ORIGINAL ARTICLE

Serum levels of angiogenic cytokines in psoriatic arthritis and SAPHO syndrome

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KEY WORDS

epidermal growth factor, fibroblast growth factor, psoriatic arthritis, SAPHO syndrome, vascular endothelial growth factor

ABSTRACT

INTRODUCTION Angiogenesis is involved in the pathogenesis of arthritis.

OBJECTIVES The aim of the study was to assess the serum levels of selected angiogenic cytokines and their association with clinical presentation in patients with psoriatic arthritis (PsA) and SAPHO syndrome.

PATIENTS AND METHODS We studied 98 patients: 80 with PsA and 18 with SAPHO syndrome. The following data were recorded: age, sex, disease duration, joint involvement, type of psoriasis, nail involvement, and treatment. The following indices used to assess the activity of PsA and SAPHO were measured: PASI, BASDAI, BASFI, BASMI, BASG, and VAS pain. We determined erythrocyte sedimentation rate, C-reactive protein (CRP), and platelet count. The serum levels of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic and acidic fibroblast growth factors (FGFb and FGFa) were determined using an enzyme-linked immunosorbent assay.

RESULTS In patients with PsA, VEGF levels were positively correlated with CRP ($P = 0.04$), BASFI ($P = 0.03$), and disease duration ($P = 0.007$). No differences were found between patients with and without nail psoriaris in the VEGF or EGF levels ($P = 0.32$ and $P = 0.85$, respectively). There were no differences between patients with the peripheral and axial forms of arthritis in VEGF or EGF levels ($P = 0.56$ and $P = 0.28$, respectively). No significant correlations were observed between EGF and FGF levels and clinical presentation in patients with PsA. In patients with SAPHO, no significant correlations were found between angiogenic cytokine levels and clinical presentation.

CONCLUSIONS Our data suggest a role of VEGF in the pathogenesis of PsA. Further studies are required to better understand the role of angiogenic cytokines in PsA.

INTRODUCTION Angiogenesis plays an important role in the pathogenesis of chronic inflammatory joint diseases.[1-9] Increased angiogenesis stimulates the formation of new vessels in the articular synovium in patients with rheumatoid arthritis (RA).[7,10] Moreover, angiogenesis participates in endothelial dysfunction in patients with RA and those with chronic kidney disease.[11,12] Synovial angiogenesis is regulated by a combination of tissue hypoxia, upregulation of endothelial growth factors, and downregulation of the inhibitors of angiogenesis.[1,2,10] Vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (FGFb), and acidic fibroblast growth factor (FGFa) are cytokines that play a key role in the initiation of angiogenesis.[1,2,10]

In chronic inflammatory joint diseases, inflammation may regulate the expression of VEGF.[4,5] In patients with psoriatic arthritis (PsA), it has been confirmed that vascular morphological changes are present in the skin and nail fold. It has also been suggested that similar vascular lesions are present in the synovial membrane in PsA.[13-15] Elevated serum levels of VEGF are correlated with disease activity and radiographic damage in RA, Wegener's granulomatosus, systemic lupus erythematosus, and spondyloarthropathies.[4,5,7,16,17] On the other hand, the +936 T allele in the *VEGF* gene may protect against the development of PsA.[18,19]

The potential roles of VEGF, EGF, FGFb, and FGFa in angiogenesis and correlations with disease activity in PsA and SAPHO syndrome (the acronym stands for synovitis, acne pustulosis,

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TABLE Clinical and laboratory characteristics of patients with psoriatic arthritis, SAPHO syndrome, and healthy controls

Parameter	Psoriatic arthritis (n = 80)	SAPHO syndrome (n = 18)	Healthy controls (n = 20)
age, y	50.1 ±12.0	51.0 ±11.1	48.1 ±14.0
sex, n (female/male)	43/37	17/1	12/8
disease duration, y	4.5 (2.0, 10.0)	2.0 (2.0, 4.0)	0
VAS pain, mm	45.0 (31.0, 67.0)	38.5 (34.0, 48.0)	0
PASI	3.0 (0.3, 8.0)	–	0
CRP, mg/l	3.9 (1.6, 9.2)	3.7 (1.2, 8.7)	–
hemoglobin, mmol/l	8.5 ±0.8	8.1 ±0.6	–
WBC, G/l	7.2 (5.9, 9.0)	7.0 (6.0, 9.0)	–
platelets, G/l	245.0 (224.0, 307.0)	254.9 ±71.1	–
ESR, mm/h	10.0 (6.0, 21.0)	13.0 (6.0, 30.0)	–
VEGF, pg/ml	288.6 (193.2, 551.0)	333.1 (205.0, 375.0)	300.1 (217.0, 437.6)
EGF, pg/ml	110.0 (60.0, 162.0)	138.0 (70.0, 228.0)	93.0 (45.0, 192.5)
FGFb, pg/ml	0 (0, 0)	0	0 (0, 0)
FGFa, pg/ml	0 (0, 0)	0	0 (0, 0)

Data are presented as number or mean ± standard deviation (Q1, Q3).

Abbreviations: CRP – C-reactive protein, EGF – epidermal growth factor, ESR – erythrocyte sedimentation rate, FGFa – acidic fibroblast growth factor, FGFb – basic fibroblast growth factor, PASI – Psoriasis Area and Severity Index, SD – standard deviation, VAS – visual analogue scale, VEGF – vascular endothelial growth factor, WBC – white blood count

hyperostosis, and osteitis) have not been well established yet. Therefore, the aim of this study was to assess the serum levels of selected angiogenic cytokines (VEGF, EGF, FGFb, and FGFa) and their associations with the clinical presentation of patients with PsA and SAPHO syndrome.

PATIENTS AND METHODS The study was approved by the local ethics committee of the Pomeranian Medical University in Szczecin, Poland. Informed consent was obtained from all patients. We studied a total of 98 Caucasian patients, including 80 patients with PsA and 18 with SAPHO. The control group consisted of 20 healthy volunteers matched for age and sex.

The diagnosis of PsA was made according to the CASPAR criteria (Classification Criteria for Psoriatic Arthritis),[20] while the diagnosis of SAPHO syndrome was based on the Kahn criteria.[21] The following data were recorded: age, sex, disease duration, presence of peripheral or axial joint involvement, type of skin psoriasis, nail involvement, and treatment.

In the PsA group, skin changes were assessed according to the Psoriasis Area and Severity Index (PASI).[22] The patient's pain due to the disease at the time of examination was assessed by the visual analogue scale (VAS). We assessed the following indices: Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Bath Ankylosing Spondylitis Global Index (BASG). These indices have a possible score of 0 to 10 with a higher score indicating greater disease activity. We regarded patients as having an active disease if the BASDAI score was higher than 4.[23-26]

Blood was taken for the assessment of erythrocyte sedimentation rate, C-reactive protein (CRP) (turbidimetric assay), platelet count (PLT), hemoglobin, and leukocyte count. Serum was stored at –70°C until the analysis of VEGF, EGF, FGFb, and FGFa using a sensitive sandwich enzyme-linked inmunosorbent assay (Human VEGF Immunoassay Quantikine® ELISA kit, Human EGF Immunoassay Quantikine® ELISA kit, Human FGF basic Immunoassay Quantikine® ELISA kit and Human FGF acidic Immunoassay Quantikine® ELISA kit, R&D System, United States). The system uses microplates with the walls coated with a monoclonal antibody and an enzyme-linked polyclonal antibody specific for VEGF, EGF, FGFb, or FGFa. All analyses and calibrations were performed in duplicate and were read using BioTek PowerWaveXS (Biotek Instruments, United States).

Data distributions were assessed using the Shapiro–Wilk test. Data were described as mean ± standard deviation and median (Q1, Q3). We used the rank Spearman's test to calculate correlations. The r values of correlations were determined and corresponding P-values of less than 0.05 were considered significant. The groups were compared using the Mann–Whitney U test and Kruskal–Wallis test. The statistical analysis was performed using the STATISTICA software, version 6.0.

RESULTS The clinical and laboratory characteristics of the patients and controls are presented in the **TABLE**. Serum VEGF, EGF, FGFb, and FGFa levels were similar between the patient groups and controls (P >0.05) (**FIGURES 1** and **2**).

In the study group, 75 patients were treated with disease-modifying antirheumatic drugs (37 received methotrexate, 2 received methotrexate in combination with cyclosporine A, and 36 received sulfasalazine) and 23 received only

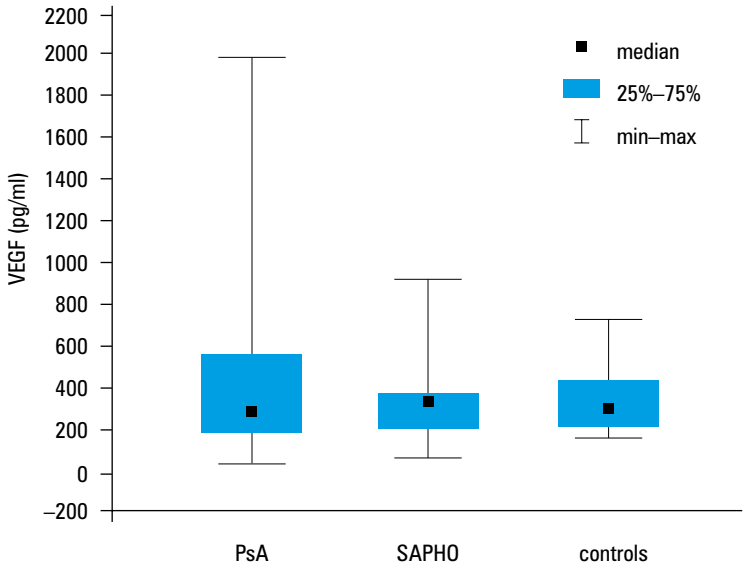

FIGURE 1 Serum concentrations of vascular endothelial growth factor (VEGF) in patients with psoriatic arthritis (PsA), SAPHO syndrome, and controls (Kruskal–Wallis test, $P = 0.97$)

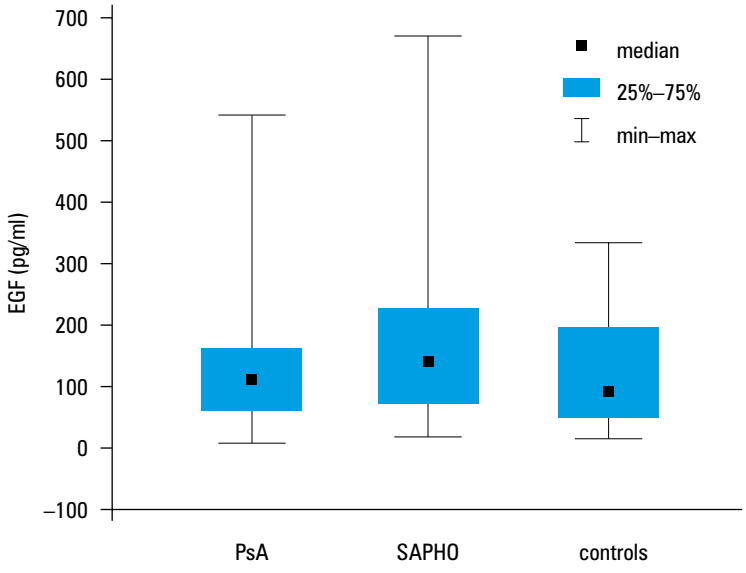

FIGURE 2 Serum concentrations of epidermal growth factor (EGF) in patients with psoriatic arthritis (PsA), SAPHO syndrome, and controls (Kruskal–Wallis test, $P = 0.43$)

nonsteroidal anti-inflammatory drugs. No patients received biological therapy.

In the PsA group, no differences were found between women and men in terms of VEGF levels ($P = 0.35$) or EGF levels ($P = 0.225$). All patients in this group had plaque-type psoriasis. No correlations were found in this group between the PASI score and the levels of VEGF ($P = 0.91$), EGF ($P = 0.63$), FGFb ($P = 0.48$), or FGFa ($P = 0.27$).

Nail psoriasis was present in 52 patients (65%). No differences were found between patients with and without nail psoriasis in terms of VEGF or EGF levels ($P = 0.32$ and $P = 0.85$, respectively).

In the PsA group, 64 patients had peripheral arthritis (44 had polyarthritis, 15 had oligoarthritis, and 5 had distal arthritis) and 16 patients had axial disease. No differences in the VEGF or EGF levels were found between patients with the peripheral and axial forms of the disease ($P = 0.56$ and $P = 0.28$, respectively). In the group of 64 patients with peripheral arthritis, there were no differences between the subgroups of patients with polyarthritis, oligoarthritis, and distal arthritis in terms of ESR, CRP, VEGF, EGF, FGFb, and FGFa levels (all $P > 0.05$).

In the PsA group, serum VEGF levels were positively correlated with CRP levels ($P = 0.04$), the BASFI score ($P = 0.03$), and disease duration ($P = 0.007$). There were no correlations between serum VEGF levels and PLT, ESR, BASDAI, BASMI, BASG, VAS, or age (all $P > 0.05$).

No correlations were found in the PsA group between EGF and CRP, ESR, PLT, BASDAI, BASFI, BASMI, BASG, VAS, age, or disease duration (all $P > 0.05$). Moreover, no differences were found in this group between the subgroups of patients receiving different treatments in terms of VEGF and EGF levels ($P > 0.05$).

Only 9 patients (11.3%) with PsA had measurable FGFb levels (above 0 ng/ml) and only 18 (22.5%) had detectable FGFa levels (above 0 ng/ml).

In patients with SAPHO syndrome, 17 patients had palmoplantar pustulosis and 1 patient had severe acne. No significant correlations were observed between VEGF and EGF levels and CRP, ESR, PLT, BASDAI, BASFI, BASMI, BASG, VAS, age, or disease duration (all $P > 0.05$). We did not calculate correlations for FGFb and FGFa in the SAPHO group because only 1 patient (5.6%) had FGFb levels over 0 ng/ml and only 4 patients (22.2%) had FGFa levels over 0 ng/ml. No differences were found in this group between the subgroups of various treatment regimens in terms of VEGF ($P = 0.99$; FIGURE 3) and EGF levels ($P = 0.80$; FIGURE 4).

DISCUSSION There are scarce data in the literature regarding a potential role of serum VEGF in disease pathogenesis and its correlations with disease activity in patients with PsA.[3,4,6,7] Moreover, no data have been found for the serum levels of VEGF or other angiogenic cytokines in SAPHO syndrome. On the other hand, there are data available on serum VEGF levels in RA patients.[7,8,10,11]

Serum VEGF may be derived from a number of sources including neutrophils, synovial fluid, inflamed synovial tissue, and platelets.[1,2] The presence of VEGF has been demonstrated in serum, synovial membrane, and synovial fluid in patients with RA. In these patients, VEGF is produced by neutrophils and its concentration in the synovial fluid is correlated with disease activity. However, no correlations have been shown between VEGF concentrations in the synovial fluid and those in serum in patients with RA.[2,7] In these patients, serum VEGF is correlated with the markers of acute disease and the number of swollen and tender joints. Previous studies reported higher serum VEGF levels in early disease in patients with RA.[7]

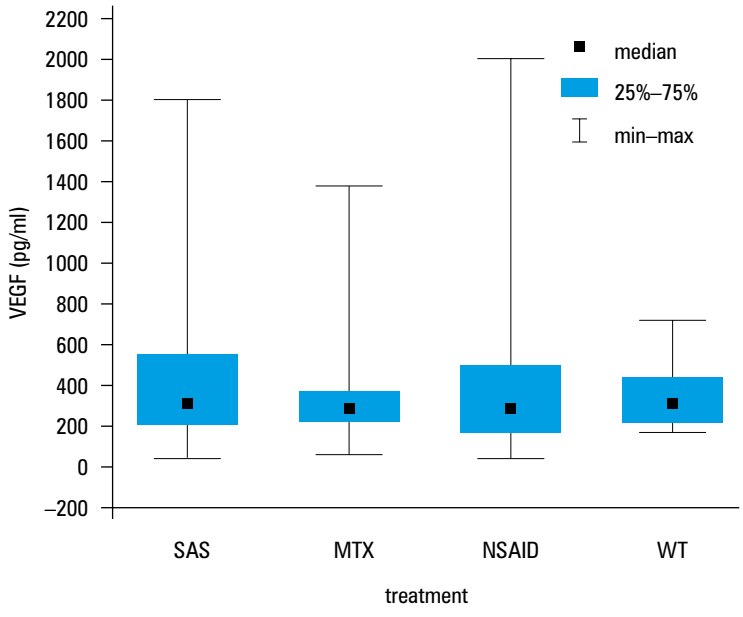

FIGURE 3 Serum concentrations of vascular endothelial growth factor (VEGF) in patients treated with sulfasalazine (SAS), methotrexate (MTX), nonsteroidal anti-inflammatory drugs (NSAIDs) and in healthy controls without treatment (WT) (Kruskal–Wallis test, $P = 0.99$)

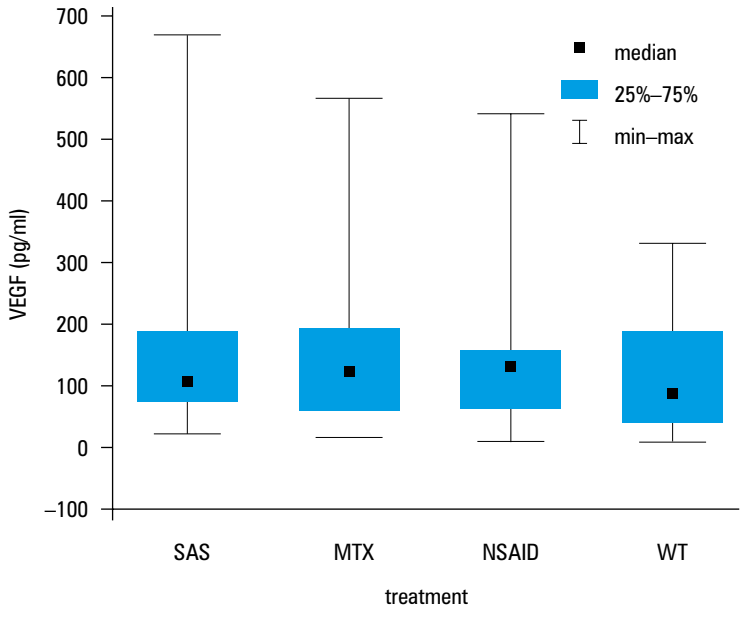

FIGURE 4 Serum concentrations of epidermal growth factor (EGF) in patients treated with sulfasalazine (SAS), methotrexate (MTX), nonsteroidal anti-inflammatory drugs (NSAIDs) and in healthy controls without treatment (WT) (Kruskal–Wallis test, $P = 0.99$)

Drouart et al.[4] demonstrated a positive correlation between serum VEGF levels and disease activity indices, such as BASDAI, ESR, and CRP in ankylosing spondylitis and PsA. In our study, we observed a correlation between serum VEGF levels and CRP only in patients with PsA. Additionally, we examined patients with SAPHO syndrome. However, no correlations were found in these patients, which may result from the small size of the group. In this study, the lack of correlations between the markers of angiogenesis and other markers of disease activity could be due to the differences in their concentrations in serum and synovial fluid.

The formation of new vessels plays an important role in the inflammatory process in the course of sacroilitis and enthesitis in ankylosing spondylitis.[4] Angiogenesis also plays a role in bone formation, especially in diseases characterized by bone formation.[2,4] Angiogenesis is stimulated by VEGF. Goldberger et al.[5] reported a positive correlation between serum VEGF levels and the BASMI in patients with ankylosing spondylitis. The absence of differences in serum VEGF levels in PsA patients with peripheral arthritis and axial disease could suggest the potential role of VEGF in synovitis and enthesitis.

In the PsA group, the lack of correlations between the markers of angiogenesis and the PASI score could be partly explained by low severity of skin lesions (mean PASI score, 7.1).

No differences between the patient groups and controls in terms of serum concentrations of angiogenic cytokines could be explained by low activity of the disease following treatment that might have affected serum concentrations of angiogenic cytokines and resulted in a decrease of their levels.

No differences between the subgroups of various treatment regimens in terms of VEGF and EGF levels could be explained by the effectiveness of treatment.

We were unable to demonstrate a relationship between the concentrations of various angiogenic cytokines and the parameters of disease activity in the SAPHO group. This may due to the small size of the group.

Our study has several limitations including a small number of study patients (especially in the SAPHO group) and no group of patients without treatment.

In conclusion, our data suggest a role of VEGF in PsA. Further studies are required to better understand the role of angiogenic cytokines in PsA.

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REFERENCES

1 Walsh DA. Angiogenesis and arthritis. Rheumatology (Oxford). 1999; 38: 103-112.

2 Ballara SC, Miotla JM, Paleolog EM. New vessels, new approaches: angiogenesis as therapeutic target in musculoskeletal disorders. Int J Exp Pathol. 1999; 80: 235-250.

3 Fink AM, Cauza E, Hassfeld W, et al. Vascular endothelial growth factor in patients with psoriatic arthritis. Clin Exp Rheumatol. 2007; 25: 305-308.

4 Drouart M, Saas P, Billot M, et al. High serum vascular endothelial growth factor correlates with disease activity of spondylarthropaties. Clin Exp Immunol. 2003; 132: 158-162.

5 Goldberger C, Dulak J, Duftner C, et al. Vascular Endothelial Growth Factor (VEGF) in ankylosing spondylitis – a pilot study. Wien Med Wochenschr. 2002; 152: 233-225.

6 Creamer D, Allen M, Jaggar R, et al. Mediation of systemic vascular hyperpermeability in severe psoriasis by circulating vascular endothelial growth factor. Arch Dermatol. 2002; 138: 791-796.

7 Ballara S, Taylor PC, Reusch P, et al. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis Rheum. 2001; 44: 2055-2064.

8 Taylor PC. Serum vascular markers and vascular imaging in assessment of rheumatoid arthritis disease activity and response to therapy. Rheumatology (Oxford). 2005; 44: 721-728.

9 Paavonen K, Mandelin J, Partanen T, et al. Vascular endothelial growth factors C and D and their VEGFR-2 and 3 receptors in blood and lymphatic vessels in healthy and arthritic synovium. J Rheumatol. 2002; 29: 39-45.

10 Paleolog EM, Fava RA. Angiogenesis in rheumatoid arthritis: implications for future therapeutic strategies. Springer Semin Immunopathol. 1998; 20: 73-94.

11 Surdacki A, Martens-Lobenhoffer J, Wloch A, et al. Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. Arthritis Rheum. 2007; 56: 809-819.

12 Wątorek E, Paprocka M, Duś D, et al. Endostatin and vascular endothelial growth factor: potential regulators of endothelial progenitor cell number in chronic kidney disease. Pol Arch Med Wewn. 2011; 121: 296-302.

13 Butt C, Lim S, Greenwood C, Rahman P. VEGF, FGF1, FGF2 and EGF gene polymorphism and psoriatic arthritis. BMC Musculoskelet Disord. 2007; 8: 1.

14 Fearon U, Griosios K, Fraser A, et al. Angiopoetins, growth b factors, and vascular morphology in early arthritis. J Rheumatol. 2003; 30: 260-268.

15 Veale DJ, Ritchlin C, FitzGerald O. Immunopathology of psoriasis and psoriatic arthritis. Ann Rheum Dis. 2005; 64: ii26- ii29.

16 Li CG, Reynolds I, Ponting JM, et al. Serum levels of vascular endothelial growth factor (VEGF) are markedly elevated in patients with Wegener's granulomatosis. Br J Rheumatol. 1998; 37: 1303-1336.

17 Navarro C, Candia-Zúñiga L, Silveira LH, et al. Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. Lupus. 2002; 11: 21-24.

18 Butt C, Lim S, Greenwood C, Rahman P. VEGF, FGF1, FGF2 and EGF gene polymorphisms and psoriatic arthritis. BMC Musculoskelet Disord. 2007; 8: 1.

19 Bowes J, Ho P, Flynn E, et al. Investigation of IL1, VEGF, PPARG and MEFV genes in psoriatic arthritis susceptibility. Ann Rheum Dis. 2012; 71: 313-314.

20 Taylor W, Gladman D, Helliwell P, et al.; CASPAR Study Group. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum. 2006; 54: 2665-2673.

21 Kahn MF, Khan MA. The SAPHO syndrome. Bailliéres Clin Rheumatol. 1994; 8: 333-362.

22 Ashcroft DM, Li Wan Po A, Williams HC, et al. Clinical measures of disease severity and outcome in psoriasis: a critical appraisal of their quality. Br J Dermatol. 1999; 141: 185-191.

23 Jenkinson TR, Mallorie PA, Whitelock HC, et al. Defining spinal mobility in ankylosing spondylitis (AS). The Bath AS Metrology Index. J Rheumatol. 1994; 21: 1694-1698.

24 Calin A, Jones SD, Garrett SL, Kennedy LG. Bath Ankylosing Spondylitis Functional Index. Br J Rheumatol. 1995; 34: 793-794.

25 Garrett S, Jenkinson T, Kennedy LG, et al. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol. 1994; 21: 2286-2291.

26 Jones SD, Steiner A, Garrett SL, Calin A. The Bath Ankylosing Spondylitis Patient Global Score (BAS-G). Br J Rheumatol. 1996; 35: 66-71.

ARTYKUŁ ORYGINALNY

Ocena stężeń w surowicy cytokin angiogennych u chorych na łuszczycowe zapalenie stawów i zespół SAPHO

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SŁOWA KLUCZOWE

czynnik wzrostu fibroblastów, czynnik wzrostu śródbłonka naczyń, naskórkowy czynnik wzrostu, łuszczycowe zapalenie stawów, zespół SAPHO

STRESZCZENIE

WPROWADZENIE Angiogeneza bierze udział w patogenezie zapalenia stawów.

CELE Celem pracy była ocena stężeń w surowicy wybranych angiogennych cytokin i ich związku z obrazem klinicznym u chorych na łuszczycowe zapalenie stawów (ŁZS) i zespół SAPHO.

PACJENCI I METODY Badaniami objęto 98 chorych: 80 chorych na ŁZS i 18 chorych na SAPHO. Zebrano następujące dane: wiek, płeć, czas trwania choroby, zajecie stawów, typ łuszczycy, łuszczycowe zmiany paznokci oraz leczenie. Oceniano następujące wskaźniki aktywności ŁZS i SAPHO: PASI, BASDAI, BASFI, BASMI, BASG i VAS. Oznaczono OB, stężenie białka C-reaktywnego (*C-reactive protein* – CRP) oraz płytki krwi. Metodą ELISA w surowicy badanych oznaczono: czynnik wzrostu śródbłonka naczyń (*vascular endothelial growth factor* – VEGF), naskórkowy czynnik wzrostu (*epidermal growth factor* – EGF), zasadowy i kwasowy czynnik wzrostu fibroblastów (*basic and acidic fibroblast growth factors* – FGFb i FGFa).

WYNIKI U chorych na ŁZS wykazano dodatnią korelację między stężeniem VEGF i CRP ($p = 0,04$), wartością BASFI ($p = 0,03$) i czasem trwania choroby ($p = 0,007$). Nie wykazano różnic między grupą chorych z łuszczycą paznokci i bez niej w stężeniu VEGF ($p = 0,32$) i EGF ($p = 0,85$). Nie wykazano różnic między grupą chorych z obwodową i osiową postacią zapalenia stawów w stężeniu VEGF ($p = 0,56$) EGF ($p = 0,28$). Nie wykazano istotnych korelacji między stężeniem EGF i FGF a obrazem klinicznym ŁZS. U chorych na SAPHO nie wykazano istotnych korelacji między stężeniem angiogennych cytokin a obrazem klinicznym.

WNIOSKI Wyniki sugerują, że VEGF bierze udział w patogenezie ŁZS. Konieczne są dalsze badania, żeby lepiej zrozumieć rolę angiogennych cytokin w ŁZS.

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