



# Assessment of serum neopterin and calprotectin as biomarkers for subclinical inflammation in patients with familial Mediterranean fever

Dilek Tezcan<sup>1</sup> · Duygu Eryavuz Onmaz<sup>2</sup> · Abdullah Sivrikaya<sup>2</sup> · Selda Hakbilen<sup>3</sup> · Muslu Kazım Körez<sup>4</sup> · Semral Gülcemal<sup>3</sup> · Sema Yılmaz<sup>3</sup>

Received: 12 September 2022 / Accepted: 21 September 2022  
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## Abstract

**Background** Familial Mediterranean fever (FMF) is the most common autoinflammatory disease characterized by short, repeated, and self-limiting attacks of fever and serositis. Subclinical inflammation can persist in the periods with no symptoms and result in amyloidosis even with colchicine treatment. Neopterin and calprotectin have been considered essential players in inflammation and immune response.

**Aim** The study was aimed to measure serum levels of neopterin and calprotectin in patients with FMF in the attack-free period.

**Methods** A total of 160 participants were recruited from the rheumatology department in this single-center, case–control study. Individuals having the inclusion criteria were divided into healthy controls ( $n = 80$ ) and FMF ( $n = 80$ ). The laboratory data were acquired from the electronic registration database. Serum calprotectin and neopterin were measured with ELISA test kits. FMF patients and healthy controls' laboratory findings were compared.

**Results** FMF patients' serum red cell distribution width (RDW), calprotectin, and neopterin values were significantly higher compared to healthy controls. There were no statistically significant differences between calprotectin and neopterin regarding gender, family history, and colchicine response of the FMF patients.

**Conclusions** Calprotectin, neopterin, and RDW can be valuable marker candidates to be used in the follow-up of subclinical inflammation in FMF patients.

**Keywords** Calprotectin · Familial Mediterranean fever · Inflammation · Neopterin · Red cell distribution width

## Introduction

Primarily in ethnic origins in the Mediterranean basin, familial Mediterranean fever (FMF) is the most frequent monogenic autoinflammatory pathology. Self-limiting

inflammatory fever attacks and polyserositis accompanying elevated acute phase reactants are the expected clinical presence in FMF [1]. The mutations within the MEFV gene of pyrin, having a part in the inflammasome responsible for inflammatory response and interleukin (IL)-1 $\beta$

✉ Dilek Tezcan  
dr\_dilekturan@hotmail.com

Duygu Eryavuz Onmaz  
duygu\_eryavuz@hotmail.com

Abdullah Sivrikaya  
biyokaya@selcuk.edu.tr

Selda Hakbilen  
seldahakbilen@gmail.com

Muslu Kazım Körez  
mkkorez@gmail.com

Semral Gülcemal  
semralgulcema@hotmail.com

Sema Yılmaz  
drsemayilmaz@hotmail.com

<sup>1</sup> Department of Internal Medicine, Division of Rheumatology, Gülhane Faculty of Medicine, University of Health Sciences Turkey, Ankara, Turkey

<sup>2</sup> Division of Department of Biochemistry, Faculty of Medicine, Selcuk University, Konya, Turkey

<sup>3</sup> Division of Rheumatology Selcuk University Faculty of Medicine, 42130 Selcuklu, Konya, Turkey

<sup>4</sup> Division of Biostatistics, Faculty of Medicine, Selcuk University, 42130 Selcuklu, Konya, Turkey

production, result in FMF [2]. Innate cells, such as granulocytes, cytokine-activated monocytes, and dendritic cells (DC), express pyrin mainly [3]. Neutrophil extracellular traps (NETs), decorated with bioactive IL-1 $\beta$ , are released by neutrophils throughout FMF attacks. Toll-like receptors (TLR) trigger intracellular signaling cascades causing gene induction, which codes the inflammatory cytokines. DC maturation is induced by TLR-signaling through upregulating co-stimulatory and HLA-molecules. Several cytokines, such as interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$ , IL-4, IL-10, and lipopolysaccharides (LPS), may upregulate the pyrin expression. Elevated IL-6, IL-10, serum-soluble IL-2 receptor, and TNF- $\alpha$  levels are determined throughout or between acute FMF attacks in various researches [4]. In addition, studies have revealed that oxidative stress deepens in FMF patients throughout the attack and the periods without an attack [5]. In spite of the apparent advancements in examining FMF pathogenesis, the reasons for its attacks' periodicity and self-limited nature are still not fully understood. Patients do not generally present with symptoms between attacks; however, subclinical inflammation still occurs in periods without an attack in some patients. Subclinical inflammation throughout these periods poses a risk for amyloidosis. Thus, preventing this subclinical inflammation is vital. Even though acute phase reactants are beneficial to diagnose the disease, they may not be sufficient to demonstrate subclinical inflammation. Biomarkers indicating subclinical inflammation are required.

Calprotectin (CLP) is calcium, and zinc-finger heterodimer originated from proteins S1008 and S1009. Primarily, granulocytes and less frequently epithelial cells, reactive macrophages, and monocytes express it. CLP is a robust antimicrobial protein, and a primary proinflammatory stimulus promotes its secretion by TLR4 from granulocytes. Its inclusion in the inflammatory course is well known. Initially, granulocytes promote the CLP secretion following inflammatory stimuli. Afterward, binding surface receptors and triggering pathways engaged in mounting immune response make CLP act as a cytokine-like protein. Furthermore, CLP promotes many cellular courses through calcium homeostasis regulation, including cytoskeletal components' rearrangement, cell cycle progression, proliferation, migration, and survival. CLP is defined as it triggers neutrophil chemotaxis and endothelial adhesion. This complex controls innate immune cells' intracellular pathways and grants the inflammatory response organization [6, 7]. Neopterin (NPT) is among the chemical group of pteridines, and macrophages synthesize it from dihydro-NPT in the presence of IFN- $\gamma$  stimulation. NPT is considered a macrophage stimulation indicator and cellular immune activation, T helper 1 (Th1) immune response, and oxidative stress marker [8]. It is produced from guanosine triphosphate (GTP) by IDO and, in parallel, by GTP cyclohydrolase I (GCH I), activated

by kynurenine production. The enzyme GCH I in activated monocytes, macrophages, dendritic cells, and endothelial cells catalyze the reaction when stimulated primarily by IFN- $\gamma$  [8]. The concentration of NPT is regarded as a systemic immune activation indicator of lymphoid (T and natural killer cells) and myeloid cells (monocytes and macrophages). The present study primarily aimed to investigate whether there was a relationship with subclinical inflammation in FMF by determining the levels of NPT and CLP and contribute to the literature.

## Materials and methods

### Study design

Eighty FMF patients in a period without an attack, diagnosed per the Tel-Hashomer criteria and followed up and had treatment in the outpatient clinic of Rheumatology on a regular basis, were included consecutively, following the criteria to include and exclude. Additionally, as the control group, 80 healthy volunteers with matching age and gender with the patient group who had no chronic diseases with non-pathological physical examination findings and normal test results and applied for regular control visits were enrolled. The patients were chosen among those in a period without an attack. At the time of admission, all participants were assessed clinically and given blood samples to be analyzed. Age, sex, age at diagnosis, disease duration, symptoms, attack period, attack frequency, dose and usage time of colchicine, and genetic mutation analyses were acquired from the hospital records throughout the follow-up period. Furthermore, patients' laboratory parameters and treatment information were recorded. Participants who smoked, used alcohol, and had a history of chronic kidney, liver, and cardiovascular, endocrine, acute or chronic infectious, other autoimmune or inflammatory, neurodegenerative or psychiatric, and other rheumatic diseases, and malignancies were excluded.

The Ethics Committee (Decision No: 2020/573) approved the present single-center study, and it was performed per the Declaration of Helsinki. Before the study, all participants provided written informed consent forms.

### Laboratory analysis

The participants provided blood samples to be analyzed. An automatic blood counting system (Beckman Coulter LH 780, Brea, CA, USA) examined the CBC results for each individual, including the platelet, lymphocyte (K/ $\mu$ L), neutrophil (K/ $\mu$ L), and monocyte (K/ $\mu$ L) count, red cell distribution width (RDW) (normal range: 11.5–14.5%), mean platelet volume (MPV) (normal 7.5–11.5 fl) levels. The erythrocyte

sedimentation rate (ESR; 0–20 mm/hour) and C-reactive protein (CRP; 0–8 mg/L) levels were determined. Serum NPT and CLP levels were measured by enzyme-linked immunosorbent assay (ELISA) test kits, in the Medical Biochemistry Department of the University Faculty of Medicine Hospital.

## Statistical analysis

R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria; <https://www.r-project.org>) performed all statistical analyses. Shapiro–Wilk’s normality test and Q–Q plots were used to assess the normality of the data and Levene’s test to check the homogeneity of the variances. Besides, Grubbs’ outlier test and box-plots were used to identify the outliers of the data. When the data included the outliers, robust statistics and descriptive statistics were used. The numerical data were presented as mean (standard deviation), trimmed mean with 10% trim proportion  $\pm$  standard error of the trimmed mean, or median with interquartile range (1st quartile–3rd quartile) values. The categorical data were expressed as count ( $n$ ) and percentage (%) values. Welch’s  $t$ -test, Pearson chi-square test, Yuen (robust) independent samples test, independent samples  $t$ -test, and Mann–Whitney  $U$  test determined the statistically significant differences between participants regarding demographical and clinical characteristics and also laboratory findings. Furthermore, Mann–Whitney  $U$  test examined the differences between CLP and NPT in terms of gender, family history, and colchicine response of the FMF patients. Spearman’s rho correlation analysis was conducted to determine the relationship between CLP, NPT, and parameters. Receiver operating characteristic (ROC) curves identified the diagnostic performance of the CLP and NPT. The optimal cutoff values were obtained with Youden index criteria. Sensitivity, specificity, PPV, and NPV values were determined with 95% confidence intervals. A  $p$ -value less than 0.05 was considered statistically significant.

## Results

The study was conducted with 160 participants, 80 of whom were FMF patients, and 80 were healthy controls. The mean age of all participants was 34.16 (10.22) (range: 18–60), with 94 females (58.8%) and 66 males (41.2%). The study groups’ demographical and clinical characteristics and laboratory findings are presented in Table 1. The age and gender distribution of the study groups were similar ( $p = .271$  and  $p = .199$ , respectively). Of the 80 FMF patients, there was a family history in 51 (63.7%), and colchicine response in 59 (73.8%). The mean attack frequency was 3.79 (4.22) (range: 1–12/year), and the mean disease time was 8.72

(7.55) (range: 1–30 years) for the FMF patients. No statistically significant difference was determined between the white blood cell, hemoglobin, platelet, lymphocyte neutrophil, monocyte, and MPV values of healthy controls and FMF disease ( $p > .05$ ). However, FMF patients’ RDW (14.05 [IQR = 13.38–14.77] vs. 13.35 [IQR = 12.90–14.30],  $p = .003$ ), CLP (133.03 [IQR = 87.52–322.11] vs. 84.78 [IQR = 64.74–198.79],  $p = .003$ ), and NPT (8.78 [IQR = 5.08–21.70] vs. 6.51 [IQR = 5.30–10.95],  $p = .003$ ) values were significantly higher. There were no statistically significant differences in CLP and NPT by sex, family history, and colchicine response of the FMF patients (Table 2). No statistically significant difference was observed between CLP, NPT and age, attack frequency, disease time, and laboratory findings in the FMF patients (Table 3). The median CRP and ESR values were 3.78 (IQR, 2–7.95) and 9.5 (IQR, 3.25–17.75), respectively. There was no statistically significant relationship between CRP, ESR, and CLP, with NPT ( $p > .05$ ). However, a significant and positive correlation was determined between CLP and NPT in the FMF patients ( $r_s = 0.797$ ,  $p < .001$ ). The area under the ROC curve values for CLP was 0.684 (95% CI, 0.616–0.764,  $p < .001$ ), and the value of 77.965 with sensitivity 93.8% (86–97.9%, specificity 43.8% (32.7–55.3%), PPV 62.5% (57.7–67.1%), and NPV 87.5% (74.3–94.4%) was considered as cutoff value to distinguish between the FMF patients and healthy controls. The area under the ROC curve values for NPT was 0.596 (95% CI, 0.516–0.673,  $p < .001$ ), and the value of 12.609 with sensitivity 46.3% (35–57.8%, specificity 78.8% (68.2–87.1%), PPV 68.5% (57.3–77.9%), and NPV 59.4% (53.7–64.9%) was considered as cutoff value to distinguish between the groups (Fig. 1).

## Discussion

The mutations in the MEFV gene encoding pyrin are held responsible for FMF pathogenesis, and recurrent attacks of fever and serositis and, sometimes, chronic subclinical inflammation define FMF. Inflammation generally develops by inflammatory cytokine secretion through macrophages and monocytes. Even though no particular serum biomarkers to diagnose FMF have been determined, prior studies have indicated that serum IL-1 $\beta$ , soluble IL-2 receptor, IL-6, TNF- $\alpha$ , IL-10, IL-12, IL-17A, and IL-18 are crucial for FMF pathogenesis. IL-1 $\beta$  promotes the gene expressions within the whole IL-1 pathway, thus boosting its secretion and supporting an inflammatory burst. Subclinical inflammation creates a hidden danger for developing FMF complications, including amyloidosis in attack-free periods [4]. ESR and acute-phase proteins, such as CRP, serum amyloid A, and fibrinogen levels elevate throughout attack periods and generally become normal throughout asymptomatic

**Table 1** The demographical and clinical characteristics, and the laboratory findings of the study groups

	Healthy controls ( <i>n</i> = 80)	FMF ( <i>n</i> = 80)	Mean diff. (95% <i>CI</i> )	<i>p</i> -value
Demographical and clinical characteristics				
Age (years)	33.49 ± 8.20	34.84 ± 11.93		.406 <sup>a</sup>
Sex				.199 <sup>b</sup>
Female	51 (54.3)	43 (45.7)		
Male	29 (43.9)	37 (56.1)		
Family history		51 (63.7)		
Colchicine response		59 (73.8)		
Attack frequency		3.79 ± 4.22 (1–12)		
Disease time (years)		8.72 ± 7.55 (1–30)		
Mutations				
M694V heterozygotes		35 (30.7)		
M694V homozygotes		18 (15.8)		
V726A heterozygotes		12 (10.5)		
M680I heterozygotes		14 (12.3)		
E148Q heterozygotes		7 (6.1)		
E148Q homozygotes		2 (1.8)		
Others		13 (11.4)		
Normal		13 (11.4)		
Laboratory findings				
Wbc (10 <sup>9</sup> /L)	7.03 ± 0.20	7.12 ± 0.18	0.10 (−0.44 to 0.63)	.725 <sup>c</sup>
Hb (g/dL)	13.85 ± 1.80	14.23 ± 1.73	0.38 (−0.17 to 0.94)	.171 <sup>d</sup>
Plt (10 <sup>9</sup> /L)	266.14 ± 6.13	269.06 ± 7.01	2.92 (−15.54 to 21.39)	.755 <sup>c</sup>
Neu (10 <sup>9</sup> /L)	3.95 (3.08–4.60)	4.10 (3.10–4.90)	0.20 (−0.20 to 0.60)	.454 <sup>e</sup>
Lym (10 <sup>9</sup> /L)	2.21 ± 0.08	2.23 ± 0.08	0.01 (−0.20 to 0.23)	.908 <sup>c</sup>
Mon (10 <sup>9</sup> /L)	0.51 ± 0.02	0.52 ± 0.02	0.02 (−0.04 to 0.07)	.561 <sup>c</sup>
RDW (%)	13.35 (12.90–14.30)	14.05 (13.38–14.77)	0.40 (0.20 to 0.80)	<b>.003<sup>e</sup></b>
MPV (fL)	8.43 ± 0.68	8.42 ± 0.77	−0.01 (−0.23 to 0.22)	.965 <sup>d</sup>
Calprotectin	84.78 (64.74–198.79)	133.03 (87.52–322.11)	40.57 (22.01 to 67.87)	< <b>.001<sup>e</sup></b>
Neopterin	6.51 (5.30–10.95)	8.78 (5.08–21.70)	1.87 (0.12 to 4.73)	<b>.036<sup>e</sup></b>

Data were expressed as mean ± standard deviation or median with interquartile range (1st quartile–3rd quartile), trimmed mean ± standard error of the trimmed mean and also were given mean difference of the groups (95% confidence intervals)

Bold values indicated that statistically significant result (*p* < .05)

FMF familial Mediterranean fever, Wbc white blood cell, Hb hemoglobin, Plt platelets, Neu neutrophils, Lym lymphocyte, Mon monocyte, RDW red cell distribution width, MPV mean platelet volume, diff. differences, CI confidence intervals

<sup>a</sup>Welch's *t*-test

<sup>b</sup>Pearson *chi-square* test

<sup>c</sup>Yuen (robust) independent sample test

<sup>d</sup>Independent sample *t*-test

<sup>e</sup>Mann–Whitney *U* test

periods. In FMF patients, various researches have been designed to determine novel indicators to detect subclinical inflammation. In the present study, revealing the CLP and NPT availability in FMF patients to determine subclinical inflammation presence, particularly throughout the attack-free period was aimed. We found that NPT and CLP were significantly higher than healthy controls in this study. They can be valuable markers for FMF patients.

CLP is a cytokine that has been identified recently that could be found in healthy individuals' cytoplasm. Nevertheless, it increases responsively following inflammation and tissue trauma [9]. Various researches have determined that fecal CLP measurement is beneficial for inflammatory bowel disease's (IBD) early diagnosis [10]. Fecal CLP levels are significantly associated with IBD's clinical or endoscopic disease activity. Similarly, various inflammatory disorders

**Table 2** The comparisons of the calprotectin and neopterin by gender, family history, and colchicine response in the FMF patients

	Categories		p-value
Sex	Female (n=43)	Male (n=37)	
Calprotectin	125.57 (87.79–303.07)	143.93 (85.56–377.18)	.851
Neopterin	8.62 (5.14–19.71)	13.74 (5.03–22.97)	.750
Family history	No (n=29)	Yes (n=51)	
Calprotectin	128.51 (87.30–315)	135.88 (89.39–344.15)	.881
Neopterin	13.65 (5.18–21.27)	8.73 (5.06–21.72)	.631
Colchicine response	No (n=21)	Yes (n=59)	
Calprotectin	130.19 (104.99–214.87)	143.93 (85.50–360.36)	.627
Neopterin	12.98 (5.47–21.27)	8.74 (4.89–21.72)	.768

Data were expressed as median with interquartile range (1st quartile–3rd quartile)  
p-values calculated by Mann–Whitney U test

beyond the gut present with increased serum and/or tissue CLP concentrations, as in psoriasis, Sjogren’s syndrome (SS), adult-onset Still’s disease, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Behçet’s disease (BD), vasculitides, gout, spondyloarthritis (SpA), periodontitis, and malignancies [7, 11–13]. Upcoming evidence suggests that CLP may also play a role in cancer development, obesity, dementia progression, and atherosclerotic plaque formation [14]. Lately, plasma and serum concentrations of CLP were suggested as prognostic biomarkers of value to assess the disease course and outcome

in hospitalized COVID-19 patients [15]. However, its part in rheumatic disease pathogenesis has been highlighted only in recent years. In pediatric rheumatic diseases, including juvenile idiopathic arthritis (JIA), Henoch-Schönlein purpura, Kawasaki disease, cryopyrin-associated periodic syndromes, and the FMF, using CLP as a marker has already been examined [16]. Lately, some researchers have put forward a protein, CLP, as a potential biomarker of disease activity. The CLP serum levels indicate the disease activity in JIA patients

**Table 3** The correlation coefficients of the parameters in the FMF patients

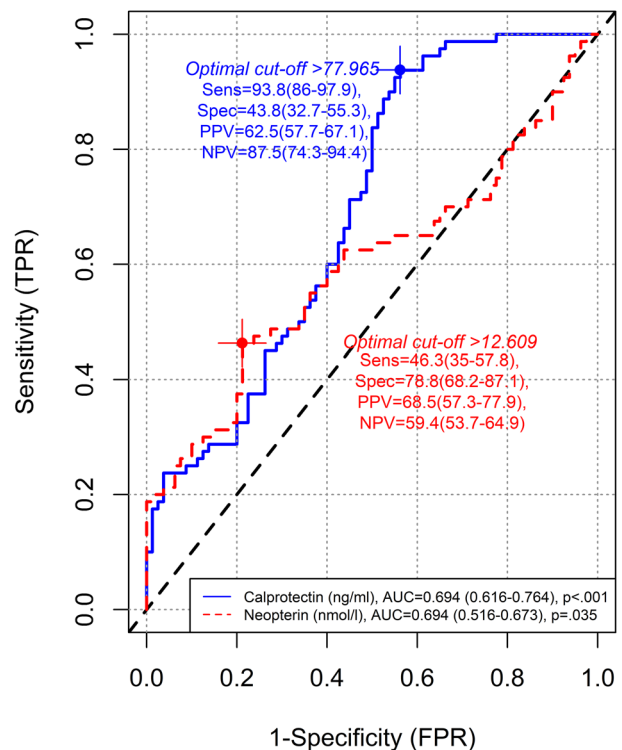
Parameters	Calprotectin		Neopterin	
	<i>r<sub>s</sub></i>	<i>p</i> -value	<i>r<sub>s</sub></i>	<i>p</i> -value
Age (years)	−0.089	.264	−0.082	.305
Attack frequency	0.112	.324	0.066	.561
Disease time	0.006	.955	−0.045	.692
WBC (10 <sup>9</sup> /L)	−0.089	.433	−0.178	.113
Hb (g/dL)	−0.053	.638	−0.029	.800
PLT (10 <sup>9</sup> /L)	0.105	.353	−0.039	.729
Neu (10 <sup>9</sup> /L)	−0.051	.652	−0.113	.320
Lym (10 <sup>9</sup> /L)	−0.177	.116	−0.205	.068
Mon (10 <sup>9</sup> /L)	0.064	.575	−0.019	.866
RDW (%)	0.054	.632	−0.087	.441
MPV (fL)	−0.190	.091	−0.032	.780
CRP (mg/L)	−0.017	.883	−0.113	.318
ESR (mm/hour)	0.115	.308	−0.050	.658
Calprotectin	–	–	0.797	<b>&lt;.001</b>
Neopterin	0.797	<b>&lt;.001</b>	–	–

Bold values indicated a statistically significant relationship between laboratory findings (*p* < .05)

*r<sub>s</sub>*, Spearman’s rho correlation coefficient

*Wbc* white blood cell, *Hb* hemoglobin, *Plt* platelets, *Neu* neutrophile, *Lym* lymphocyte, *MON* monocyte, *RDW* red cell distribution width, *MPV* mean platelet volume

**ROC Curves of Calprotectin (ng/ml) and Neopterin (nmol/l) for discriminating FMF patients from healthy controls**



**Fig. 1** The receiver operating characteristics (ROC) curves of calprotectin and neopterin in the discriminate of FMF from healthy controls

and might identify subclinical disease activity [17, 18]. Several new researches have revealed that CLP is a possibly more sensitive biomarker of rheumatic diseases compared to inflammatory markers, including ESR and CRP. Elevated CLP are also correlated to worse results in RA and, in small part, SpA [19]. Therefore, they may play a part in the treatment decision, particularly in reducing the treatment [20, 21]. Indeed, serum levels of CLP have demonstrated rapid and more pronounced responses to inflammatory changes than classical inflammatory indices; therefore, they represent a sensitive marker for the assessment of treatment response [22]. As an indication of treatment response using conventional and biological disease-modifying anti-rheumatic drugs in RA, CLP serum levels may decrease. CLP decreases quickly when effectively treated with TNF- $\alpha$ -inhibitors and secukinumab in both axial and peripheral SpA [23]. CLP can demonstrate minimal residual inflammation and predict disease relapse in several autoimmune disorders, such as SLE, BD, and anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV). In addition, elevated CLP serum levels are correlated to some severe manifestations of autoimmune diseases, including glomerulonephritis in SLE and AAV and lung fibrosis in SSc, so that it can determine patients with a need for accurate screening and close follow-up [24, 25]. Furthermore, many researchers suggest CLP in the prognosis of various inflammation-based diseases and the assessment of subclinical inflammation in patients. Thus, CLP should be included in a panel of markers to examine the inflammation state. Elevated CLP levels, even in periods without an attack, can show that there may be a persistent subclinical inflammation in FMF patients. In prior research, including FMF patients, high CLP was found. Besides, serum CLP concentrations in FMF patients before colchicine therapy were higher than after treatment [26–28]. Nevertheless, no studies have evaluated CLP along with NPT together in FMF patients. Similar to studies in the literature, we concluded here that CLP might be used as a biomarker.

NPT is in the family of pteridines and is included in several oxidation–reduction reactions in the body [8, 29]. NPT concentrations also increase during pregnancy, are very elevated in neonates, and gradually decrease with age. In the elderly, elevated NPT concentrations have also been stated to predict mortality. The NPT level reflects the activation stage of the cellular immune system, vital in the pathogenesis and progression of many diseases. Cell-mediated immunity is also activated in various neurological, cardiovascular, infectious, autoimmune diseases, and malignancies, proving this marker's increased levels [30]. Previous studies found NPT increases in the body fluids of patients such as polycystic ovary syndrome, RA, SpA, diabetes mellitus, SLE, multiple sclerosis, celiac disease, and IBD [31–34]. Elevated urinary or serum NPT levels suggest a poor prognosis in cancer patients [35]. These

data have gained importance in an era characterized by new immunotherapeutic agents' advent. Notably, NPT has been correlated to the clinical progression of patients infected with SARS-CoV [36]. NPT was also related to disease activity in RA patients. It was found that male patients with RA had a higher NPT level than female patients and that NPT plasma levels were significantly correlated with age [37]. NPT and CLP were significantly higher than healthy controls in this study, but there was no statistically significant difference between CLP, NPT, age, attack frequency, disease duration, and laboratory findings in FMF patients. Therefore, NPT predicts inflammatory status and prognosis, monitors disease progression and the effects of therapeutic interventions, and predicts exposure to toxic industrial substances. Moreover, NPT can also indicate oxidative stress because its synthesis is demonstrated to be associated with reactive oxygen species (ROS) production. Concisely, NPT is an oxidized form of dihydroneopterin throughout antioxidant reactions, where elevated NPT levels are related to an increased ROS production and oxidative stress induction throughout intense cellular immunity activation [8]. The increased oxidative stress has been demonstrated in FMF by various researches. Furthermore, ROS secreted in activated inflammatory cells through cytokines results in oxidative stress in FMF patients [5]. Nonetheless, more research examining oxidative stress markers is required to confirm this prediction. To the best of our knowledge, no study has yet been performed to investigate the serum NPT levels in patients with FMF.

The value of RDW in predicting adverse outcomes in malignant tumors, autoimmune diseases, and cardiovascular and thrombotic disorders has been reported by previous research [38]. Several studies showed that RDW was significantly higher in symptom-free FMF patients than the control groups [39, 40]. Similar to the literature, it was noteworthy that RDW levels were significantly higher in FMF patients. When considered together, the present results suggest that these parameters may be purposed as novel diagnostic parameters in FMF patients. In the future, when breakpoints are set in various rheumatic pathologies, they may replace classical markers of systemic inflammation. Therefore, they may become a potential marker to assess the inflammation status and, consequently, guide about improving risk stratification strategies for inflammation-based disorders.

However, the present study has several limitations regarding the limited number of patients and the lack of oxidative stress markers and proinflammatory cytokine measurements. The fact that all the patients were in an attack-free period was another limitation. Thus, performing further studies comparing CLP and NPT in patients during the attack and non-attack periods would be better. Accordingly, more studies, including larger populations, are required to enlighten

the CLP and NPT's roles in FMF pathogenesis and the treatment class effects on this pathway.

## Conclusion

CLP, NPT, and RDW levels show a significant difference in FMF patients than in the control group, so these molecules can be valuable marker candidates to be used in the follow-up of subclinical inflammation in FMF patients.

**Acknowledgements** We acknowledge our patients for the consent to publish this research for teaching medical professionals to help their patients better. The authors would like to thank the Selcuk University for funding this study.

**Author contribution** All authors have contributed significantly, and all authors agree with the content of the manuscript. All members of the group met the full criteria and requirements for authorship.

**Funding** This study was funded by the Selcuk University Faculty of Medicine Hospital (Proje No: 21401003).

**Data availability statement** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Declarations

The paper has not been presented, published, or submitted for publication elsewhere.

**Ethics approval** The present study was designed as a prospective study and was approved by the ethics committee of the Selçuk University Faculty of Medicine Hospital with the decision dated 30.12.2020 and numbered 2020/573.

**Consent to participate** All participants were written informed according to the principles of the Declaration of Helsinki.

**Conflict of interest** The authors declare no competing interests.

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