Opinion Paper

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Mean platelet volume in arterial and venous thrombotic disorders

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Abstract: The mean platelet volume (MPV) is an easy, rapid and inexpensive laboratory parameter which basically mirrors platelet size. Due to the essential role of platelets in hemostasis, many studies have assessed the MPV value in patients with arterial and venous thrombotic disorders. These have then been summarized in some interesting meta-analyses and recent studies that will be discussed in this narrative review. Taken together, the currently available evidence suggests that the MPV may be substantially increased in concomitance with acute episodes of coronary artery disease, venous thromboembolism, portal vein thrombosis, stroke, erectile dysfunction and preeclampsia. In many of these conditions, an increased MPV value may also be associated with unfavorable outcomes. Despite these convincing findings, some important technical issues should be considered for improving the clinical usefulness of this measure. These essentially include anticoagulant, timing of sample collection, the sample storage conditions, the influence of the analytical techniques, the approaches used for its calculation, the accurate definition of reference ranges and diagnostic cut-offs, as well as the current lack of standardization, which makes data obtained with different techniques/analyzers poorly comparable. Provided that the impact of these variables can be abated or minimized, the MPV can gain a valuable role in the laboratory workout of many arterial and venous thrombotic disorders.

Keywords: coronary artery disease; mean platelet volume; platelets; thrombosis; venous thromboembolism.

Introduction on platelet structure and biology

Platelets, also known as thrombocytes, belong to the family of corpuscular elements that are physiologically present in blood. Their main – though not univocal – function is to actively participate in the hemostatic process, namely primary hemostasis, for generating stable blood clots and stopping blood leakage from injured vessels [1–3]. Unlike leukocytes, but similar to erythrocytes, platelets are conventionally considered “terminal elements” because they lack a nucleus and cannot thereby synthesize ex-novo proteins and other molecules. Nevertheless, these small corpuscular elements not only contain a vast array of different molecules within their cytoplasm and granules, but can also actively interplay with many different cells and molecular pathways, so their actual existence is much more dynamic than what may be expected [1, 4].

Platelets exclusively originate from cytoplasmic fragmentation of megakaryocytes, their precursor cells residing in the bone marrow (each single megakaryocyte can generate as many as 5000–10,000 platelets). Each day nearly 10^{12} platelets are generated in healthy humans, thus maintaining their circulating number between 150 and $400 \times 10^9/L$ [5]. Upon generation, platelets are rapidly released into the bloodstream, where they circulate for a normal period varying between 8 and 12 days (10 days, on average) [1]. Between one-third and two-thirds of blood platelets are normally stored in the spleen, ready to be released upon necessity, and in that same organ (as well as in the liver) elderly and almost dysfunctional platelets are eliminated through phagocytosis by Kupffer cells [4]. The normal circulating platelets appear as biconvex discoid (lens-shaped) elements, with a diameter of approximately 2–3 μm , a thickness of 0.5 μm and a volume typically comprised between 6 and 13 fL (in resting conditions) [3]. Importantly, platelet size and volume (MPV) may increase

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because of two reasons: (i) bone marrow response, encompassing release of large (mostly reticulated) platelets, which can now be reliably identified by modern hematological analyzers as immature platelet fraction (IPF), and (ii) response of circulating platelets to certain stimuli.

Platelets constitutively contain two main types of secretory granules. Dense granules, which are also known as “delta granules”, contain adenosine diphosphate (ADP) and calcium which, upon secretion, trigger and/or boost platelet aggregation and surface coagulation reactions. The granules belonging to the second type are conventionally called alpha (i.e. α -granules), and represent the specific secretory apparatus for a vast array of proteins including von Willebrand factor (VWF), platelet factor 4 (PF4) and fibrinogen, among others. Notably, the α -granule content can be enriched through two different mechanisms, by either megakaryocyte biosynthesis (e.g. PF4) or endocytosis/pinocytosis (e.g. fibrinogen and immunoglobulins) [6]. The platelet surface exposes a considerable number of receptors, which are at the basis of platelet function. These typically include (i) glycoprotein (GP) IIb-IIIa, which binds fibrinogen, VWF, fibronectin, vitronectin and thrombospondin; (ii) GP Ia-IIa, which binds collagen; (iii) GP VI, which binds collagen and (iv) GP Ib-V-IX, which binds VWF along with other ligands such as thrombin and P-selectin [1, 2].

Immediately upon activation, for example, by direct contact with sub-endothelial surface or triggered by

thrombin stimulation after a vessel injury has occurred, platelets undergo a series of considerable structural changes, mutating from a conventional disc shape in resting condition, to assume a compact sphere morphology with long dendritic extensions upon activation (Figure 1). These morphological changes are enabled by the presence of actin and myosin within the platelet cytoplasm [1]. Although usually considered within the context of reducing bleeding risk by their presence, platelets may also be associated with thrombosis [7], whereby it has now been clearly established that platelets are essential elements of both arterial and venous blood clots [8]. Although the number of platelets is usually lower in venous than in arterial thrombi, their real presence has recently contributed to fuel an interesting debate as to whether antiplatelet drugs (namely aspirin) may also be effective to prevent venous thrombosis [9]. Therefore, the aim of this narrative review is to provide an update on the role of MPV value in arterial and venous thrombotic disorders.

Laboratory assessment of mean platelet volume

The conventional complete blood cell count (CBC) encompasses direct or indirect assessment of many blood cell parameters, mostly referring to their number, type, size

Figure 1: Variation of mean platelet volume in concomitance with acute thrombotic episodes.

and structure. As regards platelets, the usual parameters generated within the CBC include the number of circulating elements (reference range, $150\text{--}400 \times 10^9/\text{L}$), the plateletcrit (reference range, 0.15–0.62%), the mean platelet volume (MPV) and the platelet distribution width (PDW; reference range, 10–18%) [10–13].

Concerning MPV, this parameter is calculated (automatically) by the hematological analyzer and thus represents a rapid and virtually inexpensive measure of the average platelet size in the blood sample [14]. It was recently highlighted that MPV displays a 1.2%, 2.3% and 7.1% analytical, intra- and inter-individual variability (accounting for a reference change value of ~7%) [15], is highly dependent on genetic (i.e. polymorphisms in a large number of human genes) and metabolic (e.g. age, hypertension, smoking, presence of comorbidities and use of medications) determinants [16], as well as on the analytical technique used for its assessment and the final means used for its calculation (e.g. logarithmic data transformation, range of volume included and so forth). In a comprehensive analysis of current laboratory technology, Hoffmann has recently concluded that the reference range of MPV not only depends on the analytical technique used for platelet assessment (i.e. optic, impedance or fluorescent method), but also on the brand of the hematological analyzer [17]. More specifically, the MPV can vary from 5.6–8.9 fL using the optical technique on Siemens instrumentation (Siemens Healthineers, Erlangen, Germany), to 6.1–10.7 fL when measured with the impedance technique with Abbott analyzers (Abbott Laboratories, Chicago, IL, USA), up to 9.3–12.7 fL when assayed on Sysmex analyzers (Sysmex Corporation, Kobe, Japan) through the impedance technique. This evidence has been confirmed by a subsequent investigation, which showed variations of the reference range as high as 25% using four different commercial hematological analyzers [18]. This impressive heterogeneity has at least two important practical implications. First, longitudinal patient monitoring should always be carried out using an identical technology and – preferably – the same type of hematological analyzer, otherwise, the interpretation of platelet volume fluctuations over time may be misleading. Then, the reference ranges and the cut-offs identified in certain studies, using a specific analytical technique and a certain type of analyzer, cannot be thoughtfully translated to other clinical settings, where MPV is assayed with different instrumentation. Additional important aspects that may contribute to impair inter-study comparability encompass the time of sample collection as well as the sample storage conditions. The former issues will be more comprehensively discussed in the following sections of this article, whilst

clear evidence has now been provided that the MPV value is strongly influenced by the storage condition [19]. A recent comprehensive study, which assessed the stability of many CBC parameters over time, has concluded that MPV may only be stable up to 4 h when whole blood specimens are kept at room temperature before analysis, whilst the stability of samples can be modestly improved when whole blood is maintained refrigerated at 4 °C before analysis (i.e. up to 6–8 h) [20]. These results are in keeping with those earlier published in other studies, which also showed that analysis shall not be preferably delayed by over 4–6 h from sample collection, irrespective of the analytical technique and the storage conditions [20–23].

Regardless of these important pre-analytical and analytical issues, enhanced MPV values are usually seen in association with the release of young platelets in the bloodstream and/or presence of hyperactive platelets, whilst MPV values usually decrease when platelets are older, have secreted a large part of their granules and are hence exhausted and dysfunctional [24, 25]. Notably, reliable evidence has been provided that larger platelets are usually more functionally active than their smaller counterparts [26, 27], so that an increased MPV value shall not only be regarded as a reliable index of platelet hyper-reactivity, but also as a risk factor of hypercoagulability. The following section of this article is hence aimed at providing an updated narrative overview on the potential diagnostic applications of MVP in arterial and venous thrombotic disorders, by describing the results of recent meta-analyses and original studies in areas not covered by systematic literature reviews.

Mean platelet volume and coronary artery disease

Four meta-analyses have been published on the role of MPV in coronary artery disease (CAD) to the best of our knowledge. The first of such articles, published by Chu et al. in 2010 [28], included 16 cross-sectional studies, totaling 2809 patients. Overall, MPV was found to be significantly higher in patients with acute myocardial infarction (AMI) than in those without (mean difference, 0.92 fL; 95% confidence interval [95% CI], 0.67–1.16 fL). The MPV value was also found to be significantly higher in patients with AMI than in those with stable CAD (mean difference, 1.00 fL; 95% CI, 0.5–1.50 fL), as well as in those without known CAD (mean difference, 1.11 fL; 95% CI, 0.79–1.42 fL). An enhanced MPV value was commonplace in patients developing restenosis after revascularization compared to

those who did not (mean difference, 0.98 fL; 95% CI, 0.74–1.21 fL), and was also associated with a 65% higher risk of death after AMI (odds ratio [OR], 1.65; 95% CI, 1.12–2.52). In 2014, a meta-analysis based on results of 40 studies was published by Sansanayudh et al. [29]. Compared to healthy controls, MPV was found to be higher in all patients with CAD (mean difference, 0.70 fL; 95% CI, 0.55–0.85 fL), as well as in those with stable CAD (mean difference, 0.61 fL; 95% CI, 0.29–0.94 fL), unstable angina (mean difference, 0.85 fL; 95% CI, 0.50–1.20) or AMI (mean difference, 0.83 fL; 95% CI, 0.57–1.09 fL). The risk of having stable CAD or an acute coronary event was ~2.3-fold (OR, 2.28; 95% CI, 1.46–3.58) and ~3.8-fold (OR, 3.78; 95% CI, 1.74–8.22) higher in patients with MPV values exceeding the study cut-off. Later, in 2015, a third meta-analysis which included 30 studies was published once again by Sansanayudh et al. [30]. Patients with CAD who developed acute cardiovascular events were found to have a larger MPV than those who did not (mean difference, 0.69 fL; 95% CI, 0.36–1.01 fL). Compared to those who did not develop acute cardiovascular events, the mean MPV difference was 0.84 fL (95% CI, 0.41–1.27 fL) in patients with AMI and 0.60 fL (95% CI, 0.26–0.94 fL) in those with coronary stenosis. Notably, MPV was finally found to be significantly higher in CAD patients who developed restenosis after revascularization compared to those who did not (mean difference, 0.68 fL; 95% CI, 0.52–0.83 fL), as well as in those who died compared to those who did not (mean difference, 0.86 fL; 95% CI, 0.15–1.57 fL). Overall, a higher MPV value (i.e. exceeding the study cut-off) was associated with a 14% higher risk of mortality in patients with CAD (relative risk [RR], 1.14; 95% CI, 1.04–1.25). More recently, Moghadam et al. published the results of another meta-analysis aimed at exploring the role of MPV in patients with coronary artery ectasia [31]. A total of 14 studies accumulating 2323 subjects were analyzed, showing that patients with coronary artery ectasia had a significantly higher MPV value compared to those without this condition (mean difference, 0.58 fL; 95% CI, 0.22–0.95 fL).

Major insights into the prognostic role of MPV in arterial thrombosis have recently been published by Avci et al. [32]. The authors carried out a large retrospective population-based cohort study, including 480 consecutive patients with ST-elevation myocardial infarction (STEMI), who were followed up for a median period of 66 months. In the fully adjusted multivariate analysis, an increase of MPV value 48–72 h after hospital admission was associated with a 30% higher mortality (hazard ratio [HR], 1.30; 95% CI, 1.07–1.58), with delta MPV being associated with a diagnostic efficiency (area under the curve [AUC]) of 0.80 (95% CI, 0.75–0.85).

Mean platelet volume and venous thrombotic diseases

The possible role of MPV in patients with venous thromboembolism (VTE), encompassing either deep vein thrombosis (DVT) or pulmonary embolism (PE), has very recently been published by Kovács et al. [33]. In this meta-analysis, a total number of 18 cohort studies were included, comprising 5012 subjects (2187 VTE patients and 2825 healthy controls). Overall, patients with VTE were found to have higher MPV than those without (mean difference, 0.69 fL; 95% CI, 0.39–0.98 fL), translating into an AUC of 0.72 (95% CI, 0.63–0.81) and pooled sensitivity and specificity of 0.77 (95% CI, 0.70–0.84) and 0.57 (95% CI, 0.46–0.68), respectively. Although no specific meta-analysis has assessed the prognostic role of MPV in VTE patients so far, the results of the recent study by Díaz et al. provide some valuable contribution on this matter [34]. Briefly, the authors carried out a retrospective analysis of a cohort of nearly 600 patients diagnosed with a first episode of VTE between the years 2014 and 2015. In the fully adjusted multivariable analysis, an increased MPV value was found to be independently associated with an enhanced risk of death, as high as 2.4-fold (95% CI, 1.5–3.9) in the entire VTE population, 2.3-fold (95% CI, 1.5–4.4) in patients with DVT and 2.9-fold (95% CI, 1.6–5.6) in those with PE, respectively.

Another interesting meta-analysis has been published in 2019 by Lin et al. [35]. The authors finally selected seven case-control studies, including 239 patients with portal vein thrombosis and 616 controls, and reported that the MPV value was higher in cases than in controls (mean difference, 0.88 fL; 95% CI, 0.61–1.15 fL), especially in portal vein thrombosis patients with concomitant liver cirrhosis (mean difference, 0.96 fL; 95% CI, 0.70–1.23 fL).

Mean platelet volume in other vascular occlusive diseases

As regards additional vascular occlusive diseases, Shemirani et al. recently published the results of a systematic review and meta-analysis of the literature, aimed at defining the role of MPV in patients with acute stroke [36]. Their meta-analysis, encompassing 32 eligible articles, showed that MPV was significantly higher in patients with stroke compared to healthy controls (mean difference, 0.51 fL; 95% CI, 0.27–0.74 fL). A sub-analysis of patients with ischemic stroke generated virtually identical results (mean difference, 0.55 fL; 95% CI, 0.29–0.81 fL). Notably, the predictive role of MPV in acute ischemic stroke has

recently been hypothesized by Du and colleagues [37], who studied 281 patients with first-ever ischemic stroke and 164 with first-ever hemorrhagic stroke. Overall, the risk of both hemorrhagic and ischemic stroke in patients with increased MPV values (i.e. >13 fL) was 5.2- and 22.2-fold higher than in those with normal MPV values.

Another meta-analysis has then been published by Ren et al. [38]. The article, which included seven studies totaling 795 patients with erectile dysfunction and 524 healthy control subjects, revealed that the MPV value was significantly higher in these patients than in controls (mean difference, 0.60 fL; 95% CI, 0.38–0.82 fL). Patients with vasculogenic erectile dysfunction were also found to have higher MPV values than those with non-vasculogenic disease (mean difference, 0.71 fL; 95% CI, 0.41–1.00 fL), thus reinforcing the putative role of platelet volume in disorders or prevalently vascular origin.

Interesting evidence has then emerged from the meta-analysis of Bellos et al. [39], who explored the potential clinical significance of platelet volume in preeclampsia, a condition strongly sustained by thrombosis of utero-placental vasculature [40]. A total number of 50 studies, including 14,614 women, were included in the final analysis, which showed that MPV value was significantly higher in women with preeclampsia than in those without (mean difference, 1.04 fL; 95% CI, 0.76–1.32 fL). Notably, the mean difference of MPV between cases and controls was found to be nearly double in women with severe preeclampsia (mean difference, 1.28 fL; 95% CI, 0.75–1.80 fL) than in those with milder forms (mean difference, 0.65 fL; 95% CI, 0.19–1.11 fL).

Discussion

In a comprehensive dissertation on the advantages and drawbacks of routine MPV assessment, Noris et al. recently concluded that this parameter has no clear clinical significance for diagnosing and/or prognosticating any human disease [41]. Irrespective of the fact that medicine is not an exact science and, therefore, categorical conclusions are inappropriate [42], especially in fields where many pieces of the intricate physiopathological puzzle are still lacking (hemostasis is perhaps one of the most paradigmatic examples) [43]. Unlike Noris et al.’s conclusions, several lines of evidence now attest that MPV may retain an important clinical value in patients with arterial and venous thrombotic disorders, whereby the pooled results of meta-analyses exploring platelet size in patients with CAD, VTE, portal vein thrombosis, stroke, erectile dysfunction and preeclampsia converge to conclude that

the value of this parameter is increased in concomitance with acute episodes and may also predict unfavorable outcomes. Beyond frankly thrombotic pathologies, the recent meta-analysis performed by Pyo and colleagues also concluded that MPV is significantly increased in cancer patients compared to healthy controls (mean difference, 0.50 fL; 95% CI, 0.28–0.72 fL), whilst its value significantly decreases in post-treatment patients [44]. This is an important information that contributes to explain the considerably higher burden of thrombotic disorders in patients with malignant diseases [45], as well as the putative interplay between platelet and cancer biology [46].

What is generally acceptable concerning the potential drawbacks of using MPV in routine medical practice is that its clinical value can be strongly influenced by some important pre-analytical, analytical and post-analytical variables, as summarized in Table 1. One of the most relevant aspects here is the choice of the additive (i.e. the anticoagulant). The International Council for Standardization in Hematology (ICSH) currently recommends the use of dipotassium ethylenediaminetetraacetic acid (K₂-EDTA) as the anticoagulant of choice for routine hematological testing [47], and several lines of evidence attest that this additive is the most widely used in clinical laboratories. Nonetheless, EDTA may not be the ideal anticoagulant to assess MPV, especially for the risk of time-dependent platelet swelling, so that acid citrate-based anticoagulation may be preferable. Unfortunately, however, anticoagulating blood with citrate is unfeasible under routine conditions, though this shall be probably seen as a better option to obtain a true MPV value [48]. Another critical issue is perhaps the timing of sample collection. Platelet volume varies in parallel with

Table 1: Major pre-analytical, analytical and post-analytical variables in the assessment of mean platelet volume (MPV).

Pre-analytical variables
– Choice of <i>in vitro</i> anticoagulant
– Timing of sample collection
– Time passed between sample collection and testing
– Temperature of sample storage before testing
Analytical variables
– Influence of the analytical technique (optical, impedance, fluorescent)
– Brand of the hematological analyzer
– Approach used for calculating MPV
Post-analytical variables
– Accurately define reference ranges and diagnostic cut-offs
– Consider the time of sampling when interpreting data
– Interpret data according to analytical, intra-individual and inter-individual variation
– Do not interchange data obtained with different techniques/analyzers

platelet activation, so a bigger size typically reflects hyper-reactivity [26, 27]. As platelet activation is a key phenomenon during both arterial and venous thrombosis [7], it is reasonable to hypothesize that platelet volume may be increased immediately before or after an acute thrombotic episode (Figure 1). On the other hand, it seems also reasonable that the size of the residual platelets that do not remain entrapped within the clot (and can hence be collected with a blood draw) would gradually decrease over time after the acute event, as a result of progressive granule secretion and platelet exhaustion. It is hence not surprising that some studies have found decreased MPV values in patients with acute thrombotic events, as this is exactly what one would expect when blood samples are collected >24–48 h after the episode [49]. This would lead us to conclude that MPV shall not be preferably measured between 1 and 7 days after an acute thrombotic episode, as its value would be hardly interpretable.

The time and condition of sample storage are additional issues that should be standardized for safeguarding the clinical usefulness of the information. The impact of time and temperature of blood sample storage before MPV testing has been known for nearly 35 years [50], and it has been now clearly established that the delay between sample collection and analysis shall not be longer than 4–6 h, regardless of storage conditions. Freezing whole anticoagulated blood must be obviously avoided, as this will lead to massive breakdown of blood cells, including platelets [51]. As regards the analytical and post-analytical variables, these have been thoughtfully discussed in a previous section of this article.

Conclusions

To summarize, MPV assessment is highly dependent upon the technique, the analyzer and the algorithm used for its calculation [14, 17]. It is hence advisable that: (i) longitudinal monitoring of patient's data over time shall be always carried out with identical instrumentation, (ii) each laboratory shall adopt analyzer-specific reference ranges, (iii) cut-offs identified in clinical studies will not be straightforwardly adopted in clinical practice when using different hematological analyzers and (iv) the impact of pre-analytical variables shall be abated or minimized [23]. Accurate knowledge of analytical, intra-individual and inter-individual variation will also be essential to appropriately interpret the data over time.

In conclusion, several lines of evidence now contribute to suggest that enlarged platelets, as reflected by an increase of the easy, rapid and inexpensive laboratory MPV

parameter, interplay with many vascular occlusive disorders. Although further evidence would be needed for establishing whether larger platelets shall be considered active players or innocent bystanders in thrombosis, an enhanced MPV shall not be underestimated as it may reflect the presence of hyper-reactive, pro-thrombotic platelets.

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