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Determination of etiology in patients admitted due to isolated leukopenia

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Abstract

Patients with isolated leukopenia pose difficulties in diagnosis because there is no related guideline in the literature. In this study, our aim was to evaluate the clinical and laboratory associations of isolated, nonspecific (not related to neutropenia) leukopenia.

In this retrospective data review study, patients who were admitted to Hacettepe University Hematology Outpatient Clinic between 2014 and 2019 due to leukopenia were evaluated. The patients with anemia (other than iron deficiency) or thrombocytopenia were excluded. Clinical and laboratory data and the final diagnoses (if present) of the remaining cases and especially of those without neutropenia (the most difficult group to diagnose) were evaluated.

One hundred sixty-nine patients were included in the study. One hundred forty-four (85.2%) patients were female and 25 (14.8%) were male. One hundred ten of them had 1500/μL or higher neutrophil count. In these nonneutropenic cases, the etiological factors contributing to leukopenia were as follows: iron deficiency anemia (21.8%), other autoimmune/autoinflammatory diseases (17.3%), autoimmune thyroid disease (21.8%), autoimmune laboratory tests (2.7%), drugs (12.7%), infection (5.5%), hematopoietic disorder (2.7%), hypersplenism (2.7%), radiotherapy sequel (1.8%), and B_{12} deficiency (1.8%). No etiology was recognized in 44 patients. On the other hand, the etiological factors in patients with neutrophil count <1500/μL were as follows; iron deficiency anemia (10.2%), other autoimmune/autoinflammatory diseases (17%), autoimmune thyroid disease (5.1%), autoimmune laboratory tests (8.5%), drugs (8.5%), infection (6.8%), hematopoietic disorder (11.9%), hypersplenism (1.7%), radiotherapy sequel (1.7%), and B_{12} deficiency (1.7%). No etiology was recognized in 25 patients. Physicians ordered bone marrow examination more frequently in patients with neutropenia. If isolated antinuclear antibody positivity was also considered in favor of autoimmunity, 91/169 (53.8%) cases had an autoimmune diagnosis or laboratory finding.

In the present study, the most frequent reasons of isolated leukopenia in nonneutropenic patients are found as iron deficiency anemia, other autoimmune/autoinflammatory diseases, and autoimmune thyroid disease. In neutropenic patients, the most frequent reasons of isolated leukopenia are found as iron deficiency anemia, autoimmune/autoinflammatory diseases, and hematopoietic disorders. Therefore, autoimmunity is detected as an important factor leading to isolated leukopenia.

Keywords: autoimmunity, leukopenia

1. Introduction

Leukopenia can be defined as a condition in which the amount of white blood cells in the blood is abnormally reduced (normal white blood cells count is between 4000 and 11,000/μL).[1] Leukopenia can be caused by decreased production of white blood cells or increased use and destruction, or both. Infections, drugs, malignancies, autoimmunity, thyroid diseases, hypersplenism, nutritional factor deficiencies, and immune leukopenia are the causes responsible for leukopenia in many cases. Primary leukopenia is rare and sometimes, especially in children, it may be due to hereditary or developmental defects.[2] Leukopenia may appear as generalized leukopenia or neutropenia or lymphopenia. The pathophysiology of neutropenia includes 4 main mechanisms.[1] First, suppression of myeloid stem cells (aplastic anemia) and various infiltrative bone marrow disorders (tumors and granulomatous disease), suppression of granulocytic precursors (after exposure to certain drugs), diseases characterized by ineffective granulopoiesis (megaloblastic anemias and myelodysplastic syndromes) and some inherited conditions characterized by defects in granulocyte differentiation caused by genetic defects in specific genes may lead to the insufficient or ineffective production of neutrophils. Second, immune-mediated damage to neutrophils because of idiopathic, immunological disorders (systemic lupus erythematosus [SLE]), and drugs (alkylating agents and antimetabolites) may result in accelerated neutrophil destruction or damage. Splenic sequestration and bacterial, fungal, or rickettsial infections (increased peripheral use) are the other main mechanisms behind neutropenia.[1] The history of drug usage, lymphadenopathy, splenomegaly, ecchymoses, platelet

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counts, absolute neutrophil and lymphocyte counts, and other laboratory tests are important in making diagnosis. The blood smear provides important information about red blood cell and white blood cell morphology. In patients presenting with cytopenia, a comprehensive medical history is important to detect the underlying disease. The main purpose of the physical examination is to detect clinical findings that cannot be detected by the medical history. Tests including hemogram, basic metabolic and hepatic function tests, haptoglobulin, lactate dehydrogenase, iron parameters, etc. Peripheral smear evaluation can further narrow the differential diagnosis and decide whether a bone marrow biopsy is needed.[1]

There are difficulties in diagnosis and follow-up in patients with isolated, unrelated leukopenia who apply to hematology. The causes and frequency of leukopenia in these patients are not known exactly. There are no published or agreed guidelines for patient management in patients presenting with isolated nonspecific leukopenia. Our aim in this study is to reveal the clinical associations of isolated leukopenia and to create an algorithm that will be useful in patient management.

2. Methods

In this retrospective study, patients over the age of 18 who applied to Hacettepe University Faculty of Medicine Hematology Clinic between years 2011 and 2020 due to leukopenia were evaluated. Patients who had concomitant (non-iron deficiency) anemia or thrombocytopenia (platelet <140,000/μL) were excluded. White blood cell count <4000/μL was considered as leukopenia.[1] All leukopenic patients were divided into 2 groups as neutropenic and nonneutropenic. Neutrophil count <1500/μL was considered as neutropenia.[1]

Autoimmune thyroid diseases were evaluated separately from other autoimmune diseases. Patients with nonautoimmune thyroid disease were also excluded from this group. Patients with positive autoantibodies without any diagnosed autoimmune disease were also classified as isolated autoimmune laboratory positivity group. Those with isolated antinuclear antibody, isolated rheumatoid factor, or isolated antithyroid peroxidase/antithyroglobulin positivity were also evaluated.

2.1. Study protocol

Demographic characteristics such as age, gender, comorbid diseases (hypertension, type 2 diabetes mellitus, coronary artery disease, SLE, rheumatoid arthritis, Sjögren disease, familial Mediterranean fever, other rheumatic disease, thyroid disease, autoimmunity, iron deficiency anemia, chronic viral hepatitis, malignancy, celiac disease, gastric bypass surgery, anorexia nervosa, pernicious anemia), hemoglobin, leukocyte, lymphocyte, neutrophil, monocyte and platelet values in all patients, thyroid-stimulating hormone, thyroid autoantibodies (antithyroglobulin, antithyroid peroxidase), antinuclear antibody, rheumatoid factor, coombs, brucella antigen, hepatitis B surface antigen antibody, hepatitis B surface antigen, total hepatitis B core antibody, hepatitis B virus viral load, hepatitis C virus antibody, human immunodeficiency virus antibody, vitamin B_{12}, folic acid, iron in terms of iron deficiency anemia, iron-binding capacity, transferrin saturation, ferritin, Epstein–Barr virus immunoglobulin M, cytomegalovirus immunoglobulin M, parvovirus, bone marrow biopsy evaluation, T-clonality, examination, or imaging results for the presence of organomegaly were evaluated.

The study was approved by the local ethics committee of Hacettepe University Medical Faculty and was conducted in accordance with the Declaration of Helsinki (approval number: GO 19/1115).

2.2. Data collection tools and procedures

This study was performed in a retrospective manner. Demographic data of the patients and all other parameters were obtained from the hospital database. All of the ethical considerations were strictly handled in accordance with the Helsinki Declaration. As a standard of care/action of the hospitals of Hacettepe Medical School, it was confirmed based on patient records that all of the study patients gave informed consent at the time of hospitalization and before the administration of diagnostic/therapeutic standards of care.

2.3. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM, Armonk, NY) version 25. In descriptive statistics, numbers and percentages (%) are specified for categorical variables. The mean ± standard deviation is given for normally distributed numerical variables, and the median (minimum to maximum) is given for numerical or ordinal variables that do not show normal distribution. Comparisons in neutropenic and nonneutropenic patients, and differences in etiology between male and female genders were compared with the chi-square test. Statistical significance level was accepted as $P < .05$.

3. Results

A total of 169 patients were included in the study. When the demographic information of patients with isolated leukopenia was analyzed, 144 (85.2%) patients were female and 25 (14.8%) were male. The median age of the patients at the time of diagnosis was 49 (17–82) years (Table 1). Demographic characteristics of all patients are summarized in Table 1. Among the comorbidities of a patient included in the study, there was type 3 glycogen storage disease. In the etiology of this patient, bone marrow involvement of the depot disease was not clear; unfortunately, bone marrow evaluation of this patient was not available.

When the drug use history of the patients was examined, it was found that 104 (61.5%) patients were using at least a drug. Sixty-five (38.5%) patients were not using medication. The most commonly used drug group was antihypertensives (n: 38, 22.2%). Other frequent drugs are L-thyroxine (n: 27, 15.8%), nonsteroidal anti-inflammatory drugs and similars (n: 31, 18.1%), antidepressant/antipsychotic agents (n: 22, 12.9%), antiaggregants (n: 22, 12%), hydroxychloroquine (n: 14, 8.2%), other disease-modifying antirheumatic drugs (other than hydroxychloroquine; such as methotrexate, sulfasalazine, leflunomide) (n: 20 11.7%), colchicine (n: 8, 4.7%), and proton pump inhibitor (n: 12, 7%). Leukopenia was associated with medication in 5 (2.9%) patients. The median values of the patients' parameters are given in Table 2.

Since iron deficiency anemia was not an exclusion criterion, the minimum hemoglobin level in the woman was 10.2 g/dL. Fifty-nine (34.9%) patients among all isolated leukopenia patients, 34% (n: 49) of women, and 40% (n: 10) of men were neutropenic (<1500/μL). It was observed that the maximum follow-up period of the patients included in the study reached 19.19 years. The median follow-up was 4.4 years. The patients included in the study had a median of 16 (1–124) complete blood count values during the follow-up period. In the follow-up of these patients, a median of 7 (1–61) leukopenic values was found. Among them, there were patients who did not continue their follow-up and whose hemogram was measured only once. The number of patients with >1 (≥2) hemogram measurement was 149. Of these patients, 134 (90%) had recurrent (≥2) leukopenia. A single leukopenia was detected in 15 (10%) of the patients with >1 hemogram value.

3.1. Autoimmune disease and laboratory findings

Autoimmunity was found in 91 (53.8%) patients when patients with isolated autoimmune laboratory tests (positive

Table 1

Demographic characteristics of all patients.

	Total, n = 169 (100%)	Nonneutropenic patients, n = 110 (65%)	Neutropenic patients, n = 59 (35%)	*P*value
Gender (female/male)	144/25 (85.2%/14.8%)	95/15 (86.4%/13.6%)	49/10 (83.1%/16.9%)	.35
Age (median, minimum to maximum)	49 (17–82)	51.8 (18.3–82)	42.2 (17–81.9%)	
Comorbidities				
1. Autoimmune/autoinflammatory disease (non-thyroid)	36 (21.3%)	21 (19.1%)	15 (25.4%)	.43
Sjogren disease	4 (2.4%)	3 (2.7%)	1 (1.7%)	.56
Systemic lupus erythematosus	5 (3%)	2 (1.8%)	3 (5.1%)	.23
Rheumatoid arthritis	9 (5.3%)	4 (3.6%)	5 (8.5%)	.16
Other connective tissue diseases (ankylosing spondylitis, Behçet disease, gout, etc)	9 (5.3%)	6 (5.5%)	3 (5.1%)	.75
Familial Mediterranean fever	4 (2.4%)	2 (1.8%)	2 (3.4%)	.43
Other*	11 (6.5%)	7 (6.4%)	4 (6.7%)	.71
2. Autoimmune thyroid disease	28 (16.6%)	24 (21.8%)	4 (6.8%)	.008
3. Other thyroid disease (thyroid nodule or thyroid cancer)	19 (11.2%)	15 (13.6%)	4 (6.8%)	.13
Other comorbidities				
Type 2 diabetes mellitus	15 (8.9%)	13 (11.8%)	2 (3.4%)	.65
Hypertension	42 (24.9%)	32 (29.1%)	10 (16.9%)	.59
Coronary artery disease	12 (7.1%)	8 (7.3%)	4 (6.8%)	.52
Iron deficiency†	56 (33.1%)	37 (36.3%)	19 (32.8%)	.22
Solid organ malignancy (renal cell carcinoma, breast cancer, thyroid cancer, etc)	12 (7.1%)	9 (8.2%)	3 (5.1%)	.30
Nutrition disorder (history of gastric bypass surgery, anorexia, pernicious anemia, celiac disease, ulcerative colitis, etc)	6 (3.6%)	4 (3.6%)	2 (3.3%)	.81
Vitamin B_{12} deficiency†	6 (3.8%)	4 (4%)	2 (3.5%)	.25
Folate deficiency†	1 (0.8%)	1 (1.2%)	0	.27
Hematopoietic disorders (polycythemia vera and mycosis fungoides)	2 (1.2%)	2 (1.8%)	0	.12
Chronic viral hepatitis (hepatitis B virus/hepatitis C virus)	7 (4.1%)	5 (4.5%)	2 (3.4%)	.47
Other‡	72 (42.6%)	46 (41.8%)	26 (43.3%)	.27

* Celiac disease, multiple sclerosis, pemphigoid, Guillain–Barre syndrome, vitiligo, lichen planopilaris, lichen planus, myasthenia gravis, autoimmune hemolytic anemia, and pernicious anemia.

† Ratios were calculated among those whose laboratory parameters were examined.

‡ Other comorbid diseases that we could not group (because it is not common).

Table 2

The median value of patients' parameters.

Parameters	Median value
Leukocyte	3400/μL
Neutrophil	1700/μL
Lymphocyte	1300/μL
Monocytes	300/μL
Platelet	208 × 10^3/μL
Hemoglobin in women	12.9 g/dL
Hemoglobin in men	14.4 g/dL

antinuclear antibody, rheumatoid factor, or thyroid autoantibodies) were included, although autoimmune thyroiditis and follow-up physicians did not hold them responsible for leukopenia, as well as those with rheumatologic or other autoimmune diseases. At least 1 autoimmunity presence was detected in 54 (49.1%) of 110 nonneutropenic patients and 37 (61.7%) of neutropenic patients. Patients with at least 1 autoimmune disease and/or laboratory finding were separated by gender (Table 3). It was observed that the rate of autoimmunity detection in women was significantly higher than in men (P = .002).

The classification of autoimmunity status and its distribution in neutropenic and nonneutropenic groups are summarized in Table 4.

3.2. Bone marrow investigation

Bone marrow examination was performed in 30 (17.8%) patients included in the study. The biopsy results of 21 (70%) patients who underwent bone marrow biopsy were normal. Bone marrow examination was performed in 25% (n: 15) of patients with neutrophils <1500/μL and in 41.2% (n: 7) of patients with neutrophils <1000/μL. Abnormality was detected in 7 (46.7%) of 15 patients with neutropenia (<1500/μL) who underwent bone marrow examination. Plasmaproliferative disease was found in 1 patient, myelodysplastic syndrome in 1 patient, and hypocellularity was found in 5 (16.7%) patients as the result of biopsy. Cellularity rates were 30% in 2 patients, 20% to 30% in 2 patients, and <10% in 1 patient. These patients were evaluated as mild aplastic anemia considering their clinical and laboratory values.

Although they were not neutropenic (neutrophil ≥1500/μL), abnormality was detected in 2 (13.3%) of 15 patients who underwent bone marrow examination. Grade 2/3 fibrosis was detected in 1 patient (3.3%), and low-grade B-cell lymphoma was detected in 1 patient (neutrophils: 1700 and 1900/μL). The neutrophil count of the 9 patients who underwent bone marrow biopsy was ≥1800/μL and 1 of them was a patient diagnosed with low-grade B-cell lymphoma. In this patient, lymphadenopathies were also present in radiological examinations. Bone marrow biopsy results of the other 8 patients were normal.

3.3. Other diagnoses

When the results of clinical and laboratory evaluations performed in 169 cases were reviewed, especially in nonneutropenic cases causing diagnostic difficulties, it was thought that >1 cause was responsible for leukopenia in some patients. No responsible disease could be identified in 72 (42.6%) patients. Isolated antinuclear antibody positivity (28 patients, 16.6%) was also evaluated in favor of autoimmunity, and 91 (53.8%) of 169 patients had autoimmunity. More than 1 cause

Table 3

Autoimmunity according to the gender.

Gender		Autoimmunity (n, %) Yes	No	*P*value
All patients	Female, n = 144	85 (59%)	59 (41%)	.002
	Male, n = 25	6 (24%)	19 (76%)	
Nonneutropenic patients	Female, n = 95	51 (53.7%)	44 (46.3%)	.032
	Male, n = 15	3 (20%)	12 (80%)	
Neutropenic patients	Female, n = 49	34 (69.4%)	15 (30.6%)	.03
	Male, n = 10	3 (30%)	7 (70%)	

Table 4

Autoimmunity according to neutropenia.

Causes	Neutropenic, n = 59 (%)	Nonneutropenic, n = 110 (%)	*P*value
Autoimmune/autoinflammatory disease (non-thyroid)	15 (25%)	21 (19.1%)	.43
Autoimmune thyroid disease	4 (6.7%)	24 (21.8%)	.008
Other thyroid disease	4 (6.7%)	15 (13.6%)	.13
Isolated autoimmune laboratory positivity	20 (33.3%)	19 (17.3%)	.038
Isolated antinuclear antibody positivity	13 (21.7%)	15 (13.6%)	.24
Isolated rheumatoid factor positivity	0	1 (0.9%)	.43
Isolated antithyroid peroxidase positivity	1 (1.7%)	7 (6.4%)	.27
Isolated antithyroglobulin positivity	2 (3.3%)	2 (1.8%)	.75
Presence of any signs of autoimmunity	37 (61.7%)	54 (49.1%)	.06

was responsible for the etiology in some patients. Among all patients, there were 35 patients with >1 cause. Each of these reasons has been counted separately. The comparison of all isolated leukopenia patients and patients with or without neutropenia in terms of etiological causes according to gender is summarized in Table 5.

When the causes responsible for the etiology were evaluated, autoimmunity/autoinflammatory diseases including autoimmune thyroiditis and autoimmune laboratory tests were included in the etiology of autoimmunity in 43 (45.3%) nonneutropenic women and 2 (13.3%) men. There was no obvious difference in the gender distribution of nonautoimmunity factors.

4. Discussion

There are difficulties in diagnosis and follow-up in patients with isolated leukopenia without a specific cause and who apply to the hematology outpatient clinic, since there is no relevant guide in the literature. The causes and frequency of leukopenia in many patients are not fully known. No previous study on isolated leukopenia was found in the literature. Often neutropenia has been used instead of leukopenia. Our aim was to evaluate the clinical relevance of isolated, nonspecific leukopenia and to create an algorithm that would be useful in patient management.

The median follow-up period of 169 patients with isolated, nonspecific leukopenia included in our study was 5.3 ± 4.7 (0–19.19) years.When the demographic information was examined, it was found that leukopenia was much more common in women. In addition, the rate of autoimmunity detection was significantly higher in women (P = .002). No specific study

Table 5

Etiological causes of leukopenia in neutropenic and nonneutropenic cases.

Etiology	Neutropenic, n = 59 (%)	Nonneutropenic, n = 110 (%)	*P*value
Unidentified	28 (47.5%)	44 (40%)	?
Autoimmune/autoinflammatory disease (non-thyroid)	10 (17%)	19 (17.3%)	.43
Iron deficiency anemia	6 (10.2%)	24 (21.8%)	.22
Autoimmune thyroid disease	3 (5.1%)	24 (21.8%)	.008
Autoimmune laboratory disorder	5 (8.5%)	3 (2.7%)	.038
Drugs	5 (8.5%)	14 (12.7%)	.58
Hematopoietic disorder	7 (11.9%)	3 (2.7%)	?
Infection	4 (6.8%)	6 (5.5%)	?
Hypersplenism	1 (1.7%)	3 (2.7%)	?
Radiotherapy	1 (1.7%)	2 (1.8%)	?
Vitamin B_{12} deficiency	1 (1.7%)	2 (1.8%)	.25

has been found in the literature dealing with the relationship between gender and leukopenia. It is known that autoimmunity is more common in women than in men. Isolated antinuclear antibody positivity (29 patients) was also evaluated in favor of autoimmunity, and 91 (53.2%) of 169 patients had autoimmunity diagnosis or laboratory findings. The frequency of autoimmunity was significantly higher in women than in men in our leukopenic patient cohort. Therefore, it can be suggested that leukopenia associated with autoimmune problems was more common in women and that leukopenia could develop at a higher rate in women due to this frequent etiological reason.

Possible causes were evaluated in patients without neutropenia who were admitted to the hospital with isolated leukopenia. Etiology could not be determined in 44 (40%) of 110 nonneutropenic patients and 28 (47.5%) of 59 neutropenic patients. In other words, no disorder was detected in a significant proportion of patients included in the study. Even with isolated antinuclear antibody positivity, it is important to consider autoimmunity in determining the possible cause. Autoimmunity should be considered as the possible cause of leukopenia, even if there is isolated antinuclear antibody positivity. In humans, autoantibodies play a major opsonizing role in inducing autoimmune neutropenia.[3] There is no close relationship between the degree of neutropenia and circulating autoantibody levels, and this has been attributed to several factors. The opsonizing activity of complement activated by antinuclear antibody can increase phagocytosis. The capacity of autoantibodies to induce neutropenia is related to their ability to recognize antigenic determinants expressed by bone marrow myeloid precursors as well as mature cells.[4] In such cases, severe neutropenia is accompanied by bone marrow hypoplasia. In our patients, isolated antinuclear antibody positivity (15 patients) was evaluated in favor of autoimmunity, and 54 (49.1%) of 110 patients had autoimmunity. These data showed that autoimmunity was very common in the isolated, nonspecific leukopenic patient group.

Autoimmune thyroid disease was present in 24 (21.8%) patients included in the study. The frequency of thyroid disease in neutropenic patients may be higher than previously reported. In the literature, granulopoiesis abnormalities associated with thyroid disorders have been described. A prospective observational study of newly diagnosed Graves disease patients by Aggarwal et al showed that 14.1% of patients had neutropenia before starting antithyroid drug therapy, and this is more common in those with more severe hyperthyroidism. After the treatment of autoimmune thyroid disease, normalization of thyroid hormones resulted in normalization of neutrophil counts in all neutropenic patients. In 149 patients with available data, the change in neutrophil count after reaching euthyroidism was independently associated with a decrease in thyroid hormone

levels ($P < 0.01$). Previous studies have also reported that the prevalence of neutropenia in hyperthyroidism is between 15% and 30%.[5] Kyritsi et al showed a negative correlation between free triiodothyronine and absolute neutrophil count, a negative correlation with thyroid peroxidase antibody/thyroglobulin antibody, and an inverse correlation with $CD4^+$ counts with thyroxine and a positive correlation with thyroid-stimulating hormone. Antithyroid peroxidase titers were significantly higher in patients with positive antineutrophil antibodies ($P = 0.04$). The presence of antineutrophil antibodies and the distribution of different lymphocyte subsets among patients with different thyroid disorders demonstrated both humoral and cellular mechanisms in the pathophysiology of thyroid disease-related neutropenia.[6]

Five (4.5%) patients were found to have chronic viral hepatitis (hepatitis B virus/hepatitis C virus). Although it was evaluated in the infection group when evaluating the etiological causes, it was thought that it could be a chronic infection condition, as well as cytopenia due to cirrhotic portal hypertension and splenomegaly, or cytopenia associated with the antiviral drug used in the treatment. Cytopenia is very common in patients with hepatic cirrhosis and portal hypertensive splenomegaly.[7] Pascutti et al[8] reported that a number of viruses, including cytomegalovirus, hepatitis C virus, and human herpes viruses, can directly infect hematopoietic stem and progenitor cells.

When comorbidities were examined in nonneutropenic patients, although iron deficiency was found in 37 (36.3%) of 102 patients who were evaluated for iron deficiency, it was held responsible for the etiology of leukopenia in 24 (21.8%) patients. The reason for this is that the clinician moves away from this diagnosis when there is no improvement in the patients after iron therapy or a more dominant cause is found. In a study by Yoojoo et al[9] in 2011, a high incidence of leukopenia was found in patients with iron deficiency anemia. The severity of leukopenia is associated with decreased hemoglobin. Among the white blood cell subgroups, lymphocyte count was significantly affected.[9] B_{12} deficiency was considered as the cause of leukopenia in 2 patients (1.8%) included in a study. As Shipton et al[10] stated in their work, cobalamin deficiency leads to ineffective deoxyribonucleic acid synthesis in rapidly dividing cells, leading to megaloblastic anemia, cytopenia, and dysplastic changes in the bone marrow.

In our study, 4 (3.6%) patients had a history of disease (such as gastric bypass surgery, anorexia, pernicious anemia, celiac, ulcerative colitis) that would lead to malnutrition. Micro and macronutrient deficiencies occur after gastric bypass surgery. In the literature review published by Bal et al, nutritional deficiencies were defined after bariatric surgery. Micronutrients include essential elements, essential minerals (calcium, iodine, iron, magnesium), and water- and fat-soluble vitamins. Vitamin B_{12} deficiency is a well-defined nutritional deficiency after bariatric surgery.[11] As Sabel et al[12] stated in their study, hematological abnormalities with deficiencies in all 3 series were defined in red blood cells, white blood cells, and platelets in patients with mild-to-moderate anorexia nervosa. Pernicious anemia is a complex autoimmune disease state resulting from impaired absorption of vitamin B_{12}, which we think may be involved in the etiology of leukopenia.[13]

In our study, in 14 (12.7%) nonneutropenic patients, drugs were involved in the etiology. Numerous drugs have been reported as potential causes of leukopenia and neutropenia in the literature. There is both direct toxicity to the bone marrow and immune-mediated destruction of neutrophils.[14] Looking at the literature, drug-related neutropenia was first described by Kracke in 1931. Since then, many studies have been published on this topic. Recently, the incidence has greatly increased, primarily due to chemotherapeutic agents. Drug-induced neutropenia is most common in women and the elderly, possibly due to the more frequent use of drugs. Genetic and physiological characteristics may also contribute to the higher incidence.[15]

When the history or laboratory findings of the patients included in our study were evaluated, infection was found in 10 (5.9%) patients among all patients and in 6 (5.5%) patients who were not neutropenic. Among the patients who had only 1 hemogram value, only 1 patient was detected with an infection. Both viral and bacterial infections can cause leukopenia. Among viral infections, there are influenza, hepatitis, human immunodeficiency virus, Epstein-Barr virus, measles, varicella, parvovirus, and cytomegalovirus. Atypical infections such as malaria, typhoidal fever, brucella, tuberculosis, ehrlichiosis, and some staphylococcal infections may also be associated with neutropenia.[16]

Bone marrow examination should be considered at the diagnosis stage if initial noninvasive studies do not confirm the diagnosis or if the response to treatment is inadequate. With the severity of cytopenias, peripheral blood smear abnormalities, and the presence of complications arising from cytopenia, the decision to perform a biopsy should be taken, especially if primary hematological disease or bone marrow failure is suspected.[14] Bone marrow examination was performed in 30 (17.8%) patients included in our study. It was found that the clinician took the decision of bone marrow examination more easily in patients with neutropenia. In the absence of neutropenia, the success of bone marrow examination in diagnosing was found to be low ($P = .109$).

There are various factors in the pathogenesis of leukopenia in patients with accompanying rheumatic disease. In the previous studies with Sjögren syndrome patients, the authors have stated that although autoimmunity is the most likely explanation in the pathogenesis, studies have shown that infections and drug toxicity may also be the cause of leukopenia.[17,18] In cases of persistent neutropenia, the presence of myelodysplasia or myelofibrosis should also be evaluated.[14] When the literature studies on Sjögren syndrome were examined, studies showing its relationship with leukopenia can be found. In the study of Vivino et al,[18] leukopenia was shown in 10% to 25% of patients with Sjögren syndrome. Considering the white blood cell difference, it has been shown that neutropenia is roughly 5% to 30%, lymphopenia is about 10%, and eosinophilia is 12%.[17–20] There is also a series reporting that the cases are more related to the effects of drugs or toxins rather than the disease itself.[17]

A few studies evaluating leukopenia, lymphopenia, and neutropenia in SLE were found in the literature. In the study of Velo-Garcia et al,[21] 75% of 158 SLE patients with active disease from the time of diagnosis had lymphopenia. After disease reactivation, another 18% also developed lymphopenia. In the study conducted by Carli et al,[22] the prevalence of leukopenia was defined in 22% to 41.8% of cases, lymphopenia in 15% to 82% of cases, and neutropenia in 20% to 40% of cases. In the literature review on the hematological manifestation of rheumatoid arthritis, it was found that leukopenia is common and it is related with Felty syndrome. The study by Duquenne et al evaluated the prevalence of initial and persistent lymphopenia, underlying diagnosis, and risk of infection or malignancy in patients with early arthritis who are naive to disease-modifying antirheumatic drugs. Fifteen (35.7%) of 42 patients with initial lymphopenia developed persistent lymphopenia after 3 years, and the final diagnosis in these 15 patients was rheumatoid arthritis (n = 6), unclassified arthritis (n = 6), SLE (n = 1), spondyloarthritis (n = 1), and fibromyalgia (n = 1). The most common rheumatic cause is rheumatoid arthritis, in which significant inflammation and other cytopenias are frequent.[23] In our study, 2.4% (n = 4) of the patients investigated for leukopenia had Sjögren syndrome, 3% (n = 5) SLE, 5.3% (n = 9) rheumatoid arthritis, and 5% other connective tissue diseases (ankylosing spondylitis, Behçet disease, and gout were found a total of 9, 3 in each).

The importance of this study is that clinical and laboratory associations of isolated, nonspecific leukopenia have been described for the first time in such a large cohort in the literature. The main limitation of our study is its retrospective design.

The etiology of isolated leukopenia is very important in a clinician's every day practice. Therefore, future prospective studies with larger cohorts should be conducted to evaluate this topic.

5. Conclusions

In this study, we aimed to evaluate the clinical and laboratory associations of isolated, nonspecific (not related to neutropenia) leukopenia. In the present study, the most frequent reasons of isolated leukopenia in nonneutropenic patients are found as iron deficiency anemia, other autoimmune/autoinflammatory diseases, and autoimmune thyroid disease. In neutropenic patients the most frequent reasons of isolated leukopenia are found as iron deficiency anemia, autoimmune/autoinflammatory diseases, and hematopoietic disorders. Therefore, autoimmunity is detected as an important factor leading to isolated leukopenia.

Author contributions

Conceived and designed the analysis: YB
Collected the data: GM
Contributed data or analysis tools: GM
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