Could mean platelet volume serve as a marker of disease activity in Crohn’s disease?

Nesina Avdagić, Nermina Babić, Asija Začiragić, Almira Hadžović-Džuvo, Orhan Lepara, Emina Nakš-Ićindić

Department of Physiology
Faculty of Medicine
University of Sarajevo
Sarajevo, Bosnia and Herzegovina

Corresponding author:
Nesina Avdagić (MD, PhD)
Department of Physiology
Faculty of Medicine
University of Sarajevo
Čekaluša 90
71000 Sarajevo
Bosnia and Herzegovina
Phone: +387 33 226 472
Fax: +387 33 203 670
e-mail: avdagicn@yahoo.com

Aim: To investigate the capacity of mean platelet volume (MPV) in detecting CD disease activity and in differentiating CD patients from healthy controls. Methods: MPV values were measured in 30 CD patients and 30 healthy individuals matched for age and gender. Based on the result of Crohn’s Disease Activity Index, CD patients were subdivided into two subgroups: active and inactive phase of disease. MPV was measured by standard methods for all study participants. Results: A significant decrease in MPV was noted in CD patients compared to healthy controls (p=0.002). When active CD patients were compared with inactive CD patients, a significant decrease in MPV was also found (p=0.031). The overall accuracy of MPV in discriminating CD patients from healthy controls as well as active from inactive CD patients was 66% (cut-off level of 8.83 fL). Significant negative correlation between MPV and platelet count (PLT) (rho= -0.570; p=0.01) and significant positive correlation between MPV and platelet distribution width (PDW) (rho= 0.615; p=0.01) was observed in CD patients. Conclusion: Based on our results that have shown significant difference in MPV that was related to Crohn’s disease activity, we consider that MPV could be added to other serological markers of CD, especially in differentiating the active from the inactive phase of disease.

Key words: mean platelet volume, Crohn’s disease, inflammatory bowel disease

Introduction

Crohn’s disease (CD) is a form of inflammatory bowel disease (IBD). It usually affects the intestines, but it may occur anywhere in the gastrointestinal tract, from the oral cavity to the anus (1). Crohn’s disease is thought to be a result of an ongoing activation of the mucosal immune system leading to an inappropriate innate immune response to normal luminal factors in a genetically susceptible individual (2). Despite extensive investigation, the pathogenesis of Crohn’s disease remains unknown. Clinical and experimental studies have suggested that the pathogenesis of Crohn’s disease is multifactorial and involves genetic, environmental, immune and microbial factors (3). Various mediators have been suggested as possible participants in the pathogenesis of the inflammatory response. Platelets are one of the proposed possible factors which are involved in the pathogenesis of chronic inflammation (4).
Previous studies reported that increased platelet count has been associated with disease activity (5, 6) and has been proposed as a predisposing factor to systemic thromboembolism in IBD and to intestinal micro infarction observed in Crohn’s disease (7). Platelet aggregation and activation of the coagulation cascade are also increased in IBD (8, 9). Platelets aggravate inflammation by binding of micro infarcts to the endothelial surface which often leads to ischemic inflammation in the intestinal microvasculature (10). Mesenteric microvascular thrombosis has been observed as an early pathogenic event in Crohn’s disease, and thrombotic processes have been recognized in altered perfusion, inflammation and tissue injury in IBD (11, 12). Furthermore, platelets are capable to amplify the inflammatory response in IBD by releasing inflammatory mediators (13). Despite an increased platelet count, the mean platelet volume (MPV) in IBD patients was found to be reduced (14). The published data suggested that despite being activated, platelets in IBD are small (7) in contrast to ischemic heart (15) or diabetic vascular disease (16) where platelets are activated but large. The reason of the reduced mean platelet volume in IBD patients is still unknown, but it may be a direct consequence of the thrombopoiesis disturbance often observed in the early stage of systemic inflammatory processes (12). Alternatively, reduced MPV in the peripheral circulation of IBD patients could be explained by consumption or sequestration of large activated platelets in the intestinal vasculature (7).

Studies concerning the usefulness of MPV as disease activity marker in patients with ulcerative colitis (UC) and Crohn’s disease (CD) have given discordant results (17, 18). Güçlü et al. (17) observed that patients with active UC had lower MPV values when compared with the patients with inactive UC (p<0.001). Opposite data were obtained by Öztürk et al. (19) who found decreased MPV values in UC patients with remission. The same authors also found increased MPV values in CD patients with remission. Their results are in the accordance with the results of Liu et al. (20) who also found lower MPV values in active CD compared to inactive CD patients.

Some studies indicate that MPV may be an indicator for disease activity in patients with Crohn’s disease (4, 12). Conversely, other studies showed that MPV had a good diagnostic accuracy only in discriminating CD patients from healthy controls, while in distinguishing active from inactive CD patients MPV did not show diagnostic accuracy (19, 20).

Having in mind the controversial findings regarding this issue, the aim of the present study was to examine whether MPV could be useful in differentiating CD patients from healthy controls and in CD activity assessment.

**Materials and methods**

**Patients**

The study comprised 30 patients with Crohn’s disease of both genders. The diagnosis of CD was based on standard clinical, radiological, endoscopic and histopathological criteria. According to the Activity Index (AI) for CD patients defined by Van Hees et al. (21), CD patients were divided into active and inactive groups. Twelve patients were in the active phase, whereas 18 patients were in the inactive phase of CD. The exclusion criteria were: acute or chronic infection, endocrinological and hematological disease, heart failure, hypertension, hepatic and renal disorder, cancer and peripheral vascular disease. None of the enrolled subjects had received anticoagulant medications or contraceptives. The patients were compared with 30 healthy individuals matched for age and gender. These subjects were recruited from volunteers who were absent of clinical and biochemical features of Crohn’s disease.
or any other illness that can affect the observed parameters. Approval for the study protocol was obtained from the Ethics Committee of the Medical Faculty University of Sarajevo. Written informed consent was obtained from all subjects. Investigations were carried out in accordance with the Declaration of Helsinki as revised in 2000.

Biochemical analysis
Blood samples were obtained from the cubital vein and collected into tubes with potassium ethylenediaminetetraacetate (EDTA) as an anticoagulant. All measurements were performed within 2 hours of collection of blood because of the known effect of EDTA on platelet volume. The following hematological parameters were studied in all blood samples: mean platelet volume (MPV), platelet count (PLT), plateletcrit (PCT) and platelet distribution width (PDW). All parameters were analyzed on an automatic hematology analyzer (Siemens Healthcare Diagnosis Ltd., Camberley, UK). The range of normal values for MPV was 7.4 – 10.4 fL.

Statistical analysis
SPSS (Statistical Package for Social Science Inc., Chicago, IL, USA) version 13.0 for Windows was used for statistical analysis. Normality of continuous data was determined by the Shapiro-Wilk normality test. Data are presented as mean ± SEM for normally distributed variables and as median and interquartile ranges for skewed variables. Categorical variables are shown as frequencies. The difference in normally distributed data was tested by the independent t-test. The difference in values of parameters that showed skewed distribution was assessed by the Kruskal-Wallis test. Afterwards, the Mann-Whitney U test was used to compare differences between the two groups. The correlations between the variables were assessed by the Spearman rank sum test. To determinate the accuracy and respective best cut-off values of MPV for differentiating CD patients from healthy controls, as well as active from inactive CD patients, the Receiver Operating Characteristic (ROC) curves and their corresponding areas under the curve (AUC) were used. Accuracy rate diagnosing measures were calculated with 95% Confidence Interval (95% CI). A p value < 0.05 was considered statistically significant for all comparisons.

Results
The baseline characteristics of CD patients and healthy controls are demonstrated in Table 1. The distribution of age, gender and smoking habits were not statistically significant between groups. A significant difference (p=0.002) in body mass index was found when CD patients were compared with healthy controls. The median disease duration in CD patients was 3.75 years.

Median MPV value was statistically significantly different (p=0.009) between active CD patients, inactive CD patients and healthy controls. The MPV value of active CD patients 8.05 fL (7.32 fL-8.66 fL) was significantly lower than those of inactive CD patients [8.73 fL (7.88 fL-9.32 fL); p=0.03] and healthy subjects [9.08 fL (8.21 fL- 9.96 fL); p=0.002]. No significant difference (p=0.3) in MVP value was observed between inactive CD patients and healthy controls (Figure 1).

Comparisons of other platelet indices including: PLT, PCT and PDW are demonstrated in Table 2. A significant increase in PLT count [320.2 ± 14.48 x 10^9/L vs. 253.23 ± 8.81 x 10^9/L; p<0.0005] and PCT (2.71±0.11 ml/L vs. 2.31±0.08 ml/L; p=0.004) were noted. The values of PDW were similar in both groups [17.4% (16.88% - 17.85%) vs. 17.6 % (17.08%- 18.23%) %; p=0.49].

Table 3 demonstrates comparisons of other platelet indices in CD patients with and without active disease. PLT count [352.5 x 10^9/L (318.3 x 10^9/L - 388.8 x 10^9/L) vs.
Table 1. Baseline characteristics of Crohn’s disease patients and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>CD group (n=30)</th>
<th>HC group (n=30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages (years)</td>
<td>33 (27.5 - 43.3)</td>
<td>38.5 (30.8 - 46.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>14/16 (46.7/53.3)</td>
<td>13/17 (43.3/56.7)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.83 ± 0.82</td>
<td>25.08 ± 0.58</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Smoking</td>
<td>17 (56.7%)</td>
<td>9 (30%)</td>
<td></td>
</tr>
<tr>
<td>Active disease</td>
<td>12 (20%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>3.75 (1.0 - 8.25)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM; median and 25% - 75% interquartile ranges; n (%); CD - Crohn’s disease; HC - healthy control; BMI - body mass index; NS - not significant; p - probability

Table 2. Comparison of platelet indices between Crohn’s disease patients and healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CD group (n=30)</th>
<th>HC group (n=30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (x 10⁹/L)</td>
<td>320.2 ±14.48</td>
<td>253.23 ± 8.81</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td>PCT (ml/L)</td>
<td>2.71 ± 0.11</td>
<td>2.31 ± 0.08</td>
<td>p=0.004</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>17.4 (16.88 - 17.85)</td>
<td>17.6 (17.08 - 18.23)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM; median and 25% - 75% interquartile ranges; CD - Crohn’s disease; HC - healthy control; PLT - platelet count; PCT - plateletcrit, PDW - platelet distribution width; NS - not significant; p - probability

Figure 1. Box-and-whisker plots of MPV value (fL) in the active Crohn’s disease patients, inactive Crohn’s disease patients and healthy controls.

Each bar shows upper and lower quartile, while the square and its central bar indicate interquartile range and median. CD - Crohn’s disease; p - probability; NS - not significant; * in comparison with healthy controls; ** in comparison with inactive CD patients

289.0 x 10⁹/L (245.0 x 10⁹/L - 325.3 x 10⁹/L); p=0.031] was found to be significantly higher than PLT count in active CD patients when compared with those in the inactive period of the disease. Although PCT in active CD patients 2.86 ml/L (2.61 ml/L - 3.09 ml/L) was higher in comparison with inactive CD patients 2.53 ml/L (2.14 ml/L - 2.95 ml/L), the determined difference was not statistically significant (p=0.2).

Spearman’s correlation analysis has shown significant negative correlation between MPV and PLT count in active CD patients (rho= -0.601; p=0.05). Our data also revealed a significant positive correlation between MPV and PDW in inactive CD patients (rho= 0.751; p=0.01) and in healthy controls (rho= 0.641; p=0.01). However, there was a weak (but not significant) inverse correlation between MPV and PCT in active CD patients, inactive CD patients and in healthy controls (Table 4).
Table 3. Comparison of platelet indices in active and inactive CD patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Active CD (n=12)</th>
<th>Inactive CD (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (x 10^9/L)</td>
<td>352.5 (318.3 - 388.8)</td>
<td>289.0 (245.0 - 325.3)</td>
<td>p=0.031</td>
</tr>
<tr>
<td>PCT (ml/L)</td>
<td>2.86 (2.61 - 3.09)</td>
<td>2.53 (2.14 - 2.95)</td>
<td>NS</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>17.20 (16.55 - 17.60)</td>
<td>17.55 (17.25 - 18.47)</td>
<td>p=0.048</td>
</tr>
</tbody>
</table>

Data are presented as median and 25%-75% interquartile ranges; PLT - platelet count; PCT - plateletcrit; PDW - platelet distribution width; CD - Crohn’s disease; NS - not significant; p - probability

Table 4. Spearman’s correlation analysis between levels of mean platelet volume and platelet count, platelet distribution width, plateletcrit in active CD patients, inactive CD patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Active CD MPV (fL)</th>
<th>Inactive CD MPV (fL)</th>
<th>Healthy controls MPV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (x 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rho</td>
<td>-0.601</td>
<td>-0.441</td>
<td>-0.302</td>
</tr>
<tr>
<td>p</td>
<td>p=0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PCT (ml/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rho</td>
<td>0.312</td>
<td>0.751</td>
<td>0.641</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>p</td>
<td>p=0.01</td>
</tr>
<tr>
<td>PDW (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rho</td>
<td>0.357</td>
<td>0.039</td>
<td>0.302</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>p</td>
<td>NS</td>
</tr>
</tbody>
</table>

MPV - mean platelet volume; PLT - platelet count; PDW - platelet distribution width; PCT - plateletcrit; CD - Crohn’s disease; NS - not significant; p - probability

**ROC analysis of MPV**

We further performed an ROC analysis to investigate the capacity of MPV in differentiating CD patients from healthy controls (Figure 2A) and active CD patients from inactive CD patients (Figure 2B). As shown in Table 5., the optimal cut-off level for MPV was 8.83 fL with sensitivity of 66%, specificity of 66% and overall accuracy of 66% (area under the curve (AUC): 0.673;
Moreover, the ROC analysis also suggested 8.83 fL as optimal cut-off points for MPV (sensitivity of 50%, specificity of 92% and overall accuracy of 66% (AUC: 0.734; p=0.03) in differentiating active from inactive CD patients.

**Discussion**

Crohn's disease, one of the two major forms of inflammatory bowel disease, is a chronic inflammatory condition characterized by local and systemic inflammation. In addition to their primary haemostatic functions, platelets are involved in the pathogenesis of chronic inflammations in both forms of IBD. Platelet activation seen in the active period of the disease not only regulates coagulation but it also increases mucosal inflammation. In the inflammatory response platelets can function as inflammatory cells that produce and store enormous amounts of inflammatory mediators. Upon activation, platelets release proinflammatory mediators that control vascular permeability and regulate vasodilatation or vasoconstriction, induce neutrophil activation and degranulation, or cause degradation of subendothelial extracellular matrix facilitating leukocyte extravasations (22). A previous study by Kapsoritakis et al. (12) reported an increased platelet count in patients with clinical relapse, and proposed a platelet count as a simple method in disease activity assessment. Although abnormalities in platelet number have been suggested as an indicator of disease activity, abnormalities in mean platelets volume have also been proposed as a potential marker of clinical disease activity, being inversely proportional to the levels of classical inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate (19). Since previous studies reported that the MPV values are below the normal limit in patients with active IBD (12), the main goal of our study was to investigate the utility of MPV in the assessment of disease activity in patients with Crohn's disease.

The results of our study showed that the MPV value was significantly different between active CD patients, inactive CD patients and healthy controls. Namely, the MPV value was significantly lower in active CD patients compared to both inactive CD patients and healthy controls. No difference was found in MPV value between CD patients in the inactive phase of the disease and healthy controls.

Since only few studies so far have investigated MPV value in CD patients we were limited in comparison of our results with the results of other authors. Our results are in the accordance with the results of Douda et al. (4) who also found statistically significantly reduced MPV value.

**Table 5. Optimal cut-off, area under the curve with 95% confidence interval (AUC, 95% CI), sensitivity, specificity, positive and negative predictive value, overall accuracy of MPV in differentiating between CD patients and healthy controls and active and inactive CD patients.**

<table>
<thead>
<tr>
<th></th>
<th>Optimal Cut-off</th>
<th>AUC (95% CI)</th>
<th>SEN (%)</th>
<th>SPE (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Overall accurate</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD patients vs. healthy controls</td>
<td>MPV (≥8.83fL)</td>
<td>0.673 (0.53-0.81)</td>
<td>66%</td>
<td>66%</td>
<td>66%</td>
<td>66%</td>
<td>66%</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Active CD vs. inactive CD patients</td>
<td>MPV (≥8.83fL)</td>
<td>0.734 (0.55-0.92)</td>
<td>50%</td>
<td>92%</td>
<td>90%</td>
<td>55%</td>
<td>66%</td>
<td>p=0.03</td>
</tr>
</tbody>
</table>

AUC-Area under the curve; CI-Confidence Interval; MPV-mean platelet volume; SEN-sensitivity, SPE-specificity, PPV-positive predictive value; NPV-negative predictive value; CD- Crohn’s disease; p - probability
in CD patients during clinical relapse compared with clinical remission. Kapsoritakis et al. (12) have also reported significantly lower MPV values in active CD patients compared to inactive CD patients or healthy controls. On the other hand, the results of our research are not completely in accordance with the results of other authors such as the studies conducted by Öztürk et al. (19) and Liu et al. (20) which noted significant difference in MPV value only between CD patients and healthy controls. Contrary to our findings, these authors did not find statistically significant difference in MPV values when they compared active with inactive CD patients. The possible explanation for the observed discrepancy might be due to our small study sample. The statistically significant MPV decrease in CD patients observed in our and in previous studies (19, 20) suggests that MPV may be helpful in differentiating CD patients from healthy controls. In contrast to Öztürk et al. (19) and Liu et al. (20) who suggested that there should be caution in the use of MPV as a marker in determination of CD activity, we consider that measurement of MPV may serve as a helpful marker for monitoring disease activity in CD patients, because MPV had a fair diagnostic accuracy in distinguishing active from inactive CD patients (AUC 0.734) (18).

The results of our study showed that the PLT and PCT were significantly higher in CD patients compared to healthy controls. There was no statistically significant difference in PDW values between these two groups. Our results confirm findings from previous studies, such as the study by Öztürk et al. (19) who also observed significantly higher PLT and PCT values in CD patients compared to healthy controls. In this study, the PDW values between the compared groups were statistically similar, which is in accordance with our results related to PDW.

In our study, the mean values of PLT were significantly higher, while PDW values were significantly lower in active CD patients compared to inactive ones. Although the PCT values were higher in active CD patients compared to inactive CD patients, there was no statistically significant difference between the compared groups. Obtained results are not completely in accordance with the results of Öztürk et al. (19) who observed a significant increase of PLT values and PCT values and significant decrease of PDW values in active CD patients compared to inactive CD patients.

An association between MPV values and other inflammatory markers such as CRP, ESR, WBC has been reported in CD patients (10, 19, 20), but only few studies investigated the association between MPV and platelet indices (PLT, PCT and PDW). Our data revealed a significant inverse correlation between MPV and PLT in patients with active CD. However, in the same group of patients no significant correlation was found between MPV and PCT as well as between MPV and PDW. In inactive CD patients and in healthy controls we observed significant positive correlation only between MPV and PDW.

Our results are in accordance with Kapsoritakis et al. (12) who also observed a strong negative correlation between MPV and PLT only in patients with active CD. The results of their study suggest that, although the volume of platelets in active CD is reduced, the platelets are still activated. The fact that small platelets are activated in CD may explain the apparent paradox regarding the low MPV and high incidence of thromboembolism in this disease. These authors suggest that increased platelet activation may be responsible for the high risk of thromboembolism in CD patients.

We further investigated the ability of MPV in differentiating CD patients from healthy controls, as well as active from inactive CD patients. In our study, based on the selected optimal cut-off value by ROC
curve analysis, MPV values showed a moderate specificity and sensitivity (66% and 66%) in distinguishing CD patients from healthy controls; low specificity (50%) and a very good sensitivity (92%) in distinguishing patients with active CD from inactive CD patients. Our results are in accordance with the findings of Lui et al. (20) who found good specificity and sensitivity (78.8% and 76.6%) only in distinguishing CD patients from healthy controls. However, in their study MPV values did not show a statistically discriminative value in differentiating active from inactive CD patients (AUC: 0.504) which is not in accordance with our results.

The results of our study demonstrated that the MPV displays a moderate diagnostic accuracy in distinguishing CD patients from healthy controls (AUC: 0.673) and fair diagnostic accuracy in distinguishing active from inactive CD patients (AUC: 0.734).

Conclusion

In conclusion, the present study shows that MPV can reflect Crohn's disease activity since MPV value was significantly lower in active compared to inactive CD patients. The obtained results suggest that MPV value may be used in clinical practice as additional serological biomarker since its measurement does not require extra effort or costs. Finally, we consider that MPV could assist in the determination of CD activity, not as a stand-alone test, but in combination with other serological markers. However, due to the small sample size, our findings are warranted for further confirmation in larger studies.

Conflict of Interest: None declared.

References


