

Can mean platelet volume (MPV) be used as a predictor of vascular disease? Problems and pitfalls

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Abstract

Platelets play a pivotal role in both hemostasis and thrombosis. Increased platelet volume has a number of aetiologies and may be secondary to a genetic variant, platelet activation, increased platelet turnover or in response to inflammatory stimuli. There are several hereditary syndromes in which an increased mean platelet volume (MPV) occurs in association with a reduction in platelet number and function. An acquired increase in platelet size is often associated with increased platelet reactivity, shortened bleeding time and increased platelet aggregation. Many studies have shown that high MPV is associated with increased risk of venous and arterial thrombosis, but genome-wide association analyses found a weak inverse association between the risk of coronary heart disease and MPV. The disease associations of MPV suggest it could provide a useful contribution to risk assessment algorithms for both venous thromboembolism and arterial thrombosis. However, due to the problems associated with reliably assessing MPV, it does not yet constitute a practically useful biomarker for this purpose. Before MPV can be adopted for wider clinical use, the methodological problems involved in obtaining accurate and inter-laboratory comparable results must be resolved. In particular, a major degree of standardization is required, as are local reference ranges calculated with respect to specified time intervals from venipuncture to laboratory analysis. We discuss here the potential role of MPV in predicting the risk of acquired thrombotic disorders in addition to roles in congenital thrombocytopenic disorders, as well as the problems and pitfalls associated with use of MPV in these settings.

Key words: Platelets, MPV, thrombosis, quality control, standardization

Introduction

Platelets play a pivotal role in both hemostasis and thrombosis ¹ and also have a major role in inflammation, atherogenesis, and cancer metastasis ². Platelet count is measured as a part of the complete blood cell count (CBC) with automated blood cell counters along with in most analysers the mean platelet volume (MPV). However, unlike the analogous mean corpuscular volume (MCV), MPV is infrequently used for diagnostic or predictive purposes. Although MPV may be routinely available in both inpatient and outpatient settings at no additional cost, there are concerns regarding its accuracy and reproducibility as well uncertainty about its clinical significance. Nonetheless, MPV has been associated with a large number of clinical conditions, thereby suggesting it may have under-utilized clinical value. MPV is presently most frequently used for identification of hereditary thrombocytopenic disorders, because a distinct group of hereditary macrothrombocytopenias exist³. At the same time, many recent studies have shown the association of MPV with acquired thrombotic disorders and other cardiovascular risk factors such diabetes, hypertension and smoking ⁴⁻⁷. Mean platelet diameter (MPD) is an alternative method of assessing platelet size that has been developed to overcome some of the variables affecting MPV measurement by automated cell counters ⁸. Table 1 summarizes various platelet parameters discussed in this review and how they are measured.

The aim of this review is to discuss the problems of measuring MPV, the factors responsible for its variation and in particular its role in predicting the risk of acquired thrombotic disorders.

Measuring MPV and MPD

Techniques for measuring MPV

MPV is an analyzer-calculated measure of thrombocyte (i.e., platelet) size, determined directly from analysis of the platelet size-distribution curve. The MPV normal range (expressed in femtoliters (fL)), can vary significantly depending on the counting method, the analyzer and the statistical method⁹. Over the last 25 years, there has been a significant improvement in platelet analyzers. However, they mostly still use the same basic techniques (electrical impedance or optical methods)^{10;11} although a new fluorescence method is now available on some analyzers¹². Impedance counters determine platelet volume from the change in electrical impedance they produce while passing through an aperture. In systems using impedance technology a log transformation of the platelet volume distribution curve is used to derive a geometric mean for this parameter. Optical systems use the laser light scattering pattern of cells which depends on both their shape and reflectivity^{10;11}. These analyzers use two-dimensional platelet analysis. The volume and refractive index are simultaneously determined on a cell-by-cell basis by measuring two angles of laser light scatter. The two scatter measurements are converted to volume and refractive index (platelet density) values. The platelet scatter cytogram map determines volume up to 30 fL¹³. The new fluorescence method measures the fluorescent platelet count, MPV and immature platelet fraction (IPF) using only one fluorescent dye based on oxazine, which stains platelets more specifically. Compared with the optical method, platelets are more clearly distinguished from other blood cells using the difference in forward scattered light and the fluorescence intensity^{12;14}.

Accurate measurement of platelet size presents significant difficulties because some large platelets may not be recognized as such by the electronic impedance counters, which therefore underestimate not only the platelet count but also the mean platelet volume (MPV)^{15;16}.

Optical analyzers can measure very large platelet sizes (up to 60 fL) compared to impedance based analyzers, which have an upper threshold of platelet size between 24-36 fL. The MPV calculation usually excludes larger platelets outside of this threshold volume ¹⁷ . Therefore, MPV measurement by the two types of analyzers can produce a 20-25% variation in results⁹. Moreover, instruments operating on different principles also induce variability in the measurement of MPV within the normal range, so that comparison of MPV values obtained in different centers is difficult (table 2) and they may have different normal ranges ¹⁸.

Measuring MPD

This involves measurement of platelet diameter by optical microscopy on May–Grünwald–Giemsa-stained peripheral blood films. The mean platelet diameter (MPD) is the mean value obtained in 200 cell measurements ⁸.

Preanalytical variables and MPV

Preanalytical variables such as the anticoagulant used in the collection tube and the time taken from sampling to analysis are also significant sources of variation. In particular, ethylenediaminetetraacetic acid (EDTA)-induced platelet swelling can increase the MPV measured by impedance by 7.9% within 30min of sampling with an overall increase of 13.4% in 24h ¹⁹. In contrast, the MPV decreases over time, possibly as a result of the dilution of cytoplasmic contents, when MPV is measured by an optical light scatter system ²⁰. Although an equation allowing for correction of MPV over time from venipuncture has been published ²¹, this is not widely used due to the unpredictability in the time taken to equilibrate in individual samples. EDTA may also be responsible for a spuriously low platelet count due to EDTA-

dependent pseudothrombocytopenia occurring in 0.1% in the general population. This is attributed to the presence of EDTA-dependent antiplatelet antibodies that react best at 0°C - 4°C and recognize the cytoadhesive receptor GPIIb-IIIa. These uncertainties probably explain why many laboratories do not report the MPV to clinicians who themselves may not be familiar with the interpretation and significance of MPV.

Standardization and quality control of measuring MPV

There are no national or international standards for measuring MPV at present, and also no universally agreed external quality control material exists. There is a relatively narrow window of time after blood samples are taken before platelet activation²² and lyophilisation or fixation is not currently an option, so that external quality assurance (EQA) is not feasible, although there are internal quality assurance materials available from manufacturers. The availability of physiological, abnormal internal quality controls (IQC) is limited, due to the need for use of fresh samples. Many multi-center/national clinical studies use a centralized laboratory by shipping the samples from various distances from local centers, so resulting in significant variations in the time between venipuncture and MPV analysis²³. For a reliable estimate of MPV, confounding variables including the use of EDTA anticoagulant must be avoided. In particular an alternative anticoagulant such as citrate should be used and the time from sampling to analysis standardized. In the frequent case that it is impossible to control for these variables, the MPV should not be reported.

Microscopic examination of peripheral blood smears is a theoretical alternative for evaluating platelet size, which recognizes platelets regardless of their size and makes possible accurate

measurement of their diameters⁸. However, this method is time consuming, requires experienced operators and does not provide a numerical value of the MPV. Image capture systems such as CellaVision are available in some laboratories and²⁴ several studies have supported its accuracy and precision of CellaVision for the leukocyte differential but not yet platelet size^{25;26}. Another major problem for the use of platelet size is lack of reference values for both MPV and MPD.

In a recent editorial, Harrison et al suggested minimal requirements for studies reporting the relationship or correlation of MPV with clinical disorders or conditions²⁷ to overcome some limitations in their measurement (table 3).

Natural variation in MPV

Within an individual, platelets are heterogeneous in size and density, which is reflected in a classical log-normal distribution of platelet size²⁸. There is generally an inverse relationship between MPV and the platelet count within the normal range²⁹, which may contribute to the maintenance of normal haemostatic function. Thus the normal variation in platelet count is accompanied by a corresponding variation in MPV³⁰⁻³³. Intra and inter individual biological variations of MPV have been reported as 4.3% and 8.1% respectively (<https://www.westgard.com/biodatabase1.htm>). There are conflicting data on the effect of age and sex on MPV. It has been recognized that sex may influence platelet number and function;^{34;35} however, whilst some studies reported that MPV does not differ based on the sex of the patients,^{36;37} others found higher MPV in women^{31;38;39} or in men³⁰. There are also conflicting data relating MPV to age^{31;33;39-41}.

Relationship between platelet size and function

Some hereditary syndromes characterized by increased platelet size are clearly associated with reduced function, such as Bernard Soulier syndrome. However, when increased platelet size is an acquired characteristic, the large platelets have higher thrombotic potential⁴² and express higher levels of P-selectin and glycoprotein IIb–IIIa⁴³ per unit volume of platelet⁴⁴. Studies in rabbits have shown that thromboxane B2 production/unit volume of platelet is increased in larger platelets produced after 24 hours of thrombocytopenia compared with those produced in normal steady state. This study concluded that platelets produced in response to thrombocytopenia not only have a larger mean platelet volume but are also more reactive⁴⁵. In a study of platelet aggregation in response to ADP, collagen, or epinephrine in 34 normal subjects, a negative linear relationship was found between platelet count and platelet volume ($r = -0.53$, $p < 0.001$) and platelet aggregation velocity was directly proportional to platelet volume (ADP, $r = 0.62$, $p < 0.001$; collagen, $r = 0.59$, $p < 0.001$; epinephrine, $r = 0.53$, $p < 0.02$). It is likely that larger platelets are more reactive and cause shortened bleeding time⁴⁵ and increased platelet aggregation *ex vivo*⁴⁶ than small platelets because they express increased numbers of receptors on their surface. Larger platelets are denser, contain more secretory granules and mitochondria per unit volume of platelets, and are more reactive than their smaller counterparts⁴⁷.

Regulation of MPV

In addition to thrombopoietin, the main hormone that regulates megakaryocytopoiesis, several cytokines and growth factors interplay with platelet production and release of larger platelets

⁴⁸. Platelet volume is positively correlated with cytokines (thrombopoietin, interleukin- 6 and interleukin-3) that regulate megakaryocyte ploidy ⁴⁹⁻⁵¹. When platelet production is decreased, or in thrombocytopenia, the release of these cytokines may increase, so that young platelets become bigger, more active and the MPV increases⁵². Furthermore, changes in platelet size and shape may also occur at sites of activation ⁴⁸.

Genetic determinants of MPV in the normal population

Normal variations in platelet number, MPV and function are largely genetically controlled, and many loci associated with platelet disorders have been identified by genome-wide association studies (GWASs) ⁵³. As a consequence of these findings and availability of next generation sequencing, genetic analysis is now becoming the paradigm for diagnosis of congenital macrothrombocytopenia ⁵⁴. Panova-Noeva et al published a comprehensive analysis including known genetic and non-genetic factors that determine MPV, by evaluating 15010 individuals from the population-based Gutenberg Health Study ³³. Genotyping could identify 7 single nucleotide variants (SNVs) for females and 4 for males which were associated with higher MPV. However, all the variables studied, including both genetic and non-genetic factors, explained only 20% and 19% of the MPV variance in females and in males, respectively ³³.

Several SNVs in the proximity of the hypothetical gene FLJ 36031 on chromosome 7q22.3 were found to be associated with MPV and platelet function ⁵⁵. In this study, blood samples were processed within 10 minutes of collection and measurements were adjusted for confounders to avoid variation of MPV arising from the time between blood collection and analysis ⁵⁵. Other

genes modulating MPV levels have also been identified ⁵⁶. The influence of these loci on thrombosis risk may be modest as the percentage of MPV variance in the normal population attributable to these loci is only up to about 5%. In this study, MPV values were log transformed before analysis to approximate the normal distribution and adjusted for age and gender, and additionally for collection center within the UK sample group ⁵⁶. In a high-powered meta-analyses of GWAS for MPV and platelet count including 66,867 individuals of European ancestry (13 studies with MPV and 23 studies with platelets), Gieger et al identified 68 genomic loci reliably associated with platelet count and MPV, mapping to established or putative novel regulators of megakaryopoiesis and platelet formation ⁵⁷. Further investigation will be required to assess the direct clinical importance of these SNVs and these highlights further the need to improve the accuracy of the MPV measurement.

Acquired determinants of MPV

An increased MPV has been associated with smoking, obesity, hypertension and hyperlipidemia ⁴, which are also known to be associated with arterial or venous thrombosis. It has been suggested that the presence of raised MPV in these conditions, even in the absence of evident thrombotic events, may be due to abnormalities of arterial vessels and/or subclinical activation of coagulation, thus causing platelet activation-consumption and resulting in larger platelets ⁵⁸.

In the comprehensive analysis by Panova-Noeva et al, evidence was provided of heterogeneity in MPV determinants between genders. For example, age, cardiovascular risk factors such as

smoking, hypertension, and high glucose level were associated with higher MPV and higher risk of death in males only ³³. Intake of oral contraceptives and menstruation were strongly associated with higher MPV in females. There is some evidence that drugs such as anticoagulants, aspirin, diuretics and anti-hypertensive agents such as doxazosin and amlodipine can affect MPV ^{6;59}. Statins have been reported to lower MPV independent of their cholesterol lowering effect. However, in a pilot study of 40 patients with hypercholesterolaemia, after 12 weeks of treatment with atorvastatin the reduction in lipid levels was associated with a reduction in MPV and RDW leading to the suggestion that the MPV could be used to monitor therapy.^{60;61-62}.

MPV and platelet age

Reticulated platelets (RP) are newly released platelets containing RNA, which are analogous to immature red blood cells (i.e., reticulocytes) and are larger and so more reactive than mature platelets. The number of reticulated platelets reflects the rate of thrombopoiesis, thus increasing when platelet production rises and decreasing when it falls ⁶³. However, as with MPV, its utility is limited by problems arising from the lack of standardization of the techniques for measurement ⁶⁴. Additional analyzer, platelet-derived parameters have been developed to express RP as the immature platelet fraction (IPF) ⁶⁵. The IPF is identified by flow cytometry techniques and the use of a nucleic acid specific dye in the reticulocyte/optical platelet channel⁶³. There is a poor correlation between MPV and IPF in patients with a platelet production defect contributing to thrombocytopenia ⁶⁶. In a study to investigate whether measures of thromboelastometry and absolute immature platelet fraction (A-IPF) would

correlate better with acute bleeding score (ABS) than platelet count or MPV, MPV was not correlated with ABS, but A-IPF showed a stronger correlation with ABS than platelet count among all subjects ⁶⁷. Thromboelastometry measures of clot firmness were significantly correlated with ABS in all subjects with platelets $<60 \times 10^9/L$, and children with platelets $<60 \times 10^9/L$ and $<30 \times 10^9/L$. Based on the finding of stronger correlations of both thromboelastometry clot firmness and A-IPF than platelet count with ABS, the authors concluded that factors in addition to platelet count (e.g., platelet function), may contribute to bleeding in patients with immune thrombocytopenia (ITP) ⁶⁷.

MPV and inherited thrombocytopenia

Many forms of inherited thrombocytopenias are characterized by platelet macrocytosis ⁶⁸, and it is commonly accepted that the evaluation of platelet size is an important clue to provoke suspicion of these disorders. However, the accurate measurement of platelet size in patients with inherited thrombocytopenia is not always easy, because some forms, such as MYH9-related disease (MYH9-RD), biallelic and mono-allelic Bernard- Soulier syndrome (BSS) ⁶⁷ may have very large or giant platelets that are not recognized by the automated counters, thus leading to underestimation of platelet count and MPV ^{3;16;69;70}. Differentiating ITP from inherited thrombocytopenias is not always easy as both groups share thrombocytopenia and large platelets. Some patients with inherited thrombocytopenia have received treatment for ITP, including splenectomy, due to misdiagnosis ^{16;70-74}. Noris et al, in both a single center ⁸ and multicenter study with a larger case series of patients from six different centers from Italy ³,

identified cut-off values for MPV and MPD with a relatively good accuracy for differentiating inherited thrombocytopenia from ITP. Because MPV data varies according to the cell counter used, Noris et al ³ expressed the individual MPV not only as an absolute value, but also as percent (%) difference respect to the median value obtained in 30 healthy subjects analyzed at each center with the same instrument. The authors ^{3;8} concluded that a MPV 51% higher than that of normal controls represents an important indication of a hereditary macrothrombocytopenia. The previously identified cut-off value for MPV > 12.4 fl measured with an optical cell counter (ADVIA 120) had 91% specificity in distinguishing ITP from inherited macrothrombocytopenias (mono and biallelic Bernard-Soulier, MYH9-related disease), while its sensitivity varied greatly according to the instrument used. With an appropriate instrument, specificity was 83% ³. Because some automated analyzers do not recognize very large platelets, it is very important to combine MPV measurements with microscope evaluation of blood films in order not to miss the important diagnosis of macrothrombocytopenia.

MPV and acquired thrombotic disorders

Platelets secrete and express a large number of compounds that are crucial mediators of coagulation, inflammation, thrombosis and atherosclerosis ^{75;76}. Several studies have shown increased MPV in patients with coronary artery disease ^{77;78} and MPV has been identified as an independent risk factor for myocardial infarction (MI) and stroke ⁷⁹⁻⁸¹. In a Danish population-based study of 39,531 men and women, 1300 of whom developed MI, the risk of MI was increased by 37% (95% CI, 18-59%) in the middle and 30% (12-52%) in the upper vs. the lower

tertile of MPV after adjustment for known cardiovascular risk factors ³⁰. In a large, hospital-based cohort study with a total of 206,554 first-ever admissions between January 1996 and July 2003, an increased MPV (≥ 11.01 fL) independently predicted an increased risk of death due to ischemic heart disease; HR 1.8 (95% CI: 1.4 to 2.3), which is comparable to that reported for obesity or smoking ⁸². In a systematic review and meta-analysis ⁴ of 16 cross-sectional studies involving 2809 patients to assess the MPV as a predictor of cardiovascular risk, it was found that MPV was significantly higher in those with acute myocardial infarction (AMI) than those without AMI [mean difference 0.92 fL, 95% confidence interval (CI) 0.67–1.16, $P < 0.001$]. Pooled results from three cohort studies involving 3184 patients evaluating the risk of death following AMI demonstrated that an elevated MPV increased the likelihood of death compared to a normal MPV (11.5% vs. 7.1%, odds ratio 1.65, 95% CI 1.12–2.52, $P = 0.012$). A further pooled analysis of five cohort studies involving 430 patients undergoing coronary angioplasty revealed that MPV was significantly higher in patients who developed restenosis than in those who did not (mean difference 0.98 fL, 95% CI 0.74–1.21, $P < 0.001$). Taken together, these data suggest that MPV is a potentially useful prognostic biomarker of cardiovascular disease ⁴. A meta-analysis of MPV at presentation with suspected ischemic heart disease (IHD) found three studies for inclusion ⁸³. MPV showed good sensitivity but poor specificity for the presence of IHD but the studies showed considerable heterogeneity. Once again, standardization of studies is needed to determine whether higher MPV has predictive value.

Although there is a consistent and positive predictive relationship of MPV with risk of coronary artery disease in prospective observational studies, GWAS have found the reverse ⁸⁴. The UK Biobank and INTERVAL studies, tested 29.5 million genetic variants for association with 36 red

cell, white cell, and platelet properties in 173,480 European-ancestry participants and found hundreds of low frequency (<5%) and rare (<1%) variants with a strong impact on blood cell phenotypes. Through Mendelian randomization, authors provided evidence of shared genetic pathways linking blood cell indices with complex pathologies, including autoimmune diseases, schizophrenia and coronary heart disease. This genome-wide association analysis showed a weak inverse association between risk of coronary heart disease and MPV (OR = 0.92; 95% CI: 0.88–0.96; $p = 8.1 \times 10^{-5}$),⁸⁴ which suggests that the previously reported population associations between raised MPV and cardiovascular disease may be non-causal⁸⁴. The conflict with the previously cited observational studies may indicate that increased MPV does not play a causal role in cardiovascular disease or may be because the elevated MPVs in the two circumstances have different aetiologies. For example, congenital defects affecting platelet number and size may also have an adverse effect on platelet function.

There is also conflicting evidence on association of MPV with venous thrombosis patients. Braekkan et al, reported a study of platelet count, MPV and baseline characteristics in 25,923 subjects aged 25-96 years who participated in the Tromsø Study in 1994-1995³⁸. There was a total of 445 validated VTE events, of which 186 (42%) were unprovoked, during a mean follow-up period of 10.8 years. Subjects with $MPV \geq 9.5$ fL had a 1.3-fold [95% confidence interval (CI) 1.0-1.7] higher risk of total VTE and a 1.5-fold (95% CI 1.1-2.3) higher risk of unprovoked VTE than subjects with $MPV < 8.5$ fL in analyses adjusted for age, sex, smoking, body mass index, and platelet count. When adjusted for age and sex, increasing MPV was associated with increased VTE only in patients with unprovoked VTE ($p = 0.03$). No association was found between increasing platelet count and risk of VTE³⁸. In contrast to this finding, Biino et al

reported that MPV was not a risk factor for thrombosis in a population-based Italian study totaling 11,084 subjects³¹. In this study, MPV was negatively correlated with the time since the last thrombotic event and authors suggested that the increased MPV earlier described in subjects with acute thrombosis was an effect rather than a cause of thrombosis³¹. In a study to investigate the relationship between MPV and thrombosis recurrence in patients diagnosed with antiphospholipid syndrome (APS), MPV was found to be significantly higher in the group of patients with clinically and laboratory confirmed APS (median 7.85, range 4.73–12.2 fl) in comparison with controls⁸⁵. MPV was also higher in APS-negative thrombotic patients (7.61, range 5.21–12.3 fl) compared to healthy controls. APS patients with triple positivity for antiphospholipid antibodies (according to the classification criteria of Miyakis et al⁸⁶) had higher MPV values than other APS patients (9.69 ± 1.85 vs. 7.29 ± 1.3 fl, $p = 0.001$). In the multivariate logistic regression model, MPV >7.4 fl (OR 3.65; 95 % CI 1.38–9.64, $p = 0.009$) significantly predicted thrombosis recurrence. These results indicate the value of MPV as a prognostic factor for thrombosis recurrence in patients with APS. Overall, the findings from many observational studies support the concept that MPV has an association with the development and recurrence of VTE. However, further prospective studies are essential to establish the clinical significance and whether it can be used as a guide to therapy.

In similar studies in cancer patients, elevated MPV was found to be associated with decreased VTE risk and improved survival, the opposite finding to that in non-cancer patients⁸⁷. Similarly, Lippi et al in 2016, reported that a low MPV value was associated with the highest risk of diagnosing thrombosis in active cancer patients (relative risk, 1.29; 95% confidence interval, 1.10-1.51; $p = 0.002$)⁸⁸. In these studies, it was found that MPV was lower at the time of time of

VTE development compared to the MPV at the time of cancer diagnosis. The explanation for the different associations reported in cancer patients is not clear, but may arise from confounding due to a higher incidence of bleeding and iron deficiency in cancer, which can cause thrombocytosis and low MPV ⁸⁹. Conversely, cancer patients may have a reactive thrombocytosis due to the inflammatory effect of cancer ⁹⁰. Clarification will require further studies. Table 4 summarizes some of the reported associations of increase MPV with various diseases/conditions.

Conclusions

When identified as an acquired characteristic, larger platelets are frequently younger, more reactive and more thrombogenic, and were found to be associated with an increased risk of subsequent thrombosis in numerous studies. There is sufficient evidence to support the concept of using MPV to predict thrombotic risk in both venous and arterial systems. The lack of this relationship in genome-wide association analysis suggests the processes causing the elevated MPV may be important, directly or indirectly, and care should be taken to exclude congenital macrothrombocytosis. When reporting associations of MPV with other disorders, the problems and pitfalls in obtaining accurate and reliable estimation of MPV must be considered and these limitations mean it does not yet constitute a widely useful biomarker of disease. Before MPV can be used clinically the methodological problems must be solved and a method of quality control devised. The timing of blood collection is crucial to avoid the effects of activation and later degranulation, increasing and decreasing MPV respectively. At present, it is crucial that specific normal ranges for age and sex are calculated with precise time intervals

from venipuncture to laboratory analysis. Therefore, at the present time, studies should use a single type of analyzer with time of sampling and time from sampling to analysis carefully controlled. MPV does however, remain a useful parameter in diagnostic evaluation of thrombocytopenia.

Addendum

DRJ Arachchillage performed the literature search and wrote the first draft. M Laffan critically reviewed the manuscript and both authors approved the final manuscript.

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Conflict of interest

Authors state that they have no relevant conflict of interest

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Table 1. Summary of platelet parameters

Table 1. Summary of platelet parameters

Platelet parameter	What does it measure?	How does it measure?
Mean platelet volume (MPV)	Mean volume of detected platelets. May need log transformation to calculate geometric mean.	Measured directly from analysis of the platelet size-distribution curve Use three types of techniques 1. Electrical impedance: uses change in electrical impedance produce while passing through an aperture. 2. Optical: detects laser light scattering pattern of cells which depends on both their shape and reflectivity. The volume and refractive index are simultaneously determined on a cell-by-cell basis by measuring two angles of laser light scatter. The two scatter measurements are converted to volume and refractive index (platelet density) values. The platelet scatter cytogram map determines volume up to 30 fL 3. Fluorescence: Uses a fluorescent dye based on oxazine, which stains platelets more specifically. Compared with the optical method, platelets are more clearly distinguished from other blood cells using the difference in forward scattered light and the fluorescence intensity
Immature platelet fraction (IPF)	Automated detection reticulated platelets containing	By flow cytometry techniques and the use of a nucleic acid specific dye in the reticulocyte/optical platelet channel and fluorescence method using a fluorescent dye based on oxazine

	RNA.	
Mean platelet diameter (MPD)	An alternative method of assessing platelet size that has been developed to overcome some of the variables affecting MPV	By optical microscopy on May-Gruenwald-Giemsa-stained peripheral blood films. The mean platelet diameter (MPD) is the mean value obtained in 200 cell measurements

Table 2

Analyser	Stated Normal Range for MPV (fl)
Abbott – CELLDYN	None given
B-C – DXH 800	7.5 -11.2 (95% CI), 9.2 mean
Horiba –Pentra series of analysers	6.0-15.0
Nitron kohden (NK) Celltac	5.0-10.0
Mindray – BC5500	6.5-12.0
Siemens –Advia 2120 (OPTICAL)	7.0-9.0
Sysmex – XE5000 or 2100.	9.4-12.4

Table 3. Minimal requirements in reporting studies investigating the relationship or correlation of MPV with any clinical disorder or condition

• The study should be sufficiently powered with evidence of how the power calculations were done • Study groups that are comparing should preferably be matched age and gender. If this is not achievable the data should be analyzed for the effects of these demographic variables and adjusted appropriately • Blood sample collection, handling and processing should be done similarly to avoid or minimize the influence of pre-analytical variables between study groups • All pre-analytical variables should be mentioned, including the time delay from the sample collection to analysis samples • All samples should ideally be analyzed with same time from collection • Full description of the analyzer including manufacturer, instrument model and method principle with internal and external quality control procedures used should be mentioned

Adapted from Harrison et al, 2016 ²⁷

Table 4. Reported associations of increase MPV with various diseases/condition

Disease/condition	comments
Inherited thrombocytopenias	Many forms of inherited thrombocytopenias are characterized by platelet macrocytosis and it is commonly accepted that the evaluation of platelet size is an important clue to provoke suspicion of these disorders ^{3;16;67-70} . However, very large or giant platelets may not be recognized by the automated counters, thus leading to underestimation of platelet count and MPV ^{15;16} .
Immune thrombocytopenia	Differentiating ITP from inherited thrombocytopenias is not always easy as both groups share thrombocytopenia and large platelets ^{16;70-74} .
Coronary artery disease and arterial thrombosis	MPV has been identified as an independent risk factor for myocardial infarction and stroke ⁷⁹⁻⁸¹ . Whilst one meta-analysis has shown MPV as a predictor of cardiovascular risk ⁴ other one ⁸³ has demonstrated good sensitivity but poor specificity for the presence of IHD but the studies showed considerable heterogeneity. MPV is a potentially useful biomarker in predicting the risk of recurrence in cardiovascular events
Venous thromboembolism	There is conflicting evidence on association of MPV with venous thrombosis. Whilst some studies reported that increasing MPV was associated with increased VTE ^{38, 85} , others have shown that MPV was not a risk factor for thrombosis ³¹ . Conversely, in similar studies in cancer patients, elevated MPV was found to be associated with decreased VTE risk ⁸⁷⁻⁸⁸ . However there are numerous possible confound factors.
Smoking, obesity, and hyperlipidemia	These are known to be associated with arterial or venous thrombosis. Presence of raised MPV in these conditions, even in the absence of evident thrombotic events, may be due to abnormalities of arterial vessels and/or subclinical activation of coagulation, thus causing platelet activation-consumption and resulting in larger platelets ^{4, 33}
Hypertension	There is heterogeneity in MPV determinants between genders. Some studies reported that age; cardiovascular risk factors such as smoking, hypertension, and high glucose level were associated with higher MPV and higher risk of death in males only ³³ .

Intake of oral contraceptives and menstruation	Intake of oral contraceptives and menstruation were strongly associated with higher MPV in females ³³ .
Drugs such as aspirin, diuretics and anti-hypertensive agents such as doxazosin and statins	Studies have suggested that MPV could be used to monitor therapy with statins ^{6;59, 60;61-62} ..

