Identification of a Morphometrical Parameter that Predicts the Response to Splenectomy in Patients with Idiopathic Thrombocytopenic Purpura

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BAKKALOGLU, H., DINCCAG, A., YANAR, H., TUNCA, F., DOGAN, O., CERMIK, H. and KUCUKKAYA, R. Identification of a Morphometrical Parameter that Predicts the Response to Splenectomy in Patients with Idiopathic Thrombocytopenic Purpura. Tohoku J. Exp. Med., 2006, 210 (1), 49-55 —— Idiopathic thrombocytopenic purpura (ITP) is an isolated thrombocyte disease that has no correlation with other causes of clinical thrombocytopenia in adults. About 70% of patients with ITP were successfully treated by medical care and splenectomy, but nearly 30% of ITP patients do not respond to these treatments. The aim of this study is to evaluate the predictive factors that affect the success of treatment in 26 patients with ITP. Thirteen patients with ITP responded to splenectomy (responsive group), whereas 13 other patients were resistant to medical treatment and splenectomy (resistant group). The control group consisted of 13 patients who had undergone posttraumatic splenectomy. Age, sex, spleen weight, the number of follicles per mm², and the length of time between diagnosis and splenectomy were retrospectively analyzed. No significant difference was found between the groups with respect to these variables. Mean maximum follicle diameter (MMaFD), mean minimum follicle diameter (MMiFD), and the number and distribution of CD56 (+) cells were evaluated for each spleen specimen. There was no statistical difference in the distribution of CD56 (+) cells between the ITP patients and the control group, but the number of CD56 (+) cells was significantly higher in the control group than in patients with ITP. While MMiFD showed no statistical difference between the groups of ITP patients, a MMaFD of 350 μm and above was significantly more likely in the resistant group than in the other groups. In conclusion, this study has shown that the MMaFD is a significant predictor of the response to splenectomy.

chronic idiopathic thrombocytopenic purpura; ITP; splenic follicle; CD56

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Idiopathic thrombocytopenic purpura (ITP) is an isolated thrombocyte disease that has no correlation with other causes of clinical thrombocytopenia in adults or children. Currently 30-40% of ITP patients are asymptomatic and incidentally diagnosed (Cortelazzo et al. 1991; Frederiksen and Schmidt 1999). For more than 30 years (until the 1950’s), splenectomy was the only treatment for adult ITP (Doan et al. 1960). In 1950 glucocorticoids and, later, intravenous immunoglobulin and anti–Rh (D) were introduced. However, splenectomy is still very important in the treatment of ITP and is the only curative treatment modality. Medication and splenectomy successfully treat 70% of patients (Huber et al. 2003), but the remaining 30% do not respond to treatment. Neither the patients’ complaints nor the thrombocyte level change in this 30%.

In this study, spleen specimens of patients who were resistant to medication and splenectomy (resistant group), who responded to splenectomy (responsive group), and who had undergone splenectomy due to trauma and iatrogenic injuries (control group) were all retrospectively analyzed. Age, sex, spleen weight, minimum and maximum mean follicle diameters, the number of follicles per mm$^2$, the ratio of follicular area to section area, and CD56 (+) cell levels of these 3 groups were compared to investigate whether there is a predictive factor that correlates with the response to treatment in ITP patients.

**MATERIALS AND METHODS**

We retrospectively evaluated the files of 87 patients who had undergone splenectomy for ITP at the Istanbul Faculty of Medicine, Department of General Surgery between January 1998 and January 2004. Postoperative follow-up of these 87 patients revealed that 13 patients failed to respond to splenectomy treatment. These patients formed resistant group. Thirteen patients from the remaining 74 who responded to splenectomy were randomly chosen to form responsive group, and 13 randomly chosen patients who had undergone emergency splenectomy due to trauma or iatrogenic injuries formed control group. The protocol for this study was approved by the Ethics Committee of the Istanbul University Faculty of Medicine (Istanbul, Turkey).

All ITP patients were evaluated with The American Society of Hematology ITP Guideline Panel (George et al. 1996) in the Hematology Division of the Istanbul Faculty of Medicine. Patients with bleeding and low platelets were started on 1 mg/kg of metile-prednisolone treatment per day. The metile-prednisolone dose was increased to 2 mg/kg/day for three weeks if the bleeding was persistent or the platelet count remained less than 10,000/mm$^3$. At the end of the third week, medical treatment was considered successful if the platelet count was higher than 100,000/mm$^3$ or the bleeding symptoms had disappeared. The metile-prednisolone dose was reduced if the patient was responding to treatment. During this dose reduction period, splenectomy was performed only on patients who developed bleeding symptoms or had a platelet count of less than 50,000/mm$^3$. Splenectomy was planned in the 3rd month of medical treatment for patients who did not respond to three weeks (2 mg/kg/day) of metile-prednisolone treatment. In patients with severe bleeding, the platelet count was increased rapidly with intravenous immune globulin (IVIG) or anti-Rh D and an urgent splenectomy was performed.

The splenectomy was considered successful if the patient required no medication and the thrombocyte count was greater than 100,000/mm$^3$ for at least 3 months following the operation.

Age, sex, and spleen weight were evaluated between the three groups. The patients’ age and the length of time between ITP diagnosis and splenectomy were evaluated between resistant and responsive group. Paraffin blocks of the patients’ spleens were obtained from the archives of the Pathology Department of the Istanbul Faculty of Medicine. These blocks were labeled immunohistochemically using CD20 and CD56 antibodies.

The primary antibodies used in the immunohistochemical part of this study were mouse anti-human CD20 antibody (CD20Ab – 1, Neo-Markers Lab, Westing-house Dr., Fremont, CA, USA), mouse anti-human CD56 antibody (RTU – CD 56 - 1B6, Novo-Castro Laboratories Ltd., Eugene, UK), biotin streptavidine peroxidase kit systems (Lab Vision, CA, USA), and AEC chromogen (Lab Vision). The antibodies were incubated for one hour at room temperature. Boiling sodium phosphate buffered saline (Invitrogen, Carlsbad, CA, USA) under high pressure at pH 6 was used to retrieve the antigen.

Mean maximum follicle diameter (MMaFD, μm), mean minimum follicle diameter (MMiFD, μm), the number of follicles per mm$^2$, and the ratio of follicular
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Area to section area were morphometrically measured from the CD20 labeled slides using interactive image processing version 4.0 (SAMBA Technologies SARL, Chemin du Vieux Chene, Meylan, France) from the Pathology Dept of Gulhane Military Medical Academy Haydarpasa Training Hospital.

All patients were evaluated according to a percentage system after the tissue sections were scanned under a microscope using the low magnification 10× objective. The sections with the most activity were magnified using a 40× objective and at least 1000 CD56 (+) cells were counted.

The 5× magnification microscope image was transferred to a computer monitor for morphometric calculation. Using the computer mouse, each follicle was outlined on the image on the computer monitor. The computer calculated each follicle’s maximum diameter (µm), minimum diameter (µm), and area (µm²) (Fig. 1). Later, MMaFD, MMiFD, total follicular area (µm²), total section area (µm), and the number of follicles per mm² on each slide were calculated.

Statistical evaluations

Chi-square tests were used to analyze age, sex, CD56 (+) cell count, MMaFD, MMiFD, number of follicles per mm², the ratio of follicle area to total section area, the length of time between ITP diagnosis and splenectomy, and spleen weight for the 3 groups. Values of $p \leq 0.05$ were considered statistically significant. SPSS V11 for Windows was used for frequency analysis and statistical comparison.

RESULTS

Three groups consisting of 13 patients each were included in the study. Table 1 shows the demographic data and the spleen weight of the groups of patients.

There were no statistical differences in the sex ($p > 0.4$) and age ($p > 0.5$) between patients with ITP. No statistical difference was detected in the spleen weight among the 3 groups ($p > 0.7$). The mean length of time between ITP diagnosis and splenectomy was 47.9 months (2 – 204) in resistant group and 11.1 months (1.5 – 72) in responsive group. No statistical difference was found between the groups of ITP patients in terms of length of time from diagnosis of ITP to splenectomy ($p > 0.1$). The mean percentage of CD56 (+) cells was 3.8 (0 – 20) in resistant group, 12.2 (0 – 72) in responsive group, and 34.5 (2 – 104) in control group. The median percentages of CD56 (+) cells and the mean number of follicles are shown in Table 2. CD56 (+) cells were mostly

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Table 1. Demographic features of patients.

<table>
<thead>
<tr>
<th></th>
<th>Resistant group</th>
<th>Responsive group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>30.8 (16 – 57)</td>
<td>37.2 (17 – 83)</td>
<td>39.4 (8 – 69)</td>
</tr>
<tr>
<td>Women/Men</td>
<td>8/5</td>
<td>10/3</td>
<td>2/11</td>
</tr>
<tr>
<td>Mean spleen weight (gr)</td>
<td>133.8 (50 – 290)</td>
<td>130 (65 – 270)</td>
<td>136.3 (55 – 300)</td>
</tr>
</tbody>
</table>

There were no statistical differences in the sex and age of patients between the group of ITP patients. No statistical difference in spleen weight was detected between the 3 groups.
detected in the parafollicular area; no CD56 (+) cells were found in the germinal center and only a few CD56 (+) cells were found in the marginal zone (Fig. 2). No statistical difference was found in the distribution of CD56 (+) cells between the groups (Table 3), but the number of CD56 (+) cells was significantly higher in the control group than in patients with ITP.  

**Table 2.** Distribution of median percentage of CD56 (+) cells and mean number of follicles between the three patient groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median percentage of CD56 (+) cells</th>
<th>Mean number of follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant group</td>
<td>3 ± 5.3 (p &gt; 0.05)</td>
<td>129.1 ± 38.7</td>
</tr>
<tr>
<td>Responsive group</td>
<td>3 ± 22.5 (p &gt; 0.05)</td>
<td>120.8 ± 60.6</td>
</tr>
<tr>
<td>Control group</td>
<td>27 ± 33.7 (p &lt; 0.001)</td>
<td>139 ± 95.4</td>
</tr>
</tbody>
</table>

Median percentage of CD56 (+) cells and mean number of follicles were evaluated in each group. The number of CD56 (+) cells and the mean number of follicles were significantly higher in the control group than in patients with ITP.

**Figure 2.** CD56 (+) cells were seen in the marginal zone and parafollicular red pulp. All patients were evaluated according to a percentage system after tissue sections were scanned under the microscope using low magnification 10× objectives. The sections with the most activity were magnified using a 40× objective and at least 1000 CD56 (+) cells were counted. This figure shows a section of white pulp under 10× magnification. The section includes regressive germinal center and periarteriolar T zone. CD56 (+) cells were indicated with white arrows.

**Table 3.** Distribution of the mean number of CD56 (+) cells both the red and white pulp.

<table>
<thead>
<tr>
<th>Group</th>
<th>Red pulp (Mean ± S.E.M.)</th>
<th>White pulp (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant group</td>
<td>3.3 ± 4.2</td>
<td>0.5 ± 1.1</td>
</tr>
<tr>
<td>Responsive group</td>
<td>9.9 ± 20.9</td>
<td>2.3 ± 4.5</td>
</tr>
<tr>
<td>Control group</td>
<td>28.9 ± 28.9 (p &lt; 0.001)</td>
<td>7.2 ± 8.0 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>

The mean number of CD56 (+) cells ± s.e.m. was calculated for both the red and white pulp. No statistical difference was found in the distribution of CD56 (+) cells between the three patient groups.

**Table 4.** Evaluation of MMaFD between the three patient groups.

<table>
<thead>
<tr>
<th></th>
<th>Resistant group</th>
<th>Responsive group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMaFD &gt; 350 μm</td>
<td>12 (92.3%)</td>
<td>6 (46.2%)</td>
<td>5 (38.5%)</td>
</tr>
<tr>
<td>MMaFD &lt; 350 μm</td>
<td>1 (7.7%)</td>
<td>7 (53.8%)</td>
<td>8 (61.5%)</td>
</tr>
</tbody>
</table>

*MMaFD, Mean maximum follicle diameter.*
was statistically higher in resistant group than in the other groups ($p < 0.01$). No statistical difference was found in the MMiFD between the three groups ($p > 0.09$). The mean number of follicles per mm$^2$ was 0.9 (0.04 - 1.4) in resistant group, 1.0 (0.6 - 1.6) in responsive group, and 2.0 (0.9 - 4.4) in control group. Although there were no statistical differences between the ITP patients ($p > 0.8$), the mean number of follicles per mm$^2$ was higher in control group when compared with the groups of ITP patients ($p < 0.001$) (Fig. 3).

Analysis of the ratios of follicle area to section area showed no statistical difference between the groups ($p > 0.9$).

**DISCUSSION**

Today corticosteroids are the first line treatment for adult ITP patients (George et al. 1995; American Society of Hematology ITP Practice Guideline Panel 1997). Thirty percent of patients completely or partially respond to continuing corticosteroid treatment (McMillan 1997). Another mode of treatment, splenectomy, is successful in 70 – 85% of patients. According to the recorded literature, the rate of relapse during the first year was 10 – 20% (Picozzi et al. 1980; Najean et al. 1991; Stasi et al. 1995). This is why predicting the response to splenectomy before the operation is important. Unfortunately, predictors of the response to splenectomy have not yet been identified.

In a study performed by Radaelli et al. (2000), age and sex showed no correlation with the response to splenectomy. In a study performed by DiFino et al. (1980) and in many other studies, young age (< 50 – 60) was important in the response to splenectomy (Jiji et al. 1973; Rocco and Stein 1984; Fenaux et al. 1989; Najean et al. 1991; Naouri et al. 1993; Shiino et al. 1996; Radaelli et al. 2000). Even though 24 of the ITP patients (92.4 %) in our study were below the age of 50, no correlation with respect to patient age was found.

In a study performed by Coa (1993), a better
long-term response to splenectomy was seen in patients who suffered from a spleen that was double the size of a normal spleen. Contrary to this, Shiino et al. (1996) and Vaiphei et al. (1995) found no correlation between spleen weight and the response to splenectomy (Fabris et al. 1989). Similarly, we did not find any difference in spleen weight among the 3 groups of patients.

According to Radaelli et al. (2000), no correlation has been detected between the preoperative thrombocyte count, sites of thrombocyte breakdown, and the length of time between diagnosis and operation. In another study, Den Ottolander et al. (1984) reported a correlation between the response to splenectomy and a shorter length of time from diagnosis to splenectomy (Gratama et al. 1984). However, in our study the length of time between diagnosis and splenectomy did not have any impact on the response to splenectomy.

CD56 antigen is a 175-220 kDa glycoprotein. It was first discovered in chicken brain and was named neuronal cell adhesion molecule (NCAM). In humans the glycoprotein is not only found only in neural tissue, but can exist in the natural killer (NK) and T cells in the peripheral blood. However, its function is still unclear. Anti-CD56 and anti-CD16 antibodies can be found on up to 90% of the NK and T cells in the peripheral blood. The importance of the NK and T cells is their cytolytic activity, which is independent from the Major Histocompatibility Complex (MHC). These cells play an important role in thrombocyte cytotoxicity in the pathogenesis of ITP. According to a study performed by Garcia-Suarez et al. (1993), a significant increase in CD56 (+) cells has been observed in patients who failed to respond to corticosteroid treatment and splenectomy. Nevertheless, we found no difference between the number of CD56 (+) cells in the spleen specimens of the groups that responsive or resistant to splenectomy. In comparison to the control group, the ITP patient’s group had a significantly lower CD56 (+) cell count. This fall in the number of CD56 (+) cells was thought to be caused by the use of glucocorticoids in ITP patients before splenectomy.

Arendt et al. (1988) found a significant correlation between the spleen’s follicle diameter and the response to splenectomy. Patients did not respond to splenectomy if follicle hyperplasia (> 500 μm) was present (Vaiphei et al. 1995). In a study by Coa (1993), a spleen follicle diameter greater than 500 μm and a germinal center diameter greater than 200 μm was critical in the response to splenectomy in ITP patients. According to the literature an exact value has not been determined with respect to the MMaFD, but in our study a MMaFD of > 350 μm significantly (p < 0.01) decreased the probability of a response to splenectomy.

In conclusion, mean maximum follicle diameter seems to be a predictive factor for the response to splenectomy. However, studies including more patients are needed to investigate the factors that impact the response to splenectomy.

Acknowledgments

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References

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