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Turkish Running Head: ITP Tedavi Yanıtında NLO

Title: Neutrophil Lymphocyte Ratio in Estimating Response to Corticosteroid Treatment in Immune Thrombocytopenia Patients

Running Head: NLR in Treatment Response in ITP

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Introduction

Immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by isolated thrombocytopenia which is attributed to enhanced destruction and impaired production of platelets, often without a definable specific stimulus (1-3). Despite the implementation of novel agents such as thrombopoietin receptor agonists, corticosteroids (CSs) are still the first line recommended therapy for ITP patients who need treatment (1, 3, 4). Initial response rate to corticosteroid treatment is promising, varying from 50 to 90%, however durable platelet response could be maintained in only 10-30% of patients, when the CS treatment is tapered off or ceased (5).

Inflammation is known to have a significant role in the course of many benign (6) and malignant diseases (7). Neutrophil lymphocyte ratio (NLR), being an inexpensive and easily available parameter, has been used frequently as a marker of systemic inflammation in recent years (6). The association between elevated NLR and the disease course, prognosis and treatment response has been established in several benign (8-14) and malignant (15, 16) diseases.

Even though ITP is an autoimmune disease, the data regarding the relationship between ITP and inflammatory markers is not sufficient. Also, there is limited data with respect to the factors affecting response to CS treatment (17-19). So, we aimed to investigate the association between NLR and response, and loss of response to CS treatment in ITP patients who needed treatment.

Methods

We retrospectively analyzed the data of 47 ITP patients treated with corticosteroid therapy at XXX between 2007 and 2016. The data including age, gender, bleeding history, complete blood count at the time of diagnosis, neutrophil lymphocyte ratio (NLR) at the time of diagnosis, treatment response, loss of response, and duration of response was recorded for each patient. ITP diagnosis

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and treatment demand were defined according to The American Society of Hematology practice guidelines (1). We started treatment for patients with platelet count below $30 \times 10^9/L$ or patients with bleeding. Response to treatment was assessed according to the recommendations of the international working group (20). Response (R) was defined as a platelet count greater than $30 \times 10^9/L$ and no response (NR) was defined as a platelet count less than $30 \times 10^9/L$, while loss of response (LOR) was defined as a platelet count less than $30 \times 10^9/L$ or a less than 2-fold increase in the platelet count from baseline or the presence of bleeding. We did not further evaluate the patients according to complete remission, since the cohort was not large enough for such a detailed analysis. Forty-five patients received methylprednisolone and 2 patients received dexamethasone treatment for the first line therapy. NLR was calculated using complete blood count of patients at the time of diagnosis. The cut-off score for NLR was determined 2.5 according to median NLR level.

The study protocol has been approved by the local ethical committee and written informed consent was obtained from all patients.

Statistical Analysis

Statistical evaluation was made using SPSS 24 software. Data were described as numbers and percentage or median and range, when appropriate. χ^2 Fisher's exact test was used for evaluating categorical values and Mann-Whitney U test for continuous values in patient groups. Spearman test was used for correlation analysis. All p-values were 2-sided with statistical significance at 0.05 alpha levels

Results

Patient characteristics are summarized in Table 1. The median age of the patients at the time of diagnosis was 44 years (range, 18-74). Twenty-seven (57.4 %) patients were female and 20 (42.6 %)

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were male. Ten (21.3 %) patients had a history of bleeding at the time of diagnosis. The median white blood cell count was 7,850/mm³ (4,710-21,090/mm³), hemoglobin level was 13.2 g/dl (8.3-17.80 g/dl), platelet count was 9,000/mm³ (0-37,000/mm³), neutrophil count was 5,200/mm³ (2,210-16.900/mm³), and lymphocyte count was 1,970/mm³ (610-5,430/mm³). Thirty-five (74.5 %) patients responded to first line treatment, however loss of response developed in 19 (54.2 %) of these patients during follow-up.

The median NLR was 2.51 (range, 1.15-11.35), and the cut-off score for NLR was assigned as 2.5 according to the median NLR level. Twenty-three (48.9%) patients had NLR< 2.5 and 24 (51.1%) patients had NLR≥ 2.5. There was no statistical significant difference between two groups regarding age, gender, treatment response, response duration and loss of response (p=1, p=0.92, p=0.74, p=0.869, p=0.315) (Table 2). Also there was no correlation between NLR and response duration (p=0.918, r=0.018).

Discussion

While impaired platelet production plays a role in the pathogenesis of ITP, the fundamental step in occurrence of the disease is the production of abnormal autoantibodies specific to platelet membrane antigens. Subsequently, those antibodies bind to the membranes of circulating platelets (2, 3, 21). Autoantibody-bound platelets induce Fc receptor-mediated phagocytosis by macrophages primarily in spleen, leading to increased destruction of platelets (2, 3, 21). The triggering event in the development of antibody production remains unclear, however direct interaction of monocyte subgroups with Thelper/Tregulatory lymphocytes has been implicated in eliciting the events (22, 23).

This autoimmune nature of the disease makes CSs as the first-line therapy which exhibit their effects by decreasing the production of autoantibodies and suppressing the reticuloendothelial phagocytosis of antibody-coated platelets in ITP (2, 24). Of note that, the response rate particularly sustained

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platelet response to CSs is not excellent different than anticipated (2, 5). Nowadays, identifying risk factors has become important for tailoring individualized treatments in the majority of the diseases. Several factors such as age (17), gender (17), platelet count at diagnosis (17), abnormal platelet morphology (18) were investigated whether they affected response to corticosteroid treatment or not in ITP patients. Among them, abnormal platelet morphology was found to be associated with poor response to CS therapy. Although the data about the factors affecting the response to CS treatment is not obvious, infectious agents like H. pylori, human immunodeficiency virus (HIV), hepatitis C virus (HCV), cytomegalovirus (CMV), have been reported to augment thrombocytopenia in refractory ITP patients (3). In our study, we evaluated the association of NLR with response to CS treatment in ITP patients and we did not find an association. While 35 (74.5 %) patients responded to first line treatment, loss of response developed in 19 (54.2 %) of these patients during follow-up.

The association of elevated NLR with advanced disease and prognosis has been elucidated quite well in various malignancies (15,16). In addition, NLR has been elevated in autoimmune diseases like SLE (9, 25, 26), RA (10), Sjögren's syndrome (27), and autoimmune thyroiditis (28, 29). Also, increased NLR was shown to be an indicator of disease activity in SLE (26), RA (10, 30), and Sjögren syndrome (27). On the contrary, the data concerning the role of NLR in some other autoimmune diseases such as Behçet's disease (11, 12) and psoriasis (13, 14) is conflicting. According to our knowledge, the role of NLR in adult ITP patients has not been explored previously. On the other hand, the association of low lymphocyte and leukocyte count with disease course in pediatric ITP patients were studied in two studies (31, 32). Ahmed et al. (31) showed that low leukocyte and lymphocyte count at the time of diagnosis was a predictive parameter for persistence in pediatric ITP patients. Nevertheless, the treatment details of the patients were not noted in the study. Similarly, Deel MD et al (32) found that low lymphocyte count at 3rd month was correlated with progression to chronic ITP. Also, in that study

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majority of the patients were not treated with steroids. In the current study, when we compared ITP patients who responded to first-line therapy with non-responding patients, we did not find a significant difference in NLR values between two groups.

Accordingly, several issues can be proposed to explain the insignificant results in our study. First, we did not have adequate data concerning the infection particularly H. pylori and CMV infection history of the patients, which might have played a role in refractoriness. Secondly, subsets of lymphocytes asserted to have role in the pathogenesis of the disease constitutes only minority of the lymphocytes (33), thus any structural or quantitative abnormality in these cells would not allow alteration in lymphocyte count and also NLR. Unfortunately, we could not evaluate these factors due to the retrospective nature of the study. Lastly and the most important one according to us is that, ITP is not a systemic disease affecting the other organs leading to systemic inflammation.

Conclusion

In conclusion, although NLR was found to be associated with the prognosis and activity of various diseases in several studies, we could not verify such an association between NLR and response to corticosteroid therapy in ITP patients.

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Table 1. Patient Characteristics

Characteristic	n= 47
Gender, n, (%)	
Female	27 (57.4 %)
Male	20 (42.6 %)
Age, years, median, (range)	44 (18-74)
Bleeding, n, (%)	
Present	10 (21.3 %)
Absent	37 (78.7 %)
WBC, /10 ³ /mm ³ , median (range)	7,850 (4,710-21,090)
Hgb, /g/dl, median (range)	13,2 (8,3-17,80)
Plt, /10 ³ /mm ³ , median (range)	9,000 (0-37,000)
Neu, /10 ³ /mm ³ , median (range)	5,200 (2,210-16,900)
Lym, /10 ³ /mm ³ , median (range)	1,970 (610-5,430)
NLR, median, (range)	2,51 (1,15-11,35)
Response to corticosteroid therapy, n, (%)	
Present	35 (74.5 %)
Absent	12 (25.5 %)
Loss of response, n, (%)	
Present	19 (54.3 %)
Absent	16 (45.7 %)

WBC: white blood cell count; Hgb: hemoglobin; Plt: platelet; Lym: lymphocyte; Neu: neutrophil; NLR: neutrophil lymphocyte ratio

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Table 2. Comparison of patients with NLR < 2.5 and NLR ≥ 2.5

	NLR < 2.5 N=23	NLR ≥ 2.5 N=24	P-Value
Gender, n, (%)			
Female	13 (56.5 %)	14 (58.3 %)	1
Male	10 (43.5 %)	10 (41.7 %)	
Age, years, median, (range)	44 (18-73)	44 (26-74)	0.92
Response to corticosteroid therapy, n, (%)			
Yes	18 (78.3 %)	17 (70.8 %)	0.740
No	5 (21.7 %)	7 (29.2 %)	
Response duration to corticosteroids, median, (range), months,	8,5 (1-60)	7 (1-105)	0.869
Loss of response, n, (%)			
Yes	8 (44.4 %)	11(64.7 %)	0.315
No	10 (55.6 %)	6 (35.3 %)	

NLR: neutrophil lymphocyte ratio

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