Value of Platelet Activation Markers as Prothrombotic Risk Indicators

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Key Words

Platelet activation marker · MPV · Platelets · Haemostasis · Thrombosis

Summary

Platelets adhere to the subendothelium that is exposed at sites of vessel wall injury to form a primary haemostatic plug by aggregating together. Activated platelets support fibrin formation by exposing a procoagulant surface and encrypting circulating tissue factor. Through this process platelets are essential components of haemostasis and also of arterial thrombosis. Evidence has been provided that detection of circulating activated and/or hyperreactive platelets may indicate a high thrombotic risk. The aim of the present review is to present an overview of the various parameters that can be measured and that provide information on platelet activation and reactivity, such as changes in platelet surface membrane properties (e.g. CD62P expression, binding of PAC-1, fibrinogen or annexin V), release of stored compounds (e.g. β -thromboglobulin, platelet factor 4 or CD62P), formation of procoagulant membranous microvesicles, measurements of platelet adhesion, platelet aggregation and platelet-leukocyte conjugate formation and changes in platelet volume. The methods that can be used to measure the various parameters are briefly described. We also refer to various pitfalls in pre-analysis and data interpretation. Throughout we direct the readers' attention to published studies that indicate the potential value of the various measured parameters as indicators of thrombotic risk.

Schlüsselwörter

Thrombozytenaktivierungsmarker · MPV · Thrombozyten · Hämostase · Thrombose

Zusammenfassung

Thrombozyten adhären an subendotheliale Strukturen an der Stelle einer Gefäßverletzung, aggregieren miteinander und bilden damit den primären hämostatischen Pfropf. Aktivierte Thrombozyten unterstützen die Fibrinbildung durch Ausbildung einer prokoagulanten Oberfläche und durch Demaskierung von zirkulierenden Tissue factor. Infolge dieser Prozesse sind Thrombozyten eine entscheidende Komponente der Hämostase und tragen wesentlich zur Entstehung arterieller Thrombosen bei. Es gibt zahlreiche Hinweise dafür, dass der Nachweis von aktivierten und/oder hyperreaktiven Thrombozyten ein erhöhtes Risiko für thrombotische Ereignisse anzeigen kann. Ziel des vorliegenden Artikels ist es, einen Überblick über die verschiedenen Parameter, die zur Charakterisierung einer Thrombozytenaktivierung oder -reaktivität bestimmt werden können, zu geben. Dazu gehören Veränderungen in Eigenschaften der Plasmamembran (z.B. CD62P-Expression, Bindung von Fibrinogen, PAC-1 oder Annexin V), Freisetzung von Molekülen aus intrazellulären Speichern (z.B. β -Thromboglobulin, Plättchenfaktor 4 oder CD62P), Bildung prokoagulanten Membranvesikel, Messung der Thrombozytenadhäsion, der Thrombozytenaggregation und der Bildung von Thrombozyten-Leukozyten-Konjugaten sowie Veränderungen des Thrombozytenvolumens. Die Methoden für die Bestimmung der verschiedenen Parameter werden kurz beschrieben. Auf mögliche Fehler in der Präanalytik und der Dateninterpretation wird hingewiesen. Weiterhin werden Ergebnisse aus zahlreichen Studien, die den potenziellen Wert der verschiedenen Parameter für die Abschätzung eines thrombotischen Risikos anzeigen, vorgestellt.

Role of Platelets in Haemostasis, Thrombosis and Inflammation

Platelets are anucleated cell fragments that are derived from megakaryocytes. Due to their ability to adhere to the vessel wall at sites of vessel injury and to form a primary haemostatic plug by aggregating together, platelets are very important players in haemostasis [1, 2]. At the site of vessel injury platelets undergo initial activation via two main mechanisms:

- binding of subendothelial von Willebrand factor (vWF) and collagen to receptors on the platelet surface [3–7],
- cleavage of the platelet thrombin receptor by thrombin that is formed due to the exposure of subendothelial tissue factor (TF) to the blood stream [8–10].

The activated platelets are able to bind fibrinogen as well as vWF to glycoprotein IIb/IIIa complex (GPIIb/IIIa) on the platelet surface, resulting in platelet aggregation and the formation of a platelet-rich thrombus [11, 12]. Once activated, platelets contribute via various mechanisms to the formation of thrombin and fibrin as well as to the firmness and stability of the fibrin clot. These include:

- translocation of negatively charged phospholipids, in particular phosphatidylserine (PS), from the inner to the outer leaflet of the plasma membrane, which provide binding sites for various coagulation factors [13–17];
- production of platelet microvesicles (PMV) that are less than 1 µm in diameter as a consequence of re-organisation of the platelet membrane; PMV also expose large amounts of PS on their surface, enabling them to bind and activate coagulation factors in a similar way as native platelets [15, 16, 18, 19];
- exposure of TF on activated platelets and PMV [20–22]. Evidence has been provided that platelet-derived TF becomes activated when platelets or PMV adhere to leucocytes through a receptor-mediated mechanism that involves P-selectin (CD62P) on the surface of platelets and PMV and P-selectin glycoprotein ligand-1 (PSGL-1) on the surface of leucocytes [22–25]. On the other hand, platelets have shown to activate ‘blood-borne’ TF at the site of platelet activation, i.e. at the site of vessel wall injury [26]. Monocytes have been shown to contribute to ‘blood-borne’ TF by shedding TF-bearing microvesicles from their surface membrane. TF on monocyte-derived microvesicles seems to be encrypted and becomes decrypted when the microvesicles interact with activated platelets via PSGL-1 and CD62P [27–29]. It has also been reported that adhesion of platelets to monocytes that is mediated by CD62P and CD40 ligand (CD40L or CD154) induces a de-novo synthesis of TF in monocytes [30, 31].

As a consequence of these various functions, platelets not only play an essential role in haemostasis, but they are also major players in thrombosis, in particular in thrombi that develop in arteries and in the microcirculation. A main cause of arterial thrombosis leading to myocardial infarction, ischaemic stroke

or peripheral arterial occlusion is the presence of atherosclerotic plaques. When such plaques rupture, platelets become activated due to contact with exposed collagen fibres and vWF in the subendothelial matrix. Indirect activation is due to the exposure of plaque-associated TF and the formation of thrombin [12, 32, 33]. Today atherosclerosis is considered to be an inflammatory disease, and platelets are believed to contribute to the development of atherosclerotic plaques, mainly due to a contribution to inflammatory reactions within the vessel wall [34].

Platelets, vWF and the vWF-cleaving protease ADAMTS-13 (A Disintegrin And Metalloproteinase with a Thrombospondin type 1 motif) have now been recognised as major players in thrombotic microangiopathies. The thrombotic microangiopathies are microvascular occlusive disorders characterised by systemic or intrarenal platelet aggregation, thrombocytopenia and mechanical injury to erythrocytes. In thrombotic thrombocytopenic purpura, systemic microvascular aggregation of platelets results in ischemia in the brain and other organs [35]. The most severe form of this kind of disease, the thrombotic thrombocytopenic purpura (TTP) firstly described by Ely Moschcowitz in 1924 [36], is caused by a hereditary or acquired deficiency of ADAMTS-13. It is a consequence of a hereditary or acquired deficiency of ADAMTS-13. Due to a lack of or a markedly decreased enzyme activity, ultra large vWF multimers are insufficiently cleaved and induce activation of platelets at sites of high shear forces, i.e. within the microcirculation [35, 37].

In addition to their essential role in haemostasis and thrombosis, platelets are now recognised as an inflammatory cell. Activated platelets secrete various factors that act as signals to target cells of the innate and adaptive immune system. Some of the secreted factors are proteins and peptides, such as cytokines and chemokines, growth factors, antimicrobial peptides or bioactive amines; other factors are rapidly synthesised when platelets become activated (e.g. lipid mediators such as thromboxane A₂ or platelet-activating factor) [38–40]. Another mechanism by which platelets and PMV communicate with cells of the immune system is receptor-mediated contact. Changes in the generation of reactive oxygen species, in phagocytic and adhesion activity and in the expression of genes with relevance to the inflammatory response of the target cells have been described [41–46]. However, the mechanisms involved in the modulation of immune response by platelets or PMV are not yet completely understood.

Markers of Platelet Activation and Platelet Hyperreactivity

As outlined above, platelets are implicated in the pathophysiology of thrombosis as well as inflammation. In consequence, they also had an impact on the morbidity and mortality associated with such conditions. With respect to thrombosis, success-

ful outcomes after administration of inhibitors of platelet function such as aspirin, clopidogrel and GPIIb/IIIa antagonists have provided further evidence of the importance of platelets in cardiovascular disease. Consequently, the identification of subjects with inappropriate platelet function could help to determine patients at risk of thrombosis and also appropriate means of risk reduction. In principle there are two ways to assess the behaviour of platelets in patients at risk:

- To look for evidence of platelet hyperreactivity, i.e. to determine whether the platelet response to a given stimulus is higher when compared with that of control subjects. Such studies can be performed in vitro.
- To look for an evidence of in vivo platelet activation. For such studies platelets of patients are analysed ex vivo without any additional stimulation.

There are manifold functions of platelets, and the various aspects of platelet activation result in many and quite different tests to determine platelet reactivity. Parameters that describe platelet reactivity and/or in vivo platelet activation can be divided in several categories:

- interaction of platelets with cells or matrices
 - adhesion of platelets to artificial or natural surfaces or matrices
 - platelet-platelet aggregation
 - adhesion of platelets to leucocytes or endothelial cells,
- changes at the platelet membrane
 - translocation of molecules from intracellular compartments to the membrane surface
 - conformational changes of surface receptors
 - binding of extracellular molecules to the membrane,
- release of platelet material into the surrounding plasma
 - secretion of molecules that are stored inside platelets or that are synthesised when platelets become activated
 - shedding of surface-exposed molecules
 - shedding of membranous microvesicles,
- changes in the platelet proteome,
- changes in the platelet volume and density.

To discuss all the methods that are described in the literature would exceed the limits of this review. Therefore, only the most important and widely used methods will be reviewed. The order they will be introduced here will not follow the categorisation as given above.

Platelet Aggregation

Measurement of spontaneous or agonist-induced platelet aggregation is the most commonly used method to determine platelet reactivity. Platelet aggregation is mediated by the binding of fibrinogen to its receptor (GPIIb/IIIa, CD41) and the consequent bridging of platelets together. In 1962 Gustav Born [47] introduced an elegant method to determine platelet aggregation by simple measurement of light transmission in a stirred sample of platelet-rich plasma (PRP) or washed platelets. Although this method is still widely used today, it has the disadvantage that platelets have to be isolated from

the other blood cells. To avoid the procedure of platelet isolation and to have the opportunity to investigate the influence of other blood cells on platelet aggregation, whole blood methods were developed. One possibility to measure whole blood platelet aggregation is based on the counting of the number of single platelets [48] or on the measurement of electrical impedance of blood which changes when platelets adhere and aggregate on special platinum electrodes [49]. Platelet aggregation results in a fall in the number of single platelets or an increase in impedance [48–50]. As platelet counts already change when very small aggregates that consist of only few platelets are formed, the counting technique can also monitor the formation of microaggregates that cannot be detected by light transmission or impedance measurements [51, 52].

Another method to measure platelet micro- and macroaggregation in samples of whole blood is flow cytometry. This method also quantitates platelet aggregation by counting the number of single platelets, but microaggregates can also be directly visualised [53, 54]. According to the basic principle of flow cytometry, platelets are identified by their light scatter characteristics as well as by the binding of a fluorescence-labelled antibody directed against a platelet-specific antigen, e.g. CD41 or CD42. Formation of microaggregates is characterised by an increase in the size, i.e. the forward light scatter, as well as by a decrease in the number of the platelet-positive events. To get absolute numbers for single platelets or platelet microaggregates, it has been recommended to determine the platelet / red blood cell (RBC) ratio by flow cytometry and to determine the absolute RBC count in the whole blood sample using standard laboratory methods [54, 55].

An instrument that measures in parallel matrix-platelet adhesion and platelet aggregation is the Platelet Function Analyser PFA 100 (Dade Behring, Eschborn, Germany). The instrument measures the closure time of the aperture of the cartridge that is covered with platelet agonists, i.e. collagen + ADP or collagen + epinephrine. PFA 100 is probably more capable to detect decreased rather than increased platelet activity [56, 57].

There are also some reports in the literature describing circulating platelet aggregates in patients with high thrombotic risk. For example, there is a report of platelet aggregates in blood from TTP patients detected using flow cytometry [58].

Changes in Platelet Membrane Surface Receptors

Platelet activation results in a reorganisation of the plasma membrane that can be rather easily detected by flow cytometry and can be used for measurement of platelet activation in vivo as well as to evaluate platelet reactivity. One of the advantages of flow cytometry is that only minuscule volumes (5 µl) of blood are required. However flow cytometry has also some disadvantages when analysing in vivo activation. One of them is that blood samples have to be analysed within 30 min to avoid ex vivo platelet activation. For some but not all of the

parameters that can be determined, the time issue can be circumvented by immediate fixation of the blood sample [59, 60]. A conformational change in GPIIb/IIIa is a prerequisite for fibrinogen binding and platelet aggregation. The change on GPIIb/IIIa is associated with the appearance of a new epitope that is recognised by the monoclonal antibody PAC-1. Thus PAC-1 binds to activated but not to resting platelets [61]. Activated GPIIb/IIIa can be also detected using either fluorescence-labelled fibrinogen or an antibody against fibrinogen [62, 63]. Increased binding of PAC-1 and/or fibrinogen has been reported in patients with myocardial infarction, stroke, sepsis and anti-phospholipid syndrome as well as in post-menopausal women who are known to have an increased thrombotic risk [64–68].

The most widely studied activation marker on the platelet surface is CD62P. There are many reports on increased expression of this antigen on platelets in patients with various kinds of disease associated with thrombotic risk, and very often increased CD62P expression is associated with increased binding of PAC-1 or fibrinogen [66, 68, 69]. It has been recently reported that high CD62P expression on circulating platelets is strongly associated with adverse clinical outcomes after an early ischaemic stroke [70]. When determining platelet CD62P as a platelet activation marker, one has to consider that the molecule is also shed from circulating platelets [71–73]. Furthermore, as mentioned above, CD62P mediates the adhesion of activated platelets to monocytes and neutrophils in a process that involves PSGL-1 on the leucocyte surface [74].

There are some other receptor molecules that can be measured by flow cytometry as indicators of platelet activation, e.g.

- the α -granule membrane protein GMP-33 [75,76],
- the lysosomal protein CD63 the expression of which has been shown to be increased for example in patients with venous thrombosis or type 2 diabetes mellitus [77, 78], or
- the CD40L that is involved in the interaction of platelets with leucocytes and that is believed to trigger pro-inflammatory signalling [79–83].

Platelet-Leukocyte Conjugates

The fact that activated CD62P-positive platelets readily adhere to monocytes and neutrophils make circulating platelet-leucocyte conjugates a very sensitive marker of platelet activation. Platelet-leucocyte conjugates can be easily identified by whole blood flow cytometry as leucocytes staining positive for a platelet-specific antigen such as CD41 or CD42a. Leucocytes can be differentiated from other cells and from each other by their light scatter characteristics and also by using antibodies to cell-specific surface antigens, e.g. CD14 on monocytes. Thus it is possible to distinguish conjugates that are formed from platelets adherent to neutrophils, to monocytes or to lymphocytes [84, 85]. As mentioned above and discussed in more detail below, platelet activation also results in the shedding of membranous microvesicles from

the platelet plasma membrane. These PMV express various surface receptors similar to activated platelets. Thus, PMV can also adhere to leucocytes. However, flow cytometry cannot distinguish whether platelets or PMV adhere to leucocytes forming a platelet-leucocyte conjugate. Nevertheless, both adhesion of activated platelets or of PMV to leucocytes reflect platelet activation [86, 87]. Another aspect of determining platelet-leucocyte adhesion is related to the approach taken to evaluate the flow cytometric data obtained. Adhesion of platelets or PMV to leucocytes is characterised by an increase in the platelet-specific fluorescence signal associated with the leucocyte subspecies. There are two ways to express the amount of conjugate formation: as percentage of 'platelet-positive' leucocytes or as the fluorescence intensity of the platelet-specific fluorescence given as either mean or median fluorescence intensity per leucocyte. The number of platelet-positive leucocytes that is obtained by setting a threshold for the fluorescence intensity does not consider the number of adhering platelets or PMV. On the other hand, mean fluorescence intensity is a measure of the total 'load' of leucocytes with the platelet-specific antigen, i.e. it is a measure of the number of platelets and PMV bound to each leucocyte [85, 88]. To have both measures, the number of platelet-positive leucocytes and also the mean fluorescence per leucocyte, may be especially helpful when investigating platelet-leucocyte adhesion in patients receiving GPIIb/IIIa antagonists such as abciximab (Reopro®, Eli Lilly and Company, Indianapolis, IN, USA) or eptifibatide (Integrilin®, GlaxoSmithKline, Middlesex, UK). As such compounds are highly effective in inhibiting platelet-platelet aggregation but do not prevent platelet activation and CD62P expression, they can increase the number of platelet-positive leucocytes (due to a high number of single CD62P-positive platelets) but decrease the mean fluorescence of the platelet-positive leucocytes (no adhesion of platelet aggregates). Such phenomenon could explain the inconsistent data on the effects of GPIIb/IIIa antagonists on platelet-leucocyte adhesion [88–91].

With respect to leucocyte subspecies, platelets or PMV adhere more easily to monocytes when compared to neutrophils [71, 84, 92, 93]. Thus quantitation of circulating platelet-monocyte conjugates is considered to be a particularly sensitive means of determining platelet activation in vivo [71, 94]. The number of circulating platelet-monocyte conjugates is increased in various clinical conditions that are associated with platelet activation and increased thrombotic risk [71, 94–98].

Circulating platelet-leucocyte conjugates may not only indicate platelet activation in vivo but may be also implicated in thrombus formation. Some evidence has been provided that activated platelets as well as PMV express TF, the most important activator of intravascular coagulation, on their surface [21, 86, 99]. However, adhesion of platelets and PMV with leucocytes also seems to be important in generating actual procoagulant activity [22, 25, 100].

Platelet Procoagulant Activity and PMV

Activation of platelets is associated with a translocation of negatively charged phospholipids, in particular PS, from the inner to the outer leaflet of the plasma membrane. PS exposure at the platelet surface is not only an indicator of platelet activation, but proves essential for the binding and activation of some plasma coagulation factors resulting in the amplification and propagation of thrombin and fibrin formation [13–17]. In addition to PS, there is also some evidence for the existence of specific ‘non-lipid’ receptors for factors X(a), XI(a) and VIII(a) on the surface of activated platelets as reviewed by Hoffman and Monroe [8].

In 1985, a protein was isolated from umbilical cord arteries that inhibited blood coagulation by affecting the interaction between coagulation factors and phospholipids, and later it was identified as annexin V [101, 102]. Due to its high affinity to PS on cell surfaces, annexin V is widely used to detect PS exposure of activated platelets; annexin V binding is considered as a measure of the platelet procoagulant activity [103–105].

When measuring annexin V binding to detect platelet activation it has to be considered that PS translocation is one of the mechanisms that are involved in the shedding of PMV from the platelet plasma membrane. Thus one may speculate that most of the translocated PS is associated with PMV rather than with the activated platelets [16, 106–108]. Indeed there are considerably more reports on increased numbers of annexin V-positive PMV in various clinical conditions when compared to reports on annexin V-positive platelets.

In principle there are two ways to measure PMV in samples of whole blood or plasma: flow cytometry or enzyme-immunoassay (ELISA). Using flow cytometry, PMV can be identified by their light scatter characteristics and by the binding of an antibody that is directed against a platelet-specific antigen such as CD41 or CD42a. Additionally some authors use annexin V binding as a unique characteristic of cell-derived microvesicles and as a measure of the PMV procoagulant activity [105, 109, 110]. Using flow cytometry, PMV can be detected in samples of whole blood, PRP or platelet-poor plasma (PPP), but some authors prefer to isolate microvesicles from blood or plasma samples prior to staining with a platelet-specific antibody (and annexin V) [111, 112]. When measuring PMV in samples of whole blood or PRP, one has to define a size threshold on the forward scatter versus fluorescence dot plot or histogram to distinguish between PMV and the smallest intact platelets. To solve this problem, microspheres with a diameter of 1 µm or less can be used to set the threshold [113, 114]. Another possibility is to define the threshold in such a way that only a certain percentage (1–5%) of all platelet-positive events is below it. Of course, an unstimulated whole blood or PRP sample of healthy individuals have to be used to set the threshold [115, 116]. When measuring PMV in cell-free samples, i.e. in PPP or suspensions of washed PMV, there is no problem in discriminating between PMV and platelets. To pre-

pare PMV-containing PPP, various centrifugation protocols are used, e.g. 15 min at $700 \times g$ and up to 5 min at $5,000 \times g$. For isolating PMV from plasma high speed centrifugation, such as 1 h at $13,000 \times g$, is necessary [86, 117, 118]. A further problem concerns the enumeration of PMV. Probably the best method is to add to the sample a defined number of fluorescence-labelled counting beads that are provided by various companies. These beads can be identified and counted in the flow cytometer and used to calculate the number of PMV per sample volume, i.e. per millilitre blood or plasma [53, 118]. Another possibility is to calculate the number of PMV in the samples on the basis of the flow volume that is passed through the flow cytometer. However, this method requires to ascertain a constant flow in the instrument [112, 117]. When measurements are done in samples of whole blood or PRP, it is also possible to express the number of PMV in relation to the total number of platelet-positive events that are measured [69, 116, 119]. While a good correlation was reported between the relative and absolute numbers of PMV when measured in samples of healthy individuals or patients with activated platelets, a poor correlation was found when measured before and after in vitro platelet activation [115, 118, 120].

Flow cytometry has been widely used to demonstrate increased levels of circulating microvesicles indicating platelet activation in various clinical conditions such as myocardial infarction [112, 121, 122], percutaneous transluminal coronary angioplasty (PTCA) [117, 123], peripheral arterial disease [112, 124], sepsis [111, 125–128], TTP [120, 129, 130] or pre-eclampsia [131].

ELISA technique has been also employed to measure PMV. Osumi et al. [132] used monoclonal antibodies directed to various platelet-specific antigens to capture PMV from PPP samples and used second antibodies to quantify the amount of captured PMV. This method was successfully used to monitor changes in platelet function in patients undergoing PTCA [133].

Soluble Plasma Markers of Platelet Activation

This topic has been excellently reviewed by Gurney et al. [134] in 2002. From the huge number of molecules that are released from activated platelets only a few appeared to be reliable markers of platelet activation. There are two proteins that are released from the platelet α -granules: platelet factor 4 and β -thromboglobulin. The plasma levels of both have been shown to be elevated in clinical conditions that are associated with platelet activation and increased thrombotic risk including hypertension, coronary artery disease, diabetes mellitus and cancer [134].

Despite the fact that it can also be released from endothelial cells, soluble CD62P is also believed to a reliable marker of platelet activation [71–73, 124, 135]. Soluble CD62P in plasma samples is determined by ELISA technique, but this method cannot distinguish between free soluble CD62P and CD62P that is expressed on PMV. By exploring plasma samples ob-

tained from healthy volunteers, we could recently shown that removal of PMV and other circulating cellular microvesicles by ultracentrifugation resulted only in a partial reduction of the CD62P level, indicating that more than 50% of plasma CD62P is soluble and not bound to microvesicles [86]. Recently, soluble glycoprotein V (GPV) has attracted increasing interest as a marker of platelet activation. Expression of GPV is restricted to platelets and megakaryocytes. Together with GPIb and GPIX it forms a complex on the surface of platelets that acts as a receptor for vWF and thrombin [136, 137]. Release of GPV from activated platelets is believed to be mediated by a cleavage of the molecule by thrombin [138, 139]. Increased GPV plasma levels have been reported in patients with coronary and peripheral atherosclerosis [140–142].

Mean Platelet Volume

The mean platelet volume (MPV) as a measure of platelet size can be easily determined by haematology analysers. The consensus is that blood should be collected in a special way using sodium citrate as anticoagulant to avoid artificial swelling of the platelets [143]. In 1983 three reports were published demonstrating a relationship between MPV and myocardial infarction [144–146]. This relation was proved in many further and also in very recent studies, and evidence was provided that high MPV is an independent risk factor of myocardial infarction and a predictor of poor outcome [147–151]. Increased MPV was also shown in patients with ischaemic stroke [143, 152] and other conditions with increased thrombotic risk such as diabetes mellitus, obesity and others [143, 153–156]. Recently MPV was shown to be an independent risk factor for thromboembolic events after heart valve replacement and for stent thrombosis after PTCA [153, 157, 158].

Despite the fact that it is well accepted that high MPV is a risk indicator of thromboembolic events, the cause of high PMV is not completely understood. There is some evidence for a correlation between megakaryocyte DNA content, i.e. megakary-

ocyte polyploidy, and PMV, and both may increase under the condition of an increased platelet consumption [159–161]. PMV is also inversely correlated with platelet number which suggests an influence of platelet production on the values that are obtained. Also there are observations of persistent circulation of large activated platelets in patients after myocardial infarction [69, 158]. On the other hand large platelets are believed to be haemostatically more active and seem to be consumed at the time of thrombus formation, e.g. at time of myocardial infarction or during an acute coronary syndrome [160–162]. However, this might be expected to reduce overall MPV rather than to increase it.

Summarising, MPV is an easily and rapidly determinable parameter which appears to relate to some thromboembolic events. However, the means through which the MPV is regulated and the implications of this for thrombosis formation need further elucidation.

A Final Warning

There are many and quite different approaches to measure platelet reactivity and platelet activation, and such approaches can provide some valuable information on the thromboembolic risk. However, irrespective of the method and the parameter that is measured, it has to be considered that platelets are very reactive cells. When removed from the circulation they can spontaneously and rapidly become activated. Therefore, much attention has to be paid to pre-analytic procedures, including blood drawing and sample transportation. If one is not careful, the measured markers of platelet activation can tell us more about concomitant circumstances such as the time that has elapsed between blood sampling and sample analysis or the degree of sample agitation during transportation than about the possible thromboembolic risk in the patients under investigation.

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