PLATELET INDICES IN PATIENTS WITH DE NOVO PORTAL VEIN THROMBOSIS AND LIVER CIRRHOSIS

Irina Gîrleanu¹, Anca Trifan², Camelia Cojocariu², Mihaela Dimache², Ana-Maria Singeap², Oana Stoica², C. Sfarti², C. Stanciu²
University of Medicine and Pharmacy “Grigore T. Popa” - Iaşi
Faculty of Medicine
1. Ph.D. student
2. Discipline of Gastroenterology and Hepatology

PLATELET INDICES IN PATIENTS WITH DE NOVO PORTAL VEIN THROMBOSIS AND LIVER CIRRHOSIS (Abstract) Platelet indices are markers of platelet reactivity used for thrombotic risk assessment in patients with cardiovascular diseases, and recently in venous thrombosis. Aim: To assess the diagnostic value of platelet indices in patients with non-malignant de novo portal vein thrombosis and liver cirrhosis. Material and methods: We conducted a prospective, case-control study on patients admitted to a tertiary center in the interval January, 2010 – December, 2012. Included in the study were 54 patients with portal vein thrombosis (PVT) and 54 controls. Patients with known malignancy, sepsis, thrombophilia, on anticoagulant or antiaggregant therapy, acute or chronic inflammatory diseases, severe anemia, renal failure, acute coronary syndrome, and chronic pulmonary disease were excluded from the study. Results: Both groups were comparable for baseline characteristics. Mean platelet volume, platelet distribution width (PDW) and plateletcrit were higher in the PVT group. In a multivariate logistic regression analysis, significant predictors of the presence of PVT were mean platelet volume (MPV), PDW, and procalcitonin (PCT). Conclusion: Our data suggest that increased platelet indices contribute to the prethrombotic state in liver cirrhosis and that larger platelets may play a specific role in thrombosis despite thrombocytopenia. Keywords: PORTAL VEIN THROMBOSIS, PLATELET INDICES, LIVER CIRRHOSIS, THROMBOCYTES.

Portal vein thrombosis (PVT) is an important cause of morbidity and mortality in patients with liver cirrhosis (LC) (1). The incidence ranges between 5% and 26%, and is higher in patients on transplant waiting list (2,3). Predisposing clinical conditions for PVT are advanced LC, low portal vein velocity, malignancy, and a wide variety of acquired and inherited hematological diseases (4). Despite thrombocytopenia, platelets are important in LC, playing a major role in hemostasis and other important functions such as inflammation, host defense and angiogenesis and fibrosis (5,6). The routine platelet function tests including bleeding time and aggregometry have a low positive predicting value in differentiating between bleeding and thrombotic risk in LC, and new methods for assessing platelet function are needed (6).

Platelets are heterogeneous in size, density, and reactivity (6). Mean platelet volume (MPV) shows platelet size and may reflect platelet function and activity (7).
Since larger platelets are more reactive than the normal size ones, elevated MPV values were demonstrated to be a risk factor for arterial thrombosis and cardiovascular diseases (8,9). However, new data sustain that MPV and other parameters including platelet distribution width (PDW) and plateletcrit (PCT) may be used as indicators of platelet function and activation in patients with vein thrombosis (10). PDW directly measures the variability in platelet size and is a marker of platelet activation (11). A high PDW value could suggest a greater production of larger reticulated platelets. Of all platelet indices, PCT provides more comprehensive data about total platelet mass because it is equivalent to MPV and platelet count (PLT), where PCT=PLTxMPV/10^7 (11).

Recently it has been demonstrated that MPV is elevated in patients with deep vein thrombosis and significantly higher in patients with non-cirrhotic PVT. Therefore, in the present study we aimed at investigating whether platelet indices (MPV, PDW, and PCT) could have a diagnostic value for PVT in cirrhotic patients, despite thrombocytopenia.

MATERIAL AND METHODS

Study population
We conducted a prospective, case-control study on patients with liver cirrhosis admitted to a tertiary hospital in the interval January, 2010- December, 2012 in which a diagnosis of PVT was made during the study interval. Excluded from the study were the patients previously diagnosed with hereditary thrombophilia, hepatocellular carcinoma or other known malignancies, patients on anticoagulant or antiaggregant therapy, and patients with acute or chronic inflammatory diseases, severe anemia, renal failure, acute coronary syndrome, and chronic pulmonary disease. Between January, 2012- December 2012, 3124 cirrhotic patients were admitted to our department, of which a control group was randomly selected. After a detailed history and physical examination, all patients were subjected to hematological and biochemical blood tests and abdominal ultrasound. The study was performed in accordance with the Declaration of Helsinki and our local ethical committee approved the study. A written consent was obtained from all patients.

Portal vein thrombosis diagnosis
Portal vein thrombosis was diagnosed by abdominal ultrasound and Doppler ultrasound after 8 hours fasting. A Philips HD11XE ultrasound machine equipped with a 3.5 MHz convex transducer was used. PVT was confirmed by computed tomography with IV contrast. PVT was classified into 2 categories: complete and partial.

Laboratory data
Fasting peripheral blood sample was drawn from the antecubital vein using a 21-gauge needle with a vacutainer and minimal stasis. The blood sample was collected into tubes containing 2.7 mL EDTA for platelet count, MPV, PDW, and PCT analyses. Platelet indices were measured using an automated system (Sysmex XS-1000i), by flow cytometry (11). The measurements were performed within 30 minutes after vein puncture to rule out the possible swelling of platelets. The reference ranges in our hematology laboratory are: MPV: 8.5 to 12.0 fL, PDW: 10.0 to 18.0 %, and PCT: 0.12 to 0.40 %.

Statistical analysis
Data were analyzed using SPSS Software Version 17.0 for Windows (SPSS Inc.,
Platelet indices in patients with de novo portal vein thrombosis and liver cirrhosis

Chicago, Illinois). Continuous variables were presented as mean ± standard error and categorical variables as frequency and percentage. Student’s t test was used to compare normally distributed continuous variables, and the Mann-Whitney U test for variables without normal distribution. The χ2 test or the Fisher exact test was used to compare categorical variables. Pearson analysis was used to analyze the correlations between platelet indices. Separate logistic regression analyses were used to identify univariate predictors of PVT, and a subsequent stepwise (forward conditional) regression analysis was performed (parameters with a p value of <0.1 in univariate analysis were included in the model). Receiver-operating characteristic (ROC) analyses were used to determine the cutoff values and the sensitivity/specificity of platelet indices. The odds ratios (OR) and the 95% confidence intervals (CI) were calculated. A 2-tailed p value of <0.05 was considered statistically significant.

RESULTS

There were no significant differences between the two groups in age, sex, clinical risk factors (hypertension, diabetes, smoking), and laboratory parameters (cholesterol, fasting plasma glucose, white blood cell count, hemoglobin, platelet count, etiology of LC, MELD score and Child-Pugh class) (tab. I).

| TABLE I
Baseline demographic, clinical and laboratory characteristics of the study population |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>PVT group (n=54)</td>
<td>Control Group (n=54)</td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.5±11.0</td>
<td>62.3±12.3</td>
<td>0.471</td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>34(62.9%)</td>
<td>33(61.1%)</td>
<td>0.755</td>
<td></td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>7(12.9%)</td>
<td>8(18.4%)</td>
<td>0.438</td>
<td></td>
</tr>
<tr>
<td>Smoking, %</td>
<td>22(40.7%)</td>
<td>18(33.3%)</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td>Etiology (alcoholic/virus B/C/other)</td>
<td>23/4/25/2</td>
<td>23/6/22/3</td>
<td>0.246</td>
<td></td>
</tr>
<tr>
<td>MELD score</td>
<td>12.6±5.5</td>
<td>11.9±7.5</td>
<td>0.685</td>
<td></td>
</tr>
<tr>
<td>Score Child</td>
<td>7.6±2.3</td>
<td>7.8±2.1</td>
<td>0.724</td>
<td></td>
</tr>
<tr>
<td>Child-Pugh class (A/B/C)</td>
<td>20/10/24</td>
<td>18/14/22</td>
<td>0.111</td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>128.9±60.1</td>
<td>137±83.7</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>WBC count, per ml</td>
<td>9860±3240</td>
<td>8844±3498</td>
<td>0.229</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.2±2.07</td>
<td>11.4±2.5</td>
<td>0.584</td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>75.2±19.6</td>
<td>73.7±20.6</td>
<td>0.256</td>
<td></td>
</tr>
<tr>
<td>Platelet count x10⁹ per L</td>
<td>176±11.2</td>
<td>141±14.3</td>
<td>0.239</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PVT, portal vein thrombosis, WBC, with blood cell, AP prothrombin activity.

According to the time scale of PVT, 25 (46.2%) patients had acute and 29 (53.8%) chronic PVT. In all, PTV was complete in 15 (27.7%) cases and partial in 39 (72.3%). MPV was significantly higher in the PVT group compared with the controls (811.2 + 1.0 fL vs 9.7+1.2 fL [95% CI
Irina Gîrleanu et al.

0.07-0.99], p=0.05). PDW was also significantly higher in the PVT group compared with the control group (14.3 + 3.13 % vs 12.2 + 2.38 % [95% CI 0.10-2.48], p=0.048, respectively). PCT was, however, significantly lower in PVT group compared with the controls (0.19+0.12% vs 0.14+0.06% [95% CI 0.08-0.09], p=0.015, respectively). In addition, there was a significant inverse relationship (fig. 1).

In a multivariate logistic regression analysis, significant predictors of the presence of PVT were MPV, PDW, and PCT (tab. II). For MPV, the multiple-adjusted OR of the presence of PVT was 2.3 (95% CI, 1.77-2.37); for PDW, the multiple-adjusted OR of the presence of PVT was 2.4 (95% CI, 1.435-3.85); and for PCT, the multiple-adjusted OR of the presence of PVT was 1.79 (95% CI, 0.95-2.86).

![Fig. 1. Platelet indices in patients with liver cirrhosis with and without PVT: a-MPV, b- PDW, c- plachetocrit.](image)

The cutoff values of 11.0 fL, 11.7%, and 0.11% for MPV, PDW, and PCT, respectively, were found to be moderately sensitive and specific for predicting PVT.
TABLE II
Multivariate regression analysis of the presence of PVT

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>±SE</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV</td>
<td>-0.053</td>
<td>0.740</td>
<td>0.046</td>
</tr>
<tr>
<td>PDW</td>
<td>0.011</td>
<td>0.258</td>
<td>0.012</td>
</tr>
<tr>
<td>PCT</td>
<td>-7.584</td>
<td>5.972</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Finally, to determine the best cutoff values of platelet indices for predicting PVT, ROC analyses were performed. The areas under the ROC curves for these indices used to detect PVT were calculated (tab. III) and the sensitivity and specificity of the best cutoff values were determined (tab. IV).

TABLE III
Receiver operating characteristic analysis for platelet indices to predict PVT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area under the ROC curve</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV</td>
<td>0.610</td>
<td>0.46-0.75</td>
<td>0.022</td>
</tr>
<tr>
<td>PDW</td>
<td>0.579</td>
<td>0.43-0.71</td>
<td>0.035</td>
</tr>
<tr>
<td>PCT</td>
<td>0.576</td>
<td>0.44-0.71</td>
<td>0.038</td>
</tr>
</tbody>
</table>

The cutoff values of 11.0 fl, 11.7%, and 0.11% for MPV, PDW, and PCT, respectively, were found to be moderately sensitive and specific for predicting PVT.

TABLE IV
Sensitivity and specificity of the cutoff values of platelet indices

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cutoff value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV</td>
<td>11.0</td>
<td>53%</td>
<td>37%</td>
</tr>
<tr>
<td>PDW</td>
<td>11.2%</td>
<td>72%</td>
<td>63%</td>
</tr>
<tr>
<td>PCT</td>
<td>0.1%</td>
<td>69%</td>
<td>55%</td>
</tr>
</tbody>
</table>

DISCUSSION
In the present study, we have found that the platelet activity indices were significantly and independently associated with the presence of PVT. Platelet activity can be influenced by a multitude of variables (12), therefore it is difficult to predict the extent of platelet reactivity in an individual. A practical, reliable, and available index of platelet activation has not been discovered although considerable amount of research has been performed (11).

Various laboratory methods including platelet count and size, aggregates, and released substances from activated platelets have been introduced for the detection of platelet activity (11). However, they have different advantages and disadvantages. MPV is an inexpensive and easily available biomarker. It is routinely available in inpatient and outpatient setting at a relatively low-cost. Increased MPV values are related to various cardiovascular risk factors, disorders and inflammatory processes resulted in arterial and venous thromboses (9,10). Circulating platelets are heterogeneous in size, metabolism, functional activity, and density. Larger platelets are metabolically and enzymatically more active than smaller platelets, containing more prothrombotic material, with increased thromboxane A2 and B2 per unit volume and glycoprotein IIb-IIIa receptor expression (13). In healthy participants, there is a nonlinear inverse
correlation between MPV and platelet concentration: MPV tends to decrease in participants with higher platelet counts, as found in our study (14,15).

In previous studies, increased MPV was found to be associated with non-alcoholic fatty liver disease, hepatits C and B and advanced fibrosis (16). Recently, Braekkan et al. reported that MPV is a risk factor in venous thromboembolism (10). Our findings provide further evidence that platelet activation, measured by elevated MVP, PDW and PCT may contribute to the pathogenesis of venous thrombosis.

To our knowledge, this study has examined for the first time the relationship between all platelet indices, PVT and liver cirrhosis. Pizzuli et al. suggested that because platelets stay in the circulation 7-11 days, they might be detected days before symptoms appear (17). We believe that the same correlation might exist between platelet indices and PVT. At routine checkup a cirrhotic patient with elevated MPV, PCT and PDW might be a suspect for PVT, despite thrombocytopenia. However, to demonstrate the possible predictive value of platelet indices for PVT more prospective studies on larger patient groups should be performed.

Our data suggested that increased platelet indices contribute to the prethrombotic state in liver cirrhosis and that larger platelets may play a specific role in thrombosis despite thrombocytopenia.

Platelet indices could provide useful clinical information and be built into a risk assessment algorithm for PVT. This study supports the fact that platelet reactivity is important in the pathogenesis of PVT.

An important limitation concerning the predictive value of MPV, MPM, and MPC is the absence of well-defined limits to differentiate between activated and nonactivated platelets. Other platelet activity indices, such as radio-labeling methods, aggregometry procedures, platelet-specific eicosanoids, other release reactions such as serotonin and histamine, adhesion molecules, flow cytometric investigations, platelet function analyzers, thromboelastography, and plasma factors influencing platelet activation such as fibrinogen were not used in our study.

CONCLUSIONS

Patients with PVT and liver cirrhosis had abnormalities of platelet activation compared with the group without PVT, and these platelet abnormalities may partly contribute to the pathophysiology of PVT. Platelet activity indices including MPV, PDW, and PCT may be used in predicting PVT in patients with liver cirrhosis. However, these results have to be confirmed in larger, prospective, and more homogenous study groups.

Acknowledgements

This work was made possible by the project "Interuniversity partnership for increasing quality and interdisciplinary medical research by providing doctoral scholarships - docmed.net" POSDRU/107/1.5/s/78702.

REFERENCES

Platelet indices in patients with de novo portal vein thrombosis and liver cirrhosis


---

**LASER TREATMENT OF ONYCHOMYCOSIS**

The efficacy of in vitro laser irradiation against fungi, for treatment of onychomycosis, was investigated by Paasch et al. For this purpose, 808, 980 and 1064-nm lasers were used to heat cell culture media and nail clippings and several Trichophyton, Microsporum and Candida species were subjected to laser irradiation, followed by incubation for 6 days. The results showed a thermal effect for 980-nm and 1064-nm laser systems on cell culture media and nail clippings. Growth inhibition was detected for Candida guilliermondii and Trichophyton interdigitale and complete growth impairment was achieved at temperatures above 50°C. For other pathogens, only reduced growth was observed. (Paasch U, Mock A, Grunewald S, Bodendorf MO, Kendler M, Seitz AT, et al. Antifungal efficacy of lasers against dermatophytes and yeasts in vitro. (Int J Hyperthermia. 2013 Sep;29(6):544-50. doi: 10.3109/02656736.2013.823672)

Teodora Vremera