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Original Article

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The investigation of janus kinase 2 and calreticulin mutations in patients with essential thrombocytosis

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ABSTRACT

Aims: The aims of this study were to investigate the frequency of Janus kinase 2 (JAK2) and calreticulin (CALR) mutations in patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF) and to compare the data of each group with JAK2 and CALR mutations (+) and (-).

Methods: The research group consisted of 80 patients with chronic myeloproliferative disease (CMPD) followed in Gazi University Faculty of Medicine, Department of Hematology.

Results: Of the patients included in the study, 66.2% had ET and 33.8% had PMF. JAK2 mutation (+) was detected in 60% and CALR mutation (+) was found in 22.5% of the patients. JAK2 mutation (+) was detected in 60.4% of patients with ET and 59.3% of patients with PMF. In JAK2 (-) patients, CALR was detected as (+) in 11 patients (52.4%) with ET and 7 patients (63.6%) with PMF. CALR (+) mutation rate was higher in female patients (n=15;83.3%) than males (3;16.7%)(p=0.022).

Conclusion: Studies in the literature have shown that the incidence of CALR mutations in patients with CMPD is between 28% and 80% and that the mutation is mostly seen in patients with ET. As a result of our study, it was concluded that CALR mutations (+) were similar to those in the literature and were more common in women and ET patients.

Keywords: Essential thrombocytosis, primary myelofibrosis, JAK2, calreticulin

INTRODUCTION

Chronic myeloproliferative diseases (CMPDs) were first described by William Damashek in 1951 as "abnormal increase in cell production as a result of genetic mutations in multipotent stem cells and abnormal growth of mature cell production in peripheral blood".[1,2] CMPDs are clonal diseases characterized by uncontrolled proliferation of one or more myeloerythroid cells in the bone marrow, with anomalies of hemostasis and thrombosis due to an increased number of mature and immature cells in peripheral blood and can progress to acute leukemia. There are four diseases in the CMPDs group: chronic myeloid leukemia, polycythemia vera, primary myelofibrosis (PMF) and essential thrombocythemia (ET).[3]

PMF is also known as myelofibrosis with myeloid metaplasia, angiogenic myeloid metaplasia and chronic idiopathic myelofibrosis.[4,5] PMF is a myeloproliferative neoplasm characterized by increased clonal neoplastic cells in the megakaryocytic sequence, fibrosis in the bone marrow and non-bone marrow hematopoiesis.[6] Pathogenesis includes megakaryocyte-dominant clonal proliferation, reactive bone marrow stromal changes, and extramedullary hematopoiesis. Approximately half of the patients have the Janus Kinase 2 (JAK2)V617F mutation.[7,8]

After the discovery of the (JAK2) mutation, the CMPD classification and diagnostic criteria changed, and the treatment algorithms were reshaped. In the criteria that the World Health Organization changed in 2008, presence of the JAK2 V617F mutation in the diagnosis of polycythemia vera, ET and PMF were included in the diagnostic criteria. The relationship between the JAK2 mutation and the severity of the disease has been demonstrated.[9]

The mutation in calreticulin (CALR) has been discovered in recent years. CALR is an endoplasmic reticulum protein with chaperone activity that plays a role in calcium proliferation and differentiation in cell proliferation. CALR dysfunction has been associated with various cancers. Finally, CALR mutations have been associated with JAK 2 (-) CMPD.

The primary aim of our study was to investigate the frequency of JAK2 mutation and CALR mutation in ET and PMF patients. And the secondary aim was also to investigate

the relationship between JAK2 or CALR mutation (+) and (-) patients with thromboembolic events, bleeding, acute leukemia, myelofibrosis, age, sex and laboratory findings.

METHODS

The study was carried out with the permission of the Gazi University Faculty of Medicine Clinical Researches Ethics Committe (Date: 11.01.2016, Decision No: 13). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients and Methods

The study included 80 patients with ET and PMF who were treated at Gazi University Medical Faculty Hospital Hematology Clinic. An approved consent form was obtained from all patients in writing and the study was performed in accordance with the principles of the Helsinki Declaration.
Apart from the CALR mutation analysis, all of the parameters used in the research were routinely required. JAK2 mutation analysis, age, sex, spleen and liver size, complete blood count, biochemistry, bone marrow examination, the presence of structural symptoms, additional diseases, bleeding, thrombosis, leukemic transformation, and itching were used in the parameterized study. Peripheral blood samples of the patients were taken. DNA was isolated from patient samples and the DNA obtained from the CALR mutation was examined.

Calreticulin Analysis

DNA was isolated from peripheral blood samples using the Qiagen-QIAmp DNA Blood Mini Kit(50) (Reference No. 51104) in accordance with the kit instructions.Using a NanoDrop device, the quality of the DNA samples was checked to ensure that approximately 100 ng of DNA was obtained and the Real-Time PCR mix was prepared. Qiagen CALR RGQ PCR Kit (24) was used for Real-Time PCR. For each sample, 3 controls were used and 7 mutations, 2 of which were major, were scanned. The analyses were carried out according to the kit instructions and the results were given qualitatively. s

Data Analysis

The data were analyzed with SPSS 22.0 (Statistical Package for Social Science) program. The normal distribution of the variables was evaluated with the Kolmogorov Smirnov (KS) test and the variables with normal distribution were compared with T-test. Variables that did not meet the normal distribution were compared with Kruskal Wallis H (KW-H) test. Chi-square test was used for the analysis of categorical variables. Data are shown as averages, standard deviations, standard errors of mean, minimum and maximum values in percentages. Results were compared with a 95% confidence level and p <0.05 was considered statistically significant.

RESULTS

Demographic Features

The distribution of demographic characteristics according to the diagnoses of the cases included in the study was shown in Table 1. As seen in the table, 66.2% of 80 cases were diagnosed as ET and 33.8% were diagnosed with PMF.

Table 1. Distribution of gender and age averages with essential thrombocytosis (ET) and primary JAK2 mutation distribution

Demographic Features		Number of People (n) (%) ET-PMF	Diagnosis Total and Rate		
Gender	Female	n	35	16	51
		%	66	59.3	63.8
	Male	n	18	11	29
		%	34	40.7	36.2
Total		n	53	27	80
		%	66.2	33.8	100
Age (year)	Mean±SD		55.8±15.1	63.9±11.8	58.6±14.5

JAK: Janus kinase , ET: Essential thrombocytosis, PMF: Primary myelofibrosis

The mean age at the time of diagnosis was 58.6 ± 14.5 years. There was no statistically significant difference between the sexes of the cases and ET and PMF diagnoses (p> 0.05). However, the mean age of patients with PMF was higher than patients with ET and this difference was statistically significant (p<0.05).

JAK2 mutation (+) was found in 60% (n=48) of 80 patients included in the study and JAK2 mutation (-) in 40% (n=32). JAK2 mutation (+) was detected in 60.4% (n=32) of the cases with ET. JAK2 mutation (+) was found in 59.3% (n=16) with PMF.

The mean age of the patients with JAK2 mutation (+) was 59.7 ±14 years and was higher than the mean age of the patients with JAK2 mutation (-) (56.9±15.2 years). However, no significant difference was found in gender and age for cases with and without JAK2 mutation (p=0.492 and p=0.777, respectively).

The Relationship Between JAK2 Mutation and Laboratory/ Clinical Findings

There was no statistically significant difference (p> 0.05) between platelet, white blood cell, lactate dehydrogenase (LDH)and survival time in ET patients with JAK2 mutation (+) and (-); however, ET patients with JAK2 mutation (+) had higher hemoglobin and hematocrit values compared to mutation (-) cases and this difference was statistically significant (p=0.030 and p=0.008, respectively).
In patients with PMF, no statistically significant difference was found between the laboratory values of JAK2 mutation (+) and (-) patients (p>0.05).

There was no statistically significant difference between patients with JAK2 mutation (+) and (-) in patients with ET, splenomegaly, hepatomegaly, bleeding, thrombosis, structural symptoms, treatment, itching and transformation to leukemia (p> 0.05). The presence of fibrosis in the bone marrow was higher in the JAK2 mutation (+) cases (59.4%); mutation was less observed in (-) cases (28.6%). However, this difference was not statistically significant (p=0.084). There was no statistically significant difference between the patients with JAK2 mutation (+) and (-) in patients with PMF, bone marrow fibrosis, splenomegaly, hepatomegaly, bleeding,

thrombosis, structural symptoms, treatment, itching, disease return to leukemia and additional diseases (p>0.05).

Calreticulin Mutation and Its Distribution

Of the 32 patients with JAK2 mutation (-), 18(56.2%) had a CALR mutation (+) and 14(43.8%) had a CALR mutation (-). The rate of patients with CALR mutation (+) was 22.5% of all subjects (n=80) and 17.5% of patients with CALR mutation (-). When a comparison was made according for sexes, it was observed that CALR (+) mutation rate was higher in female patients (n=15;83.3%) than males (3;16.7%) and this difference was statistically significant (p=0.022).

Laboratory and Clinical Findings in Patients With JAK2 Mutation (+), CALR Mutation (+) and Both Mutations (-)

There was no statistically significant difference between platelet, white blood, hemoglobin, LDH values and survival times of the patients with JAK2 mutation (+), CALR mutation (+) and JAK2 (-)CALR mutations (-) (p>0.05). However, there was a statistically significant difference between the groups with the hematocrit values of the CALR mutation (+) group lower than the other two groups (p=0.048).

Bone marrow fibrosis, splenomegaly, hepatomegaly, bleeding, thrombosis, structural symptoms, treatment, pruritus, transformation to leukemia, and the incidence of comorbidities was not statistically significant in patients with a JAK2 mutation (+), a CALR mutation (+) and a JAK2(-) CALR(-) mutation (p> 0.05). Frequency of CALR mutations were mentioned in Table 2.CALR mutation (+) was found in 11(52.38%) patients with JAK2 mutation (-), and CALR (-) in 10(47.61%) patients. 7 (63.63%) patients with PMF were CALR mutation (+) and 4 (36.36%) CALR (-) (Figure).

Table 2. Frequency of Calreticulin (CALR) mutation types.

Result (n,%)	In JAK2 (-) (n=32) Frequancy / Rate		Totally (n=80) Rate	CALR Mutation Positive (n=18)
CALR Mutation (-)	14	43.8	17.5	
CALR Mutation (+)	18	56.2	22.5	
Minor Mutation	4	12.5	5	22,2
Type 1 Mutation and Minor Mutation	8	25	10	44,4
Type 2 Mutation and Minor Mutation	2	6.2	2,5	11,1
Type 1 and Type 2 Mutation and Minor Mutation	1	3.1	1,3	5,6
Type 1 Mutation	2	6.2	2.5	11.1
Type 2 Mutation	1	3.1	1.3	5.6

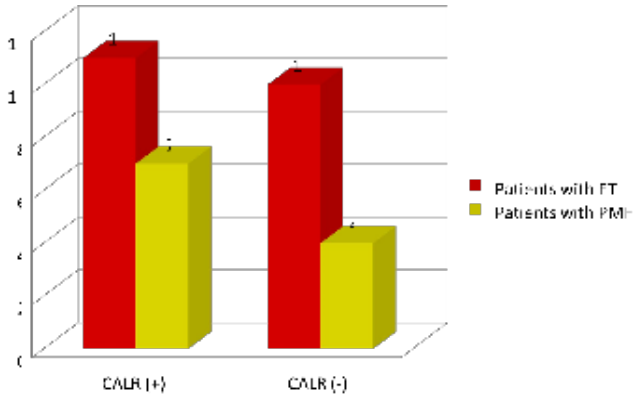

Figure. CALR numbers of ET and PMF with JAK2(-)

CALR types and frequency data of ET and PMF patients mentioned above did not show a statistically significant difference (p>0.05) (Table 3).Table 4 shows the frequency of (-) and (+) CALR mutations diagnosed with 21 ET and 11 PMF patients with a (-) JAK2 mutation.

Table 3. Frequency of Calreticulin (CALR) mutation types in ET and PMF patients with JAK2 (-).

CALR (+) Numbers and Types	ET (n=21)		PMF (n=11)		
	n	%	n	%	p
CALR 1 Major	5	23.8	6	54.5	0.123
CALR 2 Major	3	14.28	1	9.1	1
CALR Clamp 1 Minor	5	23.8	4	36.4	0.681
CALR Clamp 2 Minor	5	23.8	3	27.3	1
CALR Clamp 3 Minor	2	9.5	-	-	0.534
CALR Clamp 4 Minorss	2	9.5	1	9.1	1
CALR Clamp 5 Minor	7	33.3	1	9.1	0.209

DISCUSSION

The presence of JAK2 mutation in ET and PMF patients is an important finding for diagnosis and has led to an increase in the knowledge of the genetic basis of these diseases.[10] The rate of JAK2 mutation (+) in ET and PMF patients is different in the literature. This rate was 23.2%-79.2% in ET patients; PMF patients ranged from 37% to 78%.[9,10-16] In two studies in Turkiye, JAK2 mutation in ET patients (+) was at a rate of 40% and 47.6%.[2,16] In our study, JAK2 mutation (+) was found in 60% of patients with ET and 59.3% of patients with PMF. Our findings are consistent with most of the information in the literature. The presence of JAK2 mutation (+) in ET and PMF patients was found to be associated with elevated hemoglobin levels, high leukocyte count and low platelet count in ET patients, except for high age of diagnosis.[17] Similarly, the JAK2 mutation (+) was found to increase the tendency to itch in polycythemia vera and PMF patients.[18] In our study, it was determined that most of ET and PMF patients with JAK2 (+) and (-) did not have pruritus, and it was found that there was no statistically significant difference between the groups according to pruritus. This finding differs from the literature findings.

Studies have shown that there is a statistically significant relationship between JAK2 mutation in ET and PMF patients with high hemoglobin levels, increased leukocyte count, low platelet count at diagnosis, increased arterial/venous thrombosis risk and presence of splenomegaly.[19,20] Demir et al.[21] found higher leukocytes, hemoglobin and hematocrit values and incidence of thrombosis in ET patients with JAK2 mutation, in their research which was conducted in Turkiye. In our study, no significant difference was found in platelet, leukocyte, hemoglobin, LDH values and mean survival time of JAK2 mutation (+) and (-) patients. However, there was a significant difference between hemoglobin and hematocrit values and hemoglobin and hematocrit values of ET patients with JAK2 mutation (+), which were higher (p=0.030 and p= 0.008). This finding is consistent with the literature. In ET patients, old age (≥60 years), leukocytosis (≥11.000/mm^3) and thrombosis (arterial or venous) are risk factors for survival. Similarly, anemia and an excessively high platelet count (>1.500.000/mm^3) were found to increase the risk of

Table 4. Frequency of Calreticulin (CALR) mutation types in ET and PMF patients with JAK2

Result	Number of People / Rate	Diagnosis		Total
		ET	MF	
Mutation (-)	n	10	4	14
	% (In Group)	71.4	28.6	100
	% (In ET and PMF Patients)	47.6	36.4	43.8
	% (In JAK2 Mutation (-) ones)	31.2	12.5	43.8
Type1 Mutation, Minor Mutation	n	4	0	4
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	19.0	0	12.5
	% (In JAK2 Mutation (-) ones)	12.5	0	12.5
	n	4	4	8
	% (In Group)	50	50	100
	% (In ET and PMF Patients)	19	36.4	25
	% (In JAK2 Mutation (-) ones)	12.5	12.5	25
Type2 Mutation, Minor Mutation	n	1	1	2
	% (In Group)	50	50	100
	% (In ET and PMF Patients)	4.8	9.1	6.2
	% (In JAK2 Mutation (-) ones)	3.1	3.1	6.2
Type1 ve Type2	n	1	0	1
Type1 Mutation	n	0	2	2
	% (In Group)	0	100	100
	% (In ET and PMF Patients)	0	18.2	6.2
	% (In JAK2 Mutation (-) ones)	0	6.2	6.2
Type2 Mutation	n	1	0	1
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	4.8	0	3.1
	% (In JAK2 Mutation (-) ones)	3.1	0	3.1
Total	n	21	11	32
	% (In Group)	65.6	34.4	100
	% (In ET and PMF Patients)	100	100	100
Mutation, Minor Mutation	% (In JAK2 Mutation (-) ones)	65.6	34.4	100
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	4.8	0.0	3.1
	% (In JAK2 Mutation (-) ones)	3.1	0	3.1

leukemic transformation.[22] Passamonti et al.[23] reported that 10.6% of 605 patients died during the study period, and the median survival was 22.3 years. In our study, the majority of patients with JAK2 mutation (93.8%) had no transformation to leukemia, 2 patients (6.2%) transformed to AML; there were no significant differences in the number of JAK2 mutations (+) (95.8%), no transform to leukemia, and transform to AML in 1 patient (4.2%) and there was no statistically significant difference between the mutation (+) and (-) patients in return Ito leukemia.

In the literature, the CALR mutation (+) ratio in ET and PMF patients varies between 20% and 100%. In one study, 20% to 25% of CALR somatic mutations were detected in all exon studies with new generation sequencing in ET and PMF patients with JAK2 mutation (-), respectively.[24] In another study, the frequency of CALR and JAK2 in patients with PMF and the effects of this mutation on prognosis and clinical status were investigated and CALR mutation (+) was detected in 22.7% of 617 patients.[25] In the study of Klampfl et al.,[16] 60% of 311 patients with CMPD CALR mutation (+) were JAK2 (-) for all patients with ET (100%). The CALR mutation (+) was determined for 72 patients with polycythemia vera.[26] In the study of Nangalia et al.,[27] CALR mutation was detected in 84% of patients with ET and MF with JAK2 mutation (-). In another study conducted by Tefferi et al,[22] 407 of 1027 patients with PMF had ET and 111 (28%) of these ET patients had a CALR mutation.

In our study; CALR mutation (+) was found in 18 (22.5%) of 80 patients. The number of female patients with CALR mutation (+) was found to be higher than the number of male patients and it was found that this difference was statistically significant. The CALR mutation (+) or (-) did not differ according to age. CALR mutation (+) was found in 52.38% of patients with ET with JAK2 (-). CALR (+) was found in 63.63% of patients with PMF. There was no statistically significant difference between the groups in terms of (+) and (-) CALR frequency.

CALRdel52 (type 1 mutation) and CALRins5 (type 2 mutations) are the most common mutations in ET and PMF patients and type 1 mutation has been reported to be more frequent in PMF than in type 2.[28] In our study, it was detected that among patients with PMF; there were CALR mutation (-) in 4 patients (36.4%), Type 1 and minor mutation in 4 (36.4%), Type 2 and minor mutation in 1 (9.1%) patients Type 1 mutation in 2 patients (18.2%).

There are some limitations in our study. Our most important limitation is that it is a retrospective study. Our other limitation is the lack of generalizability of the findings due to the single center experience.

CONCLUSION

In conclusion, the findings of the CALR mutation are similar to the findings in patients with JAK2 mutation, as in the literature, and it is thought that the presence of CALR mutation in patients with JAK2 mutation (-) will support the diagnosis. This study is the first study in our country to investigate the presence of CALR mutation in ET and PMF. Therefore, we believe that this study will serve as an example for other studies in terms of using ET and PMF patients for diagnostic purposes in the investigation of JAK2 and CALR mutation.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Gazi University Faculty of Medicine Clinical Researches Ethics Committe (Date: 11.01.2016, Decision No: 13).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

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Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Causes of acquired isolated neutropenia in adulthood: a single center study

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ABSTRACT

Aims: Neutropenia, associated with several hereditary and acquired causes, is detected in a significant portion of patients presenting to the hematology outpatient clinic today. We conducted this study since we failed to find any Turkish publication on acquired isolated neutropenia in adults.

Methods: This retrospective study examined data belonging to patients aged 18 years and older who applied to the adult hematology outpatient clinic whose absolute neutrophil count (ANC) was below the normal reference values (≤ 1.5x109/L).

Results: 103 adult patients, 93 women and 10 men, were included in the study. Our study detected the cause of acquired isolated neutropenia as chronic idiopathic neutropenia (CINA) in 39.8% (n=41) of patients, autoimmune diseases in 18.4% (n=19), drugs in 14.6% (n=15), folate deficiency in 13.6% (n=14), infection in 9.7% (n=10), and hematological malignancy in 2.9% (n=3) and cyclic neutropenia in 1% (n=1). 92 patients (89.3%) were found to display mild neutropenia while 9 (8.7%) displayed moderate neutropenia and 2 (2%) displayed severe neutropenia. Interestingly, folate deficiency was detected in 6 of 9 (66.7%) moderately neutropenic patients.

Conclusion: In our study, CINA was determined as the most common cause of acquired isolated neutropenia in adults, while autoimmune neutropenia was the second most common. In addition, the detection of folate deficiency in 6 of 9 patients (66.7%) with moderate neutropenia and the fact that these patients were in the advanced age group [mean 68 (range 62-81)] was a major finding of the study.

Key words: Adult neutropenia, acquired isolated neutropenia, chronic idiopathic neutropenia, folate deficiency, autoimmune neutropenia

INTRODUCTION

Neutropenia is a condition in which the peripheral blood absolute neutrophil count (ANC) determined based on geographic region is more than two standard deviations below the normal mean.[1] It has been known for a long time that a number of hereditary and acquired diseases cause neutropenia, except for the fact that healthy individuals of African descent have lower neutrophil counts than those of European descent.[1] Neutropenia is associated with decreased neutrophil production, increased sequestration or peripheral destruction depending on the etiology. While the causes of hereditary neutropenia include Kostmann syndrome, X-linked agammaglobulinemia, Shwachman–Diamond syndrome, Chédiak–Higashi syndrome, acquired causes mainly include bacterial, fungal and viral infections, vitamin B12 and folic acid deficiency, exposure to drugs and chemical agents, bone marrow infiltration and autoimmune diseases.[2-5] Despite its wide-spectrum etiology, only a few available publications have investigated acquired neutropenia in the adult age group.[6,7] The aim of this study, designed as a single-center study from Turkiye, was to present the causes of acquired isolated neutropenia in adults living in central anatolia to the literature.

METHODS

In this study, we retrospectively evaluated the data of patients aged 18 years and older who applied to the Erciyes University Faculty of Medicine Adult Hematology Outpatient Clinic between 2015 and 2020 and whose neutrophil count was below normal reference values. The study was approved by

the Kayseri Erciyes University Ethics Committee. Kayseri Erciyes University Faculty of Medicine Ethics Committee (Date:24.06.2020, Decision No: 2020-319). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Demographic and clinical features of the patients were recorded. Furthermore, the use of legal and illegal drugs and patients' diet programs, if any, were recorded. Since the neutrophil count may be affected by ethnicity, only the data of patients from Central Anatolia were included in the study, and the data of refugees from other countries were excluded. We also excluded patients with hereditary neutropenia and a family history of neutropenia from the study. Also, individuals with previous diagnosis of splenomegaly, portal hypertension, anemia or thrombocytopenia were excluded from the study. Available existing tests were recorded using the patient files such as complete blood count (CBC), serum folate, vitamin B12 level, iron and ferritin, total biochemistry, serological exams for hepatitis B and C virus, Cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, adenovirus, parvovirus B19, antinuclear and anti-DNA antibodies and rheumatoid factor, and bone marrow biopsy. Isolated neutropenia was defined as ANC ≤ 1.5 x 109/L, not multiple cytopenias. In addition, according to the ANC count, neutropenia is classified as *mild* (ANC:1-1.5x109/L), *moderate* (ANC: 0.5-1 x 109/L), and *severe* (ANC < 0.5 x 109/L).[8] The infectious, autoimmune and hematological diseases related neutropenia were defined when diagnoses of the diseases were established by specific laboratory exams. Also, drug-induced neutropenia was diagnosed in patients under treatment with drugs associated with idiosyncratic neutropenia, in whom the neutrophil counts reached normal values when the treatment was withdrawn.[9,10] Cyclic neutropenia was characterised by regularly recurring episodes of neutropenia, as described literature.[11,12] Neutropenia lasting at least 3 months and not attributable to drugs or a specific infectious, inflammatory, autoimmune or malignant cause was termed chronic idiopathic neutropenia of adults (CINA).[13]

Statistical Analysis

Descriptive statistics were expressed using mean, standard deviation, median minimum and maximum values. The distribution of variables was measured with the Kolmogorov-Smirnov test. Data analysis was performed using SPSS 26.0 program.

RESULTS

The study population consisted of 103 persons (93 females, 10 males) (Table 1). The median age of the patients was 47 (18-81) years. The mean ANC count was 1.3 ± 0.5 x 109/L. Other blood count parameters were within the normal limits. The detected causes of neutropenia are presented in Table 2. CINA and autoimmune neutropenia were identified in 39.8 and 18.4% (n=41 and 19), respectively, of our cohort of individuals. Their mean ANC counts were 1.3±0.2 x 109/L and 1.2±0.2x109/L, respectively. The autoimmune neutropenia group included 8 patients (7.8%) diagnosed with mixed connective tissue disease, 6 patients (5.8%) with systemic lupus erythematosus, and 5 patients (4.8%) with rheumatoid arthritis. Approximately forty percent of patients presented neutropenia associated with drugs (14.6%, n=15)(mean ANC, 1.2±0.1x109/L), folate deficiency (13.6%, n=14) (mean ANC, 1.1±0.3 x 109/L), and infection (9.7%, n=10) (mean ANC, 1.3±0.7x109/L). The drug-induced neutropenia group included 7 patients (6.8%) with a history of non-steroidal anti-inflammatory drug use (3 diclofenac, 2 ibuprofen, 1 naproxen, 1 etodolac), 6 patients (5.8%) with antibiotic use (4 amoxicillin-clavulanic acid, 2 levofloxacin), and 2 patients (1.9%) with antiepileptic use (1 carbamazepine, 1 sodium valproate). The infection-related neutropenia group included 4 patients (3.9%) with Hepatitis B, 2 patients (1.9%) with Cytomegalovirus and parvo virus B19, and 1 patient (1%) with Epstein-Barr virus and Hepatitis C. The hematological malignancy group, in which 3 patients (2.9%) were diagnosed using bone marrow biopsy, included 2 patients (1.9%) diagnosed with myelodysplastic syndrome (MDS) and 1 patient (1%) with acute myeloid leukemia (AML). Another patient in the group was diagnosed with cyclic neutropenia.

Table 1. Clinical characteristics and blood counts of 103 patients with neutropenia

Paremeters	n	Reference range
Age (years)[a]	47 (18-81)	
Gender (male/female)	10/93	
Hemoglobin (g/dl)[b]	12.5 ± 1.5	12-16
WBC (x109 /L)[b]	4.2± 0.5	4.0-10.5
ANC (x109 /L)[b]	1.3 ± 0.5	2.2-4.8
Lymphocytes (x109 /L)[b]	1.8± 0.4	1.3-2.9
Monocytes (x109 /L) [b]	0.6± 0.2	0.3-0.8
Eosinophils (x109 /L)[b]	0.1± 0.1	0-0.2
Basophils (x109 /L)[b]	0.03± 0.01	0-0.1
Platelets (x109 /L)[b]	211 ± 48.1	130-400

WBC: White blood cell, n:Number, ANC: absolute neutrophil count aMedian (range), bMean ± standard deviation

Table 2. Causes of neutropenia in 103 patients enrolled in study

Neutropenia	n (%)
Chronic idiopathic neutropenia of adults	41 (39.8)
Autoimmune neutropenia	19 (18.4)
Mixed connective tissue disease	8 (7.8)
Systemic lupus erythematosus	6 (5.8)
Rheumatoid arthritis	5 (4.8)
Drug-induced neutropenia	15 (14.6)
Nonsteroidal anti-inflammatory drugs	7 (6.8)
Antibiotics	6 (5.8)
Antiepileptic	2 (1.9)
Folate deficiency	14 (13.6)
Infection-related neutropenia	10 (9.7)
Hepatitis B	4 (3.9)
Cytomegalovirus	2 (1.9)
Parvo B19	2 (1.9)
Epstein-Barr virus	1 (1)
Hepatitis C	1 (1)
Hematological malignancy	3 (2.9)
Myelodsyplastic syndrome	2 (1.9)
Acute myeloid leukemia	1 (1)
Cyclic neutropenia	1 (1)

As shown in the results, 92 (89.3%) of the patients had mild neutropenia, while 9 (8.7%) had moderate and 2 (2%) had severe neutropenia. Two patients with severe neutropenia were

in the drug-induced neutropenia group. One of these patients had a history of 3-month antiepileptic (carbamazepine) use, while the other had a history of 7-month nonsteroidal anti-inflammatory drugs (diclofenac) use. On the other hand, 6 out of 9 (66.7%) moderately neutropenic patients had folate deficiency (≤4 ng/ml), 2 (22.2%) had CINA, and 1(11.1%) had hematological malignancy (AML). The mean age of 14 patients with folate deficiency was 62 (58-74), while the mean age of 6 patients with moderate neutropenia was 68 (62-81) years.

DISCUSSION

In the literature, existing studies mostly focus on the childhood age group in which hereditary diseases, infections and CINA are reported as the major causes.[14] Acquired isolated neutropenias in adults may include a wide range of disorders with variable clinical significance. Although drugs, infections, autoimmune diseases, folate and B12 deficiency are considered as major causes associated with isolated neutropenia in adults, CINA is still considered to be the most common cause today.[11,10,15] During our literature review, we have not encountered an extensive study on the etiology of acquired isolated neutropenia in adulthood in Turkiye. CINA was the most common neutropenia seen in our study, comprising 39.8% (n=41) of the cohort of individuals. CINA is a benign granulocytic disorder characterized by an "unexplained" decrease in the ANC to below the lower limit of the normal distribution for a prolonged period of time. This condition was firstly described by Kyle and Linman in 1968,[16] and its natural history was reviewed some years later by Kyle.[17] There is no history of exposure to irradiation, chemical compounds, or drugs capable of causing neutropenia, or evidence for any underlying disease to which neutropenia might be ascribed, and a genetic predisposition or cyclic fluctuation of neutrophils cannot be demonstrated in these patients.[8] In adult women, CINA are relatively common, with a female to male ratio of about 5:1. In our study, 93 of the patients were female and 10 were male, and all patients with CINA were female. CINA was also seen as a common disorder among apparently healthy persons by Lima et al.[12] and Papadaki et al.[18]

Autoimmune neutropenia (18.4%, n=19) was detected as the second most common cause in our study. Autoimmune neutropenia, caused by neutrophil-specific autoantibodies is a common sign in autoimmune disorders such as systemic lupus erythematosus. In this situation, antineutrophil antibodies can affect neutrophil function causing qualitative abnormalities such as defective response to chemotaxis.[19] As per our results, we identified mixed connective tissue disease as the major cause of autoimmune neutropenia in 8 (7.8%) patients, systemic lupus erythematosus in 6 (5.8%) patients, and rheumatoid arthritis in 5 (4.8%) patients, which is consistent with the literature.[6,5]We found neutropenia associated with drugs 14.6% (n=15) of our patients. In the literature, the incidence of drug-induced neutropenia varies between 2.4-15.4 per million.[11] The pathogenesis of drug-induced neutropenia is a heterogeneous process which is not yet fully understood. In many cases, neutropenia occurs after prolonged drug exposure, resulting in decreased granulocyte production by hypoplastic bone marrow. In other cases repeated, intermittent exposure is implicated. This suggests an immune mediated mechanism, although this hypothesis is not entirely confirmed.[11,20] As per our results, neutropenia-inducing drugs were identified as nonsteroidal anti-inflammatory drugs (3 diclofenac, 2 ibuprofen, 1 naproxen, 1 etodolac) in 7 patients, antibiotics (4 amoxicillin-clavulanic acid, 2 levofloxacin) in 6 patients, and antiepileptics (carbamazepine, sodium valproate) in 2 patients. One of the two patients with severe neutropenia had a history of antiepileptic drug use (carbamazepine for 3 months) and the other had a history of nonsteroidal anti-inflammatory drug use (diclofenac for 7 months). In the study of Andres et al.,[21] antibiotics (49.3%), especially β-lactams and cotrimoxazole, antithyroid drugs (16.7%), neuroleptic and anti-epileptic agents (11.8%), antiviral agents (7.9%), and ticlopidine and acetylsalicylic acid (6.9%) were identified as the main drugs associated with neutropenia.

We found neutropenia associated with folate deficiency 13.6% (n=14) of our patients. Detection of moderate neutropenia in 6 of these 14 patients (42.9%) was considered a significant finding. Folate deficiency associated with nutritional deficiency may cause neutropenia. It is especially of great importance to assess the elderly population in this respect.[8] Different studies report folate deficiency between 6.4% and 9.3% in individuals over 65 years of age.[23] In our study, the mean age of 14 patients with folate deficiency was 62 (58-74), while the mean age of 6 patients with moderate neutropenia was 68 (62-81)years.

We found neutropenia associated with infections 9.7% (n=10) of our patients. As infectious agents, hepatitis B were detected in 4 (3.9%) patients, cytomegalovirus and parvovirus B19 in 2 (1.9%) patients, and Epstein-Barr virus and hepatitis C in 1 (1%) patient. Viral infections can supress bone marrow either directly or via an immune-mediated process.[4] In the study of Andersen et al.,[24] HIV (35.6%), viral hepatitis (16%), cytomegalovirus (4.8%) and Ebstein-Barr virus (10.6%) were reported at higher prevalence among neutropenic patients. Also, there are a few reports of isolated neutropenia caused by viral infections, mainly involving parvovirus B19 and cytomegalovirus.[25,26] Our results are consistent with the literature.

We found neutropenia associated with hematological malignancy 2.9% (n=3) of our patients. Two patients (1.9%) were diagnosed with MDS and one patient (1%) with AML upon bone marrow biopsy. At the onset of acute leukemia in adults, the detection of isolated neutropenia may be considered an important warning in the presence of other blood count values within the normal range.

We found cyclic neutropenia 1% (n=1) of our patients. Cyclic neutropenia has been generally diagnosed in children. Yılmaz et al.[14] investigated the causes of severe neutropenia in the pediatric age group and detected the prevalence of cyclic neutropenia as 13.3%. There is insufficient data on the prevalence of cyclic neutropenia in adults.[27]

Limitations

Our study's limitation might be the small number of patients in all groups. Large-scale multicenter studies are needed to confirm our findings especially in adult patients. Bone marrow biopsy could not be performed in the entire study group.

CONCLUSIONS

Consequently, acquired isolated neutropenia may occur due to a wide variety of causes in adults. The results of this study, which was designed as a single-center study from Türkiye, demonstrate that folate deficiency may be an important cause of neutropenia, especially in the elderly group. Folate deficiency may frequently occur due to nutritional deficiency in elderly individuals. Therefore, folate deficiency should be considered in the differential diagnosis if isolated neutropenia is detected even in the absence of anemia in laboratory tests.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Kayseri Erciyes University Faculty of Medicine Ethics Committee(Date: 24.06.2020, Decision No: 2020-319).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Follow-up of patients given isoniazid prophylaxis in multiple myeloma patients underwent autologous stem cell transplantation

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ABSTRACT

Aims: With the developments in recent years, multiple myeloma (MM) a plasma cell discrazy has become a disease whose life expectancy is expressed in 10 years. Stem cell transplantation still maintains its place in this disease and post-transplant prophylaxis is important. One of these is antituberculosis prophylaxis. We wanted to introduce this barren subject.

Methods: A retrospective screening of patients with MM who were started on isoniazid (INH) prophylaxis and underwent autologous stem cell transplantation was planned.

Results: Antituberculosis prophylaxis was given to 25 patients (6.5%) out of 380 MM transplant patients. Purified protein derivative skin test was positive in 20 of the patients who were given INH prophylaxis. Fifteen patients had pulmonary findings compatible with latent tuberculosis infection. Ten patients had both. No patients progressed to active tuberculosis. There was no difference between those who used INH/not in terms of obtaining complete remission or very good partial complete remission and other responses (p=0.220). And there was no survival difference between INH users and others.

Conclusion: Follow-up of patients receiving INH prophylaxis is also important. Histopathological findings obtained with autopsy of myeloma patients who died with pneumonia after autologous transplantation with INH prophylaxis will answer the question of whether it was tuberculosis.

Keywords: Myeloma, transplantation, INH prophylaxis

INTRODUCTION

Multiple myeloma (MM) is a malignant hematological monoclonal plasma cell disease. Autologous stem cell transplantation (ASCT) after high-dose chemotherapy still maintains its place in consolidation despite new drug options in MM patients.

In the 20 years preceding the last 5 years, the 5-year life expectancy of MM patients has increased approximately 2 times (from 27.2% to 50.2%).[1]

In myeloma patients with a higher incidence of tuberculosis than in the normal population,[2] an increased risk can be expected after ASCT.

The chest diseases department is one of the key units that is consulted for pre-transplant eligibility assessment of patients undergoing ASCT. As a result of this consultation, it is recommended that patients who have a positive image on thorax CT and/or PPD test that may be compatible with past pulmonary tuberculosis disease should undergo transplantation with anti-tuberculosis prophylaxis. The most recommended prophylactic drug is isonicotinic acid hydrazide (INH).

In this study, we aim to determine how many myeloma patients who underwent ASCT received prophylaxis and whether there were any problems in their follow-up.

METHODS

The study was carried out with the permission of Ethics Committe of Kayseri Erciyes Universtiy (Date: 22.09.2021, Decision No: 2021-622). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Gathering data from 491 Multiple Myeloma ASCTs that were received from patients who underwent hematopoietic stem cell transplantation at Kayseri Erciyes University between December 2008 and December 2019, a total of 380 patients, 240 men, and 140 women, were evaluated retrospectively. File records of how many of these patients we gave antituberculosis prophylaxis treatment were scanned and recorded.

Internationally established criteria were used to determine the risk of reactivation for tuberculosis. According to the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC), 5 mm and above are considered positive in immunocompromised individuals. Immunocompromised individuals: HIV positivity, acquired immunodeficiency syndrome, chronic renal failure on dialysis, individuals who have taken high-dose corticosteroids for a long time [Steroid doses equivalent to 15 mg and above prednisone dose per day for 2-4 weeks are considered high enough dose], those who have undergone organ or haematological transplantation and other conditions where immunosuppressive therapy is given are those with reticuloendothelial system malignancies.[3]

Statistical Analysis

The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk test, histogram, and q-q graphs. Two-sample independent t-test was used in the analysis of quantitative data. Fisher-Freeman-Halton exact and Pearson chi-square tests were used to compare categorical data. Mean, standard deviation statistics, frequency, and percentage statistics were used to summarize the data. Significant risk factors on survival were examined with Cox regression analysis, and significant variables were determined by the forward selection method (Forward LR). The hazard ratios obtained from the model were evaluated with a 95% confidence interval. Analysis of the data was performed in TURCOSA (Turcosa analytics Ltd Co, Turkiye, (www.turcosa.com.tr) statistical software. The statistical significance level was accepted as $p<0.05$.

RESULTS

It was determined that a total of 25 patients used INH prophylaxis. Of these patients, 19 were male and 6 were female ($p>0.05$).

In addition, there was no difference between those who received INH and those who did not, in terms of gender, age at the time of transplantation, comorbidity, the last pre-transplant chemotherapy regimen, transplantation conditioning melphalan dose, transplantation response, and follow-up time. As the last chemotherapy regimen before transplantation, the majority of patients in both groups used a regimen containing bortezomib drug. The rate of two or more comorbid diseases was approximately 10% percent and was similar in both groups.

Baseline International Staging System (ISS) stage and pre-transplant remission status differed between groups. Approximately 50% of those who did not use INH had the initial disease stage of ISS at stage 3, while the majority of those who received INH had stage 2 diseases ($p=0.053$, Fischer 0.039). Those who did not use INH had higher remission rates at the time of transplantation, and this difference was statistically significant ($P=0.047$)

Pretransplant baseline characteristics are shown below in Table 1. PPD was positive in 20 of the patients who were given INH prophylaxis. 15 patients had pulmonary findings. Ten patients had both PPD positivity and pulmonary findings. The acid resistance in mycobacterium tuberculosis test was suspicious in the bronchoalveolar lavage sample obtained in one patient.

Table 1. Evaluation of intergroup characteristics before transplantation

Parameters compared	INH use		p
	No	Yes	
Age	63.50±9.10	63.16±8.03	0.855
Gender			
Male	221 (62.3)	19 (76.0)	0.168
Female	134 (37.7)	6 (24.0)	
Stage			
Stage1	63 (21.6%)	6 (28,6%)	0.053
Stage2	69 (23.6%)	9 (42.9%)	Fischer 0.039
Stage3	160 (54.8%)	6 (28.6%)	
Comorbidities			
Less than 2 chronic diseases	321 (90.4%)	22 (88%)	0.702
>2 or more chronic diseases	34 (9.6%)	3 (12.0%)	
Last chemotherapy regimen pretransplant			
VAD	67(18.9%)	3 (12.0%)	0.187
BOR	233 (65.8%)	21 (84.0%)	
LEN	40 (11.3%)	0 (0.0%)	
Pretransplant-Condition			
CR or VGPR	262 (82.9%)	16 (66.7%)	0.047
Other(PR and less)	54 (17.1%)	8 (33.3%)	
Melphalan dose			
200 mg/m^2	299 (84.2%)	23 (92.0%)	0.296
140 mg/m^2	56 (15.8%)	2 (8.0%)	

INH: Isonicotinic acid hydrazide, VAD: Vincristine-doxorubicin-dexamethasone, BOR: Bortezomib, LEN: Lenalidomid, CR: Complete remission, VGPR: Very good partial complete remission, PR: Partial remission

Quadruple antituberculosis (INH+Rifampicin+etambutol+pirazinamid) treatment was started in one patient out of 25 patients, Rifampicin + INH was started in another patient, and only INH prophylaxis was started in all the remaining patients. In the follow-ups, liver function test disorder was evaluated as INH-related in 5 patients and it was interrupted. None of the patients progressed to active tuberculosis. A summary of patients at risk of tuberculosis reactivation is shown in Table 2.

Table 2. Patients at risk of tuberculosis reactivation

TST with PPD	Radiographic chest signs		
	Positive	Negative	Total
PPD positive	10	10	20
PPD negative	5	355	360
Total	15	365	380

TST: tuberculin skin test, PPD: purified protein derivative

There was no difference in terms of using INH with or without renal function test disorder. While 24% of INH users were hepatic serologic test positive, 12.1% of those who did not use INH were positive ($p=0.087$). CMV PCR positivity was 20% (5pts) in INH users and 6.2% (22pts) in non-users ($p=0.024$).

After transplantation, there was no difference between those who used INH and those who could not, in terms of obtaining CR or VGPR and rates of other responses ($p=0.220$). However,

when evaluated in detail, the CR rate is 40.8% in those who do not use INH, while it is 20% in those who do; while the rate of VGPR was 36.8% in non-users, it was 45% in users. Stable disease was found in 7.1% of those who did not use INH and 25% of those who used it (p=0.031).

Initially, despite the apparent favor towards non-users of INH in terms of life expectancy, there was no discernible disparity in overall survival between individuals who utilized INH and those who did not. (Figure).

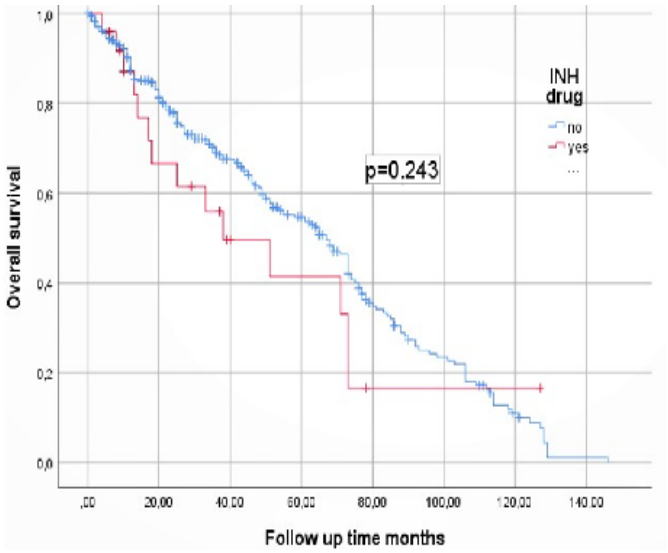

Figure . Overall Survival (months) graphic of INH users or not by Kaplan-Meier plot

DISCUSSION

New drugs for myeloma attract a lot of attention, but prophylaxis remains in the shadows. In the prophylactic and antimicrobial treatment consensus reports regarding high-dose chemotherapy and autologous stem cell transplantation, although CMV, herpes, and hepatitis b prophylaxis are included in the guidelines, antituberculosis prophylaxis is not mentioned.[4] According to a recently published review about consensus and recommendations for myeloma patient infection prevention, bacterial viral and fungal infections have taken a large place.[5] Tuberculosis is mentioned in only one sentence, perhaps because it is thought to be infrequent. However, tuberculosis disease is more common in MM patients compared to the normal population. In the study in which the tuberculosis risk in MM was compared with the healthy cohort; age 65 and over, alcohol use, and daily use of prednisone 5 mg or more were found to be factors associated with increased risk.

Eighty-three (2.1%) of 3979 MM patients were tuberculosis-positive, more than the normal population (1.5%). In addition, the mortality of these positive MM patients was found to be higher than negative myeloma patients.[2]

In our study, there was no difference in terms of age at the time of transplantation of patients who were not given INH prophylaxis. In our patients, the rate of patients with PPD-positive or pulmonary findings who were started prophylaxis was 6.5% (25/380). While the rates of patients diagnosed with tuberculosis were mentioned in the above publication, patients at risk of tuberculosis reactivation were discussed in our article. No patient progressed to active tuberculosis. Besides, there was no difference in the survival of patients who received or did not receive INH.

Gitman et al.[6] reported that they investigated the tuberculosis risk in 170 myeloma patients; 26 patients were found to be at high risk for reactivation and prophylactic treatment was started whereas 14 of them had positive tuberculin skin test. The novelty of the current article is due to a retrospective review of patients who started prophylaxis due to the risk of tuberculosis reactivation in myeloma transplant patients, and it is a survey conducted with more patients. As stated in this study and the study of Gitman et al., a substantial number of patients receive INH prophylaxis, and the use of this drug, which also has side effects and drug interactions, in haematology is a task that requires special attention.

There are conflicting publications about the effect of drugs used in the treatment of myeloma on tuberculosis. Thalidomide is found effective in drug-resistant tuberculosis, however, a case is reported after new generation immunomodulatory drug lenalidomide maintenance in MM patients.[7,8] None of the patients who were given antituberculosis prophylaxis had a previous history of lenalidomide use.

Since INH is a molecule that interacts with other drugs, the duration of use after transplantation is also an issue that should be noted. In our study, 5 patients out of 25 patients using INH had liver function test disorders, so INH was interrupted and hepatic serological tests were also performed. It was started again when the liver function test improved in the follow-ups. Although 24% of patients using INH were hepatic serology positive, these patients were not the same patients who developed liver function test abnormalities and had to interrupt INH.

Another significant finding of the present study is that there is a positive relationship between INH use and CMV positivity, which is raw data that needs to be supported by larger studies. Since it may take up to 6 months for the immune system to fully recover after autologous transplantation, it will be a period open to opportunistic infections.

CONCLUSION

Some of our autologous stem cell transplant patients with myeloma who receive INH prophylaxis die from pneumonia. Determining whether there is tuberculosis activation or not by post-mortem studies will shed light on this area. In countries where tuberculosis is endemic, tuberculosis should be kept in mind in the differential diagnosis in case of unexplained infection in hematological malignant patients with or without post-transplant fever.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ethical Committe of the Medicine Kayseri Erciyes Universtiy (Date: 22.09.2021, Decision No: 2021-622).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
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Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The role of therapeutic plasma exchange in the treatment of patients with septic shock- single center experience

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ABSTRACT

Aims: Sepsis is a life-threatening organ dysfunction resulting from an excessive inflammatory response in the host due to infection. Due to the activation of the coagulation system in sepsis, the search for treatment has shifted in this direction and therapeutic plasma exchange (TPE). This study, it was aimed to compare the hemogram and biochemical values and inflammatory markers of patients who underwent TPE before and after TPE.

Methods: The research data were obtained retrospectively from the records on the hospital system of the patients who were hospitalized in Malatya İnönü University Faculty of Medicine Intensive Care Clinic and were treated with TPE.

Results: A total of 25 patients were included. It was observed that platelet count (p=0.427), hemoglobin (p=0.545), leukocyte (p=0.527) and neutrophil (p=0.657) counts decreased statistically after TPE. It was observed that C-reactive protein (p=0.065) and procalcitonin (p=0.267) values also decreased after TPE procedure. Among the direct bilirubin (p=0.326), total bilirubin (p=0.397), AST (p=0.840) and alanine transferase (ALT) (p=0.122) values, it was determined that the aspartate transferase (AST) value increased after the TPE procedure and the others decreased. It was observed that the blood urea nitrogen (p=0.326) value increased after TPE procedure, while creatinine (p=0.677) value decreased.

Conclusion: TPE process can reduce harmful components in plasma. Since it is of vital importance to reduce harmful components in the plasma in sepsis, TPE can be applied by evaluating patient-specifically. Our study is important in terms of showing that TPE can be an alternative treatment modality in patients with sepsis and septic shock.

Keywords: Sepsis, septic shock, therapeutic plasma exchange, inflammatory markers

INTRODUCTION

Sepsis is a life-threatening organ dysfunction as a result of an excessive inflammatory response observed in the host because of infection.[1] Despite the identification of the factors leading to sepsis and the administration of specific antibiotic treatment, the death rate due to sepsis in intensive care patients is still quite high.[2,3] This increases the tendency to try new therapies and develop novel methods in the treatment of sepsis.[4]

Bacterial toxins and cytokines released as a result of sepsis may cause endothelial dysfunction. Disseminated intravascular coagulation and multiple organ failure syndrome may also develop. It is thought that reducing the level of these toxic substances in blood will affect the prognosis of sepsis positively.[1]

The quest for new treatments has shifted towards the coagulation system which is activated in sepsis, and therapeutic plasma exchange (TPE), a procedure followed for the treatment of sepsis, has been highlighted. The said procedure is based on the principle of removing the plasma and replacing it with albumin or plasma.[5]

TPE has been thought to be a treatment alternative that could be used in the treatment of patients with sepsis and septic shock, and this study aimed to compare the pre- and post-

TPE whole blood counts (WBC), biochemical values, and inflammatory markers of patients.

METHODS

The study was carried out with the permission of The study was carried out with the permission of the Malatya İnönü University Faculty of Medicine Ethics Committe (Date: 09.02.2021, Decision No: 2021-1607). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The research data were obtained retrospectively from the hospital records of the inpatients in Malatya İnönü University Faculty of Medicine Intensive Care Clinic who were treated with TPE. This is a cross-sectional study conducted between January 2021 and October 2021 by way of evaluating the data of 25 patients who were determined to meet the study inclusion criteria between January 2019 and June 2021.Patients that presented with sepsis criteria, patients with septic shock or severe sepsis, and patients admitted to intensive care were included in the study. Exclusion criteria included end-stage malignant disease, liver cirrhosis, use of medication that may alter blood coagulation, previously known coagulopathy, pregnancy, and nephrotic syndrome.Therapeutic plasma exchange procedures were performed using a Fresenius AS 204TM automatic apheresis machine and veno-venous access. Acid-citrate dextrose solution was used as an anticoagulant. In the plasmapheresis process, fresh frozen plasma was used as replacement fluid in accordance with the blood groups of the patients, and the fresh frozen plasma volume was set as 1-1.5 times the total plasma volume calculated for each patient.

All of the replacement fluid was made with FFP. No sepsis column was used for TPE. Steroid premedication may change leukocyte levels. Extra avil was applied as premedication.

Study Variables

In this study, we compared the patients' pre- and post-TPE platelet counts, C-reactive protein (CRP), hemoglobulin (HB), WBC, neutrophil, procalcitonin, PT, APTT, INR, fibrinogen, total bilirubin, direct bilirubin, blood gas lactate, BUN (blood urea nitrogen), creatinine, AST (Aspartate Aminotransferase test), and ALT (Alanine aminotransferase) values.

SPSS version 22.0 was used to perform the statistical analyses. Descriptive statistics were expressed as numbers and percentages, and the Shapiro-Wilk normality test showed that the quantitative variables did not follow a normal distribution. The Wilcoxon test was used to compare the medians of the dependent group. Mann-Whitney U test was used to compare the medians in independent groups p <0.05 was accepted as statistically significant.Before the TPE procedure, 52% of the patients had not undergone any surgical operation. Prior to the procedure, inotropes were administered to all patients, and they were given anticoagulants and fresh frozen plasma (FFP). 60% of the patients were not mechanically ventilated before TPE.

RESULTS

A total of 25 patients were included in the study. 10 (%40) of them died.Of the patients included in the study, 68% were male, 56% were hospitalized in the organ transplant intensive care unit, and it was found that sepsis was a hospital-acquired infection in 87.5% of the patients. Demographic data of the study participants are provided in Table 1. The clinical characteristics of the study participants are given in Table 2.

Table 1. Patients' sociodemographic and infection details

Variable		Number of participants (n)	Percentage (%)
Gender	Female	8	32.0
	Male	17	68.0
The intensive care clinic the patient is admitted to	Reanimation	2	8.0
	Hematology	9	36.0
	Organ transplant	14	56.0
Presence of septicshock	Yes	14	56
	No	11	44
Site of infection	Urological	1	4.0
	Unknown	1	4.0
	Skin and soft tissue	2	8.0
	Other endocarditis	2	8.0
	Abdominal	6	24.0
	Lungs	13	52.0
Sepsis source	Community-acquired	3	12.0
	Hospital-acquired	21	84.0
	Unknown	1	4.0
Pathogenic feature	Bacterial gram positive	8	32.0
	Bacterial gram negative	5	20.0
	Fungal	1	4.0
	Mix	9	36.0
	Undefined	2	8.0

Table 2. Patients' age, body mass index, APACHE2 score, TPE session quantity, post-TPE follow-up days

Variable	Median ± SD	Minimum-Maximum
Age	42,80±18,48	18-80
Body mass index	23,66±3,56	13,4-30,5
APACHE 2 score	14,48±4,64	7-23
Number of TPE sessions	3,76±1,98	1-10
Number of post-TPE follow-up days	22,68±28,95	1-104

SD: Standard deviation, APACHE: Acute physiology and chronic health evaluationscoring, TPE: Therapeutic plasma exchange

Although the findings of the study are statistically non-significant, the following were the observations after the TPE procedure: The number of platelets (p:0.427), hemoglobin (p: 0.545), WBC (p:0.527), and neutrophils (p:0.657) in the blood, which are the formed elements of blood, decreased. CRP (p:0.065) and procalcitonin (p:0.267) levels beneficial in infection follow-up also diminished after the TPE procedure. PT (p:0.333), aPTT (p:0.367), INR (p:0.319) and fibrinogen (p:0.123) levels used to examine coagulation dropped after the procedure.From among direct bilirubin (p:0.326), total bilirubin (p:0.397), AST (p:0.840), and ALT (p:0.122), the AST level elevated following the procedure while the others declined. On the other hand, BUN (p:0.326) increased after TPE whereas creatinine (p:0.677) lessened.It would be appropriate to monitor and evaluate more patients with a

prospective study plan in order to determine the statistically significant difference. Table 3 provides changes in laboratory values before and after the TPE procedure. The changes in kidney functions before and after TPE are given in Table 4. The limitations of the study are that it is retrospective and the number of patients is small

Table 3. Comparison of the median values of pre- and post-TPE platelet count, CRP, hemoglobin, WBC, neutrophil count, procalcitonin, PT, aPTT, INR (International Normalized Ratio), fibrinogen, direct bilirubin, indirect bilirubin, AST, and ALT

Variable	Mean±SD	Median	Min-Max	p
Pre-TPE CRP	12.10±9.99	10.58	0.7-0.3	0.065
Post-TPE CRP	8.68±7.29	6.89	0.3-22.8	
Pre-TPE procalcitonin	17.66±29.68	3.70	0.03-100.10	0.277
Post-TPE procalcitonin	8.17±17.22	1.47	0.47-73.76	
Pre-TPE platelet count	94.00±135.65	42.00	3-647	0.427
Post-TPE platelet count	78.92±103.71	38.00	2-368	
Pre-TPE HB	12.95±19.03	9.60	5.9-104.0	0.545
Post-TPE HB	9.07±1.82	9.20	5.5-13.5	
Pre-TPE WBC	8.94±11.53	4.82	0.02-51.7	0.527
Post-TPE WBC	6.67±8.25	4.30	0.14-37.30	
Pre-TPE neutrophil	7.16±10.54	4.03	0.00-46.60	0.657
Post-TP Eneutrophil	5.17±7.52	3.23	0.05-35.76	
Pre-TPE PT	18.27±8.01	16.5	10.2-41.4	0.333
Post-TPE PT	16.36±4.26	15.7	11.5-28.1	
Pre-TPE aPTT	34.26±23.58	27.2	20.8-142.0	0.367
Post-TPE aPTT	28.1±5.28	26.3	22.1-44.6	
Pre-TPE INR	1.55±0.72	1.41	0.8-3.7	0.319
Post-TPE INR	1.38±0.40	1.28	0.95-2.57	
Pre-TPE fibrinogen	356.79±204.86	336.00	51.6-791.0	0.123
Post-TPE fibrinogen	251.18±78.45	242.15	72.0-392.7	
Pre-TPE total bilirubin	7.56±7.08	6.40	0.44-33.74	0.397
Post-TPE total bilirubin	6.13±5.40	4.99	0.69-19.87	
Pre-TPE direct bilirubin	5.10±5.08	4.60	0.18-23.99	0.326
Post-TPE direct bilirubin	4.02±3.60	3.32	0.34-13.99	
Pre-TPE lactate	3.37±2.50	2.30	1.1-12.1	0.884
Post-TPE lactate	4.70±5.75	3.00	0.7-27.0	
Pre-TPE AST	80.32±82.40	47.0	9-315	0.840
Post-TPE AST	97.48±145.37	57.0	6-704	
Pre-TPE ALT	90.40±111.88	57.0	8-501	0.122
Post-TPE ALT	63.68±64.67	41.0	5-241	

TPE: Therapeutic plasma exchange, CRP: C-reactive protein, HB: Hemoglobulin, WBC: White blood cell, PT:Prothrombin Time,aPTT:Activated partial thromboplastin Time, INR:International normalized ratio, AST:Aspartate aminotransferase ALT: Alanine aminotransferase

Table 4. Comparison of the median values of pre- and post-TPE BUN and serum creatinine

Variable	Mean ± Standard Deviation	Median Value	Minimum-Maximum	p value
Pre-TPE BUN	36.80±26.13	29.90	8.07-119.80	0.326
Post-TPE BUN	42.68±32.77	33.33	3.2-124.0	
Pre-TPE creatinine	1.16±0.94	0.83	0.43-4.67	0.677
Post-TPE creatinine	1.23±0.93	0.84	0.45-3.65	

TPD: Therapeutic Plasma Exchange, BUN: Blood urea nitrogen

DISCUSSION

Caused by the dysregulated inflammatory response by the host, sepsis is defined as a clinical condition that progresses with the development of cytokine storm and organ dysfunctions in the host.[1] Sepsis is one of the most common causes of death in intensive care units. This illustrates the importance of sepsis treatment approaches aiming to reduce ICU mortality rates.[6] Whilesepsis and septic shock mortality rates vary across countries, the international literature shows that these rates are around 30-50%.[7,8]

It was determined that sepsis and septic shock mortality rates in Türkiye are higher than the mentioned figures. This paves the way for the review of the currently implemented treatments for sepsis and the trial of new therapies.[9,10]

The TPE procedure allows reducing the harmful components in the plasma and performing a large-volume plasma replacement without causing a volume load.[11] Since the reduction of harmful components in the plasma is of vital importance in sepsis, the TPE procedure is implemented in a patient-specific manner. TPE is known as a treatment method used to removetoxic materials from the plasma in sepsis.[12]

The research which cannot be generalized represents only its own sample. The retrospective nature of the study allowed for the evaluation of only certain variables. Prospective furtherstudies will enable a more comprehensive evaluation and follow-up.

In this study, the mortality rate following the TPE procedure was 40%. In a study performed at another center, on the other hand, the rate was 36.4%.[13] This difference may be due to the fact that in that center, TPE was performed in Category I and Category II patients in accordance with the American Society for Apheresis (ASFA) indication classification. In our study, sepsis patients in Category III according to ASFA plasmapheresis indication classification underwent the TPE procedure. It is crucial to perform a therapeutic plasmapheresis procedure by adopting a case-specific approach in category III diseases.

Although TPE is a procedure with a certain level of risk, the rate of its fatal side effects is quite low. Common side effects after TPE are anaphylactoid reactions such as urticaria and shivering; hypocalcemia symptoms such as headache, hypotension, hypovolemia, nausea, vomiting, muscle twitching, and paresthesia may also be observed.[14,15,16]

It was determined that 72% of the patients included in our study did not develop any complications after the TPE procedure. 1 person developed an allergic reaction, 1 person had decreased HB, 2 people had hypocalcemia, and 3 people developed serious reactions that could lead to life-threatening risks. In a study in another center, it was observed that the patients who underwent the TPE procedure most commonly developed catheter site-related complications, hypocalcemia, chills and shivering.[17] This difference in the frequency of complications may be due to the procedures performed.

Calculated according to the APACHE II scoring system in which body temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial pH, venous HCO3, sodium, potassium, serum creatinine, hematocrit, leukocytes, and glasgow coma score were evaluated as physiological variables, the mean score of the patients in our study was 14.48±4.64. It was determined that in the APACHE II scoring

which is a way to evaluate acute physiology, age, and chronic health, mortality was 25.0% when the total score was 25 but it rose to 80% once the total score was 35 and above.[18] A crucial weakness of the said scoring system employed in this study is the inability to evaluate hemodynamic support therapy and mechanical ventilation; these two variables can be analyzed in further studies.

Although the findings of the study are statistically non-significant, it demonstrated that platelets, hemoglobin, WBC, and neutrophils, which are the formed elements of blood, decreased following the TPE procedure. In another study, the number of platelets in patients who underwent TPE due to multiple organ failure and sepsis showed a statistically significant increase. Our study might not have provided the same results because we did not examine patients only with multiple organ failure and sepsis but evaluated patients with sepsis and septic shock altogether.[19] It was seen that there was a drop in platelet and WBC counts of patients who had TPE as part of another study; however, similar to our study, the decrease was not statistically significant.[20]

It was also detected that CRP and procalcitonin levels used in infection follow-up also diminished after the TPE procedure. A study compared procalcitonin and CRP levels in 21 patients before and after 42 TPE procedures and showed a 31.0% and 64.0% decrease in procalcitonin and CRP levels respectively.[21]

Similar to previous research, our study exposed a drop in CRP and PCT levels. A 48.0% and 28.2% decrease in procalcitonin and CRP levels respectively were found after the TPE procedure in the present study. In another research, survival following the TPE procedure in sepsis and septic shock patients was compared, and it was seen that the surviving group had higher levels of procalcitonin. This result suggests that the decrease in procalcitonin levels following the TPE procedure also has an effect on survival.[22] Nevertheless, a comparison of mortality and procalcitonin levels in our study did not provide statistically significant results while the mean of procalcitonin levels of patients who died was found to be higher than those who survived. It would be appropriate to perform further research to evaluate survival using a larger sample. PT, aPTT, INR, and fibrinogen levels used to evaluate coagulation were also observed to have dropped at a statistically non-significant rate after the TPE procedure. From among direct bilirubin, total bilirubin, AST, ALT, and blood gas lactate, the AST level was found to have elevated following the procedure while the others declined. In another study, patients'total bilirubin, AST, and ALT levels decreased statistically significantly after the TPE procedure. This difference may be due to dissimilarities in study samples.[19]It was seen that BUN increased after TPE whereas creatinine lessened. In a different study, patients' BUN and creatinine levels were found to have reduced statistically significantly after the TPE procedure.[19] Another study, on the other hand, demonstrated that BUN and creatinine levels rose but statistically significantly, unlike the resultsof our study.[20] It would be appropriate to monitor and evaluate more patients with a prospective study plan in order to determine the statistically significant difference.

CONCLUSION

The TPE procedure performed in the clinical course of the patients provided longer-term antibiotic use with improvement in survival. It is predicted to provide an advantage in agent control.

A total of 25 patients were included. It was observed that platelet count (p=0.427), hemoglobulin (p=0.545), WBC (p=0.527) and neutrophil (p=0.657) counts decreased statistically after TPE. It was observed that CRP (p=0.065) and procalcitonin (p=0.267) values also decreased after TPE procedure. Among the direct bilirubin (p=0.326), total bilirubin (p=0.397), AST (p=0.840) and ALT (p=0.122) values, it was determined that the AST value increased after the TPE procedure and the others decreased. It was observed that BUN (p=0.326) value increased after TPE procedure, while creatinine (p=0.677) value decreased.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Malatya İnönü University Faculty of Medicine Ethics Committe (Date: 09.02.2021, Decision No: 2021-1607).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

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Current status and potential future of PAI-1 inhibitors

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ABSTRACT

PAI-1 is an important factor in the fibrinolytic system. It is also involved in the etiopathogenesis of atherosclerotic processes, metabolic syndrome, obesity, and a significant number of solid and some of the hematological malignancies and even has valuable prognostic value. In this article, we review involvement of PAI-1 in areas other than the fibyrinolytic system, its roles, and its potential contribution to regulatory mechanisms and inhibition pathways. Many important studies proved that PAI-1 is significantly increased in obesity, metabolic syndrome, a significant proportion of malignancies. Although cell culture studies, in vivo studies and animal experiments have provided data on PAI-1 inhibition and models that block the pathways through which PAI-1 is metabolized and acts, suggesting that this blockade can reverse tumor progression, improve metabolic syndrome parameters and improve atherosclerotic processes, the results of these inhibitory agents in humans are still unknown and worth investigating. The upcoming phase 1-2 studies will answer these questions.

Keywords: PAI-1, PAI-1 inhibitors, malignancies, potential, metabolic syndrome

INTRODUCTION

Fibrinolysis is vital for mammalian physiology. The main component of fibrinolysis is plasmin, which breaks down fibrin. Existing other components form the main stem of the system.

Plasminogen is the pro-enzyme for plasmin and can be converted to plasmin by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). tPA is synthesized mainly by endothelial cells through multiple special stimuli and receptors. Plasminogen activator inhibitor type 1 (PAI-1), an antagonizing structure to this mechanism, acts as an inhibitor of tPA and Upa.[1,2]

PAI-1 is a prominent member of the serine protease inhibitor family (SERPIN). PAI-1 is a single-chain glycoprotein produced primarily by endothelial cells and platelets, but can also be produced by peritoneum, endometrium, megakaryocytes, mesenchymal stem cells, monocytes/macrophages, cardiomyocytes, hepatocytes, smooth muscle cells, fibroblasts and adipocytes.[3-5]

Similarly, although previous research has mainly focused on the function of PAI-1 in the coagulation and fibrinolytic system, a growing number of studies have shown that abnormal expression of PAI-1 is also associated with various pathological conditions such as blood diseases, obesity, metabolic syndrome and tumors.[6]

Since PAI-1 is recognized as a risk factor in the development of various pathological conditions, there has been an intense effort to develop inhibitors of PAI-1.

PAI-1 inhibitors have a potential therapeutic role in both for etiopathogenesis and treatment of chronic complex inhibitors.

STRUCTURE OF PAI-1

Features of the three-dimensional structure of PAI-1 have provided insight to the function and activity status of the molecule. PAI-1 is unstable in the absence of a carrier protein vitronectin.[7]

PAI-1 has a flexible reactive center loop (RCL) at its surface, which contains the substrate-mimicking peptide sequence. The structure of the PAI-1 has been the target for developing its inhibitors. Understanding the structure and function of the PAI-1 protein is crucial to develop its inhibitors.

PAI-1 blocks the actions of t-PA and u-PA. Additionally, PAI-1 interacts with cofactors of vitronectin or the glycosaminoglycan heparin to inhibit thrombin.

There have been intensive studies on the interaction of PAI-1 with these cofactors at the tissue level. Experimental and clinical studies have elucidated the significance of the binding

of PAI-1 to intact fibrin for the mechanism of t-PA-mediated fibrinolysis.[8]

DEVELOPMENT OF PAI-1 INHIBITORS

PAI-1 inhibitors consist of 5 main groups: small molecules, synthetic peptides, RNA aptamers, monoclonal antibodies (mAbs), and antibody derivatives. The working principle of these groups can be summarized as follows:

- Directly blocking the initial formation of specific complexes between PAI-1 and Plasminogen activators (PA),
- Preventing the formation of the final complex, or
- Causing/accelerating the transition of the active PAI-1 molecule into a latent or inactive form.

Although numerous well-equipped and extensive in vitro and in vivo studies, clinical phase studies for various indications have been performed, no PAI-1 inhibitor is currently approved for therapeutic use in humans. Tremendous potential exists for the future.

We need to resolve the affinity and specificity issues, which are common, especially when using small molecules.

In addition, the structural plasticity of PAI-1 and its counteraction to other potential binding partners pose a real challenge for developing PAI-1 inhibitors. Therefore, a better drug design and deeper understanding of PAI-1 inhibition at the molecular level are essential to be able to use these drugs in humans to produce real and effective inhibition.[9,10]

From the hematologic point of view, PAI-1 is a negative regulator of the fibrinolytic system in the bone marrow and is also thought to act as an inhibitor of hematopoietic regeneration. After myeloablative irradiation, PAI-1 concentration is significantly increased in hypocellular bone marrow (BM) containing abundant bone marrow adipocytes (BMA). The BM-rich microenvironment harboring obese individuals is thought to be associated with the higher PAI-1 concentrations observed in this population.

Therefore, Harata et al.[11] hypothesized that PAI-1 produced by BMAs would be associated with impaired hematopoietic regeneration observed in BMA-rich microenvironments and examined whether blocking PAI-1 activity using TM5614, a PAI inhibitor, facilitates hematopoietic regeneration after HSCT in BMA-rich recipients. At the end of this investigation, they showed that higher PAI-1 concentration inhibited hematopoietic regeneration and that blocking PAI-1 activity facilitated hematopoietic regeneration in BMA-rich microenvironments after HSCT.[11-13]

In addition to this, chronic myeloid leukemia (CML) also attracts attention as an area where PAI-1 inhibitors are intensively studied. Yahata et al.[14] showed that; inhibiting PAI-1 activity can increase hematopoietic stem cell (HSC) motility, and this process may result in HSC dissociation from their niche.

These cardinal observations brought insight information that stimulated the research community to look for answers for the following questions:

- Can we improve the PAI-1 inhibition safely?
- Which of the above is the most effective method to inhibit PAI-1?
- What is the fine border between the efficacy versus safety for PAI-1 inhibitors?
- Can PAI-1 inhibition have a potential antitumor effect?
- Will PAI-1 inhibition result in CML-defective cells leaving their niches and being exposed to tyrosine kinase inhibitors more intensely and incrementally?
- Will PAI-1 inhibition result in systemic side effects?

To answer all these questions, Tohoku University group and Sasaki et al.[15] performed an in vitro experiment in which they aimed to evaluate the effect of TM5614, a PAI-1 inhibitor, on CML cells. PAI-1 inhibition has a direct and profound anti-tumor activity on CML cells. These seminal observations led to the landmark phase and phase 2 trials.

Another area of intensive research has been the use of PAI-1 inhibitors in solid tumors. Studies on experimental models report that PAI-1 plays an essential role in tumor growth, invasion, metastasis, and angiogenesis.[16,17]

PAI-1 generally plays a tumor-promoting role in cancer development and tumorigenesis. PAI-1 is a biomarker of poor prognosis. Medical community is expecting to see a future role for PAI-1 inhibitors in solid tumors based on these significant associations. Potential types of cancer for therapeutic role of PAI-1 inhibitors, include breast cancers, gastric, head and neck cancers and ovarian cancers.[18-21]

PAI-1 level serves as a biomarker target or follow-up parameter in cancer treatment. The PAI-1 inhibitors that have been evaluated previously in preclinical trials are PAI-039, SK-216, SK-116, TM5441, TM5275 and TM5614. These studies have yielded promising results and are still a matter of curiosity.[22-26]

Other types of PAI-1 inhibitors have been intensely studied. Fortenberry et al.[27] aimed to inhibit intracellular PAI-1 using RNA aptamers in their study by transfecting human breast cells. Aptamer-expressing cells exhibited decreased cell migration and invasion. Furthermore, intracellular PAI-1 and urokinase plasminogen activator (uPA) protein levels decreased, while PAI-1/uPA complex increased.

Another PAI-1 inhibitor that attracts attention with its antiangiogenic properties is SK-216. Its significant feature is that it contains an antitumor effect regardless of PAI-1 levels. In the study conducted by Masuda et al.[28] with melanoma and lung carcinoma cells; they showed that SK-216 reduced the size of subcutaneous tumors and the extent of metastases regardless of PAI-1 secretion levels from the tumor cells.

In the study conducted by Tzekaki et al.[29] on breast cancer cells using oleuropein, a natural PAI-1 inhibitor, they obtained a promising response in Er-/PR- tumors.

PAI-1 IN METABOLIC AND CARDIOVASCULAR DISEASES

PAI-1 has long been a therapeutic target in metabolic and cardiovascular diseases. High plasma concentrations of PAI-1 are well known to be associated with the development

of coronary artery disease and other vascular thrombotic diseases.[30-33] The potential effects of agents with demonstrated cardiovascular benefits on PAI-1 concentrations, particularly in type 2 diabetic patients with cardiovascular disease or increased cardiovascular risk factors, are of interest.

Sakurai et al.[34] investigated the treatment-related change in plasma PAI-1 from baseline to week 12 in type-2 DM patients treated with empagliflozin and they showed that empagliflozin decreased PAI-1 concentration.

Studies on obesity and fibrinolytic processes have helped us to understand PAI-1 better. McGill et al.[35] showed that obese diabetic subjects had a threefold increase in PAI-1 concentrations, but no significant difference in (t-PA) plasma concentrations compared to healthy lean subjects and attributed this significant increase in PAI-1 to an increase in immunoreactive insulin and C-peptide concentrations, indicating a stimulatory effect of insulin. This and similar studies in this field suggest that enlarged adipose tissue is closely associated with impaired fibrinolytic processes.[35,36]

Pharmacological agents such as thiazolidinediones, metformin and AT1-receptor antagonists have been shown to reduce adipose expression of PAI-1.[37-40] PAI-1 can explain the cardiovascular benefit of these therapeutic agents.

In addition, weight loss through dietary restriction or lifestyle modification has been shown to be effective in reducing PAI-1 plasma levels.[41] These studies suggest that increased PAI-1 expression in adipose tissue may be a cause of impaired fibrinolysis in obesity.

CONCLUSION

As a result despite the fact that PAI-1 is an important factor in the fibrinolytic system, it is also involved in the etiopathogenesis of atherosclerotic processes, metabolic syndrome, obesity, and a significant number of solid cancers, and even has valuable prognostic value. PAI-1 is likely in the crossroad of metabolic diseases and cancer. In this article, we review PAI-1 inhibitors. We summarize the role of PAI-1 in areas other than the fibrinolytic system, and its potential contribution to regulatory mechanisms and inhibition pathways. Most studies confirm that PAI-1 is significantly increased in obesity, metabolic syndrome, a significant proportion of solid cancers and some hematologic malignancies. Cell culture studies, in vivo studies and animal experiments have provided data on PAI-1 inhibition with novel agents. Studies are performed on several models that block the pathways through which PAI-1 operates. PAI-1 blockade can halt/reverse tumor progression, improve metabolic syndrome parameters and improve atherosclerotic processes.

Currently, thrombolytic agents represent the only direct way of augmenting fibrinolytic activity in humans. The results of clinical trials with novel PAI-1 inhibitors in the upcoming phase 1-2-3 studies will answer these questions.

Cancer and metabolic diseases are age related conditions. Age-related diseases are associated with inhibition of the fibrinolytic system. In the aging populations, there is tremendous potential for PAI-1 inhibitors in cardiovascular disease, arterial and venous thrombosis, aging, amyloidosis, obesity, and type 2 diabetes mellitus.

ETHICAL DECLARATIONS

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Case Report

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A case report of relapsed/refractory primary central nervous system lymphoma

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ABSTRACT

Primary central nervous system lymphoma is a rare and aggressive subtype of non-Hodgkin lymphoma. The disease presents with neurologic findings at the time of diagnosis and responds rapidly to first-line chemo-radiotherapy but frequent and early relapses are observed. Because of the blood-brain barrier, many drugs cannot pass into the central nervous system, limiting effective treatment options. We will try to discuss a relapsed-refractory primary central nervous system lymphoma that exhausted many treatment options

Keywords: Primary central nervous system lymphoma, diffuse large B cell lymphoma, medical and radiation therapy

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is a rare aggressive non-Hodgkin's lymphoma outside the lymph nodes. Currently, high-dose chemotherapy based on methotrexate (MTX) is the standard induction treatment for newly diagnosed PCNSL but effective treatment of relapsed/refractory and elderly PCNSL is still unclear. With the advancement of clinical trials, new drugs and combination therapies such as rituximab and ibrutinib are constantly emerging, increasing the remission rate of resistant and relapsed patients.[1] In this case report, we will try to present a case of relapsed/refractory primary central nervous system lymphoma.

CASE

A 42-year-old male patient, was diagnosed with PCNSL in 2019. (the patient's mass was in the right frontal, radiologic image was compatible with central nervous system (CNS) lymphoma but biopsy was performed and the diagnosis was confirmed because the mass was also suitable for definitive diagnosis). The patient went into remission with high dose and cytarabine(2,000 mg/m^2 every 12 hours total of 4 doses) + radiotherapy (RT) (44 Gy) and started to be followed up. In 2020, the patient whose disease relapsed was given a high-dose methotreaxte-based regimen received an autologous hematopoietic stem cell yerine hematopoietic stem cell transplant. The patient in remission was followed up without treatment until 2022. In November 2022, off-label ibrutinib was approved and ibrutinib was started for the patient who relapsed again. The patient initially responded to ibrutinib and progression in the lesion was detected on magnetic resonance imaging (MRI) after an increase in central nervous system complaints in the 3rd month of treatment. Since the patient had previously responded to a high-dose MTX regimen and more than 2 years had passed since then, temozolamide+high-dose MTX+rituximab regimen was started in January 2023. After the first course of treatment, the patient's right frontal mass significantly decreased in size and symptoms significantly decreased and the same regimen was continued. While the patient was receiving the 4th course, central nervous system complaints developed again and MRI revealed new lesion in the left parietal region and near the hypothalamus and lesion in the old frontal region. The patient who was bradycardic due to edema + mass was first given anti-edema treatment with dexamethasone+mannitol. The patient with partial reduction in complaints was investigated for ongoing clinical trial. He was wanted to be included in the epcoritamab study but was not eligible for inclusion due to age and neurologic side effects. The dose of RT the patient had received previously was calculated. The necrosis dose was not reached. The patient, who had previously benefited from cytarabine treatment and more than 2 years after the end of treatment, was given cytarabine and RT again. Necrosis dose was not reached in RT. The patient benefited significantly

with the first course of cytarabine+RT. After the 3rd course of cytarabine, the patient's complaints increased again. After MRI revealed an enlarged mass, rituximab+lenalidomide was started with off-label consent. In the 2nd cycle, the patient's symptoms increased and his mass progressed and he died before completing the 2nd cycle.

DISCUSSION

PCNSL is a rare subtype of aggressive non-Hodgkin's lymphoma located in the brain, leptomeninges, spinal cord, cerebrospinal fluid or vitreoretinal compartment without overt systemic disease.[2] It accounts for less than 3% of all cases of non-Hodgkin's lymphoma and almost 3% of all primary central nervous system tumors.[2] Symptoms may start as focal neurologic deficits, personality changes, nausea and vomiting due to increased intracranial pressure. The patient should be rapidly evaluated and then CNS imaging is required.[3] Contrast-enhanced brain MRI is the best imaging option to evaluate patients with PCNSL.[4] Our case; a 42-year-old male patient, was admitted to the neurology outpatient clinic in 2019 with neurological findings at the time of the first diagnosis and it was observed that there was a 5 cm mass in the right parietal lobe on the contrast-enhanced cranial MRI and he was referred to the neurosurgery department and the diagnosis was made by biopsy. Age and performance status (PS) are important prognostic factors but some other details such as comorbidity, organ function, frailty and risk of neurotoxicity should also be taken into account when choosing treatment.[3] Modern treatment of PCNSL includes two phases: induction and consolidation. High dose MTX-based regimens and autologous hematopoietic stem cell transplantation in appropriate patients form the basis of treatment.[4] Whole brain RT is an alternative to autologous hematopoietic stem cell transplantation in case of failure of hematopoietic stem cell harvesting or complications during induction.[3] In our case, cytarabine followed by high-dose MTX-based regimens with induction chemotherapy and RT were administered and autologous hematopoietic stem cell transplantation was performed. Following indication and consolidation treatments, the disease relapsed or developed resistance to treatment in approximately half of the patients.[5] In addition, the presence of the blood-brain barrier prevents polypharmacy in PCNSL.[2] A standard of care for patients with relapsing or refractory PCNSL has not yet been established. Clinical trials should be the preferred treatment option as this is the best strategy to identify new active drugs and strategies that can then be investigated as part of first-line treatment.[4] In the case of late relapse (>24 months) and prior response to high dose-MTX-based regimens, reintroduction of high dose-MTX is a safe and effective strategy.[3] Ibrutinib, a Bruton tyrosine kinase (BTK) inhibitor, can cross the blood-brain barrier. Rapid and impressive responses have been achieved in CNS lymphoma patients when given as a single agent or in combination.[3,6] Immunomodulatory drugs (lenalidomide and pomalidomide) have been investigated in patients with recurrent or refractory PCNSL, achieving an objective response in half of patients but the response was usually short-lived.[7] Our patient, who went into remission and relapsed approximately 26 months later. MRI revealed involvement in the right fronto-tempo-occipital and left occipital regions consistent with PCNSL (Figure). It was first started on ibrutinib and a response was obtained but when the disease progressed, first a high-dose MTX-based regimen + RT was started, then high-dose cytarabine was given when the disease progressed. When no suitable clinical trial was found, the patient was started on rituximab+lenalidomide. Anti-CD19 chimeric antigen receptor (CAR)-T cells have recently been approved for the treatment of relapsed/refractory diffuse large B cell lymphoma(DLBCL) and have shown promising results in other B-cell lymphomas. This approach deserves to be evaluated in patients with relapsed or refractory PCNSL, given the encouraging preliminary results reported in patients with secondary CNS DLBCL.[8] In our case, the CAR-T treatment option could not be reached and the patient who did not respond to rituximab-Lenalidomide treatment died.

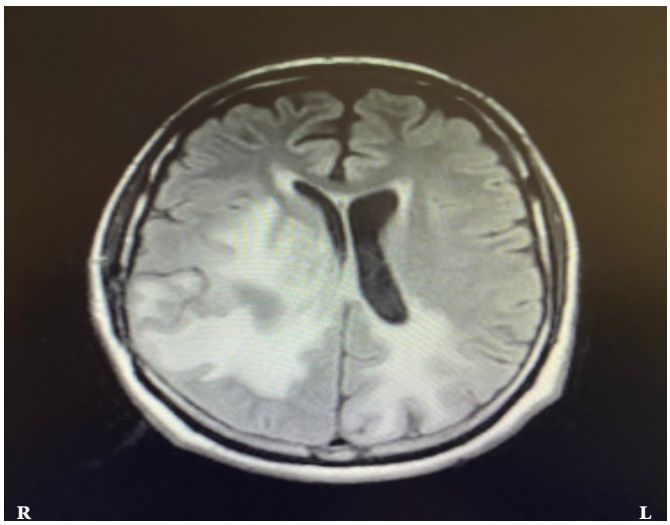

Figure. Right fronto-tempo-occipital involvement on MRI, left occipital appearance compatible with PCNSL
MRI: Magnetic resonance imaging,
PCNSL: Primary central nervous system lymphoma

CONCLUSION

In the treatment of PCNSL, the disease responds to treatment, but relapses occur frequently. The need for new treatment options for patients who have exhausted current treatment options and are progressing/refractory remains current.

ETHICAL DECLARATIONS

Informed Consent

The patient signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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