Serum PADI4 Levels in Neutropenia and Febrile Neutropenia

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ABSTRACT

Peptidyl arginine deiminase (PADI) enzymes catalyze the conversion of peptidylarginine to peptidylcitrulline and are involved in the extracellular neutrophil trapping (NET) process. PADI4 is one of the most important PADI isoforms since it is the only PADI isotype with a nuclear localization signal and is found in tissues such as granulocytes, monocytes, and CD34(+) bone marrow cells. It contributes to the genesis of certain illnesses, such as autoimmune inflammatory diseases and cancer. In this investigation, we assessed PADI4 levels in patients with hematological malignancies that are neutropenic and compared them to hospitalized patients' neutropenic fever periods. PADI4 levels were lower in patients with neutropenic hematological malignancies than in the control group (4.44 ± 2.76 ng/ml (med: 3.98, IQR: 3-4.58) vs. 6.14 ± 6 .39 ng/ml (med: 2.7, IQR: 2.32-9.5); p= 0.049). It was shown that the patients with neutropenic fever had serum PADI4 levels that were statistically substantially lower than those of the control group (4.65 ± 2.54 ng/ml (med: 3.98, IQR: 3.24-5.17) vs. 6.14± 6.39 (med: 2.7, IQR: 2.32-9.5; p= 0.05). There was no statistically significant difference in PADI4 level between the neutropenic group and the neutropenic fever group (p= 0.734). PADI4 might be significant because of its potential to be a target in the management of infectious complications and neutropenic hematological malignancies. We require further research in this area.

Keywords: PADI4 protein, Neutropenia, Febrile neutropenia, Hematologic malignancies

INTRODUCTION

Peptidyl arginine deiminase (PADI) enzymes provide citrullination, that is, the calcium-dependent conversion of peptidylarginine to peptidylcitrulline. This enzymatic conversion is crucial for immune system function and gene regulation. In humans, there are five calcium-dependent PADIs known as PADIs 1-4 and PADI6.[1,2,3] The PADI family may also participate in the formation of extracellular neutrophil traps (NETs), secretion of inflammatory cytokines, energy metabolism and release of extracellular vesicles, and other important physiological processes. Recent research shows that it plays a role in the etiology of various diseases, including cancer and inflammatory autoimmune diseases.[2] PADI4 (also known as PAD4) is the only PADI isotype with a nuclear localization signal and is present in a variety of tissues like granulocytes, macrophages, monocytes, CD34(+) bone marrow cells, and multipotent progenitor cells, making it one of the most crucial PADI isoforms.[1,2,4] This protein has a molecular weight of 74 kDa, a length of 663 amino acids, and is encoded by the PADI4 gene.[5]

PADI4 participates in post-translational modifications of histones, one of the main focus areas of epigenetic research. Citrullination of histones, particularly histone H3, is one of the key points for inflammatory signals that trigger the neutrophil response to infections. Citrulline histone H3 has an essential function in the formation of NETs. NETs are extracellular strands of DNA condensed in complex with histones and granular proteins expelled from dying neutrophils to trap and kill microbes.[6] NETs play a role in a wide range of diseases, from autoimmune to cancer. High NET levels in plasma have been linked to multi-organ failure and sepsis in trauma patients.[1]

Uncontrolled activation of PADI4 followed by citrullination, i.e. protein dysregulation, has been linked to a variety of illnesses including rheumatoid arthritis, multiple sclerosis, and Alzheimer's disease. PADI4 is also found in rheumatoid arthritis patients' synovial tissues.[4,7,8] PADI4 expression is aberrant in a variety of malignant tumors and may influence tumor development and metastasis, such as hepatocellular carcinoma.[9,10] The underlying cellular processes could be epithelial-mesenchymal transition, apoptosis, and NETs.[4,7,8] The information available on NETs development in patients with hematological malignancies, such as acute leukemia, is limited.[11,12] Malignant progenitor cell overproduction is a hallmark of acute leukemias. Patients with leukemia are more vulnerable to infectious consequences, which account for the majority of non-relapsed mortality, due to reduced blood cell formation.

In many hematological malignancies, neutropenia is present at the time of diagnosis or due to chemotherapy or radiotherapy. Neutropenic fever is a condition that we avoid but frequently encounter in neutropenic patients. Severe sepsis and septic shock during the follow-up could result in serious morbidity and mortality. Patients with neutropenic fever are routinely monitored in the clinic using a variety of diagnostic approaches, including vital signs, physical examination, and high-resolution computed tomography (HRCT). C-reactive protein (CRP) and procalcitonin monitoring are additional frequently utilized biochemical markers. However, sometimes these parameters are insufficient in the early diagnosis and follow-up of neutropenic fever.

There are not enough publications in the literature regarding PADI4 level in hematological malignancies. Therefore, in our study, we evaluated PADI4 levels in patients with hematological malignancies, and compared PADI4 levels in neutropenic and neutropenic fever in hospitalized patients with hematological malignancies. Since there is no similar study so far, we aimed to contribute to the literature.

PATIENTS AND METHODS

The study comprised both healthy control volunteers and neutropenic patients who were hospitalized in the hematology unit at the Pamukkale University Hospital in Denizli, Turkey, due to hematological malignancy.

Neutropenia was defined as an absolute neutrophil count of less than 1500/l. Patients were diagnosed of "neutropenic fever" in accordance with ESMO recommendations. Serum PADI4 levels were investigated in the neutropenic and control groups, and additionally in the neutropenic group during fever. Neutropenic fever was defined as two consecutive >38.0^{0}C for at least two hours or an absolute neutrophil count of 38.3^{0}C. Patients were questioned about their medical problems, a physical examination was undertaken to rule out infection, and cultures were sent from potential foci. The culture findings were retrieved from the HIS (hospital information system).

Fasting venous blood samples were collected from neutropenic and control group participants in the morning. When fever developed, venous blood samples were once again requested. Serum obtained by the centrifugation of the blood samples was aliquoted and stored in -20^{0}C until serum PADI4 levels (ng/ml) were measured. Complete blood count results, CRP (0-0.5 μg/dl), and procalcitonin levels (ng/ml) were all measured the same day that the samples were collected.

To assess PADI4 in human serum, 5 cc of venous blood was collected in a serum separator tube, the samples were held at room temperature for about 15 minutes, and then centrifuged at 3500 rpm for 10 minutes. Human PADI4 levels were detected using BT Lab (Bioassay Technology Laboratory,

Shanghai, China) commercial kits. Before analysis, all samples and kits were brought to room temperature. The standards were added to the wells in the microplate after the standards and chemicals of the kits used in the study were prepared. The samples were then colored in accordance with the concentrations of the tests, as instructed in the package insert. After seeing the color creation, the Biotek Elx800 Microplate reader (BioTek Instruments Inc., USA) was used to read the absorbance values of the wells at 450 nanometers (nm). The serum absorbance readings were used to calculate concentrations using the Gen5 data analysis program. The units used to measure the PADI4 levels were ng/ml.

Before the study, approval was obtained from the local Ethics Committee of Pamukkale University Medical Faculty (25.01.2022 /number 02) and was carried out in accordance with the legal and regulatory requirements, outlined in Helsinki Declaration.

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 [IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)]. The mean ± standard deviation defined continuous variables, and categorical variables were defined by number and percent. Shapiro-Wilk test was used for the determination of normal distribution. For dependent group comparisons, paired samples t-test was used when parametric test assumptions were provided; and Wilcoxon signed rank test was used, otherwise. Spearman correlation analysis was used for analysing the relationships between continuous variables. Mann-Whitney U-test was used in comparison with the control group. Statistical significance was determined as $p< 0.05$.

RESULTS

Thirty patients diagnosed with hematological malignancy (11 women, 36.7% and 19 men, 63.3%) were included in the study. The mean age of the patients included in the study was 58.73 ± 16.85 years. The majority of the patients had acute myeloid leukemia (AML) (20, 66.6%). There were four cases of multiple myeloma, three cases of myelodysplastic syndrome, and one case each of ALL (acute lymphoblastic leukemia), diffuse large B cell lymphoma, central nervous system lymphoma.

In 26 patients (86.7%), neutropenia was secondary to chemotherapy, and 4 patients (13.3%) were neutropenic due to hematological malignancy itself. The average absolute neutrophil count of the included neutropenic patient group was 320/μl (IQR:77.5- 972.5; ranging from 0 – 1980).

During the neutropenic fever period, the average body temperature was 38.42 ± 0,43^{0}C. Vital signs were stable in most patients during neutropenic fever. Only hypotension occurred in two patients, tachycardia in two patients, and hypoxia occurred in one patient each during neutropenic fever. There was no tachypnea in any of the patients.

Microorganism isolation (36.7%) was achieved in some of the cultures. Isolation was primarily obtained from blood cultures (5 patients). In addition, growth of pathogenic microorganism (data not shown) growth was recorded in the sputum culture of two patients, in the urine culture of two patients, and in the catheter culture of one patient.

PADI4 was found to be significantly lower in the neutropenic group than the control group (4.44 ± 2.76 ng/ml (med: 3.98, IQR: 3-4.58) vs. 6.14 ± 6.39 ng/ml (med: 2.7, IQR: 2.32-9.5); p= 0.049). No statistically significant correlation was found between PADI4 level and CRP, ferritin and procalcitonin values in the neutropenic group [CRP (r= 0.032; p= 0.866), ferritin (r= -0.001; p = 0.997) and procalcitonin (r= 0.259; p= 0.258)]. Likewise, no statistically significant correlation was detected between PADI4 and white blood cell (WBC) and absolute neutrophil count (ANC) [WBC (r= -0.107; p= 0.572) and ANC (r = 0.031; p= 0.871)]. In the neutropenic fever group, no statistically significant correlation was found between PADI4 level and CRP, ferritin and procalcitonin values [CRP (r= 0.183; p= 0.343) and procalcitonin (r= 0.119; p= 0.609)]. In the neutropenic fever group, no statistically significant correlation was detected between PADI4 and WBC and ANC [WBC (r= 0.113; p= 0.551) and ANC (r= 0.222; p= 0.238)] (Table 1).

Compared with the control group and the neutropenic or neutropenic fever group, hemoglobin

Table 1. Relationship of peptidyl arginine deiminase 4 (PADI4) and white blood cell (WBC), absolute neutrophil count (ANC), C-reactive protein (CRP), ferritin and procalcitonin in neutropenia and neutropenic fever period

		PADI4 (ng/ml) in neutropenia period	PADI4 (ng/ml) in neutropenic fever period
White blood cell (WBC) (/µl)	r	-0.107	0.113
	p	0.572	0.551
Absolute Neutrophil Count (ANC) (/µl)	r	0.031	0.222
	p	0.871	0.238
C-reactive protein (CRP) (mg/dl)	r	0,032	0.183
	p	0.866	0.343
Ferritin (µg/l)	r	-0.001	-
	p	0.997	-
Procalcitonin	r	0.259	0.119
	p	0.258	0.609

r: Spearman correlation coefficient
PADI4: Peptidyl arginine deiminase 4, WBC: White blood cell, ANC: Absolute neutrophil count, CRP: C-reactive protein

(Hgb), hematocrit, WBC, ANC, absolute lymphocyte count, platelet values were statistically significantly lower. During neutropenic fever, WBC (p= 0.001), ANC (p= 0.005), absolute lymphocyte count (p= 0.022) were significantly lower than neutropenic period. However, no significant difference was found between the two groups at the Hgb level (p = 0.476) and platelet level (p= 0.092) (Table 2). One cause for this might be that the Hgb or platelet value has not yet dropped in certain individuals due to the short interval between neutropenia and neutropenic fever. Another possibility is that the patient got erythrocyte or thrombocyte transfusions prior to developing neutropenic fever. The neutrophil/lymphocyte ratio (NLR) was statistically significantly lower in the neutropenic fever group than in the neutropenic group (3.36 ± 14.43 (med: 0.09, IQR: 0- 1.5) vs. 4.26 ± 12.39 (med: 1.08, IQR: 0.13- 3.03); p= 0.049).

In neutropenic fever group, procalcitonin was found significantly higher than neutropenic group (med: 1 ng/ml, IQR: 0 – 5 ng/ml vs. med: 0 ng/ml, IQR: 0 ng/ml; p= 0.007). CRP was also found to be higher in the neutropenic fever group compared to the neutropenic group (med: 6.16 mg/dl, IQR: 2.53-19.97 mg/dl vs. med: 2.01 mg/dl, IQR: 0.76-4.3 mg/dl; p= 0.001). Serum PADI4 level was found to be statistically significantly lower in the neutropenic fever patient group than in the control group (4.65 ± 2.54 ng/ml (med: 3.98, IQR: 3.24-5.17 vs. 6.14 ± 6.39 (med:2.7, IQR: 2.32-9.5); p= 0.05). However, there was no significant difference in neutropenic fever group in terms of PADI4 compared to the neutropenic group (4.44 ± 2.76 ng/ml vs. 4.65 ± 2.54 ng/ml; p= 0.734) (Table 2).

Prothrombin time (PT) was statistically significantly longer in the neutropenic fever group than in the neutropenic group (14.12 ± 1.84 (med: 13.75, IQR: 12.88- 14.95) vs. 12.74 ± 1.4 (med: 12.55, IQR: 11.68-13.55); p= 0.0001). While there was no significant difference between the two groups in terms of d-dimer and activated partial thromboplastin time (aPTT), the fibrinogen value was statistically significantly higher in the neutropenic fever group than in the neutropenic group (508,9 ± 224,55 (med: 478, IQR: 358.5- 625.25) vs. 379.24 ± 162.07 (med: 349, IQR: 243 – 489.5); p= 0.008).

When patients in remission were compared with neutropenic patients newly diagnosed or diagnosed with AML that were not in remission, no significant difference was found between PADI4 levels (p= 0.559). Also no significant difference was found in terms of PADI4 levels in the neutropenic fever period of these patients (p= 0.444). No significant difference was found between the neutropenic group and the neutropenic fever group in terms of PADI4 levels in AML patients in re-

Table 2. Comparison of peptidyl arginine deiminase 4 (PADI4) and the other parameters in neutropenic period and neutropenic fever groups

		Neutropenic period	Neutopenic fever period	Intra Group p
Hemoglobin				
(Hgb) (g/dL)	Mean ± SD	8.48 ± 1.16	8.31 ± 1.26	0.476 (t=0.722)
	Med (IQR)	8.1 (7.7 - 8.93)	8.25 (7.4 - 8.9)	
White blood cell				
(WBC) (/µl)	Mean ± SD	1500.33 ± 1149.86	1015 ± 1040.59	0.001* (z=-3.199)
	Med (IQR)	1190 (535 - 2175)	635 (277.5 - 1427.5)	
Absolute neutrophil count				
(ANC) (Neu#) (/µl)	Mean ± SD	644.33 ± 662.05	383 ± 653.56	0.005* (z=-2.836)
	Med (IQR)	320 (77.5 - 972.5)	45 (0 - 490)	
Absolute lymphocyte count				
(Lymph#) (/µl)	Mean ± SD	705 ± 600.09	500.33 ± 467.73	0.022* (z=-2.284)
	Med (IQR)	575 (190 - 1112.5)	430 (162.5 - 795)	
Neutrophil to lymphocyte				
ratio (NLR)	Mean ± SD	4.26 ± 12.39	3.36 ± 14.43	0.049* (z=-1.97)
	Med (IQR)	1.08 (0.13 - 3.03)	0.09 (0 - 1.5)	
Platelet (/µl)	Mean ± SD	58800 ± 71360.9	40166.67 ± 45163.37	0.092 (z=-1.687)
	Med (IQR)	31000 (16250 - 66000)	25000 (14750 - 47750)	
PADI4 (ng/ml)	Mean ± SD	4.44 ± 2.76	4.65 ± 2.54	0.734 (z=-0.339)
	Med (IQR)	3.98 (3 - 4.58)	3.98 (3.24 - 5.17)	
C-reactive protein (CRP)	Mean ± SD	3.03 ± 2.96	11.14 ± 10.93	0.001* (z=-3.362)
(mg/dl)	Med (IQR)	2.01 (0.76 - 4.3)	6.16 (2.53 - 19.97)	
Procalcitonin	Mean ± SD	0.06 ± 0.24	4.35 ± 8.97	0.007* (z=-2.692)
	Med (IQR)	0 (0 - 0)	1 (0 - 5)	

SD: Standard Deviation; Med (IQR): Median (25th – 75th percentiles); t: Paired samples t test; z: Wilcoxon Signed Rank Test
PADI4: Peptidyl arginine deiminase 4, Hgb: Hemoglobin, WBC: White blood cell, ANC: Absolute neutrophil count, CRP: C-reactive protein, NLR: Neutrophil to lymphocyte ratio

mission (p= 0.686). No significant difference was also found between the neutropenic group and the neutropenic fever group in newly diagnosed and non-remission AML patients (p= 0.177).

DISCUSSION

In a substantial percentage of hematological malignancies, neutropenia is frequently evident upon diagnosis or during treatment. Infection monitoring and treatment in neutropenic individuals should be done with caution because it can lead to morbidity and mortality later on. It is critical to diagnose and treat neutropenic fever as soon as possible. The literature does not contain adequate articles about PADI4 level in hematological malignancies. Thus, in our investigation, we began by assessing the PADI4 level in patients who had hematological malignancies. Furthermore, we examined PADI4 levels in hospitalized patients with hematological malignancies during the neutropenic phase and the neutropenic fever period. In the present study, the neutropenic patient group's serum PADI4 level was shown to be statistically substantially lower than that of the control group (4.44 ± 2.76 ng/ml (med: 3.98, IQR: 3-4.58) vs. 6.14 ± 6.39 ng/ml (med: 2.7, IQR: 2.32-9.5); p= 0.049). Additionally, it was discovered that the serum PADI4 level in the group of patients with neutropenic fever was statistically significantly lower than in the control group (4.65 ± 2.54 ng/ml (med: 3.98, IQR: 3.24-5.17) vs. 6.14± 6.39 (med: 2.7, IQR: 2.32-9.5; p=

0.05) (Table 2). No statistically significant difference was found between the neutropenic group and the neutropenic fever group in terms of PADI4 level (p= 0.734). PADI4 levels were observed to be lower in our study among patients with hematological malignancies who experienced neutropenia as compared to the control group. Our research further demonstrated that this reduction continued in tandem with lower neutrophil counts when neutropenic fever was present.

Since PADI4 protein is derived by granulocytes, it was lower in neutropenic patients with hematological malignancies in our study compared to controls. However, although the absolute neutrophil count decreased significantly during the neutropenic fever period, the PADI4 level remained stable. At this point, the source may be the small number of neutrophils present. Although we gave very broad-spectrum parenteral antibiotics in the treatment of neutropenic and infected patients, we always witness that a few newly emerged neutrophils in the peripheral blood in the patient's follow-up cause much more positive changes in the patient's clinic. Perhaps even if the patient is neutropenic, a small number of neutrophils may be strongly acting on PADI4 and NET formation. Another hypothesis is that PADI4 may originate from another cell or structure, just like PADI4 expression in synovial tissues in rheumatoid arthritis.[4] In this respect, additional and new studies are needed.

It has been shown that PADI4 may play a role in the differentiation of NB4 cells and upregulation of cytokines induced by ATRA. It has been stated that PADI4 overexpression can induce the secretion of inflammation-related cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL)-8, IL-1β, CC chemokine receptor 1 (CCR1), CCL4 and intercellular adhesion molecule in these cells.[13,14] It has also been shown that PADI4 accelerates metastasis by promoting IL-8 expression in gastric cancer cells.[15] PADI4 concentration, but not PADI4_89, PADI4_94, and PADI4_104 polymorphisms have been shown to be associated with intensive care unit mortality in septic shock patients.[1] In our study, PADI4 level was found to be lower in patients with neutropenic hematological malignancies compared to the control group. However, no statistically significant difference was found between the neutropenic fever period and the neutropenic period in terms of PADI4 level. The fact that we take blood samples as soon as fever occurs in our patients may have an effect on this. During this period, most of the patients' vital signs were stable. If we took a blood sample during the period when the infection progressed, its severity increased, vital signs deteriorated, and findings such as hypotension, tachycardia, and hypoxia were accompanied, the PADI4 level might have been lower.

Neutrophils develop different strategies to apply for their antimicrobial activities such as phagocytosis and degranulation. NETs are extracellular network-like structures made up of histone-coated chromatin filaments and antimicrobial proteins. The recently studied process called NETosis is noteworthy. Two types of NETosis have been identified: "lytic/suicidal" NETosis (characterized by slow cell death) and an alternative pathway called "vital" NETosis (involving rapid release of NETs into the extracellular environment).[16]

NETs are formed in vivo during infection by mechanisms different from those originally described in vitro. Citrullination of histones by PADI4 is central to NET formation in vivo. NETs can promote autoantibody formation and also act as scaffolds for thrombosis, thus providing a link between infection, autoimmunity, and thrombosis.[12] The kinetics of "classical" NETosis are slow, delayed hours after stimulation, require reactive oxygen species (ROS), and peak in a non-viable cell. Rapid 'vital' NETosis occurs within minutes of stimulation independent of ROS, leaving a viable nucleated cell likely still capable of migration and phagocytosis. NETs are "traps" because their physical separation function inhibits microorganism spread. The limiting function of NETs is important in the early stages of infection and may limit progression to sepsis and/or sepsis severity.[6]

While NETosis and NETs have been discovered as processes responsible for trapping and killing pathogens, recent evidence also suggests that they play an important role in cancer progression, in line with the role of cancer-associated neutrophils. It is highlighted that NETs could be helpful biomarkers for the diagnosis of cancers such head and neck, small bowel, and colorectal cancers.[16,17]

It has been shown that PADI4 expression occurs in the cells of a variety of cancers, including lung, ovarian, and uterine adenocarcinomas, colorectal, hepatic, and breast cancers. PADI4, on the other hand, is only weakly expressed in benign and normal tissue.[17] As a treatment target, studies are carried out on PADI4 and NETosis. A study conducted in 2022 showed that a compound called 'berberine' can regulate PADI4-related macrophage function to prevent lung cancer.[18] In another study conducted in the same year, it was observed that the PADI4 antibody had a therapeutic effect on breast tumors by inhibiting the citrullination of fibronectin to change the tumor tissue microenvironment.[19]

Additionally, there is strong proof that PADI4 expression, by regulating the expression of c-myc, controls the proliferation of multipotent progenitors in the bone marrow.[20,21] PADI4 was discovered as a protein caused in human promyelocytic leukaemia HL-60 cells after they differentiated into granulocytes and monocytes. PADI4 is expressed primarily in haematopoietic organs such as bone marrow and spleen, as well as neutrophils, eosinophils, and monocytes in peripheral blood in mice and humans.[14,22,23] All blood cells are produced from haematopoietic stem cells (HSCs) found in adult bone marrow, via a series of myeloid and lymphoid progenitor cells. Deregulation of the proliferation and differentiation of haematopoietic progenitors is well known to produce catastrophic illnesses such as leukemia. The proto-oncogene c-myc encodes c-myc, which has been linked to the control of several biological processes, including cell division, apoptosis, cellular growth, and differentiation. C-myc expression is tightly regulated during haematopoiesis and plays key functions in the regulation of HSC and progenitor cell proliferation and differentiation.[14,24-26]

Normal hematopoietic cells are repressed in acute leukemias, which has a serious impact on immunity and blood cell formation. Therefore, patients with acute leukemia are highly susceptible to infectious complications, both from the disease itself and from treatment-related factors (eg, chemotherapy-associated neutropenia). However, it has been shown that the inadequate immunity observed in patients with acute leukemia may be independent of the neutrophil count and this may contribute to secondary immunodeficiency in this population. At this point, it is argued that the decrease in NET formation may be important. In a study, it was shown that while NET formation is sufficient in normal humans, NET formation is low at the time of diagnosis in acute leukemia, increases slightly in the case of infection and during the induction period, and improves after hematopoietic stem cell transplantation.[27] In a study, hydrogen peroxide, neutrophil elastase, phagocytosis and myeloperoxidase enzymatic activity and NET formation were studied in 10 pediatric ALL and 7 AML patients after induction therapy. Median neutrophil elastase activity and NET formation were found to be significantly lower in AML patients compared to ALL (41% vs. 90%, p= 0.005 and 51% vs. 94%, p= 0.008). In addition, it has been found that AML patients had more febrile neutropenia attacks in the first two blocks of treatment and tended to have more invasive bacterial and fungal infections.[11] In our study, most of the patients were acute leukemia patients. Consistent with the study in the literature; it was shown in our study that the level of PADI4 required for NET formation decreased as the patients were neutropenic. In this respect, since the decrease in PADI4, NETosis may not have been sufficient. We were unable to investigate NET development in our research. This may be the subject of another study.

NETs can act as a scaffold for thrombus formation through the binding of platelets, red blood cells, and procoagulant molecules. In addition, the extracellular activity of PADI4 has been suggested to be a trigger of diseases through uncontrolled citrullination of proteins. PADI4 has been detected at high levels in unstable carotid plaques, representing the presence of NETs.[20,28,29] We observed thrombophlebitis of the superficial vein in one patient, ischemia surrounding the cranial mass in one patient with central nervous system lymphoma, and an ischemic lesion in the spleen in one patient when they were hospitalized for neutropenic fever.

In a study, it was shown that homocysteine provides a strong interaction between neutrophils and platelets by inducing platelet aggregation and NETosis, which may lead to type 2 diabetes mellitus-related vascular diseases.[29,30] In a study conducted in 2023, it was determined that NETs delay diabetic

wound healing by inducing the transition from the endothelium to the mesenchyme via hippo.[31] All this information shows that NETosis, and therefore PADI4, is important in many diseases with infection and inflammation in its pathogenesis.

The main limitation of this study was the heterogeneity of the hematologic malignancy types among the participants, and for some, the insufficiency in patient numbers. The majority of the patients had AML as their diagnosis.

In conclusion, hematological malignancy patients with neutropenia had a lower PADI4 level than the control group. Our investigation further shown that, in contrast to the neutropenic group, no appreciable extra decline in PADI4 levels was observed during the neutropenic fever episode. As a result, it does not appear to be appropriate for use in neutropenic fever. However; PADI4, which is thought to have a role in tumorigenesis, may be important in terms of its potential to be a target in the prognosis and treatment of hematological malignancies and infectious complications. In this respect, new studies are needed.

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