of procoagulant phosphatidylserine [50]. This so-called procoagulant response is accomplished *via* the ion channel anoctamin-6 [22], and accompanied by marked morphological changes involving swelling, bleb and balloon formation [51,52]. Phosphatidylserine exposure facilitates the binding of multiple coagulation factors and promotes thrombin generation [19,20]. Remarkably, the majority of collagen-bound platelets is refrained from phosphatidylserine exposure and swelling.

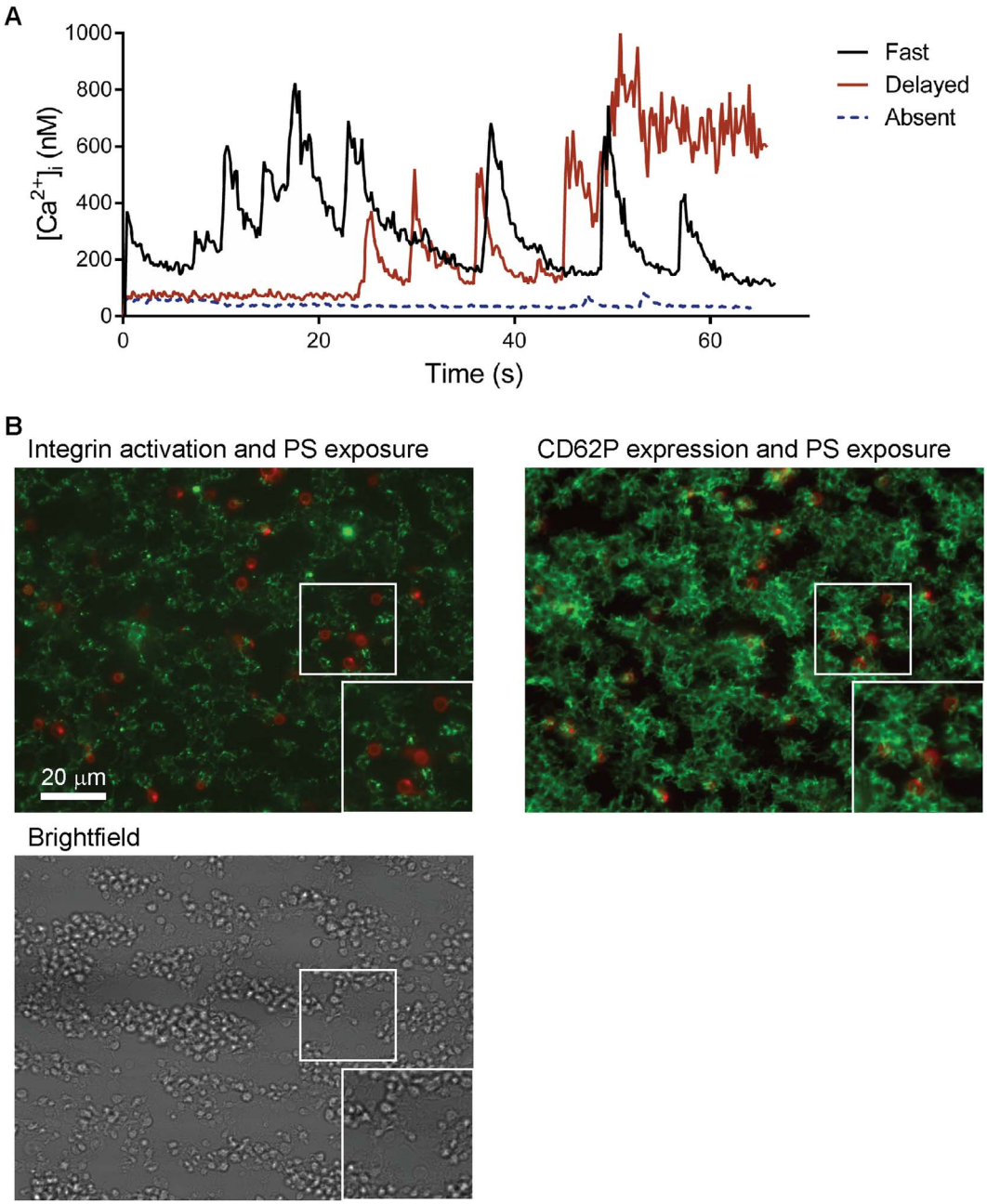

Fig. 2. Heterogeneity of platelet responses.
(A) Distinct calcium fluxes of single platelets adhered under flow to collagen. Based on the time between adhesion and increase in calcium signal, platelets were classified as having a fast, delayed or absent activation response. (B) Platelet populations in thrombi formed on collagen: aggregating platelets characterized by integrin activation and CD62P expression (green) and procoagulant platelets forming blebs/balloons and exposing PS (red).

3.2. Platelet heterogeneity within a thrombus

Heterogeneity in platelet activation properties progresses during collagen-dependent thrombus formation in flowing blood, both *in vitro* and *in vivo*. At high, arterial wall-shear rates, platelet adhesion to collagen/VWF is accomplished *via* GPVI, integrin $\alpha_2\beta_1$ and the VWF receptor, GPIb-V-IX [53,54]. Single-cell studies of adhered platelets from flowing blood again show marked differences in Ca^{2+} fluxes (Fig. 2A) [55]. Thrombi formed under high-shear flow show clusters of aggregated platelets (staining for activated integrin $\alpha_{IIb}\beta_3$ and P-selectin), alternated with patches of ballooned phosphatidylserine-exposing platelets (binding annexin A5 and coagulation factors) (Fig. 2B) [56,57]. The population of aggregated platelets also undergoes contraction [58,59]. It is not quite clear how platelets 'decide' to either aggregate or expose phosphatidylserine. Given that adjacent platelets can form gap junctions [60], and communicate with each other [61], it is possible that cross-communication in the aggregate protects against anoctamin-6-dependent procoagulant activity. In a study where platelet adhesion to multiple surfaces was examined, the response heterogeneity in thrombus formation with different populations of platelets formed was a constant finding, regardless of the surface type and adhesive receptors involved (GPIb-V-IX, GPVI, CLEC-2, $\alpha_6\beta_1$, $\alpha_{IIb}\beta_3$ or CD36) [62].

Recent *in vivo* studies with mice, where laser-induced injury was applied to cremaster or femoral arterioles, have pointed to another level of platelet heterogeneity within thrombi, *i.e.* an inner core of aggregated and contracted platelets, surrounded by an outer shell of loosely adhered platelets [63,64]. Two specific platelet populations were distinguished, *i.e.* platelets in the thrombus core having

undergone secretion and CD62P expression, and platelets in the outer shell without secretion [63,64]. Several factors may contribute to the heterogeneous thrombus buildup, including local levels of soluble platelet agonists (ADP, thromboxane A_2) and the local wall shear gradients [65]. Also, this type of platelet heterogeneity in thrombi is likely to be influenced by the type of injury, vascular bed location and the physiological or pathological state of the vessel.

3.3. Coagulation and thrombus heterogeneity

Under conditions favoring coagulation, *e.g.* with tissue factor present, the formation of thrombin and fibrin in a thrombus appear to be highly localized processes. In mouse microvascular thrombosis models, thrombin activity and fibrin deposits appeared to be concentrated at the thrombus base, *i.e.* close to the vascular localization of tissue factor [66]. *In vitro* models of thrombus formation showed that the population of phosphatidylserine-exposing platelets was responsible for the binding of coagulation factors (prothrombin, factors Va, IXa and Xa), and also for the formation of thrombin and fibrin [46,57]. A potent feedback loop was identified, in that the local formation of thrombin enhanced thrombin-mediated phosphatidylserine exposure of nearby platelets, thus increasing the population of procoagulant platelets [46]. Fibrin formation appeared to be initially linked to PS exposure, and secondarily dependent on integrin $\alpha_{IIb}\beta_3$ activation and transglutaminase-dependent fibrin cross-linking [17,67]. *In vitro* models further indicated that fibrin preferentially localized near the sites of tissue factor and on procoagulant (coated) platelets [68]. Other identified elements of heterogeneity in a thrombus are the local secretion of polyphosphate clusters (activating factor XII) [69], and the phosphatidylserine-dependent accumulation of plasmin (activating the fibrinolytic system) [70]. Taken together, during thrombus formation, the coagulation process appears to enhance the formation of distinct platelet populations, and increase the heterogeneity in platelet functions.

4. Positive and negative priming of platelets

Multiple bioactive molecules in the blood can enhance or suppress platelet functionality. In psychological and neurobiological sciences, the implicit memory effect by which exposure to a first stimulus influences the response to a next stimulus, is called 'priming' [71]. By analogy, we can consider the exposure of platelets to enhancing or suppressing agents as positive or negative priming events. Fig. 3 gives an illustration of potential priming biomolecules that are either derived from the vascular wall or found in plasma.

4.1. Positive priming: key roles of plasma derived factors

Several hormones enhance platelet activation processes (shape change, secretion, aggregation), when applied in combination with other agonists. Epinephrine, binding to the Gi-coupled α_2-adrenergic receptor, reduces intracellular cyclic AMP levels which, *e.g.* enhances Ca^{2+} fluxes raised by agonists like ADP and thrombin [16,72]. Thrombopoietin, a growth factor for megakaryocytes, also sensitizes platelets to agonists at threshold concentrations [73,74], and at shear stress [75]. Similarly, insulin-like growth factor-1 (IGF1) enhances platelet activation provided that other agonists are present [76]. *In vitro* studies indicate that positive priming of platelets with thrombopoietin or insulin-like growth factor-1 can overcome the inhibitory effects on platelet function by aspirin or $P2Y_{12}$ receptor treatment [77]. This suggests that positive priming of platelets can be a mechanism of resistance to antiplatelet therapy.

Other positive priming molecules are Gas6 (plasma-derived) and soluble CD40L (platelet-derived), both of which stabilize and extend platelet aggregation [78,79]. TNFα, a pro-inflammatory cytokine which is synthesized in high amounts during inflammatory conditions, is capable of inducing platelet activation mainly indirectly *via* endothelial activation [80], although some authors report an enhancing effect of TNFα on collagen-induced aggregation [81].

Besides the positive enforcement of such plasma molecules on platelet function, the wall shear stress is of great influence as well. Rapid changes in laminar blood flow and shear rate, which occur at sites of stenosis promote platelet aggregate formation [58,65,82]. This wall-shear effect is highly dependent on VWF activity and autocrine platelet stimulation [65]. As such, disturbances in blood flow can be considered as a positive priming condition.

Several dietary nutrients are known to positively modulate platelet responsiveness. In addition, gut microbes may contribute to platelet hyperreactivity *via* the generation of trimethylamine *N*-oxide (TMAO) from dietary sources of trimethylamine, present in a western diet. The generated TMAO is proposed to prime platelet activation by enhancing Ca^{2+} release from intracellular stores [83].

4.2. Negative priming: key roles of endothelial mediators

Not all platelets are freely circulating, as part of the platelets are kept in the spleen and lungs [84,85]. In mice the lungs also significantly contribute to platelet biogenesis [86]. Whether this also applies to the human situation, remains to be explored. Clearly, these pools of marginated platelets in the lungs and spleen are exposed to different environments. Unclear is at which rate and to what extent the platelet pools exchange, as well as how these different locations alter the activation properties. Adaptation in properties can also arise, when platelets are flowing through the micro- and macro-vascular beds lined with specialized endothelial cells.

At least three endothelial-derived substances – likely there are more – are accountable for the major negative priming effect of the vessel wall on platelet activation. These are prostacyclin, nitric oxide and CD39/CD73 [87]. Prostacyclin (prostaglandin I_2), a short-lived eicosanoid produced by endothelial cells, binds to the platelet IP receptor, which couples to the Gs GTP-binding protein, stimulates adenylate cyclase and thereby elevates the inhibitory second messenger cyclic AMP [88]. Continuous exposure of circulating platelets to prostacyclin leads to cyclic AMP elevation and thereby platelet inhibition. In freshly isolated platelets, supra-basal levels of cyclic AMP have indeed been measured [72]. However, IP receptors can desensitize and the functional effects of prostacyclin on platelets may be most prominent locally, *i.e.* after platelet adhesion [87]. Prostaglandin E_2 (EP receptor agonist) also has an inhibitory, though more complex action mechanism [88]. The effect of PGE_2 depends on the receptor isotype that is activated. Activation of EP3 isoforms by PGE_2 leads to sensitization of platelets, while binding of PGE_2 to EP2 or EP4 receptors causes platelet inhibition. It has been proposed that specifically the blocking of EP3 could be beneficial for the prevention of atherothrombosis [89].

The unstable free radical nitric oxide is membrane-permeable, and interacts with intracellular guanylate cyclase, raising the inhibitory second messenger cyclic GMP in platelets [90]. Nitric oxide is produced by endothelial nitric oxide synthase isoforms in response to the pulsatile flow of blood. Together, elevated levels of cyclic AMP and cyclic GMP accomplish strong platelet inhibition *via* a flood of protein phosphorylation reactions, mediated by the protein kinases A and G, respectively [91,92]. The levels of both cyclic nucleotides are balanced *via* cyclic nucleotide-dependent phosphodiesterases. The hormone insulin can also affect platelet reactivity *via* cyclic AMP and GMP elevation. In diabetic patients, who are resistant to insulin, a loss of these inhibitory pathways might contribute to platelet hyperreactivity [93,94].

The endothelial-expressed ecto-nucleotidases CD39 and CD73 degrade intra-vascular ATP/ADP and AMP, respectively, to ultimately produce adenosine [87]. These activities convert potent platelet-activating autacoids (ATP, ADP) into a platelet inhibitor (adenosine, activating Gs) [87]. Both mouse and human studies point towards a modulating role of ecto-nucleotidases in thrombus formation and thrombosis. In addition to the inhibitory A_2-adenosine receptor,

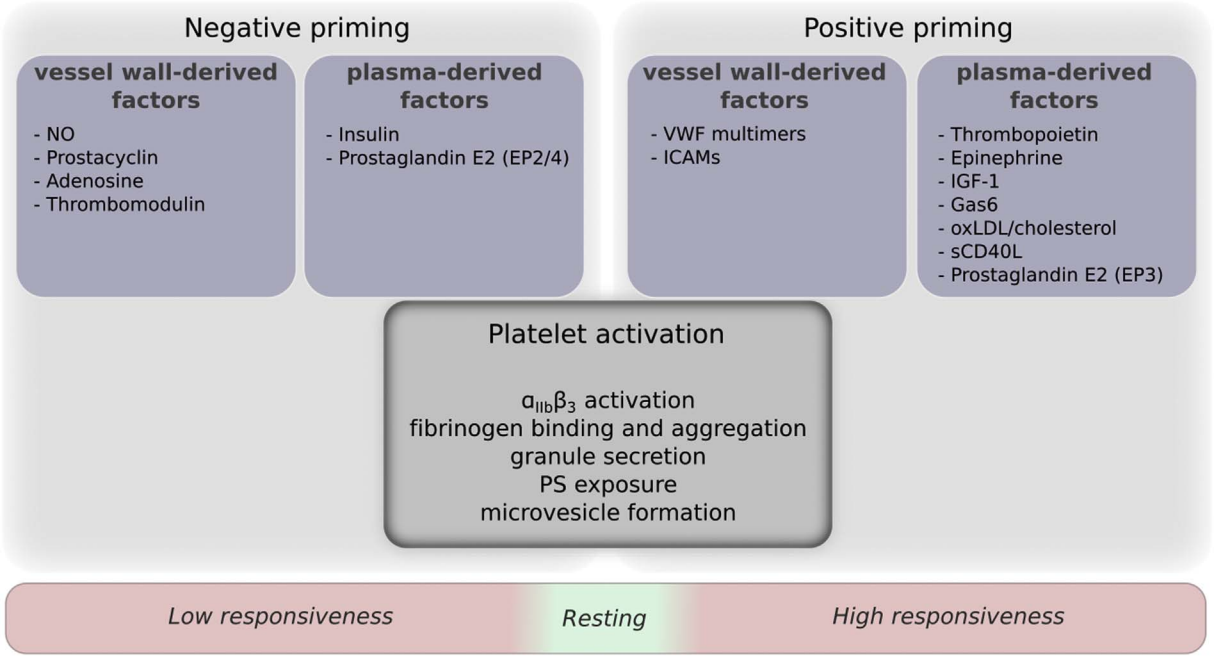

Fig. 3. Concept of negative and positive priming of platelets.
The indicated factors can cause negative or positive priming of platelet activation processes, either plasma-derived or from the vessel wall.

platelets possess receptors linked to protein-tyrosine phosphatases, which also suppress activation processes [95]. The physiological ligands of these are mostly unknown. Indirectly, also the thrombin-inactivating activity of endothelial thrombomodulin contributes to platelet suppression, *i.e.* by preventing thrombin-induced activation [96]. Some nutritional components can contribute to negative priming, *e.g.* a mild to moderate alcohol consumption may lower platelet reactivity by inhibiting thromboxane formation [97].

Given that circulating platelets are exposed to a plethora of positive and negative priming molecules, their net effect must be prevention of platelet adhesion and activation, unless positive stimuli prevail, such as at a damaged vessel wall. The implicit assumption is that a shift or disturbance in the balance of positive and negative priming molecules causes a change in the threshold of platelet activation. This could for example explain why the population of coated platelets increased in a variety of pathophysiological conditions [18].

5. Pathology induced alterations in platelet populations

Considering that multiple bioactive mediators present in the blood or derived from the vessel wall influence the tendency of platelet activation, it can be expected that disease conditions associated with vascular abnormalities or blood changes enhance the positive priming of platelets. An increasing number of reports points to such increased priming events, often ascribed to the formation of different 'types' of platelets, especially in patients with high-burden inflammatory, prothrombotic or hematological diseases (Table 1). Albeit different terms are used to annotate the platelet changes, the majority of papers refers to either a gain-of-function (high-responsive platelets) or a loss-of-function (low-responsive platelets). A still unresolved question is how these functional changes relate to the formation of specific platelet populations.

5.1. Negatively primed platelets in disease

The genetic makeup of a subject co-determines platelet responsiveness. Rare genetic modifications of over 25 different platelet proteins have been described that result in low-responsive platelets, and associate with bleeding disorders [98,99]. Loss-of-function mutations, often accompanied by thrombocytopenia, in particular concern genes of transcription factors, the actin-myosin and tubulin cytoskeletons, signaling proteins, and granule secretion-regulating proteins [98,99]. Genetic polymorphisms in these and other genes may also influence platelet responsiveness.

A clear example how a genetic deficiency can imbalance the formation of platelet populations is the Scott syndrome, a mild bleeding disorder. Herein, mutations in the *ANO6* gene result in the near absence of the procoagulant, phosphatidylserine-exposing platelet population due to inability of phospholipid scrambling [21,22], resulting in a severely compromised fibrin formation [68].

In addition to genetics, there is accumulating evidence for acquired forms of low-responsive platelets. A primary loss-of-function is observed in patients with hematological malignancies and thrombocytopenia. For instance, platelets from patients with acute leukemia can be impaired in adhesion, aggregation and secretion. These functional defects are likely a consequence of dysfunctional megakaryocytopoiesis [100,101].

In patients with renal failure and associated uremia, it is considered that impaired platelet adhesion to the vessel wall is the main cause of bleeding. Several indicators of low platelet responsiveness are described, including impaired Ca^{2+} fluxes, reduced secretion and low platelet aggregation [102]. On the other hand, also impaired platelet adhesion to the vessel wall due to anemia may explain the hemorrhagic complications in these patients. A low hematocrit limits the margination of platelets towards the vessel wall, and might furthermore restrict the availability of red blood cell-derived ADP and the scavenging effect of hemoglobin on nitric oxide as a platelet inhibitor [102]. Other papers report that renal failure increases the risk of thrombosis, *e.g.* by increasing the population of apoptotic, phosphatidylserine-exposing platelets [103].

5.2. Positively primed platelets in disease

Diabetes has often been associated with platelet hyperreactivity, *i.e.* enhanced platelet adhesion, activation, aggregation and platelet-dependent thrombin generation [104]. This increased responsiveness of

Table 1
Platelet phenotypes and activation markers in hematological and other diseases, induced by negative or positive priming. Representative examples from the literature are given.

Clinical condition	Hemostatic balance	Type of PLT	PLT activation markers *in vivo*	PLT activation testing *in vitro*	Ref.
Negatively primed (low responsive)					
Congenital defects: Scott syndrome	Mild bleeding	Procoagulant	*n.d.*	PS ↓ fibrin formation ↓	[22,68]
Hematological malignancies	Bleeding	Hypo-responsive	PLT count ↓ TPO ↑	$\alpha IIb\beta_3$, CD62P, CD63, GPIb ↓	[100,101]
Renal failure and uremia	Bleeding (thrombosis)	Dysfunctional	NO ↑	CD62P, Ca^{2+} ↓ PS, caspase ↑	[102,103]
Positively primed (high responsive)					
Diabetes type 2	Prothrombotic	Angry	sCD62P, sCD40L ↑ PF4, MPV ↑, NO ↓	GPIb, CD41, GPVI ↑ CD63, CD62P, CAT ↑	[104,105,129,130]
Hypercholesterolemia	Prothrombotic	Hyper-reactive	PLT turnover, MPV ↑	LTA ↑	[108,109,131–133]
Myeloproliferative neoplasms (ET, PCV)	Prothrombotic	Hyper-reactive	PLT count ↑	LTA, CD62P, CAT ↑	[110,111]
Positively primed and secondarily inactive (low responsive)					
Cancers (solid tumors)	Prothrombotic	Hyper-reactive (*exhausted*)	sCD62P, PEV ↑ PLT count ↑	CD62P, CD63, GPIb ↑ TxB_2 ↓	[112–115]
Sepsis	Thrombosis, bleeding	Hyper-reactive/ exhausted	PLT count ↓ sCD62P, sTLT, PEV ↑	CD62P ↑ LTA, fibrinogen binding ↓ (severe sepsis)	[116,119]
Stroke	Bleeding	Exhausted	sCD62P, βTG ↑ TxB_2 (urine) ↑	CD62P, CD63 ↓ mepacrine ↓	[120–122]
Major trauma (brain)	Bleeding	Hypo-responsive	PEV↑ CD62P, PAC mAb ↑ (unstimulated)	LTA ↓	[123,124]
Viral hemorrhagic fever	Thrombosis (bleeding)	Exhausted/reticulated	sCD62P, sGPVI ↑ TPO, IPF, MPV ↑	$\alpha IIb\beta_3$, CD62P, CD63 ↓	[125,126]

Abbreviations: CAT, calibrated automated thrombin generation in PRP; ET, essential thrombocytopenia; IPF, immature platelet fraction; LTA, light transmission aggregometry; MPV, mean platelet volume; NO, nitric oxide; PCV, polycythemia vera; PEV, platelet-derived extracellular vesicles; PF4, platelet factor 4; PLT, platelet; PS, phosphatidylserine; s, soluble; β–TG, β-thromboglobulin; s, soluble; TLT, TREM-like transcript-1; TPO, thrombopoietin.

platelets is explained by high blood-glucose levels, oxidative stress and increased vascular shear stress [105]. Under these hyperglycemic conditions, advanced glycation end products (AGEs) are generated, which activate platelets *via* the CD36 pathway [106]. Further, activation of the receptor of AGEs (RAGE) by S100A8/A9 may lead to enhanced production of TPO, thereby increasing platelet production from megakaryocytes upon hyperglycemia [107]. In diabetic patients, antiplatelet treatment strategies were shown to be effective for the secondary prevention of ischemic complications [104].

Plasma cholesterol levels can positively prime platelets, such as manifested by the enhanced activity and larger size of platelets from patients with familial hypercholesterolemia, in whom total lipid and low-density lipoprotein levels are severely elevated. Accumulation of cholesterol in the platelet membrane enhances platelet aggregation by influencing the membrane structure and signaling *via* surface receptors [108,109]. Hypercholesterolemia is also linked to increased platelet production and turnover [108]. Similarly, oxidation of low-density lipoproteins in the intima of atherosclerotic lesions is considered to result in enhanced platelet activation [108].

In the myeloproliferative neoplasms, essential thrombocytosis and polycythemia vera, quantitative as well as qualitative platelet abnormalities have been identified. Particularly in patients carrying the $JAK2^{V617F}$ mutation, for whom aspirin treatment is recommended, platelet responsiveness is increased (secretion, aggregation and procoagulant activity), which effect is in part associated with thromboembolic complications [110,111].

5.3. Positively primed and secondarily inactivated platelets in disease

Despite the presence of positive priming molecules *in vivo*, platelet activation *in vitro* may appear to be impaired. This can be a direct consequence of prior activation in the circulation resulting in secondary loss of function. In such cases, authors describe the dysfunctional platelets often as exhausted platelets.

In patients with different types of solid tumors, apart from a high platelet count, platelet activation can be enhanced *in vivo*, as deduced from the presence of platelet-derived soluble (s)CD62P in the blood plasma [112]. This associates *in vitro* with a low-level of platelet activation, leading to the assumption that these platelets are 'exhausted', *i.e.* displaying a secondarily down-regulated responsiveness to agonists (CD62P, CD63 expression), although the primarily enhanced activation may predispose patients to an increased risk of thromboembolic complications [113,114]. Another marker of ongoing platelet activation in cancer patients is the accumulation of platelet-derived extracellular vesicles, interestingly, with almost no effect on mean platelet volume [115]. Strikingly, in those patients a high mean platelet volume associates (though with low odds ratio) with a decreased risk of venous thromboembolism [113].

In sepsis, as a life-threatening condition of systemic inflammation, platelets are positively primed as a result of endothelial damage or activation in combination with enhanced coagulant activity. This leads to increased plasma levels of platelet secretion products as well as platelet-derived extracellular vesicles [116]. It has been demonstrated that several pathogens can interact with platelets *via* Toll-like receptors, FcγRIIa and/or integrin $\alpha_{IIb}\beta_3$, thus inducing platelet activation [116–118]. In severe sepsis, also secondary defects in platelet function have been observed, often concomitantly with a lowering in platelet count [116,119].

Papers studying patients who suffered a stroke, report increased levels in the circulation of platelet activation markers, including sCD62P, β-thromboglobulin and thromboxane B_2, in those patients with a high risk of bleeding [120,121]. Since this is accompanied by an impaired platelet activation tendency *in vitro*, the authors type such platelets as exhausted [122]. A similar platelet phenotype is expected in patients with acute coronary syndrome before the initiation of antiplatelet therapy. An exhausted platelet type has also been observed after major trauma, in particular brain injury, where plasma markers of platelet activation are elevated, while the platelets after isolation are hypo-responsive in terms of aggregation [123,124]. The suggestion was made that sub-endothelial tissue exposure upon injury causes a low

Table 2
Markers of the different platelet populations. For abbreviations and explanation, see text and Table 1.

Platelet population	Markers
Reticulated	mRNA (immature platelet fraction)
Secretory	PF4, βTG release
	CD62P, CD63 expression
	TxB_2 formation, serotonin
Aggregating	Fibrinogen binding
	PAC1 mAb binding (activated integrin $\alpha_{IIb}\beta_3$)
	Platelet-leukocyte aggregate formation
Procoagulant	PS exposure, coagulation factor binding
	Extracellular vesicle formation
Coated	PS exposure
	Fibrin binding, transglutaminase activity
	Extracellular vesicle formation
Apoptotic	PS exposure
	Extracellular vesicle formation

level of platelet activation, resulting in dysfunctional circulating platelets with an activation history.

Higher fractions (populations) of reticulated platelets have been detected in patients with viral hemorrhagic fever due to hantaviral disease, which was accompanied by increased levels of activation markers such as sCD62P and sGPVI, followed by a secondary loss in platelet activity *in vitro* [125]. A similar phenotype of exhausted platelets has been described for patients with a severe dengue infection [126].

It is currently unclear how the different pathologies induce specific changes in platelet function. The pathological environment may alter one specific platelet population or it rather affects all circulating platelets and thereby elicit a shift in the distribution of the different platelet populations. It can be reasonably presumed that this change is specific for the pathological environment. Furthermore, the impact of a pathological condition on platelet function may be multifactorial (*e.g.* diabetes) and therefore even more complex.

6. Platelet heterogeneity and risk assessment for bleeding and thrombosis: recommendations

For diagnosing a known or suspected risk of thrombosis or bleeding, current routine measurements include (*i*) platelet count and mean volume, (*ii*) plasma markers of *in vivo* platelet activation, and (*iii*) *in vitro* platelet activation tests. Particularly, the latter tests are used for the monitoring of anti-platelet therapy. Table 2 presents an outline of how different test outcomes relate to distinct platelet populations, provided that sample processing is up to standards.

The proposed concept of partly overlapping platelet populations that are subjected to positive/negative priming can shed new light on the often paradoxical information obtained from different diagnostic platelet function and activity tests. Fig. 4 shows a flow chart aiming to structure the information obtained from such tests for a risk estimate of hemorrhage or thrombosis. From the figure, it will be clear that information on both activation markers *in vivo* and platelet responsiveness *in vitro* is needed to obtain a complete picture. It is noted though that platelets are deprived of endothelial inhibitors when performing *in vitro* measurements, which influences their responsiveness. Assay measurements should preferably include markers of different platelet populations, given their specific contribution to hemostasis or thrombosis. Understanding of the redistribution of platelet populations in patients with particular hemorrhagic or thrombotic tendencies will also aid in designing an optimized treatment. One such an example is the administration of DDAVP to patients with a mild congenital platelet function defect which selectively augments the formation of coated platelets by enhancing Na^+/Ca^{2+} mobilization [127].

Practice points

- Platelet heterogeneity can originate from differences between megakaryocytes, variation in proplatelet formation, unequal platelet priming and environmental conditions, as well as platelet ageing. Changes in the physiological environment upon disease can hence influence platelet populations and platelet responses.
- Combining of both *in vivo* and *in vitro* markers of platelet activation and activity permits better assessment of a platelet-related thrombotic or hemorrhage risk.

Research agenda

- How the pathophysiological environment affects platelet populations and their responsiveness is incompletely understood and warrants further investigation.
- The causes and consequences of platelet heterogeneity have only partly been resolved. Characterization of the preferential response spectrum of certain platelet populations may contribute to risk factor assessments for prediction of thrombotic or hemorrhagic complications.

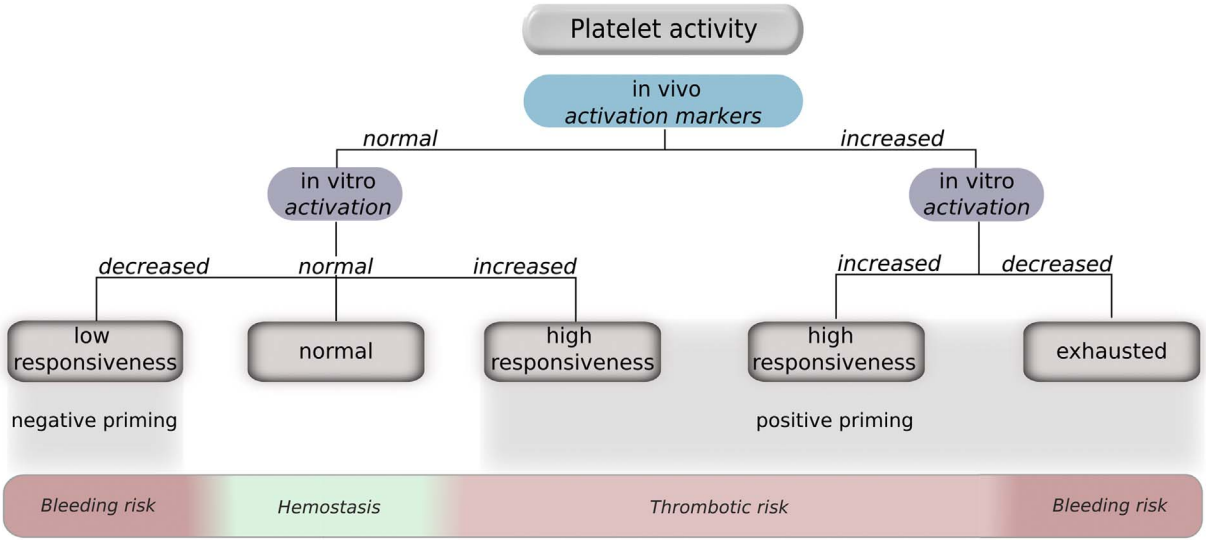

Fig. 4. Concept of negative and positive platelet priming resulting in changed platelet activation markers *in vivo* and altered platelet activities *in vitro*. Flow chart for assessing changes in platelet functions in patients with thrombosis or bleeding risk.

Funding

PvdM was supported by an EHA-ISTH Research Fellowship award granted by the European Hematology Association and the International Society of Thrombosis and Haemostasis (2014).

Conflict of interest

The authors report no conflicts of interest.

References

[1] Long MW. Population heterogeneity among cells of the megakaryocytic lineage. Stem Cells 1993;11(1):33–40.
[2] Paulus JM, Levin J, Debili N, Albert A, Vainchenker W. Genesis of clone size heterogeneity in megakaryocytic and other hemopoietic colonies: the stochastic model revisited. Exp Hematol 2001;29(11):1256–69.
[3] den Dekker E, van Abel M, van der Vuurst H, van Eys GJJM, Akkerman JWN, Heemskerk JW. Cell-to-cell variability in the differentiation program of human megakaryocytes. Biochim Biophys Acta 2003;1643(1–3):85–94.
[4] Moreau T, Evans AL, Vasquez L, Tijssen MR, Yan Y, Trotter MW, et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. Nat Commun 2016;7:11208.
[5] Rondina MT, Weyrich AS. Regulation of the genetic code in megakaryocytes and platelets. J Thromb Haemost 2015;13(Suppl. 1):S26–32.
[6] Chaabane C, Dally S, Corvazier E, Bredoux R, Bobe R, Ftouhi B, et al. Platelet PMCA- and SERCA-type Ca^{2+}-ATPase expression in diabetes: a novel signature of abnormal megakaryocytopoiesis. J Thromb Haemost 2007;5(10):2127–35.
[7] Lood C, Amisten S, Gullstrand B, Jönsen A, Allhorn M, Truedsson L, et al. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. Blood 2010;116(11):1951–7.
[8] Machlus KR, Italiano Jr. JE. The incredible journey: from megakaryocyte development to platelet formation. J Cell Biol 2013;201(6):785–96.
[9] Jackson CW, Jennings LK. Heterogeneity of fibrinogen receptor expression on platelets activated in normal plasma with ADP: analysis by flow cytometry. Br J Haematol 1989;72(3):407–14.
[10] Munnix ICA, Cosemans JMEM, Auger JM, Heemskerk JWM. Platelet response heterogeneity in thrombus formation. Thromb Haemost 2009;102(6):1149–56.
[11] Heemskerk JW, Willems GM, Rook MB, Sage SO. Ragged spiking of free calcium in ADP-stimulated human platelets: regulation of puff-like calcium signals in vitro and ex vivo. J Physiol 2001;15(535):625–35.
[12] Eckly A, Rinckel JY, Proamer F, Ulas N, Joshi S, Whiteheart SW, et al. Respective contributions of single and compound granule fusion to secretion by activated platelets. Blood 2016;128(21):2538–49.
[13] Ollivier V, Syvannarath V, Gros A, Butt A, Loyau S, Jandrot-Perrus M, et al. Collagen can selectively trigger a platelet secretory phenotype via glycoprotein VI. PLoS One 2014;9(8):e104712.
[14] van Geffen JP, Kleinegris MC, Verdoold R, Baaten CC, Cosemans JM, Clemetson KJ, et al. Normal platelet activation profile in patients with peripheral arterial disease on aspirin. Thromb Res 2015;135(3):513–20.
[15] Mattheij NJA, Gilio K, Van Kruchten R, Jobe SM, Wieschhaus AJ, Chishti AH, et al. Dual mechanism of integrin αIIbβ3 closure in procoagulant platelets. J Biol Chem 2013;288(19):13325–36.
[16] Versteeg HH, Heemskerk JWM, Levi M, Reitsma PS. New fundamentals in hemostasis. Physiol Rev 2013;93(1):327–58.
[17] Mattheij NJ, Swieringa F, Mastenbroek TG, Berny-Lang MA, May F, Baaten CC, et al. Coated platelets function in platelet-dependent fibrin formation via integrin αIIbβ3 and transglutaminase factor XIII. Haematologica 2016;101(4):427–36.
[18] Dale GL. Coated-platelets: an emerging component of the procoagulant response. J Thromb Haemost 2005;3(10):2185–92.
[19] Heemskerk JWM, Mattheij N, Cosemans JMEM. Platelet-based coagulation: different populations, different functions. J Thromb Haemost 2013;11(1):2–11.
[20] Podoplelova NA, Sveshnikova AN, Kotova YN, Eckly A, Receveur N, Nechipurenko DY, et al. Coagulation factors bound to procoagulant platelets concentrate in cap structures to promote clotting. Blood 2016;128(13):1745–55.
[21] Solari F, Mattheij NJ, Burkhart JM, Swieringa F, Collins P, Cosemans JM, et al. Combined quantification of the global proteome, phosphoproteome, and proteolytic cleavage to characterize altered platelet functions in the human Scott syndrome. Mol Cell Proteomics 2016;15(10):3154–69.
[22] Mattheij NJA, Braun A, van Kruchten R, Castoldi E, Pircher J, Baaten CCFMJ, et al. Survival protein anoctamin-6 controls multiple platelet responses including phospholipid scrambling, swelling and protein cleavage. FASEB J 2016;30(2):727–37.
[23] Van Kruchten R, Mattheij NJ, Saunders C, Feijge MA, Swieringa F, Wolfs JL, et al. Both TMEM16F-dependent and TMEM16F-independent pathways contribute to phosphatidylserine exposure in platelet apoptosis and platelet activation. Blood 2013;121(10):1850–7.
[24] Thompson CB, Eaton KA, Princiotta SM, Rushin CA, Valeri CR. Size dependent platelet subpopulations: relationship of platelet volume to ultrastructure, enzymatic activity, and function. Br J Haematol 1982;50(3):509–19.
[25] Jakubowski JA, Thompson CB, Vaillancourt R, Valeri CR, Deykin D. Arachidonic acid metabolism by platelets of differing size. Br J Haematol 1983;53(3):503–11.
[26] Robinson MSC, Machin SJ, Mackie IJ, Harrison P. In vivo biotinylation studies: specificity of labelling of reticulated platelets by thiazole orange and mepacrine. Br J Haematol 2000;108(4):859–64.
[27] McBane II RD, Gonzalez C, Hodge DO, Wysokinski WE. Propensity for young reticulated platelet recruitment into arterial thrombi. J Thromb Thrombolysis 2014;37(2):148–54.
[28] Hoffmann JJ, van den Broek NM, Curvers J. Reference intervals of reticulated platelets and other platelet parameters and their associations. Arch Pathol Lab Med 2013;137(11):1635–40.
[29] Hoffmann JJ. Reticulated platelets: analytical aspects and clinical utility. Clin Chem Lab Med 2014;52(8):1107–17.
[30] Robinson MSC, Mackie IJ, Machin SJ, Harrison P. Two colour analysis of reticulated platelets. Clin Lab Haematol 2000;22(4):211–3.
[31] Harrison P, Goodall AH. "Message in the platelet" - more than just vestigial mRNA!. Platelets 2008;19(6):395–404.
[32] Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol 2004;126(1):93–9.
[33] Alhasan AA, Izuogu OG, Al-Balool HH, Steyn JS, Evans A, Colzani M, et al. Circular RNA enrichment in platelets is a signature of transcriptome degradation. Blood 2016;127(9):e1–11.
[34] Simon LM, Edelstein LC, Nagalla S, Woodley AB, Chen ES, Kong X, et al. Human platelet microRNA-mRNA networks associated with age and gender revealed by integrated plateletomics. Blood 2014;123(16):e37–45.
[35] Pujol-Moix N, Vazquez-Santiago M, Morera A, Ziyatdinov A, Remacha A, Nomdedeu JF, et al. Genetic determinants of platelet-large-cell ratio, immature platelet fraction, and other platelet-related phenotypes. Thromb Res 2015;136(2):361–6.
[36] McManus DD, Freedman JE. MicroRNAs in platelet function and cardiovascular disease. Nat Rev Cardiol 2015;12(12). (717-2).
[37] Lindsay CR, Edelstein LC. MicroRNAs in platelet physiology and function. Semin Thromb Hemost 2016;42(3):215–22.
[38] Li R, Hoffmeister KM, Falet H. Glycans and the platelet life cycle. Platelets 2016;27(6):505–11.
[39] Lebois M, Josefsson EC. Regulation of platelet lifespan by apoptosis. Platelets 2016;27(6):497–504.
[40] Josefsson EC, James C, Henley KJ, Debrincat MA, Rogers KL, Dowling MR, et al. Megakaryocytes possess a functional intrinsic apoptosis pathway that must be restrained to survive and produce platelets. J Exp Med 2011;208(10):2017–31.
[41] Pleines I, Lebois M, Au AEL, Lane RM, Henley KJ, Corbin J, et al. Extended platelet in vivo survival results in exhausted platelets. Blood 2015;126(23):416.
[42] Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? Blood 2010;116(12):2011–8.
[43] Vogler M, Hamali HA, Sun XM, Bampton ETW, Dinsdale D, Snowden RT, et al. BCL2/BCL-X_L inhibition induces apoptosis, disrupts cellular calcium homeostasis, and prevents platelet activation. Blood 2011;117(26):7145–54.
[44] Grozovsky R, Begonja AJ, Liu K, Visner G, Hartwig JH, Falet H, et al. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK-STAT3 signaling. Nat Med 2015;21(1):47–54.
[45] Bevers EM, Comfurius P, van Rijn JL, Hemker HC, Zwaal RF. Generation of prothrombin-converting activity and the exposure of phosphatidylserine at the outer surface of platelets. Eur J Biochem 1982;122(2):429–36.
[46] Swieringa F, Kuijpers MJE, Lamers MME, Van der Meijden PE, Heemskerk JW. Rate-limiting roles of the tenase complex of factors VIII and IX in platelet procoagulant activity and formation of platelet-fibrin thrombi under flow. Haematologica 2015;100(6):748–56.
[47] Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, et al. Glycoprotein VI but not α2β1 integrin is essential for platelet interaction with collagen. EMBO J 2001;20(9):2120–30.
[48] Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor? Blood 2003;102(2):449–61.
[49] Gibbins JM. Platelet adhesion signalling and the regulation of thrombus formation. J Cell Sci 2004;117(Pt 16):3415–25.
[50] Siljander PRM, Farndale RW, Feijge MAH, Comfurius P, Kos S, Bevers EM, et al. Platelet adhesion enhances the glycoprotein VI-dependent procoagulant response: involvement of p38 MAP kinase and calpain. Arterioscler Thromb Vasc Biol 2001;21(4):618–27.
[51] Heemskerk JW, Kuijpers MJ, Munnix IC, Siljander PRM. Platelet collagen receptor and coagulation. A characteristic platelet response as possible target for antithrombotic treatment. Trends Cardiovasc Med 2005;15(3):86–92.
[52] Agbani EO, van den Bosch MT, Brown E, Williams CM, Mattheij NJ, Cosemans JM, et al. Coordinated membrane ballooning and procoagulant spreading in human platelets. Circulation 2015;132(15):1414–24.
[53] Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 1998;94(5):657–66.
[54] Siljander PRM, Munnix ICA, Smethurst PA, Deckmyn H, Lindhout T, Ouwehand WH, et al. Platelet receptor interplay regulates collagen-induced thrombus formation in flowing human blood. Blood 2004;103(4):1333–41.
[55] Auger JM, Kuijpers MJE, Senis YA, Watson SP, Heemskerk JW. Adhesion of human and mouse platelets to collagen under shear: a unifying model. FASEB J 2005;19(7):825–7.
[56] Gilio K, Van Kruchten R, Braun A, Berna-Erro A, Feijge MAH, Stegner D, et al. Roles of platelet STIM1 and Orai1 in glycoprotein VI- and thrombin-dependent procoagulant activity and thrombus formation. J Biol Chem 2010;285(31):23629–38.

[57] Munnix ICA, Kuijpers MJE, Auger JM, CMLGD Thomassen, Panizzi P, van Zandvoort MAMJ, et al. Segregation of platelet aggregatory and procoagulant microdomains in thrombus formation. Regulation by transient integrin activation. Arterioscler Thromb Vasc Biol 2007;27(11):2484–90.
[58] Jackson SP. Arterial thrombosis: insidious, unpredictable and deadly. Nat Med 2011;17(11):1423–36.
[59] de Witt SM, Verdoold R, Cosemans JM, Heemskerk JW. Insights into platelet-based control of coagulation. Thromb Res 2014;133(Suppl. 2):S139–48.
[60] Vaiyapuri S, Jones CI, Sasikumar P, Moreas LA, Munger SJ, Wright JR, et al. Gap junctions and connexins hemichannels underpin hemostasis and thrombosis. Circulation 2012;125(20):2479–91.
[61] Nesbitt WS, Giuliano S, Kulkarni S, Dopheide SM, Harper IS, Jackson SP. Intercellular calcium communication regulates platelet aggregation and thrombus growth. J Cell Biol 2003;160(7):1151–61.
[62] de Witt SM, Swieringa F, Cavill R, Lamers MME, van Kruchten R, Mastenbroek T, et al. Identification of platelet function defects by multi-parameter assessment of thrombus formation. Nat Commun 2014;5:4257.
[63] Stalker TJ, Welsh JD, Brass LF. Shaping the platelet response to vascular injury. Curr Opin Hematol 2014;21(5):410–7.
[64] Welsh JD, Poventud-Fuentes I, Sampietro S, Diamond SL, Stalker TJ, Brass LF. Hierarchical organization of the hemostatic response to penetrating injuries in the mouse macrovasculature. J Thromb Haemost 2017;15(3):526–37.
[65] Westein E, van der Meer AD, Kuijpers MJE, Frimat JP, van den Berg A, Heemskerk JW. Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. PNAS 2013;110(4):1357–62.
[66] Ivanciu L, Krishnaswamy S, Camire RM. New insights into the spatiotemporal localization of prothrombinase in vivo. Blood 2014;124(11):1705–14.
[67] Cosemans JM, Schols SEM, Stefanini L, de Witt SM, Feijge MAH, Hamulyák K, et al. Key role of glycoprotein Ib/V/IX and von Willebrand factor in platelet activation-dependent fibrin formation at low shear flow. Blood 2011;117(2):651–60.
[68] Swieringa F, Baaten CC, Mastenbroek T, Rijnveld N, van der Laan K, Collins P, et al. Platelet-dependent control of fibrin distribution and micro-elasticity in thrombus formation under flow. Arterioscler Thromb Vasc Biol 2016;36(4):692–9.
[69] Verhoef JJ, Barendrecht AD, Nickel KF, Dijkxhoorn K, Kenne E, Labberton L, et al. Polyphosphate nanoparticles on the platelet surface trigger contact system activation. Blood 2017;129(12):1707–17.
[70] Whyte CS, Swieringa F, Mastenbroek T, Lionikiene AS, Lancé MD, van der Meijden PE, et al. Plasminogen associates with phosphatidylserine-exposing platelets and contributes to thrombus lysis under flow. Blood 2015;125(16):2568–78.
[71] Henson R, Shallice T, Dolan R. Neuroimaging evidence for dissociable forms of repetition priming. Science 2000;287(5456):1269–72.
[72] Keularts IMLW, van Gorp RMA, Feijge MAH, Vuist MWJ, Heemskerk JWM. α_{2A}-Adrenergic receptor stimulation potentiates calcium release in platelets by modulating cAMP levels. J Biol Chem 2000;275(3):1763–72.
[73] Montrucchio G, Brizzi MF, Calosso G, Marengo S, Pegoraro L, Camussi G. Effects of recombinant human megakaryocyte growth and development factor on platelet activation. Blood 1996;87(7):2762–8.
[74] Pasquet JM, Gross BS, Gratacap MP, Quek L, Pasquet S, Payrastre B, et al. Thrombopoietin potentiates collagen receptor signaling in platelets through a phosphatidylinositol 3-kinase-dependent pathway. Blood 2000;95(11):3429–34.
[75] Oda A, Miyakawa Y, Druker BJ, Ozaki K, Yabusaki K, Shirasawa Y, et al. Thrombopoietin primes human platelet aggregation induced by shear stress and by multiple agonists. Blood 1996;87(11):4664–70.
[76] Hers I. Insulin-like growth factor-1 potentiates platelet activation via the IRS/PI3Ka pathway. Blood 2007;110(13):4243–52.
[77] Blair TA, Moore SF, Hers I. Circulating primers enhance platelet function and induce resistance to antiplatelet therapy. J Thromb Haemost 2015;13(8):1479–93.
[78] Cosemans JM, van Kruchten R, Olieslagers S, Schurgers LJ, Verheyen FK, Munnix IC, et al. Potentiating role of Gas6 and Tyro3, Axl and Mer (TAM) receptors in human and murine platelet activation and thrombus stabilization. J Thromb Haemost 2010;8(8):1797–808.
[79] Kuijpers MJ, Mattheij NJ, Cipolla L, van Geffen JP, Lawrence T, Donners MM, et al. Platelet CD40L modulates thrombus growth via phosphatidylinositol 3-kinase β, and not via CD40 and IkB kinase α. Arterioscler Thromb Vasc Biol 2015;35(6):1374–81.
[80] Pircher J, Merkle M, Wörnle M, Ribeiro A, Czermak T, Stampnik Y, et al. Prothrombotic effects of tumor necrosis factor alpha in vivo are amplified by the absence of TNF-alpha receptor subtype 1 and require TNF-alpha receptor subtype 2. Arthritis Res Ther 2012;14(5):R225.
[81] Pignatelli P, De Biase L, Lenti L, Tocci G, Brunelli A, Cangemi R, et al. Tumor necrosis factor-alpha as trigger of platelet activation in patients with heart failure. Blood 2005;106(6):1992–4.
[82] Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, et al. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. Nat Med 2009;15(6):665–73.
[83] Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. Cell 2016;165(1):111–24.
[84] Weyrich AS, Zimmerman GA. Platelets in lung biology. Annu Rev Physiol 2013;75:569–91.
[85] Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 1966;45(5):645–57.
[86] Lefrançais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 2017;544(7648):105–9.
[87] Rex S, Freedman JE. Platelets Chapter 13 In: Michelson AD, editor. Inhibition of platelet function by the endothelium2. Academic press; 2006. p. 251–79.
[88] Swieringa F, Kuijpers MJE, Heemskerk JW, Van der Meijden PE. Targeting platelet receptor function in thrombus formation: the risk of bleeding. Blood Rev 2014;28(1):9–21.
[89] Mawhin MA, Tilly P, Fabre JE. The receptor EP3 to PGE2: a rational target to prevent atherosthrombosis without inducing bleeding. Prostaglandins Other Lipid Mediat 2015;121(Pt A):4–16.
[90] Naseem KM, Roberts W. Nitric oxide at a glance. Platelets 2011;22(2):148–52.
[91] Smolenski A. Novel roles of cAMP/cGMP-dependent signaling in platelets. J Thromb Haemost 2012;10(2):167–76.
[92] Beck F, Geiger J, Gambaryan S, Veit J, Vaudel M, Nollau P, et al. Time-resolved characterization of cAMP/PKA-dependent signaling reveals that platelet inhibition is a concerted process involving multiple signaling pathways. Blood 2014;123(5):e1–10.
[93] Trovati M, Mularoni EM, Burzacca S, Ponziani MC, Massucco P, Mattiello L, et al. Impaired insulin-induced platelet antiaggregating effect in obesity and in obese NIDDM patients. Diabetes 1995;44(11):1318–22.
[94] Randriamboavonjy V, Fleming I. Insulin, insulin resistance, and platelet signaling in diabetes. Diabetes Care 2009;32(4):528–30.
[95] Senis YA. Protein-tyrosine phosphatases: a new frontier in platelet signal transduction. J Thromb Haemost 2013;11(10):1800–13.
[96] Ruf W, Dorfleutner A, Riewald M. Specificity of coagulation. J Thromb Haemost 2003;1(7):1495–503.
[97] Phang M, Lazarus S, Wood LG, Garg M. Diet and thrombosis risk: nutrients for prevention of thrombotic disease. Semin Thromb Hemost 2011;37(3):199–208.
[98] Nurden AT, Nurden P. Inherited disorders of platelet function: selected updates. J Thromb Haemost 2015;13(Suppl. 1):S2–9.
[99] Bianchi E, Norfo R, Pennucci V, Zini R, Manfredini R. Genomic landscape of megakaryopoiesis and platelet function defects. Blood 2016;127(10):1249–59.
[100] Leinoe EB, Hoffmann MH, Kjaersgaard E, Johnsen HE. Multiple platelet defects identified by flow cytometry at diagnosis in acute myeloid leukaemia. Br J Haematol 2004;127(1):76–84.
[101] Foss B, Bruserud O. Platelet functions and clinical effects in acute myelogenous leukemia. Thromb Haemost 2008;99(1):27–37.
[102] Mannucci PM, Tripodi A. Hemostatic defects in liver and renal dysfunction. Hematology Am Soc Hematol Educ Program 2012;2012:168–73.
[103] Lutz J, Menke J, Sollinger D, Schinzel H, Thürmel K. Haemostasis in chronic kidney disease. Nephrol Dial Transplant 2014;29(1):29–40.
[104] Rollini F, Franchi F, Muñiz-Lozano A, Angiolillo DJ. Platelet function profiles in patients with diabetes mellitus. J Cardiovasc Transl Res 2013;6(3):329–45.
[105] Arthur JF, Jandeleit-Dahm K, Andrews RK. Platelet hyperreactivity in diabetes: focus on GPVI signaling - are useful drugs already available? Diabetes 2017;66(1):7–13.
[106] Zhu W, Li W, Silverstein RL. Advanced glycation end products induce a prothrombotic phenotype in mice via interaction with platelet CD36. Blood 2012;119(25):6136–44.
[107] Kraakman MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. J Clin Invest 2017;127(6):2133–47.
[108] Wang N, Tall AR. Cholesterol in platelet biogenesis and activation. Blood 2016;127(16):1943–53.
[109] Icli A, Aksoy F, Nar G, Kaymaz H, Alpay MF, Nar R, et al. Increased mean platelet volume in familial hypercholesterolemia. Angiology 2016;67(2):146–50.
[110] Moscardó A, Latorre A, Santos MT, Bonanad S, Vallés J. Platelet function in malignant hematological disorders. Curr Opin Oncol 2015;27(6):522–31.
[111] Panova-Noeva M, Marchetti M, Russo L, Tartari CJ, Leuzzi A, Finazzi G, et al. ADP-induced platelet aggregation and thrombin generation are increased in essential thrombocythemia and polycythemia vera. Thromb Res 2013;132(1):88–93.
[112] Riedl J, Pabinger I, Ay C. Platelets in cancer and thrombosis. Hamostaseologie 2014;34(1):54–62.
[113] Riedl J, Kaider A, Marosi C, Prager GW, Eichelberger B, Assinger A, et al. Decreased platelet reactivity in patients with cancer is associated with high risk of venous thromboembolism and poor prognosis. Thromb Haemost 2017;117(1):90–8.
[114] Boneu B, Bugat R, Boneu A, Eche N, Sie P, Combes PF. Exhausted platelets in patients with malignant solid tumors without evidence of active consumption coagulopathy. Eur J Cancer Clin Oncol 1984;20(7):899–903.
[115] Falanga A, Tartari CJ, Marchetti M. Microparticles in tumor progression. Thromb Res 2012;129(Suppl. 1):S132–6.
[116] de Stoppelaar SF, van't Veer C, van der Poll T. The role of platelets in sepsis. Thromb Haemost 2014;112(4):666–77.
[117] Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zéni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. Clin Immunol 2012;145(3):189–200.
[118] Fälker K, Klarström-Engström K, Bengtsson T, Lindahl TL, Grenegård M. The toll-like receptor 2/1 (TLR2/1) complex initiates human platelet activation via the src/Syk/LAT/PLCγ2 signalling cascade. Cell Signal 2014;26(2):279–86.
[119] Lundahl TH, Petersson J, Fagerberg IH, Berg S, Lindahl TL. Impaired platelet function correlates with multi-organ dysfunction. A study of patients with sepsis. Platelets 1998;9(3–4):223–5.
[120] Iwamoto T, Kubo H, Takasaki M. Platelet activation in the cerebral circulation in different subtypes of ischemic stroke and Binswanger's disease. Stroke 1995;26(1):52–6.
[121] McConnell JP, Cheryk LA, Durocher A, Bruno A, Bang NU, Fleck JD, et al. Urinary 11-dehydro-thromboxane B2 and coagulation activation markers measured within

24 h of human acute ischemic stroke. Neurosci Lett 2001;313(1–2):88–92.
[122] Jurk K, Jahn U, van Aken H, Schriek C, Droste DW, Ritter MA, et al. Platelets in patients with acute ischemic stroke are exhausted and refractory to thrombin, due to cleavage of the seven-transmembrane thrombin receptor (PAR-1). Thromb Haemost 2004;91(2):334–44.
[123] Jacoby RC, Owings JT, Holmes J, Battistella FD, Gosselin RC, Paglieroni TG. Platelet activation and function after trauma. J Trauma 2001;51(4):639–47.
[124] Kutcher ME, Redick BJ, McCreery RC, Crane IM, Greenberg MD, Cachola LM, et al. Characterization of platelet dysfunction after trauma. J Trauma Acute Care Surg 2012;73(1):13–9.
[125] Connolly-Andersen AM, Sundberg E, Ahlm C, Hultdin J, Baudin M, Larsson J, et al. Increased thrombopoiesis and platelet activation in hantavirus-infected patients. J Infect Dis 2015;212(7):1061–9.
[126] Michels M, Alisjahbana B, de Groot PG, Indrati AR, Fijnheer R, Puspita M, et al. Platelet function alterations in dengue are associated with plasma leakage. Thromb Haemost 2014;112(2):352–62.
[127] Colucci G, Stutz M, Rochat S, Conte T, Pavicic M, Reusser M, et al. The effect of desmopressin on platelet function: a selective enhancement of procoagulant COAT platelets in patients with primary platelet function defects. Blood 2014;123(12):1905–16.
[128] Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood 2012;120(15):e73–82.
[129] Israels SJ, McNicol A, Dean HJ, Cognasse F, Sellers EAC. Markers of platelet activation are increased in adolescents with type 2 diabetes. Diabetes Care 2014;37(8):2400–3.
[130] Soma P, Swanepoel AC, du Plooy JN, Mqoco T, Pretorius E. Flow cytometric analysis of platelets in type 2 diabetes mellitus reveals 'angry' platelets. Cardiovasc Diabetol 2016;15:52.
[131] Calkin AC, Drew BG, Ono A, Duffy SJ, Gordon MV, Schoenwaelder SM, et al. Reconstituted high-density lipoprotein attenuates platelet function in individuals with type 2 diabetes mellitus by promoting cholesterol efflux. Circulation 2009;120(21):2095–104.
[132] Carvalho AC, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. N Engl J Med 1974;290(8):434–8.
[133] Pathansali R, Smith N, Bath P. Altered megakaryocyte-platelet haemostatic axis in hypercholesterolaemia. Platelets 2001;12(5):292–7.