ORIGINAL ARTICLE

Distribution of peripheral blood cells after splenectomy in immune thrombocytopenia patients

Distribución de glóbulos periféricos después de la esplenectomía en pacientes

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Abstract

Background: *There are some difficulties regarding the evaluation of the post-splenectomy state.* **Objective:** *The objective of the study is to compare the post-splenectomy blood changes of immune thrombocytopenia (ITP) patients with those of trauma patients, 1 month and ≥ 6 months after surgery.* **Methods:** *Medical records of patients, who had undergone total splenectomy for ITP and trauma at a tertiary center between January 2009 and December 2019, were retrospectively reviewed.* **Results:** *The current study included 52 patients, who had undergone splenectomy for ITP (57.7%), and trauma (42.3%). Splenectomy, irrespective of the indications, resulted in an increase in hemoglobin concentration, hematocrit, and platelet levels. Neutrophils were responsible for the preoperative leukocytosis in ITP patients, and neutrophilia was ameliorated by splenectomy and also withdrawal of the steroid therapy in some patients. Decreased neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio supported the finding that splenectomy ameliorated inflammation in ITP patients. Splenectomy resulted in a change in percentages of leukocytes in favor of basophils in ITP patients.* **Conclusions:** *Splenectomy, irrespective of the indications, resulted in an increase in hemoglobin concentration, hematocrit and platelet levels, lymphocyte, monocyte, and eosinophil counts. Splenectomy ameliorated inflammation in ITP patients and resulted in a change in percentages of leukocytes in favor of basophils.*

Keywords: *Immune thrombocytopenia. Neutrophil-to-lymphocyte ratio. Peripheral blood cells. Splenectomy. Trauma.*

Resumen

Antecedentes: *Existen algunas dificultades con respecto a la evaluación del estado post-esplenectomía.* **Objetivo:** *Comparar los cambios sanguíneos post-esplenectomía de pacientes con PTI con los de pacientes traumatizados, 1 mes y ≥ 6 meses después de la cirugía.* **Métodos:** *Se revisaron retrospectivamente las historias clínicas de los pacientes que habían sido sometidos a esplenectomía total por PTI y trauma en un centro terciario entre enero de 2009 y diciembre de 2019.* **Resultados:** *El presente estudio incluyó a 52 pacientes, que habían sido sometidos a esplenectomía por PTI (57.7%) y traumatismo (42.3%). La esplenectomía, independientemente de las indicaciones, resultó en un aumento de la concentración de hemoglobina, hematocrito y niveles de plaquetas. Los neutrófilos fueron responsables de la leucocitosis preoperatoria en pacientes con PTI, y la neutrofilia mejoró mediante esplenectomía y también la suspensión de la terapia con esteroides en algunos pacientes. La disminución de NLR y PLR apoyó el hallazgo de una disminución de la inflamación en la esplenectomía en pacientes con PTI. La esplenectomía resultó en un cambio en los porcentajes de leucocitos a favor de los basófilos en*

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Date of reception: 15-03-2021
Date of acceptance: 25-05-2021
DOI: 10.24875/CIRU.21000246

Cir Cir. 2022;90(2):157-164
Contents available at PubMed
www.cirugiaycirujanos.com

pacientes con PTI. **Conclusiones:** *La esplenectomía, independientemente de las indicaciones, resultó en un aumento de la concentración de hemoglobina, niveles de hematocrito y plaquetas, recuentos de linfocitos, monocitos y eosinófilos. Una disminución de la inflamación en la esplenectomía en pacientes con PTI resultó en un cambio en los porcentajes de leucocitos a favor de los basófilos.*

Palabras clave: *Trombocitopenia inmune. PTI. NLR. Glóbulos periféricos. Esplenectomía. Trauma.*

Introduction

As a major lymphoid organ, receiving 5% of cardiac output every minute, the spleen has a fundamental role in the reticuloendothelial system. It removes antigens, aging platelets, and erythrocytes from the circulation and participates in iron recycling, as well as in the production of lymphocytes, immunoglobulins, and opsonins[1]. It serves as a storage site for blood components and supplies erythrocytes and platelets in increased demand, such as trauma[1].

The knowledge of splenic functions is mostly based on murine studies. However, morphological and physiological differences between humans and other non-primate species may obscure understanding of splenic functions[1,2]. *Ex-vivo* studies are preferred, due to limitations in *in-vivo* human testing, to analyze the interactions between the functioning spleen and circulating blood components. *Ex-vivo* studies are performed in splenectomized patients by comparing preoperative conditions with postoperative ones. However, there are some difficulties regarding the evaluation of the post-splenectomy state. Along with surgical stress, any indication itself for splenectomy may interfere with the evaluation process by promoting cellular abnormalities[3].

Immune thrombocytopenia (ITP) is an acquired form of thrombocytopenia that is characterized by autoantibody-mediated destruction of platelets and suppression of platelet production[4]. As a primary site of platelet removal, the spleen plays a critical role in the pathogenesis of ITP. It also participates in the production of anti-platelet antibodies[4]. Although splenectomy is associated with a better outcome than any other therapy, it is generally reserved (as second-or third-line therapy) for patients who fail medical therapy, as it poses short and long-term risks[4]. Although several efforts have been made to predict splenectomy responses, they have not been fully successful[4].

This study aimed to compare the post-splenectomy blood changes of ITP patients with those of trauma patients, 1 month and ≥ 6 months after surgery. The comparison of ITP patients with trauma patients, who are hematologically normal people, may clarify the post-splenectomy changes in patients with ITP.

Material and method

Medical records of patients, who had undergone total splenectomy for ITP and trauma at a tertiary center between January 2009 and December 2019, were retrospectively reviewed. Exclusion criteria included partial splenectomies, patients younger than 18 years of age, patients with congestive heart failure, patients with chronic pulmonary diseases, patients with chronic liver diseases, patients with malignant diseases, and patients with autoimmune diseases other than ITP. The patients, who had not had a blood test in the 1st month and/or ≥ 6 months after surgery and/or who had died before the blood samplings were also excluded.

The diagnosis of ITP was based on isolated thrombocytopenia in the absence of other causes of thrombocytopenia. In cases where platelet counts were $< 30 \times 10^9$/L and/or patients had signs and symptoms of bleeding, first-line therapy was administered with 1 mg/kg/day oral methylprednisolone. In addition to steroid therapy, some patients had taken danazol, azathioprine, cyclosporine, cyclophosphamide, vincristine, thrombopoietin receptor agonists, intravenous immunoglobulin. For the management of ITP, medical treatment was applied initially, and splenectomy was indicated when medical treatment was exhausted in long-term follow-up. Appropriate preparation of patients for surgery was performed.

The data included demographics, clinical findings, laboratory findings, and therapeutic interventions. The following characteristics were recorded:

- Demographics: Age and gender;
- Clinical findings: Comorbid factors, postoperative hospital stay, and perioperative complications;
- Laboratory findings: Preoperative and postoperative (1st month and/or ≥ 6 months after surgery) complete blood counts (hematocrit, hemoglobin, white blood cell, platelet, and mean corpuscular volume [MCV], and percentages of neutrophils, lymphocytes, eosinophils, basophils, and monocytes) and

inflammatory markers (neutrophil-to-lymphocyte ratio [NLR] and platelet-to-lymphocyte ratio [PLR]), which were calculated based on complete blood count results; and
– Therapeutic interventions: Surgical technique (open or laparoscopic), and transfusion of blood components.

The study was conducted according to the principles set forth by the Helsinki Declaration of 1975. Approval from the Human Ethics Committee of the Institution was obtained.

Statistical analysis

Data were analyzed using SPSS 17.0 for Windows. Continuous variables were presented as means with SDs; categorical variables were presented as numbers with percentages. The Shapiro-Wilk test was used to analyze the normality of the groups. For intergroup comparison, the Chi-squared test or Fisher's exact test was used for categorical variables. The Student's t-test was used for continuous variables with normal distribution. The Mann-Whitney U-test was applied for non-normally distributed variables. The paired-sample t-test was used to compare the changes of laboratory values, at the 1st month and ≥ 6 months after surgery. The level of significance was accepted as 0.05.

Results

The current study included 52 patients, who had undergone splenectomy for ITP (57.7%), and trauma (42.3%). The mean age was 37.1 ± 15.4 (range: 18-76) years. Of the 52 patients, 33 (63.5%) had one or more systemic diseases including ITP (n: 30), hypertension (n: 2), diabetes mellitus (n: 2), coronary artery diseases (n: 1), and psychiatric disease (n: 1, depression). All trauma cases were operated on with open technique. Of the ITP cases, 80% were operated with laparoscopic technique. Transfusion of blood components was required in 48.1% of the cases. The mean length of hospitalization was 9.9 ± 15.8 (range: 2-96 days) (Table 1). All ITP patients had a complete response to splenectomy, which means platelet count > 100×10^9/L and the absence of bleeding without any treatment after splenectomy.

Hematocrit and hemoglobin count

One month after surgery, we did not find any significant change in hematocrit and hemoglobin levels in both groups (Table 2). However, at least 6 months after surgery, there was a significant increase in hematocrit and hemoglobin levels in both groups (Table 2). There was not a significant difference between the groups in terms of preoperative and postoperative hematocrit levels (Table 1). Although preoperative and postoperative 1st-month hemoglobin concentration was not different between the groups, it was significantly lower in the ITP group 6 months after surgery (Table 1).

MCV

The preoperative and postoperative MCVs were lower in the ITP group compared to the trauma group (Table 1). One month after surgery, there was a significant increase in MCVs in the trauma group. However, these declined to preoperative values after 6 months (Table 2).

Platelet count

There was a significant increase in postoperative platelet counts in both groups (Table 2). One month after surgery, the increase in platelet count was more prominent in the trauma group but declined slightly overtime. The preoperative and postoperative 1st-month platelet counts were statistically higher in the trauma group. However, the difference disappeared after 6 months (Table 1).

Leukocyte count

The preoperative leukocyte count was statistically higher in the trauma group. There was no statistical difference between the groups on postoperative courses (Table 1). There was a significant decrease in postoperative leukocyte counts in the trauma group, although they were still above the normal range (Table 2). However, we did not find any significant change in leukocyte count in the ITP group.

Neutrophil count

The pre-operative neutrophil count was statistically higher in the trauma group (Table 1). There was no statistical difference between the groups on post-operative courses (Table 1). There was a significant decrease in post-operative neutrophil counts in both groups (Table 2).

Table 1. The characteristics of the patients and comparison of the laboratory values of ITP patients with those of trauma patients

Laboratory findings	Total (n: 52) mean ± SD or n (%)	Trauma (n: 22) mean ± SD or n (%)	ITP (n: 30) mean ± SD or n (%)	p
Age (years)	37.1 ± 15.4	35.6 ± 18.8	38.2 ± 12.5	0.176
Gender				
Male	25 (48.1)	17 (77.3)	8 (26.7)	0.000
Female	27 (51.9)	5 (22.7)	22 (73.3)	
Pre - operatıve				
Hematocrit value	37.6 ± 6.2	35.5 ± 6.6	39.2 ± 5.5	0.035
Hemoglobin value	12.5 ± 2.07	11.9 ± 2.2	12.9 ± 1.9	0.112
Platelet value	189.7 ± 101.8	247 ± 64.9	147.7 ± 104.2	0.000
White blood cell count	15.2 ± 6.5	19.4 ± 6.2	12.1 ± 4.9	0.000
Neutrophil count	11.47 ± 6.4	15.4 ± 6.3	8.56 ± 4.8	0.000
Lymphocye count	2.75 ± 1.4	2.8 ± 2.05	2.7 ± 0.76	0.304
Neurophil percentage	70.4 ± 16.6	77.5 ± 15.07	65.2 ± 15.9	0.005
Lymphocye percentage	20.6 ± 11.9	15.5 ± 12.7	24.4 ± 9.9	0.005
Eisonphil percentage	1 ± 1.29	0.37 ± 0.38	1.46 ± 1.52	0.011
Basophil percentage	0.53 ± 0.78	0.54 ± 1.13	0.53 ± 0.37	0.032
Monocyte percentage	6.35 ± 5.6	6.03 ± 8.43	6.6 ± 1.9	0.000
Neutrophil-to-lymphocyte ratio	6.1 ± 6.1	9.4 ± 7.6	3.72 ± 3.2	0.005
Platelet-to-lymphocyte ratio	94.1 ± 97.2	141.2 ± 115.8	59.6 ± 49.7	0.001
Mean corpuscular volume	84.6 ± 6.7	87 ± 6.4	82.8 ± 6.5	0.002
Post- operative 1st month				
Hematocrit value	39.5 ± 5.2	38.3 ± 4.6	40.4 ± 5.58	0.156
Hemoglobin value	12.8 ± 1.7	12.5 ± 1.4	13.1 ± 1.86	0.279
Platelet value	496.7 ± 298.7	692.4 ± 301.7	353.26 ± 201.7	0.000
White blood cell count	11.6 ± 4.3	12.4 ± 5.1	11.05 ± 3.64	0.251
Neutrophil count	6.8 ± 4.08	8.03 ± 5.1	6.06 ± 2.9	0.067
Lymphocye count	3.15 ± 1.06	2.8 ± 1.03	3.4 ± 0.96	0.027
Neurophil percentage	56.6 ± 12.9	60.1 ± 14.4	53.1 ± 10.85	0.021
Lymphocye percentage	28 5 ± 11.6	24.6 ± 11.8	31.4 ± 10.7	0.036
Eisonphil percentage	3.02 ± 2.68	2.8 ± 3.1	3.1 ± 2.3	0.541
Basophil percentage	1.36 ± 1.79	1.3 ± 2.04	1.4 ± 1.6	0.224
Monocyte percentage	9.7 ± 2.72	9.5 ± 3.1	9.9 ± 2.4	0.664
Neutrophil-to-lymphocyte ratio	2.6 ± 2.4	3.5 ± 3.3	1.89 ± 0.98	0.009
Platelet-to-lymphocyte ratio	187.8 ± 152.5	292.7 ± 171	110.9 ± 72.6	0.000
Mean corpuscular volume	85.9 ± 6.1	89.05 ± 3.7	83.6 ± 6.5	0.001
Post- operative ≥ 6 month				
Hematocrit value	42.6 ± 5.3	44.3 ± 5.3	41.4 ± 5.16	0.052
Hemoglobin value	13.9 ± 1.9	14.6 ± 1.7	13.4 ± 1.92	0.021
Platelet value	375.2 ± 138.7	412 ± 75.7	348.3 ± 167.1	0.295
White blood cell count	11.2 ± 2.9	12.1 ± 3.4	10.57 ± 2.4	0.071
Neutrophil count	5.6 ± 1.9	6.1 ± 2.1	5.26 ± 1.6	0.122
Lymphocye count	4.1 ± 1.4	4.3 ± 1.6	3.9 ± 1.2	0.447
Neurophil percentage	49.8 ± 8.3	50.2 ± 8.5	49.4 ± 8.2	0.731
Lymphocye percentage	36.8 ± 8.4	36.3 ± 8.9	37.1 ± 8.1	0.754
Eisonphil percentage	2.4 ± 1.8	2.1 ± 1.8	2.58 ± 1.85	0.364
Basophil percentage	0.9 ± 0.6	0.86 ± 0.75	1.04 ± 0.55	0.038
Monocyte percentage	9.9 ± 2.8	10.2 ± 3.37	9.7 ± 2.5	0.774
Neutrophil-to-lymphocyte ratio	1.5 ± 0.74	1.5 ± 0.89	1.46 ± 0.6	0.704
Platelet-to-lymphocyte ratio	104.7 ± 62.4	105.5 ± 38.9	104.1 ± 75.9	0.604
Mean corpuscular volume	85.9 ± 7.33	88.6 ± 4.6	83.7 ± 8.29	0.012
Surgical technique				
Open	28 (53.8)	22 (100)	6 (20)	0.000
Laparoscopic	24 (46.2)	-	24 (80)	
Transfusion of blood components				
Yes	25 (48.1)	19 (86.4)	6 (20)	0.000
No	27 (51.9)	3 (13.6)	24 (80)	
Lenght of hospitalization (day)	9.9 ± 15.8	17.8 ± 22	4.1 ± 2.2	0.000

Lymphocyte count

The pre-operative lymphocyte counts were not different between the groups (Table 1). One month after surgery, there was no change in lymphocyte count in the trauma group. However, it increased significantly after 6 months (Table 2). There was a significant increase in post-operative lymphocyte counts in the ITP group, which was predominant 6 months after surgery (Table 2). The post-operative 1st-month lymphocyte count was significantly higher in the ITP group, but the difference disappeared within 6 months (Table 1).

The percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils

The pre-operative percentages of neutrophils and basophils were significantly higher in the trauma group. The pre-operative percentages of lymphocytes, eosinophils, and monocytes were significantly higher in the ITP group (Table 1).

In the trauma group, there was a significant decrease in the percentage of neutrophils, while an increase in the percentages of lymphocytes and eosinophils. Although the percentage of monocytes increased, it was statistically significant 6 months after surgery. The changes in the percentages of neutrophils, lymphocytes, and monocytes were more prominent 6 months after surgery. Although the percentage of basophils increased, it was not statistically significant (Table 2).

In the ITP group, there was a significant decrease in the percentages of neutrophils, while there was an increase in the percentages of lymphocytes, eosinophils, basophils, and monocytes. The changes in the percentages of eosinophils, basophils, and monocytes were more prominent 1 month after surgery, while the changes in the percentages of neutrophils and lymphocytes were more prominent 6 months after surgery (Table 2).

The percentage of neutrophils decreased, while the percentage of lymphocytes increased in both groups (Table 2). The percentage of neutrophils was significantly higher in the trauma group, while the percentage of lymphocytes was significantly higher in the ITP group 1 month after surgery (Table 1). There were no statistical differences between the groups in terms of postoperative percentages of eosinophils and monocytes (Table 1). Six months after surgery, there was no significant difference between the groups in terms of neutrophils, lymphocytes, eosinophils, and monocytes. Only the percentage of basophils was significantly higher in the ITP group 6 months after surgery (Table 1).

NLR

The preoperative NLR was significantly higher in the trauma group (Table 1), but it decreased over time in both groups (Table 2). The NLR of the trauma group was significantly higher 1 month after surgery, but the difference disappeared over time (Table 1).

PLR

The preoperative PLR was significantly higher in the trauma group (Table 1). It increased in both groups 1 month after surgery and was more prominent in the trauma group (Table 2). Although the change in PLR in the ITP group remained stable over time, it declined to preoperative values in the trauma group (Table 2). Thus, PLR values were comparable between the groups 6 months after surgery (Table 1).

Discussion

It was not surprising that hematocrit and hemoglobin values increased within the normal range after therapy in both groups. The post-operative 1st-month increase in MCVs in trauma patients was thought to be a compensatory mechanism for blood loss. ITP, as a chronic inflammatory disease, had lower hematocrit, hemoglobin, and MCV levels compared to those of trauma patients in the post-operative period even after iron repletion.

Thrombocytosis is defined as a platelet count >500×109/L, and extreme thrombocytosis is defined as a platelet count >1,000×109/L. Thrombocytosis is either a reactive process (secondary to infection, trauma, surgery, and malignancy) or is caused by a clonal bone marrow disorder[5]. It is a normal physiologic response to splenectomy, which may persist in up to 30% of splenectomized patients, and cessation of splenic sequestration is thought to be a major participatory mechanism[6]. In the current study, 1 month after surgery, the increase in platelet count was more prominent in the trauma group but declined slightly overtime. The preoperative and postoperative 1st-month platelet counts were statistically higher in the trauma group. However, the difference disappeared ≥ 6 months after. All ITP patients had a complete response to splenectomy.

Leukocytes constitute the cellular components of the immune system and participate in host defense.

Table 2. The paired-sample t-test for comparison of the changes of laboratory values, at the 1st month and ≥ 6 months after surgery

Laboratory findings	Pre-operative mean ± SD	Post-operative 1st month mean ± SD	p	Post-operative ≥ 6 month mean ± SD	p
Trauma Patients					
Hematocrit value	35.5 ± 6.6	38.3 ± 4.6	0.118	44.3 ± 5.3	0.000
Hemoglobin value	11.9 ± 2.2	12.5 ± 1.4	0.288	14.6 ± 1.7	0.000
Platelet value	247 ± 64.9	692.4 ± 301.7	0.000	412 ± 75.7	0.000
White blood cell count	19.4 ± 6.2	12.4 ± 5.1	0.000	12.1 ± 3.4	0.000
Neutrophil count	15.4 ± 6.3	8.03 ± 5.1	0.000	6.1 ± 2.1	0.000
Lymphocye count	2.8 ± 2.05	2.8 ± 1.03	0.927	4.3 ± 1.6	0.006
Neurophil percentage	77.5 ± 15.07	60.1 ± 14.4	0.001	50.2 ± 8.5	0.000
Lymphocye percentage	15.5 ± 12.7	24.6 ± 11.8	0.007	36.3 ± 8.9	0.000
Eisonphil percentage	0.37 ± 0.38	2.8 ± 3.1	0.001	2.1 ± 1.8	0.000
Basophil percentage	0.54 ± 1.13	1.3 ± 2.04	0.156	0.86 ± 0.75	0.289
Monocyte percentage	6.03 ± 8.43	9.5 ± 3.1	0.082	10.2 ± 3.37	0.049
Neutrophil-to-lymphocyte ratio	9.4 ± 7.6	3.5 ± 3.3	0.001	1.5 ± 0.89	0.000
Platelet-to-lymphocyte ratio	141.2 ± 115.8	292.7 ± 171	0.002	105.5 ± 38.9	0.206
Mean corpuscular volume	87 ± 6.4	89.05 ± 3.7	0.047	88.6 ± 4.6	0.124
ITP Patients					
Hematocrit value	39.2 ± 5.5	40.4 ± 5.58	0.123	41.4 ± 5.16	0.005
Hemoglobin value	12.9 ± 1.9	13.1 ± 1.86	0.449	13.4 ± 1.92	0.050
Platelet value	147.7 ± 104.2	353.26 ± 201.7	0.000	348.3 ± 167.1	0.000
White blood cell count	12.1 ± 4.9	11.05 ± 3.64	0.342	10.57 ± 2.4	0.092
Neutrophil count	8.56 ± 4.8	6.06 ± 2.9	0.034	5.26 ± 1.6	0.001
Lymphocye count	2.7 ± 0.76	3.4 ± 0.96	0.002	3.9 ± 1.2	0.000
Neurophil percentage	65.2 ± 15.9	53.1 ± 10.85	0.003	49.4 ± 8.2	0.000
Lymphocye percentage	24.4 ± 9.9	31.4 ± 10.7	0.006	37.1 ± 8.1	0.000
Eisonphil percentage	1.46 ± 1.52	3.1 ± 2.3	0.001	2.58 ± 1.85	0.012
Basophil percentage	0.53 ± 0.37	1.4 ± 1.6	0.008	1.04 ± 0.55	0.000
Monocyte percentage	6.6 ± 1.9	9.9 ± 2.4	0.000	9.7 ± 2.5	0.000
Neutrophil-to-lymphocyte ratio	3.72 ± 3.2	1.89 ± 0.98	0.007	1.46 ± 0.6	0.001
Platelet-to-lymphocyte ratio	59.6 ± 49.7	110.9 ± 72.6	0.002	104.1 ± 75.9	0.012
Mean corpuscular volume	82.8 ± 6.5	83.6 ± 6.5	0.302	83.7 ± 8.29	0.435
Total					
Hematocrit value	37.6 ± 6.2	39.5 ± 5.2	0.030	42.6 ± 5.3	0.000
Hemoglobin value	12.5 ± 2.07	12.8 ± 1.7	0.186	13.9 ± 1.9	0.000
Platelet value	189.7 ± 101.8	496.7 ± 298.7	0.000	375.2 ± 138.7	0.000
White blood cell count	15.2 ± 6.5	11.6 ± 4.3	0.001	11.2 ± 2.9	0.000
Neutrophil count	11.47 ± 6.4	6.8 ± 4.08	0.000	5.6 ± 1.9	0.000
Lymphocye count	2.75 ± 1.4	3.15 ± 1.06	0.088	4.1 ± 1.4	0.000
Neurophil percentage	70.4 ± 16.6	56.6 ± 12.9	0.000	49.8 ± 8.3	0.000
Lymphocye percentage	20.6 ± 11.9	28 5 ± 11.6	0.000	36.8 ± 8.4	0.000
Eisonphil percentage	1 ± 1.29	3.02 ± 2.68	0.000	2.4 ± 1.8	0.000
Basophil percentage	0.53 ± 0.78	1.36 ± 1.79	0.005	0.9 ± 0.6	0.003
Monocyte percentage	6.35 ± 5.6	9.7 ± 2.72	0.000	9.9 ± 2.8	0.000
Neutrophil-to-lymphocyte ratio	6.1 ± 6.1	2.6 ± 2.4	0.000	1.5 ± 0.74	0.000
Platelet-to-lymphocyte ratio	94.1 ± 97.2	187.8 ± 152.5	0.000	104.7 ± 62.4	0.508
Mean corpuscular volume	84.6 ± 6.7	85.9 ± 6.1	0.031	85.9 ± 7.33	0.121

Normal levels of leukocytes are 4.000-11.000 per microliter of blood. Leukocytosis is an expected finding after splenectomy; thus, the diagnosis of postoperative infection in those patients may be a challenge[7-9]. Leukocyte count rises, irrespective of the reason for splenectomy[7]. The increase is initially confined to neutrophils. However, neutrophil count returns to within the normal range within weeks or months, and lymphocyte and monocyte counts rise steadily[7].

Leukocytosis is a well-known response of the body to trauma. It is mainly due to neutrophilia, caused by redistribution of neutrophils from the storage pool to the circulation, and not due to increased production[10]. Neutrophils are one of the first defenders of inflammatory cells to migrate toward the site of injury. So, leukocytosis, especially neutrophilia, is an expected finding in trauma patients as in the current study. In trauma patients, the leukocyte count decreased compared to that

recorded preoperatively, but it was still above the normal range. In ITP patients, the leukocyte count did not change statistically following splenectomy, though leukocytosis was present already before the surgery. Neutrophil counts decreased permanently in both groups during the period of recovery from surgery. The change in lymphocyte and monocyte counts followed a different pattern between the groups. In trauma patients, lymphocyte and monocyte counts initially did not change but increased ≥ 6 months after surgery. However, in ITP patients, lymphocyte and monocyte counts increased gradually overtime. The postoperative 1st-month lymphocyte count was significantly higher in the ITP group, but the difference disappeared within 6 months.

Which mechanism, therefore, is responsible for leukocytosis after splenectomy? The spleen participates in the regulation of leukocytes. Along with the preservation of lymphoid tissue, it may also sequester leukocytes produced elsewhere[7]. Thus, the removal of the spleen causes the leukocyte count to rise. Could there be any mechanism other than the cessation of sequestration that could cause leukocytosis? In order to determine this, several questions must be answered. First: why does the leukocyte count not change significantly in ITP patients, while it remains stable above the normal range in trauma patients? Second: why do lymphocyte and monocyte counts in trauma patients increase several months later than those of ITP patients? Third: could the high basophil level in ITP patients be a unique finding?

ITP is an autoimmune disease, and inflammation plays a central role in pathogenesis. It was clear that neutrophils were responsible for the preoperative leukocytosis in ITP patients, and neutrophilia was ameliorated by both splenectomies, and also the withdrawal of steroid treatment in some patients. Thus, despite the increase in lymphocytes, monocytes, eosinophils and basophiles, total leukocyte count, which was already above the normal range, did not change in this group. The NLR and PLR have been found to be potential markers of systemic inflammation and used to predict prognosis and treatment response in various benign and malign diseases[11-13]. The NLR was used in a small number of studies for the prediction of treatment response to medical therapy in ITP patients, and their results were contradictory[14,15]. In a recent study, it was concluded that splenectomy might improve the weakened immune system of patients with cirrhosis, possibly by reducing suppressive cell fractions and amplifying the effector cell population. In that study, splenectomy was found to decrease NLR[16]. We supposed that, based on the decrease in the NLR and PLR, splenectomy did not only eliminate sequestration but also ameliorated the inflammation in ITP patients.

In ITP patients, the effect caused by the cessation of sequestration on lymphocytes and monocytes is likely to emerge several weeks to months later as in the trauma patients. It seems there was another mechanism responsible for the early increase in lymphocyte and monocyte counts. This mechanism, causing lymphocyte and monocyte counts to increase without inflammatory effects, must be particularly complex, and its effect must not have lasted longer than a few months as the leukocyte counts were comparable with those of the trauma patients, ≥ 6 months after splenectomy. Furthermore, it must have been negated in the presence of a spleen. Although the mechanism that triggers inflammation in ITP has not yet been determined, direct interaction of monocytes with stimulated T- lymphocytes might be a major pathway for the production of proinflammatory cytokines and their inhibitors in monocytes, which has been postulated as a possible mechanism in the pathogenesis of any chronic inflammation[17,18]. Depending on T-cell type and T-cell stimulus, multiple ligands/counter-ligands are involved in the contact-mediated activation of monocytes, and the outcome of the inflammatory process is determined by the balance between proinflammatory cytokines and their inhibitors, which are produced in the monocytes. We suppose that ITP, as a chronic inflammatory disease, might possess similar inflammatory processes[17,18]. Further research is needed to identify inhibitory and/or stimulatory molecules involved in the inflammatory process.

Basophils are the least common type of leukocytes, constituting about 0.5-1% of circulating leukocytes. In the current study, basophils were the only leukocyte type, which was significantly higher in the ITP group ≥ 6 months after surgery. Basophils participate in the regulation of immune response and allergic reactions by producing histamine and serotonin. They also secrete endogenous heparin for anticoagulation[19]. Low basophil percentages or counts are thought to be responsible for the increased risk of thrombosis[20]. Although patients with ITP have a low platelet count with a tendency to bleeding, they occasionally develop venous and/or arterial thromboembolic events by a mechanism, which has not yet been fully elucidated. It may occur as a result of inflammatory processes acting on thrombosis, or as a result of complications of medical and/or surgical treatment. Comorbid diseases may also increase the risk for thromboemboli in ITP patients[21]. It

was not known whether the increase in basophil percentage was a compensatory mechanism in the protection of ITP patients against thromboembolism or whether it was a participant in the inflammatory process itself. Only three patients of the study population, two patients with ITP and the other with trauma, developed thromboembolic complications following splenectomy. Further research is needed for clarification.

Small case numbers and a retrospective design were weaknesses of the current study, while the comparison with a normal population (trauma patients) was a strength.

Conclusions

Splenectomy, irrespective of the indications, resulted in an increase in hemoglobin concentration, hematocrit, and platelet levels. It did not alter leukocyte count in ITP patients but led to leukocytosis in trauma patients. Although lymphocyte, monocyte, and eosinophil counts increased after splenectomy in both groups, total leukocyte count, which was already above the normal range, did not change in ITP patients. Neutrophils were responsible for the preoperative leukocytosis in ITP patients, and neutrophilia was ameliorated by splenectomy and also withdrawal of the steroid therapy in some patients Decreased NLR and PLR supported the finding that splenectomy ameliorated inflammation in ITP patients. Splenectomy resulted in a change in percentages of leukocytes in favor of basophils in ITP patients, but did not result in any change in trauma patients. It is not known whether the increase in basophil percentage was a compensatory mechanism to protect ITP patients against thromboembolism or whether it was a participant in the inflammatory process itself. There are many issues that require clarification with regard to the inhibitory and/or stimulatory molecules involved in the inflammatory process in ITP patients. Our knowledge of the spleen is encompassed by the literature, and everything we don't yet know falls outside of that repository. Further research is needed to illuminate this mysterious organ.

Funding

The author reports no sources of funding.

Conflicts of interest

The author reports no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The author declares that no experiments were performed on humans or animals for this study.

Confidentiality of data. The author declares having followed the protocols in use at their working center regarding patients' data publication.

Right to privacy and informed consent. Patients were informed that their data could be used for research and informed consents were obtained from all patients prior to surgery. The corresponding author is in possession of this document.

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