



Hepatocyte growth factor is a potential biomarker for osteoproliferation and osteoporosis in ankylosing spondylitis

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Received: 5 May 2018 / Accepted: 26 September 2018 / Published online: 10 October 2018
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Abstract

Summary We explored relations between serum hepatocyte growth factor (HGF), disease activity, osteoproliferation, and bone mineral density (BMD) in ankylosing spondylitis (AS), in comparison with healthy controls. HGF was increased especially in male AS patients and smokers and associated with both lower BMD and more chronic radiographic changes in the spine.

Introduction Ankylosing spondylitis (AS) is characterized by both osteoproliferation and increased bone loss. Biomarkers are requested to predict the processes. The aims of this study were to compare serum levels of hepatocyte growth factor (HGF), matrix metalloproteinase-3 (MMP-3), and vascular endothelial growth factor (VEGF) in AS patients with healthy controls (HC) and to explore the associations with disease activity, osteoproliferation, and bone mineral density (BMD).

Methods Serum from AS patients (modified NY-criteria) and HC was analyzed for HGF, MMP-3, and VEGF with ELISA. Disease activity parameters were collected. Osteoproliferation was assessed with modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) and BMD was measured in femoral neck.

Results Totally, 204 AS patients and 80 sex and age matched HC were included. Serum HGF was higher in the AS patients compared with the HC, whereas serum MMP-3 and VEGF were not. Serum HGF was also higher in smokers and in the male AS patients positively correlated with age, BASMI, and mSASSS, and negatively correlated with BMD. The biomarkers were all positively associated with ESR, CRP, and WBC. In multiple linear regression analysis serum HGF remained associated with higher mSASSS and lower BMD, after adjusting for age, sex, CRP, smoking, and body mass index.

Conclusions Serum HGF was increased in male AS patients and associated with higher mSASSS and lower BMD. In addition, serum HGF was positively associated with risk factors for osteoproliferation such as age, CRP and smoking. HGF could be a potential biomarker of importance for the bone metabolism in AS.

Trial registration NCT00858819.

Keywords Ankylosing spondylitis · Hepatocyte growth factor (HGF) · Matrix metalloproteinase-3 (MMP-3) · Osteoporosis · Osteoproliferation · Vascular endothelial growth factor (VEGF)

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterized by sacroiliitis, accompanied by

inflammation of the spine and the entheses. Involvement of peripheral joints is common. Extra skeletal manifestations include anterior uveitis and intestinal inflammation. AS is also characterized by osteoproliferation, which can be seen as an enhanced reparative response to inflammation, mechanical stress or micro-damage. At the same time, AS patients are at increased risk of developing osteoporosis.

Disease activity assessment in AS is limited to patient self-reported questionnaires and standard laboratory measures such as acute phase reactants. Erythrocyte sedimentation (ESR) and C-reactive protein (CRP) do not entirely reflect the disease process in patients with AS [1]. There is a need for biomarkers assessing disease activity, disease progression, and response to therapy.

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Hepatocyte growth factor (HGF), originally discovered as a mitogen of hepatocytes, is a multi-functional cytokine involved in embryogenesis, organogenesis, wound healing, and tissue repair, but it also plays a role in tumorigenesis and cancer invasion [2]. HGF is mainly expressed by stromal cells and binds to receptor-tyrosine kinase c-MET. HGF-MET signaling leads to a variety of cellular responses including morphogenesis, proliferation, cell survival, regeneration, and tissue protection in inflammatory diseases [3, 4]. In the immune system, HGF mainly has anti-inflammatory effects [5]. Interestingly, c-MET is also expressed on osteoblasts and osteoclasts indicating that HGF may regulate bone metabolism [6].

Matrix metalloproteinases (MMPs) are proteins involved in the breakdown of extracellular matrix proteins and active during tissue remodeling in normal physiological processes as well as disease processes like arthritis and tumor metastasis. MMPs are produced in response to pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) [7, 8]. There have been promising results on the association between disease activity in AS and MMP-3 [9].

Vascular endothelial growth factor (VEGF) is a signaling protein involved in angiogenesis, an important process in the pathogenesis of chronic inflammatory disorders such as rheumatoid arthritis [10]. Histopathological examinations of sacroiliitis and peripheral arthritis in AS have also demonstrated increased vascularity in the synovial tissues, indicating the importance of angiogenesis in this disease [11, 12]. Earlier studies have reported significant correlations between serum VEGF and disease activity in AS [13, 14].

Aims

The aims of this study were to compare the serum levels of HGF, MMP-3, and VEGF in AS patients with healthy controls and to explore the associations between the biomarkers and disease activity, osteoproliferation, and bone mineral density in the AS patients.

Materials and methods

Patients and controls

A total of 204 patients were recruited from three rheumatologic sites in the west of Sweden, the Sahlgrenska University Hospital in Gothenburg, and the Rheumatology Departments in Borås and Alingsås. All patients fulfilled the modified New York criteria for AS [15]. Exclusion criteria were psoriasis, inflammatory bowel disease, dementia, pregnancy, and other concomitant rheumatologic diseases. Due to extensive

questionnaires included in the study, patients with language difficulties were also excluded.

The healthy control group consisted of 80 blood donors, who were recruited when giving blood at the Sahlgrenska University Hospital. All blood donors answered a questionnaire stating that they were in full health and not on any medication, before blood samples were collected.

Written informed consent was obtained from each participant in the study. The study was approved by the regional ethics committee in Gothenburg and carried out in accordance with the Helsinki Declaration.

Clinical assessment

All patients underwent physical examination by the same physician (EK) and blood samples were drawn. Body weight and height were measured and body mass index (BMI) was calculated. Disease activity and function was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Ankylosing Spondylitis Disease Activity Score based on CRP (ASDAS-CRP), the Bath Ankylosing Spondylitis Patient Global Score (BAS-G), the Bath Ankylosing Spondylitis Functional Index (BASFI), and the Bath Ankylosing Spondylitis Metrology Index (BASMI) [16].

Laboratory assessment

The levels of ESR, CRP, hemoglobin (Hb), white blood cell count (WBC), and platelet count (PLT) were analyzed using standard laboratory techniques at the hospitals. Serum was stored at -80° until analysis. The biomarkers, HGF (pg/mL), MMP-3 (ng/mL), and VEGF (pg/mL) were analyzed in serum using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA, R&D Systems, Inc., Minneapolis, MN, USA) following the manufacturer's instructions. Serum carboxyterminal telopeptide of type 1 collagen (CTX-1), a biomarker for bone resorption, and serum osteocalcin, a marker of bone formation, were also measured in order to study their association with HGF, MMP-3, and VEGF. Serum CTX-1 and osteocalcin were analyzed with ELISA (Immunodiagnostic Systems, Tyne and Wear, UK). Absorbance was read at 450 nm in a spectrophotometer (SpectraMax 340PC³⁸⁴, Molecular Devices). The software SoftMax Pro 5.2 was used to calculate the biomarker concentrations. The limits of detection (LoD) and lower limits of quantification (LLoQ) for each biomarker were as follows: HGF: LoD = 40 pg/mL, LLoQ = 125 pg/mL. MMP-3: LoD = 0.002 ng/mL, LLoQ = 0.156 ng/mL. VEGF: LoD = 9 pg/mL, LLoQ = 31.2 pg/mL. CTX-1: LoD = 0.020 ng/nL, LLoQ = 0.020 ng/nL. Osteocalcin: LoD = 0.5 ng/mL, and LLoQ = 0.5 ng/mL.

Bone mineral density (BMD) and radiographic assessment

BMD was measured using dual-energy X-ray absorptiometry (DXA) (Hologic Discovery A, Hologic Inc., Bedford, MA, USA) in the femoral neck of the non-dominant hip. BMD of femoral neck was used since it is less affected by osteoproliferation than BMD of lumbar spine in AS and therefore more adequately reflects bone loss [17]. Lateral radiographs of the cervical and lumbar spine were acquired. Chronic radiographic changes related to AS in the spine were assessed by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) [18].

Statistical analysis

All data was analyzed using SPSS Statistics version 25 (SPSS Inc., IBM, Chicago, IL, USA). Descriptive statistics are presented as median and inter-quartile range (IQR). In comparisons between groups, the Mann–Whitney *U* test was used for continuous variables and the chi-square test for categorical variables. Correlations were calculated using Spearman's correlation (r_s). In dichotomous variables, an event was coded 1 and no event was coded 0. Sex was coded 1 for women and 2 for men. Due to its skewed distribution, mSASSS was log-transformed before used as an outcome in linear regression analysis and patients with only sacroiliitis (mSASSS = 0) were excluded, leaving data from 148 patients. Linear regression with a stepwise method was run with 10Log mSASSS and BMD of femoral neck as outcomes. Covariates in the regression analyses were the studied biomarkers and variables known to be associated with osteoproliferation and osteoporosis: sex, age, BMI, smoking, and CRP. All tests were two-tailed and $p < 0.05$ was considered statistically significant.

Results

Characteristics of the participants

The characteristics of the 204 patients with AS are shown in Table 1. The median age of the AS patients was 49 (IQR, 41–62) years, median disease duration was 24 (13–34) years, and 57% ($N = 117$) were men. The healthy control group consisted of 80 blood donors, free from underlying disease and medication, with median age 48.5 (IQR 41–57) years and 66% ($N = 54$) were men. The distribution of age ($p = 0.100$) and sex ($p = 0.202$) was not significantly different between the patient and the healthy control group.

Table 1 Characteristics of the 204 patients with ankylosing spondylitis

	Patients' number (%) median (IQR)
Demographic variables	
Sex women/men	87 (43%)/117 (57%)
Age, years	49 (41–62)
BMI, kg/m ²	25.2 (22.8–28.2)
Smokers	24 (12%)
HLA-B27 positive	178 (87%)
Disease-related variables	
Symptoms, years	24 (13–34)
Diagnosis, years	12 (5–23)
History of anterior uveitis	102 (50%)
History of peripheral arthritis	120 (59%)
History of coxitis	17 (8%)
BASDAI, score	3.50 (1.72–5.35)
BASFI, score	2.30 (1.00–3.86)
BASMI, score	3.00 (2.00–4.00)
BAS-G, score	2.90 (1.20–5.38)
ASDAS-CRP, score	2.30 (1.70–3.10)
Bone-related variables	
mSASSS, score	5.5 (0–20)
BMD femoral neck, g/cm ²	0.78 (0.68–0.88)
Laboratory values	
ESR, mm/h	11 (7–19)
CRP, mg/L	5 (5–7)
Hb, g/L	140 (130–148)
WBC $\times 10^9/L$	6.7 (5.4–8.2)
PLT $\times 10^9/L$	288 (249–348)
Treatment	
Glucocorticoids	7 (3%)
NSAID	158 (77%)
TNFi monotherapy	10 (5%)
TNFi and cDMARD in co-medication	32 (16%)
csDMARD monotherapy	30 (15%)

BMI, body mass index; *BASDAI*, Bath Ankylosing Spondylitis Disease Activity Index; *BASFI*, Bath Ankylosing Spondylitis Functional Index; *BASMI*, Bath Ankylosing Spondylitis Metrology Index; *BAS-G*, Bath Ankylosing Spondylitis Patient Global Score; *ASDAS-CRP*, Ankylosing Spondylitis Disease Activity Score based on CRP; *mSASSS*, modified stoke ankylosing spondylitis spinal score; *BMD*, bone mineral density; *ESR*, erythrocyte sedimentation rate; *CRP*, c-reactive protein; *Hb*, hemoglobin; *WBC*, white blood cell count; *PLT*, platelet count; *NSAID*, non-steroidal anti-inflammatory drugs; *cDMARD*, conventional synthetic disease-modifying anti-rheumatic drugs; *TNFi*, tumour necrosis factor inhibitor

Serum levels of biomarkers in AS patients and controls

The male AS patients had higher serum levels of HGF, CTX-1 and osteocalcin compared with the healthy male controls,

whereas no such differences were found between the female patients and female controls (Table 2).

Serum MMP-3 and VEGF were both evenly distributed over AS patients and controls. Men had however higher serum levels of MMP-3 than women both among the AS patients [28.64 (21.12–36.71) vs. 16.12 (12.41–22.11) ng/mL $p < 0.001$] and the healthy controls [23.31 (19.44–35.99) vs. 14.73 (11.06–19.54) ng/mL $p < 0.001$].

As shown in Table 3, the serum levels of HGF correlated positively with MMP-3 and VEGF in AS patients of both sexes, whereas serum MMP-3 was positively correlated with VEGF only in the female patients. Serum CTX-1 was positively correlated with serum osteocalcin (AS all patients $r_s = 0.493$, $p < 0.001$; AS women $r_s = 0.520$, $p < 0.001$; AS men $r_s = 0.469$, $p < 0.001$), but no significant correlation was found between the serum levels of CTX-1 or osteocalcin and HGF, VEGF, or MMP-3.

Age and biomarkers

Increasing age was correlated with higher levels of serum HGF ($r_s = 0.316$, $p = 0.001$) in the male AS patients, but not in the female AS patients. Serum VEGF was positively

Table 2 The serum levels of biomarkers in patients with AS ($N = 204$) and healthy controls ($N = 80$)

	Patients median (IQR)	Healthy controls median (IQR)	p value
HGF, pg/mL			
All	1486 (1251–1759)	1373 (1108–1657)	0.024
Women	1504 (1235–1839)	1346 (1090–1999)	0.333
Men	1469 (1281–1711)	1375 (1136–1562)	0.043
MMP-3, ng/mL			
All	23.3 (16.1–32.2)	21.5 (14.7–29.9)	0.207
Women	16.1 (12.4–22.1)	14.7 (11.1–19.5)	0.155
Men	28.6 (21.1–36.7)	23.3 (19.4–36.0)	0.058
VEGF, pg/mL			
All	338.8 (216–529)	378.0 (229–5067)	0.524
Women	337.3(231–636)	347.7 (184–471)	0.731
Men	340.0 (222–526)	387.5 (241–512)	0.280
CTX-1, ng/mL			
All	0.25 (0.18–0.35)	0.24 (0.17–0.32)	0.098
Women	0.23 (0.16–0.35)	0.21 (0.17–0.34)	1.000
Men	0.27 (0.20–0.36)	0.23 (0.16–0.30)	0.026
Osteocalcin, ng/mL			
All	15.8 (11.6–22.3)	14.3 (10.1–18.7)	0.009
Women	15.3 (10.9–21.7)	14.2 (10.4–19.8)	0.414
Men	16.0 (11.6–22.5)	13.6 (10.0–18.3)	0.004

HGF, hepatocyte growth factor; MMP-3, matrix metalloproteinase 3; VEGF, vascular endothelial growth factor; CTX-1, carboxyterminal telopeptide of type 1 collagen

Table 3 The correlation between the serum levels of HGF, MMP-3 and VEGF in the 204 patients with ankylosing spondylitis

	VEGF	MMP-3
	All	All
	Women	Women
	Men	Men
HGF, pg/mL		
All	$r_s = 0.268$ $p = < 0.001$	$r_s = 0.198$ $p = 0.005$
Women	$r_s = 0.253$ $p = 0.018$	$r_s = 0.262$ $p = 0.014$
Men	$r_s = 0.293$ $p = 0.001$	$r_s = 0.198$ $p = 0.033$
MMP-3, ng/mL		
All	$r_s = 0.110$ $p = 0.119$	
Women	$r_s = 0.214$ $p = 0.046$	
Men	$r_s = 0.029$ $p = 0.753$	

HGF, hepatocyte growth factor; MMP-3, matrix metalloproteinase 3; VEGF, vascular endothelial growth factor

correlated with age in both the male ($r_s = 0.206$, $p = 0.026$) and in the female AS patients ($r_s = 0.255$, $p = 0.017$), whereas serum MMP-3, CTX-1, and osteocalcin were not associated with age in the AS patients. In the healthy control group, osteocalcin was negatively correlated with age among women ($r_s = -0.494$, $p = 0.010$), but none of the other biomarkers were associated with age in the healthy control group.

Biomarkers and disease activity in AS

The associations between disease activity parameters and the biomarkers are shown in Table 4. ESR, CRP, and WBC were all positively correlated with serum levels of HGF, MMP-3, and VEGF. Furthermore, both serum HGF and MMP-3 were positively correlated with swollen joints count. Serum HGF was positively associated with BASMI among the male patients ($r_s = 0.323$, $p < 0.001$), but not among the female patients. Serum CTX-1 and osteocalcin were not associated with any parameters reflecting disease activity.

Biomarkers and smoking among AS patients

Smoking AS patients had higher serum HGF and CTX-1 than non-smokers [1715 (1431–2086) vs. 1458 (1235–1706) pg/mL; $p = 0.002$] and [0.31 (0.24–0.39) vs. 0.24 (0.17–0.35) ng/mL] $p = 0.032$] respectively, whereas serum MMP-3, VEGF, and osteocalcin were not associated with smoking status.

Table 4 The correlation between serum HGF, MMP-3, and VEGF and parameters reflecting disease activity, osteoproliferation, and bone mineral density in patients with ankylosing spondylitis ($N = 204$)

	HGF (pg/mL)	MMP-3 (ng/mL)	VEGF (pg/mL)
Age, years	$r_s = 0.227$ $p = 0.001$	NS	$r_s = 0.222$ $p = 0.001$
BMI, kg/m ²	$r_s = 0.252$ $p < 0.001$	NS	NS
ESR, mm/h	$r_s = 0.355$ $p < 0.001$	$r_s = 0.140$ $p = 0.047$	$r_s = 0.236$ $p = 0.001$
CRP, mg/L	$r_s = 0.258$ $p < 0.001$	$r_s = 0.170$ $p = 0.015$	$r_s = 0.188$ $p = 0.007$
WBC $\times 10^9/L$	$r_s = 0.554$ $p < 0.001$	$r_s = 0.174$ $p = 0.013$	$r_s = 0.214$ $p = 0.002$
Swollen joints count	$r_s = 0.169$ $p = 0.016$	$r_s = 0.182$ $p = 0.009$	NS
ASDAS-CRP, score	NS	NS	NS
BASDAI, score	NS	NS	NS
BASFI, score	NS	NS	NS
BASMI, score	$r_s = 0.205$ $p = 0.003$	NS	NS
BAS-G, score	NS	NS	NS
mSASSS, score	$r_s = 0.164$ $p = 0.019$	$r_s = 0.286$ $p = < 0.001$	NS
BMD fem. neck g/cm ²	$r_s = -0.179$ $p = 0.011$	NS	$r_s = -0.155$ $p = 0.027$

HGF, hepatocyte growth factor; MMP-3, matrix metalloproteinase 3; VEGF, vascular endothelial growth factor; BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; WBC, white blood cell count; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score based on CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BAS-G, Bath Ankylosing Spondylitis Patient Global Score; mSASSS, modified stoke ankylosing spondylitis spinal score; BMD fem. neck, bone mineral density of femoral neck

Biomarkers and osteoproliferation and BMD in AS

The AS men had significantly higher mSASSS than the AS women [8 (2–34) vs. 2 (0–10); $p < 0.001$].

mSASSS was positively correlated with serum HGF and MMP-3 (Table 4).

In the male patient group, serum HGF correlated with mSASSS ($r_s = 0.302$, $p = 0.001$), but the association was non-significant among the female patients.

Serum HGF and VEGF were also correlated with lower BMD in the femoral neck. Whereas, serum CTX-1 and osteocalcin were not correlated with mSASSS or BMD of femoral neck.

Medication and biomarkers

The medication of the patients is demonstrated in Table 1. Totally, 76% of the patients on a tumor necrosis factor inhibitor (TNFi) were co-medicated with a conventional synthetic disease-modifying anti-rheumatic drug (csDMARD), mostly methotrexate 15 mg weekly.

Users of glucocorticoids had higher levels of HGF compared with non-users [2382 (1706–2927) vs. 1475 (1245–1714) pg/mL; $p = 0.001$]. Serum VEGF was significantly lower in users of TNFi compared non-users [249.4 (178.1–457.4) vs. 352.0 (224.3–558.4) pg/mL; $p = 0.042$]. Serum MMP-3 was not associated with use of glucocorticoids or TNFi and none of the biomarkers were associated with use of non-steroidal anti-inflammatory drugs (NSAIDs) or csDMARDs. No association was found between serum CTX-1, osteocalcin, and medication.

Multivariate analyses

The results of the linear regressions are demonstrated in Table 5. The analysis showed that 10 Log mSASSS was positively associated with male sex, age, and serum HGF. BMD of femoral neck was positively associated with male sex and BMI and negatively associated with age and serum HGF.

Table 5 Linear regression with serum 10Log mSASSS and BMD of femoral neck as out-comes in patients with ankylosing spondylitis

	10Log mSASSS			BMD of femoral neck		
	$R^2 = 0.366$			$R^2 = 0.286$		
	$N = 148$			$N = 204$		
	B	S.E.	<i>p</i> value	B	S.E.	<i>p</i> value
Age, years	0.021	0.003	<0.001	−0.004	0.001	<0.001
Male sex	0.293	0.074	<0.001	0.016	0.123	0.047
BMI, kg/m ²				0.011	0.002	<0.001
CRP, mg/L						
Current smoking						
HGF, pg/mL	0.000197	0.000079	0.014	−0.000053	0.000018	0.005
MMP-3, ng/mL						
VEGF, pg/mL						

Covariates in both regression analyses were serum HGF, MMP-3, VEGF, and the following variables known to be associated with bone metabolism: age, sex, BMI, CRP, and smoking. Empty spaces signify that the covariate was excluded in the stepwise linear regression analysis

HGF, hepatocyte growth factor; *MMP-3*, matrix metalloproteinase 3; *VEGF*, vascular endothelial growth factor; *BMI*, body mass index; *CRP*, c-reactive protein; *mSASSS*, modified stoke ankylosing spondylitis spinal score; *BMD femoral neck*, bone mineral density femoral neck

Discussion

The main findings of this study were that the serum levels of HGF were higher in the male AS patients than in the male healthy controls and associated with higher mSASSS and lower BMD of femoral neck after adjusting for other risk factors for osteoproliferation and osteoporosis. The serum levels of HGF increased with age in the male AS patients, but not in the male controls. The male AS patients also had higher serum levels of CTX-1 and osteocalcin than the male healthy controls, indicating both increased bone resorption and new bone formation in male AS patients.

AS is characterized by both osteoproliferation and increased bone loss. HGF also has dual effects on bone. Both osteoblasts and osteoclasts express c-MET and HGF is synthesized by osteoclasts [6]. HGF has been shown to promote osteoblastic differentiation of mesenchymal stem cells, osteoblast proliferation, and osteogenesis [19]. HGF treatment has led to enhanced fracture healing in animal models [20]. On the other hand, HGF also stimulates migration, cell division, and morphological changes in osteoclasts [21]. The association between serum HGF and male sex, age, smoking, and elevated CRP is intriguing, since these are all risk factors for osteoporosis and osteoproliferation in AS [17, 22]. Biomarkers for bone resorption, CTX-1, and new bone formation osteocalcin were however not associated with serum HGF, BMD, or mSASSS in the AS patients.

The elevated serum HGF in AS could also be a response to inflammation, since HGF was positively correlated with inflammatory parameters, especially WBC. HGF has pleiotropic effects in immunomodulation and inflammation. HGF is

secreted by stromal cells of lymphoid organs and HGF and c-MET expression is enhanced by pro-inflammatory cytokines. HGF has been shown to have anti-inflammatory effects on monocytes, macrophages, dendritic cells, and B- and T-lymphocytes [5]. Animal models have also demonstrated that HGF mainly has anti-inflammatory effects, since HGF therapy in different animal models ameliorated collagen-induced arthritis, autoimmune encephalomyelitis, experimental colitis, pulmonary fibrosis, and renal injury in chronic renal disease and also prevented lupus nephritis [5, 23–26]. In addition, HGF has angiogenic effects and counteracts the effects of TGF- β and other fibrogenic cytokines [3].

The results from the present study indicate that HGF could be involved in the process of osteoporosis and osteoproliferation in AS, although the pathophysiologic mechanisms are unknown. The increase in serum HGF in the AS patients was however modest and the study gives no support for serum HGF as a diagnostic tool.

One earlier study on AS assessing multiple cytokines and MMPs in serum showed that HGF was positively correlated with BASDAI, BASFI, and BAS-G in univariate analyses and that HGF together with MMP-8, MMP-9, and CXCL8 from a principal component analysis was associated with higher BASDAI and smoking status in multivariate analyses [27]. Smokers had however not higher HGF than non-smokers in the study.

Earlier studies on rheumatoid arthritis (RA) have shown increased levels of HGF in serum and synovial fluid in RA and that an elevated serum HGF is predictive of joint damage and radiographic progression [28–30]. Similar to our study, it is unclear if HGF contributes do damage or if the increased

serum HGF is a response to inflammation. In patients with systemic lupus erythematosus (SLE), a high HGF expression and a low TGF- β expression in kidney biopsies was associated with a favorable response to cyclophosphamide and less chronic tubulointerstitial damage [31]. Interestingly, elevated serum HGF and mucosal overexpression of HGF and c-MET has also been reported in inflammatory bowel disease in young adults and children [32].

In the field of cancer research, aberrant HGF-MET signaling from cancer-associated fibroblasts has been shown to promote growth and survival of cancer cells, drug resistance, and metastatic spreading [33]. Elevated levels of serum HGF have been associated with poor prognosis in several tumor types and HGF and MET have emerged as interesting therapeutic targets in cancer treatment with several ongoing clinical trials [34–36].

In the present study, serum MMP-3 was positively associated with mSASSS, swollen joints count, ESR, CRP, and WBC. Previous studies have indicated that serum MMP-3 could be a useful marker for disease activity in AS, particularly in patients with peripheral synovitis [37–39]. Maksymowych et al. reported that higher serum levels of MMP-3 could be predictive of greater radiographic progression in AS, especially in patients with pre-existing syndesmophytes [40]. Other studies found that MMP-3 levels are reduced in response to treatment with TNFi [37, 38, 41, 42].

Serum VEGF was associated with higher ESR, CRP, and WBC and lower BMD of femoral neck in the present study. Serum VEGF was also lower in users of TNF-blockers compared with non-users. The finding is in accordance with the previous studies by Tošovský et al. [43] and Pedersen et al. [44] who also reported that AS patients treated with TNF inhibitors had lower serum VEGF compared to untreated patients. The association between VEGF and BMD has not previously been found in AS patients. Serum VEGF analyzed as a measure for progression of new bone formation has produced conflicting results [45, 46].

The strengths of the present study were the large and well-characterized cohort of AS patients and that the patients were evaluated with both radiography and DXA. A limitation was the cross-sectional design of the study. A longitudinal study is needed to show if serum HGF is a predictor for radiologic progression and osteoporosis. Despite being statistically significant, the increased in serum HGF in the AS patients was modest and the study gives no so support for serum HGF as a diagnostic tool.

Another limitation is the lack of information about smoking status in the healthy control group, since this may have affected the level of serum HGF in the control group. The results also need to be confirmed in other cohorts of patients with AS.

Conclusions

We show that serum HGF was higher in male AS patients and independently associated with higher mSASSS and lower BMD of femoral neck. Serum HGF was also positively associated with CRP, WBC, and smoking status. The findings suggest that HGF could be of importance for osteoproliferation and bone loss in AS, but an elevated HGF may also reflect the level of inflammatory activity.

In contrast, the serum levels of MMP-3 and VEGF were not elevated in the AS patients, but positively associated with standard inflammatory laboratory parameters. In addition, MMP-3 was associated with swollen joints count, and VEGF with lower BMD of femoral neck.

Acknowledgments We wish to thank all the patients who participated in the study.

Funding This work was supported by grants from The Health and Medical Care Executive Board of the Västra Götaland, Rune and Ulla Amlövs foundation for Rheumatology Research, Göteborg's Association Against Rheumatism, the Swedish Association Against Rheumatism, the Medical Society of Göteborg, and the Region Västra Götaland (agreement concerning research and education of doctors), COMBINE, the Margareta Rheuma research foundation and the Swedish Society of Medicine.

Compliance with ethical standards

Ethical approval All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest None.

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