RESEARCH LETTER

Serum VEGF, EGF, basic FGF, and acidic FGF levels and their association with disease activity and extra-articular symptoms in ankylosing spondylitis

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Introduction Ankylosing spondylitis (AS) is a seronegative spondyloarthritis and a chronic inflammatory disease.[1] The role of angiogenesis has not been clearly established in patients with AS.[2-6] Cytokines involved in angiogenesis include, among others, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and acidic fibroblast growth factor (aFGF).[7] The source of VEGF in serum is unclear; it may be derived from platelets, inflamed synovial tissue, or other sources.[7]

Serum interleukin 6 (IL-6) may be a marker of disease activity in inflammatory joint diseases. Serum IL-6 levels were shown to be elevated in patients with AS, along with factors associated with poor prognosis such as positive HLA-B27, inflammatory low back pain, and arthritis. Correlation between serum IL-6 levels, serum C-reactive protein (CRP) levels, and erythrocyte sedimentation rate (ESR) were shown in patients with AS.[8] There are also some data showing that interleukin 23 (IL-23) plays an important role in the pathogenesis of spondyloarthritis.[9]

To our knowledge, there have been no published studies evaluating serum EGF, bFGF, and aFGF levels or their correlations with disease activity and extra-articular symptoms in AS. Therefore, the aim of this study was to examine the serum levels of selected angiogenic cytokines (VEGF, EGF, bFGF, and aFGF) and their association with disease activity and extra-articular symptoms in patients with AS.

Patients and methods This study was approved by an institutional review board at Pomeranian Medical University in Szczecin, Poland. Informed consent was obtained from all patients. We studied 80 Caucasian patients with AS. The diagnosis of AS was made according to the modified New York criteria.[1] The following data were recorded: age, sex, disease duration, presence of peripheral arthritis, HLA-B27 positivity, and extra-articular symptoms including uveitis, inflammatory bowel disease (IBD), and psoriasis. Patients were treated with nonsteroidal anti-inflammatory drugs (NSAIDs) only or in combination with sulfasalazine (2g/d) or methotrexate (15 mg/wk).

The patient's pain due to the disease at the time of examination was assessed by a visual analogue scale (VAS). We also assessed the following indices: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Metrology Index (BASMI). These indices have a possible score of 0 to 10, with a higher score indicating greater disease activity. We regarded patients as active if the BASDAI score was higher than 4.[10,11] Blood samples were collected for the assessment of ESR, CRP (turbimetric nephelometry, rate reaction), and platelet count (PLT). The Ankylosing Spondylitis Disease Activity Score (ASDAS) was assessed using CRP and ESR parameters. The ASDAS-CRP and ASDAS-ESR were calculated using an online calculator available at the website of the Assessment of SpondyloArthritis international Society.

Serum was stored at –70°C until analysis. VEGF, EGF, bFGF, aFGF, IL-6, and IL-23 were analysed by means of a sensitive sandwich enzyme-linked immunosorbent assay using the Human VEGF Immunoassay Quantikine® ELISA kit, Human EGF Immunoassay Quantikine® ELISA kit, Human FGF basic Immunoassay Quantikine® ELISA kit, Human FGF acidic Immunoassay Quantikine® ELISA kit, Human

TABLE 1 Demographic, clinical, and laboratory characteristics of patients with ankylosing spondylitis and healthy controls

Parameter		Ankylosing spondylitis (n = 80)	Healthy controls (n = 21)
age, y		50.9 ±12.8	48.2 ±13.5
sex (female/male), n		16/64	8/13
disease duration, y		15.0 (9.0–23.0)	0
HLA-B27 (+), n (%)		70 (92.1)[a]	0 (0)
VAS pain, mm		60.0 (40.0–70.0)	0.0
BASDAI		4.86 (3.4–6.6)	0
BASMI		4.0 (4.0–3.6)	0
CRP, mg/l		9.5 (4.5–15.6)	0.0
ESR, mm/h		16.0 (7.0–30.0)	8.0 (2.0–16.0)
ASDAS-CRP		2.8 (2.3–3.2)	–
ASDAS-ESR		2.5 (2.0–2.9)	–
PLT, $10^3/mm^3$		260.5 (210–340)	225.8 (210–242)
VEGF, pg/ml		396.1 (221.6–676)	238.1 (209.5–420.9)
EGF, pg/ml		121.0 (78.0–187.0)	88.0 (38.0–172.0)
bFGF, pg/ml		0.0 (0.0–0.0)	0.0 (0.0–0.0)
aFGF, pg/ml		0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-23, pg/ml		0.3 (0.0–2.8)	0.0 (0.0–0.0)
IL-6, pg/ml		4.4 (2.5–5.9)	1.1 (0.7–1.4)
peripheral arthritis, n (%)		37 (46.3)	0 (0)
uveitis, n (%)		17 (21.3)	0 (0)
IBD, n (%)		7 (8.8)	0 (0)
history of psoriaris, n (%)		1 (1.25)	0 (0)
treatment, n (%)	NSAIDs only	17 (21.3)	0
	NSAIDs and sulfasalazine	48 (60)	0
	NSAIDs and methotrexate	15 (18)	0

Data are presented as mean ± standard deviation or median (interquartile range) unless otherwise stated.

a the measurement was not performed in 4 patients

Abbreviations: aFGF, acidic fibroblast growth factor; ASDAS-CRP, ankylosing spondylitis disease activity score using CRP; ASDAS-ESR, ankylosing spondylitis disease activity score using ESR; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; bFGF, basic fibroblast growth factor; CRP, C-reactive protein; EGF, epidermal growth factor; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; IL-6, interleukin 6; IL-23, interleukin 23; NSAIDs, nonsteroidal anti-inflammatory drugs; PLT, platelet count; VAS pain, visual analogue scale of patient's pain; VEGF, vascular endothelial growth factor

IL-6 Immunoassay Quantikine® ELISA kit, and Human IL-23 Immunoassay Quantikine® ELISA kit, respectively (R&D Systems, Minneapolis, Minnesota, United States). The system uses microplates with the wells coated with a monoclonal antibody and an enzyme-linked polyclonal antibody specific to VEGF, EGF, bFGF or aFGF, IL-6, or IL-23. All analyses and calibrations were performed in duplicate and were read using a BioTek PowerWaveXS (BioTek, Winooski, Vermont, United States).

The controls were 21 healthy volunteers, matched to patients for age and sex.

Data distributions were assessed using the Shapiro–Wilk test. Data were presented as mean ± standard deviation and median (Q1, Q3). We used the rank Spearman's test to calculate correlations. *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 and Kruskal–Wallis tests. To assess parameters associated with serum VEGF, EGF, bFGF, and aFGF levels, the Pearson's χ^2 test, logistic regression analysis, and step-wise analysis were performed.

The level of significance was set at a *P* value of less than 0.05. Statistical analysis was performed using STATISTICA version 6.0 (StatSoft Inc., Tulsa, Oklahoma, United States).

Results The clinical and laboratory characteristics of the patients and controls are presented in TABLE 1. Serum VEGF and bFGF levels were higher in AS patients than in controls ($P = 0.02$ and $P = 0.03$, respectively). Serum EGF and aFGF levels were similar in AS and controls ($P = 0.07$ and $P = 0.41$, respectively).

No differences were found between women and men in terms of VEGF ($P = 0.83$); however, men had higher EGF levels than women ($P = 0.02$). In addition, no differences were found between the various treatment regimens in terms of VEGF ($P = 0.90$) and EGF levels ($P = 0.48$) in AS patients.

Serum VEGF levels were positively correlated with disease duration ($r = 0.27$; $P = 0.01$). Additionally, serum VEGF levels were positively correlated with ESR ($r = 0.21$; $P = 0.05$) and BASMI ($r = 0.21$; $P = 0.06$).

Serum EGF levels were negatively correlated with IL-23 levels ($r = –0.29$; $P = 0.02$); serum bFGF levels were negatively correlated with PLT ($r = –0.26$; $P = 0.04$); and serum aFGFa levels were negatively correlated with disease duration ($r = –0.26$; $P = 0.04$) (data not shown).

There were no correlations of serum angiogenic cytokines with CRP, IL-6, IL-23, VAS, BASDAI, BASMI, ASDAS-CRP, and ASDAS-ERS (all *P* values >0.05) (data not shown).

The results of the univariate and multivariate logistic regression analyses and step-wise analysis showed no associations between serum VEGF and EGF levels in AS patients after adjustment for CRP, VAS, BASDAI, uveitis, IBD, peripheral arthritis, and use of NSAIDs. The adjusted odds ratio (OR) for serum VEGF levels of 420.9 pg/ml or higher in AS patients with a BASMI of 4 or higher was 2.89 (95% confidence interval [CI], 1.16–7.23; $P = 0.02$). The adjusted OR for serum EGF levels of 172 pg/ml or higher in AS patients on sulfasalazine treatment was 0.21 (95% CI, 0.08–0.59; $P = 0.003$). Finally, the adjusted OR for serum EGF levels of 172 pg/ml or higher in AS patients on methotrexate treatment was 4.7 (95% CI, 1.24–17.8; $P = 0.02$) (data not shown).

Discussion There are scarce data on the potential role of serum VEGF levels in patients with AS.[2-6] In the available literature, we did not find

any study evaluating serum EGF, bFGF, or aFGF levels in AS.

In our study, we confirmed the observations of other authors who reported that patients with AS had higher serum VEGF levels compared with controls.[2,3,6] Previous studies demonstrated a positive correlation between serum VEGF levels and disease activity indices such as BASDAI, ESR, and CRP in patients with spondyloarthritis.[3,4,6] Likewise, other investigators have demonstrated positive correlations between serum VEGF and CRP and BASDAI in AS.

In our previous study, we found a positive correlation between serum VEGF levels and CRP in patients with psoriatic arthritis.[12] However, in this study, we did not demonstrate a relationship in the AS group between the concentrations of angiogenic cytokines and disease activity measured by the VAS, BASDAI, ASDAS-CRP, ASDAS-ESR, and CRP.

In our previous study, we found a positive correlation of serum IL-6 levels with CRP and ESR, but no correlation of serum IL-23 levels with CRP and ESR in patients with AS.[8] In the current study, we did not demonstrate a relationship between the concentrations of angiogenic cytokines and disease activity measured by IL-6 and IL-23.

As in other studies, in the present study, we did not demonstrate a relationship between serum VEGF levels and uveitis.[6] In addition, we showed no relationship between serum VEGF levels and IBD and peripheral arthritis in patients with AS. We also observed no correlations between extra-articular symptoms and serum EGF, FGF, bFGF, and aFGF levels.

Poddubnyy et al[5] demonstrated that elevated serum levels of VEGF are a highly specific predictor of radiographic spinal progression. Goldberger et al[2] showed a significant correlation between plasma VEGF levels and the BASMI score in patients with AS. In our study, elevated serum VEGF levels were associated with an increased risk of a higher BASMI score, the value of which is affected by spinal progression.

The lack of difference in VEGF levels between the different treatment groups suggests a similar effect of these drugs on the inhibition of angiogenesis stimulated by VEGF in AS.

Sulfasalazine therapy was associated with a reduced risk of high serum EGF levels, while methotrexate therapy was associated with an increased risk of high serum EGF levels in AS. The results of our study indicate a lack of the effect of MTX on the inhibition of angiogenesis stimulated by EGF.

Conclusions Serum VEGF levels were increased in patients with AS. There were no correlations of serum angiogenic cytokines with disease activity and extra-articular symptoms in this patient group. Increased serum VEGF levels were associated with progression of the disease assessed by the BASMI. Serum levels of EGF, aFGF, and bFGF do not play a significant role in AS.

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