Original article

Elevated serum level of hepatocyte growth factor predicts development of new syndesmophytes in men with ankylosing spondylitis

Anna Deminger [1,2], Eva Klingberg[1,2], Merja Nurkkala[1], Mats Geijer[3,4,5], Hans Carlsten[1,2], Lennart T. H. Jacobsson[1] and Helena Forsblad-d'Elia[1,6]

Abstract

Objectives. To study baseline serum hepatocyte growth factor (s-HGF) as a predictor of spinal radiographic progression overall and by sex and to analyse factors correlated to changes in s-HGF in patients with AS.

Methods. At baseline and the 5-year follow-up, s-HGF was analysed with ELISA. Spinal radiographs were graded according to modified Stoke Ankylosing Spondylitis Spinal Score. Radiographic progression was defined as ≥2 modified Stoke Ankylosing Spondylitis Spinal Score units/5 years or development of ≥1 syndesmophyte. Logistic regression analyses were used.

Results. Of 204 baseline participants, 163 (80%) completed all examinations at the 5-year follow-up (54% men). Baseline s-HGF was significantly higher in men who developed ≥1 syndesmophyte compared with non-progressors, median (interquartile range) baseline s-HGF 1551 (1449–1898) vs 1436 (1200–1569) pg/ml, P = 0.003. The calculated optimal cut-off point for baseline s-HGF ≥1520 pg/ml showed a sensitivity of 70%, a specificity of 69% and univariate odds radio (95% CI) of 5.25 (1.69, 14.10) as predictor of development of ≥1 new syndesmophyte in men. Baseline s-HGF ≥1520 pg/ml remained significantly associated with development of ≥1 new syndesmophyte in men in an analysis adjusted for the baseline variables age, smoking, presence of syndesmophytes and CRP, odds radio 3.97 (1.36, 11.60). In women, no association with HGF and radiographic progression was found. Changes in s-HGF were positively correlated with changes in ESR and CRP.

Conclusion. In this prospective cohort study elevated s-HGF was shown to be associated with development of new syndesmophytes in men with AS.

Key words: AS, hepatocyte growth factor, outcomes research

CLINICAL SCIENCE

Rheumatology key messages

- There is a lack of prognostic biomarkers of spinal radiographic progression in patients with AS.
- Higher levels of s-HGF predicted development of new syndesmophytes in men with AS.
- Whether the moderate prediction of s-HGF improves if combined with other biomarkers is not elucidated.

---

[1]Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, [2]Region Västra Götaland, Sahlgrenska University Hospital, Department of Rheumatology, [3]Department of Radiology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, [4]Region Västra Götaland, Sahlgrenska University Hospital, Department of Radiology, Gothenburg, [5]Department of Clinical Sciences, Lund University, Lund and [6]Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Correspondence to: Anna Deminger, Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Box 480, 405 30 Gothenburg, Sweden and Region Västra Götaland, Sahlgrenska University Hospital, Department of Rheumatology, Gothenburg, Sweden. E-mail: anna.deminger@vgregion.se

Introduction

AS is a chronic, inflammatory disease affecting the spine and sacroiliac joints, and is associated with increased spinal bone formation and development of syndesmophytes. The spinal bone formation contributes to the limited mobility and impaired physical function often affecting patients with AS [1, 2], a process only partly understood. The strongest predictor for spinal radiographic progression is the presence of baseline syndesmophytes [3–5]. However, radiographic progression can be slow and highly variable between patients [6], and

other prognostic factors such as biomarkers are warranted. Elevated CRP, the biomarker most studied in this regard, is independently associated with spinal radiographic progression in AS [5, 7, 8]. Several other biomarkers have been studied but are not in clinical use [9].

Hepatocyte growth factor (HGF) is a potent growth factor essential for organ development. HGF signalling is required for self-repair of injuries in various organs such as liver, kidney, muscles and skin [10]. Further, HGF can modulate various immune cell types and has anti-inflammatory effects in animal models [11], promotes angiogenesis [12] and contributes to tumour invasion and metastatic growth [13]. HGF signals through its only receptor, cellular MET (cMET) receptor, expressed on both osteoclasts and osteoblasts [14]. Knowledge of the role of HGF in bone biology is limited.

Previously, we have shown that serum HGF (s-HGF) was higher in patients with AS than in controls and independently associated with higher modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) [15]. The aims of the present study were to assess (i) baseline s-HGF as a predictor for spinal radiographic progression over 5 years overall and by sex, and (ii) if demographic characteristics, disease activity and medications are correlated with changes in s-HGF.

Methods

Patients and controls

Patients with AS fulfilling the modified New York criteria [16] were recruited at baseline in 2009 from three rheumatology clinics in western Sweden. Exclusion criteria were diagnosed IBD, malignancy, psoriasis, dementia, ongoing pregnancy and difficulties in understanding the Swedish language [17]. In total, 204 patients completed the baseline protocol. After 5 years, they were invited to a follow-up. The enrolment from baseline to follow-up has been described in detail in a previous report for 166 patients; all with baseline mSASSS <72 (three patients with maximum baseline mSASSS were excluded) [18]. Of these 166 patients, three did not have s-HGF analysed at follow-up.

For comparison of baseline s-HGF levels, 80 healthy controls (HCs) were recruited among blood donors while giving blood at the Sahlgrenska University Hospital, Gothenburg. The blood donors answered a questionnaire stating they were in full health and not on any medication. Written informed consent was obtained from all participants and was approved by the regional ethics committee in Gothenburg, Sweden (reference numbers: Dnr: 597-08 and Dnr: 690-13) and the study complies with the Declaration of Helsinki.

Physical examinations and questionnaires

The patients were assessed with the same methods at baseline and at the 5-year follow-up. Physical examination included BASMI [19]. Questionnaires included medical history, lifestyle factors, medications, BASFI, BASDAI and AS Disease Activity Score based on CRP [19]. Based on baseline occupation, the patients were categorized as blue-collar workers (manual labour), white-collar workers (less physical activity and more formal education) [20] or no work. NSAID consumption from baseline to the follow-up was quantified according to the recommendations of the Assessment of SpondyloArthritis international Society [21].

Laboratory tests

CRP, ESR and white blood cell count (WBC) were analysed using standard laboratory techniques on both occasions. Serum for analysis of HGF (pg/ml) was collected at baseline for patients and HC and at follow-up for patients and stored at −80°C. Baseline and follow-up s-HGF were analysed on two separate occasions using the same ELISA kit (Quantikine ELISA, R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. Absorbance was read at 450 nm with a SpectraMax 340PC^{384} spectrophotometer (Molecular Devices, San Jose, CA, USA). The software SoftMax Pro 5.2 (Molecular Devices) was used for calculating HGF concentrations. The limit of detection for HGF was 40 pg/ml and the lower limit of quantification was 125 pg/ml. All patients and HCs had levels above the limit of quantification. Since baseline and follow-up s-HGF were analysed on separate occasions with the serum samples being stored in the freezer for different length of time, the association for the average s-HGF (derived from the baseline and the follow-up values) and spinal radiographic progression was also analysed.

Radiography

Cervical and lumbar lateral spinal radiographs were obtained at baseline and at the 5-year follow-up and graded according to mSASSS. The total score ranges from 0 to 72 [22]. All radiographs were scored simultaneously by the same musculoskeletal radiologist with known chronological order but blinded to clinical data. Definite radiographic progression was defined by an increase in mSASSS over 5 years by ≥2 points or as development of ≥1 new syndesmophyte over 5 years, defined as mSASSS of ≥2 points at follow-up at a vertebral corner with 0 or 1 at baseline [4]. Further details of the scoring process, the intraclass correlation coefficient and smallest detectable change have been reported for this cohort previously [18].

Statistics

Descriptive statistics are presented as numbers (percentage) or median (interquartile range). The Mann–Whitney U-test, χ^2 test or Fisher's exact test were used to compare differences between groups. The Wilcoxon signed-rank test was used to compare s-HGF at baseline and follow-up. The average s-HGF was calculated from baseline and follow-up values. A receiver operating characteristic curve was plotted for men, with baseline

s-HGF as the test variable and development of ≥1 new syndesmophyte as the categorical state variable. Youden's index was used to identify an optimal cut-off point for a predictive s-HGF value. Positive likelihood ratio (LR+) and negative likelihood ratio (LR−) for developing ≥1 syndesmophyte were calculated for the cut-off point of s-HGF. Univariate and multivariable logistic regression analyses with progression of ≥2 mSASSS over 5 years or development of ≥1 new syndesmophyte as dependent variable (yes = 1, no = 0) were conducted to analyse the association for baseline s-HGF, baseline optimal cut-off point for s-HGF and the average s-HGF and definite spinal radiographic progression. Multivariable analyses (backward method) were conducted if the HGF-covariate was significantly associated with the dependent variable in the univariate analysis. The multivariable analyses were adjusted for variables significantly associated with spinal radiographic progression previously reported for this cohort—for new syndesmophyte: age, smoking and baseline syndesmophytes for men, and additionally, sex and exposure to bisphosphonates during follow-up for the total group; and for progression ≥2 mSASSS points for men: baseline BMI, ever smoker, mSASSS and CRP [18]. Two separate sensitivity analyses were conducted for baseline CRP and type of occupation for development of ≥1 new syndesmophyte, even though the variables were not significantly associated with development of new syndesmophytes in this cohort. Correlations were calculated using Spearman's correlation coefficient (r_s). The tests were two-tailed and $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA), except calculation of s-HGF cut-off point where SAS software version 9.4 (SAS Institute, Cary, NC, USA) was used.

Results

Characteristics of participants

Of the 204 patients completing the baseline protocol, 163 (80%) patients completed the examinations including analysis of s-HGF at the 5-year follow-up. There were no significant differences between patients that participated in the 5-year follow-up *vs* those who did not participate regarding baseline s-HGF, mSASSS, age or sex (*P*-value 0.13, 0.70, 0.46 and 0.17, respectively).

Of the 163 patients, 88 (54%) were men. Some sex differences were found: men had higher BMI and mSASSS and lower ESR compared with women, more men had at least one syndesmophyte and more men were HLA-B27 positive (Table 1).

The 80 HCs consisted of 54 (67.5%) men and 26 (32.5%) women with median (IQR) age 48.5 (41–57) years. The sex distribution or age of HCs did not differ significantly from the AS cohort, ($P = 0.061$ and $P = 0.25$, respectively).

HGF serum levels

Patients with AS had significantly higher median (IQR) s-HGF [1493 (1247–1706) pg/ml, compared with HCs, 1375 (1112–1665) pg/ml, $P = 0.050$]. However, in analyses stratified by sex, s-HGF did not differ between patients and controls (Supplementary Fig. S1, available at *Rheumatology* online). Also, s-HGF did not differ between sexes in patients (Table 1) or HCs.

At the 5-year follow-up, median s-HGF had decreased in the total group from 1493 (1247–1706) to 1225 (1077–1427) pg/ml ($P < 0.001$). Both sexes had significant decreases (Supplementary Fig. S2, available at *Rheumatology* online). Median average s-HGF was 1378 (1189–1609) pg/ml and did not differ between men and women [1383 (1180–1527) *vs* 1369 (1189–1641) pg/ml respectively, $P = 0.55$].

HGF and spinal radiographic progression

Spinal radiographic progression was more common among men compared with women; 32 (36%) men *vs* 15 (20%) women had progression of ≥2 mSASSS over 5 years ($P = 0.034$) and 27 (31%) men *vs* 9 (12%) women had developed ≥1 new syndesmophyte ($P = 0.005$).

Comparisons of patients with either type of radiographic progression *vs* non-progression showed that baseline s-HGF was higher in men with development of ≥1 new syndesmophyte. Also, the average s-HGF was higher in men with both types of progression (Table 2).

In the receiver operating characteristic analysis done to assess the predictive value of baseline s-HGF for development of new syndesmophytes in men, the area under the curve was 0.70 (95% CI: 0.58, 0.82) ($P = 0.003$, Fig. 1). The optimal HGF cut-off level was defined as 1520 pg/ml, which was found in 38 (43%) of the men and showed a sensitivity of 70%, a specificity of 69%, a LR+ of 2.26 and LR− of 0.43 for development of ≥1 new syndesmophyte in men. The cumulative probability of mSASSS progression in men over 5 years stratified by HGF cut-off point is shown in Fig. 2.

In the multivariable logistic regression analyses adjusted for age, smoking and baseline syndesmophytes, baseline s-HGF per 1 s.d., baseline s-HGF ≥1520 pg/ml and the average s-HGF were significantly associated with development of ≥1 new syndesmophyte in men. In the multivariable analyses for men, odds radio (OR) (95% CI) for s-HGF per 1 s.d. was 1.90 (1.01, 3.59) ($P = 0.048$), for s-HGF ≥1520 pg/ml OR was 3.97 (1.36, 11.60) ($P = 0.012$) and for the average s-HGF OR was 2.07 (1.11, 3.85) ($P = 0.022$). The point estimates for ORs did not change if baseline CRP or type of occupation was included in the models (Table 3).

Variables correlated with baseline s-HGF and changes in HGF

Correlation analyses showed that baseline s-HGF was still positively correlated with older age and higher BMI, ESR, CRP, WBC, BASMI, mSASSS and swollen joint count despite patients being lost to follow-up. The highest r_s was found for HGF and WBC. In addition, s-HGF was still higher in current smokers and users of prednisolone whereas no

TABLE 1 Baseline characteristics and medications at baseline and during follow-up for 163 patients with ankylosing spondylitis

	Total group (n = 163)	Men (n = 88)	Women (n = 75)	P-value
Demographic variables				
Age, median (IQR), years	49 (40–62)	49 (39–61)	49 (42–63)	0.33
BMI, median (IQR), kg/m^2	24.8 (22.8–27.7)	25.6 (23.4–28.3)	24.0 (21.8–27.7)	**0.034**
Current smoker, n (%)	17 (10)	8 (9)	9 (12)	0.73
Ever smoker, n (%)	78 (48)	43 (49)	35 (47)	0.90
Type of occupation, n (%)				0.98
Blue collar	38 (23)	20 (23)	18 (24)	
White collar	77 (47)	42 (48)	35 (47)	
No work	48 (29)	26 (29)	22 (29)	
Disease-related variables				
Symptom duration, median (IQR), years	22 (12–34)[a]	20 (11–31)[b]	24 (14–34)	0.31
HLA-B27 positive, n (%)	140 (86)	81 (92)	59 (79)	**0.026**
History of anterior uveitis, n (%)	84 (52)	49 (56)	35 (47)	0.32
BASMI, median (IQR), score	2.8 (2.0–4.0)	3.0 (1.7–4.0)	2.8 (2.2–3.8)	0.87
BASFI, median (IQR), score	2.2 (1.0–3.7)[c]	1.8 (1.0–3.2)	2.5 (1.0–4.5)[d]	0.25
BASDAI, median (IQR), score	3.1 (1.7–5.1)[a]	2.5 (1.4–4,9)[e]	3.6 (1.9–5.5)[d]	0.065
ASDAS_CRP, median (IQR), score	2.0 (1.4–2.8)	1.9 (1.3–2.9)	2.0 (1.5–2.6)	0.55
CRP, median (IQR), mg/l	2.0 (1.0–6.0)	3.0 (1.0–7.0)	2.0 (1.0–5.0)	0.21
WBC, median (IQR), ×10^9/l	6.5 (5.2–8.1)	6.4 (5.1–7.8)	6.5 (5.3–8.4)	0.55
mSASSS, median (IQR), score	5.0 (0–20.0)	8.5 (2.0–34.75)	2.0 (0–11.0)	**<0.001**
≥1 syndesmophyte, n (%)	75 (46)	50 (57)	25 (33)	**0.005**
HGF, median (IQR), pg/ml	1493 (1247–1706)	1490 (1244–1685)	1511 (1247–1842)	0.45
Medications at baseline, n (%)				
NSAID	126 (77)	65 (74)	61 (81)	0.34
TNFi and/or csDMARD[f]	57 (35)	32 (36)	25 (33)	0.81
Bisphosphonates	7 (4)	1 (1)	6 (8)	0.049
Prednisolone	6 (4)	3 (3)	3 (4)	1.00
Medications during follow-up, n (%)				
NSAID-index, 0–100	16 (3–67)	19 (3–81)	16 (3–42)	0.27
TNFi and/or csDMARD[g]	69 (42)	39 (44)	30 (40)	0.69
Bisphosphonates	30 (18)	11 (13)	19 (25)	0.057
Prednisolone	17 (10)	8 (9)	9 (12)	0.61

Significant differences between men and women are shown in bold typeface. [a]n = 161. [b]n = 86. [c]n = 162. [d]n = 74. [e]n = 87. [f]In the total group, 32 (20%) patients were using TNFi in monotherapy or in combination with a csDMARD, 21 (24%) men and 11 (15%) women. [g]In the total group, 48 (29%) patients were exposed to TNFi in monotherapy or in combination with csDMARD, 29 (33%) men and 19 (25%) women. ASDAS_CRP: Ankylosing Spondylitis Disease Activity Score based on CRP; csDMARD: conventional synthetic DMARD; HGF: hepatocyte growth factor; HLA-B27: HLA B27; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score; IQR: interquartile range; TNFi: TNF inhibitor; WBC: white blood cell count.

difference was found between users and non-users of TNFi. The only difference from the baseline study was use of NSAID now being correlated with lower s-HGF (Supplementary Table S1, available at *Rheumatology* online).

Correlation analyses for changes in HGF (ΔHGF) showed that increasing HGF was correlated with increases over 5 years in ESR, CRP and WBC. Neither medication at baseline nor medication during follow-up was associated with ΔHGF (Table 4).

Discussion

In the present study, we found an elevated baseline s-HGF to be associated with development of new syndesmophytes in men, also after adjustment for variables previously shown to predict new syndesmophytes in men in this cohort. Also, an increased average s-HGF was associated with development of new syndesmophytes in men. s-HGF in relation to AS-related spinal alterations has, to our knowledge, only been evaluated before in our baseline, cross-sectional study, which demonstrated that higher s-HGF was associated with higher mSASSS [15]. The pathophysiological mechanisms behind the relationship between new bone formation and HGF in AS are not known, but there are several possibilities. Human osteoclasts and osteoblasts express cMET indicating that HGF participates in regulating bone metabolism [14]. Also, osteoclasts and osteoblasts have been found to synthesise HGF [14, 23,

TABLE 2 Comparing baseline serum-HGF and the average serum-HGF in patients with and without radiographic progression

	Baseline serum HGF, median (IQR), pg/ml		P-value	Average-serum HGF, median (IQR), pg/ml[a]		P-value
	Yes	No		Yes	No	
Progression ≥2 mSASSS						
Total group (n = 163)	1527 (1315–1711)	1484 (1235–1693)	0.34	1408 (1236–1688)	1367 (1161–1584)	0.18
Men (n = 88)	1534 (1373–1711)	1458 (1196–1572)	0.064	**1455 (1263–1676)**	**1348 (1129–1466)**	**0.025**
Women (n = 75)	1405 (1183–1832)	1517 (1264–1862)	0.53	1314 (1164–1728)	1374 (1196–1640)	0.71
Development ≥1 syndesmophyte						
Total group (n = 163)	1537 (1393–1805)	1475 (1242–1706)	0.21	**1465 (1302–1685)**	**1358 (1163–1591)**	**0.031**
Men (n = 88)	**1551 (1449–1898)**	**1436 (1200–1569)**	**0.003**	**1478 (1381–1722)**	**1289 (1147–1453)**	**<0.001**
Women (n = 75)	1230 (1103–1573)	1527 (1278–1898)	0.11	1297 (1172–1442)	1377 (1183–1651)	0.30

[a]Significant differences are shown in bold. Progression ≥2 mSASSS, in total group $n = 47/163$, men $n = 32/88$, women $n = 15/75$. Development ≥1 syndesmophyte, in total group $n = 36/163$, men $n = 27/88$, women $n = 9/75$. The average serum-HGF is calculated from baseline and follow-up values. HGF: hepatocyte growth factor; IQR: interquartile range; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score.

FIG. 1 ROC curve for baseline serum-HGF and development of new syndesmophytes in 88 men with AS
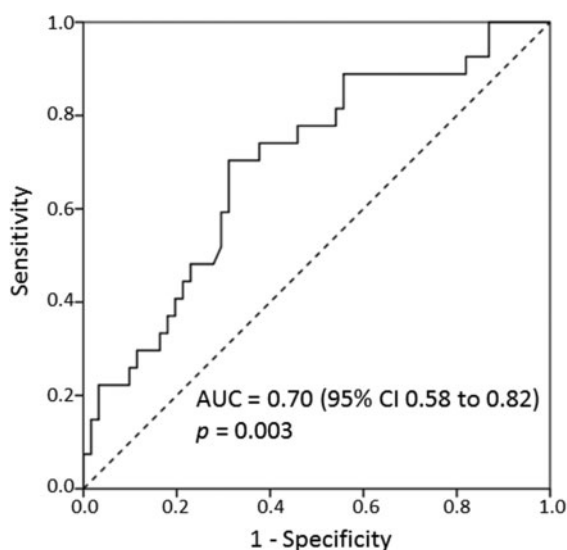
ROC curve for association of baseline s-HGF and development of ≥1 new syndesmophyte over 5 years in 88 men with ankylosing spondylitis. AUC: area under the curve; s-HGF: serum hepatocyte growth factor; ROC: receiver operating characteristic.

FIG. 2 Cumulative probability of mSASSS progression over 5 years in men stratified by HGF cut-off point
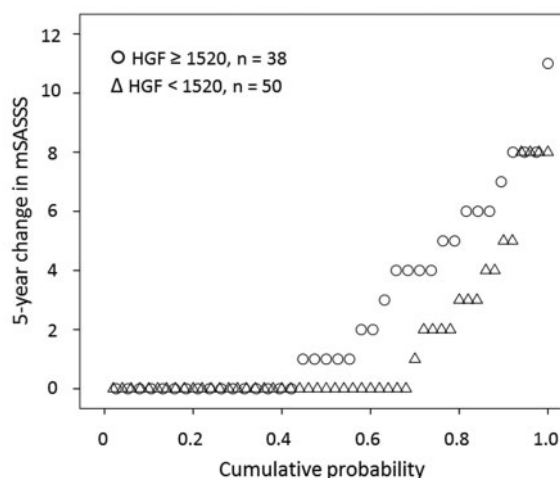
The cumulative probability for the change in mSASSS from baseline to the 5-year follow-up in 88 men with ankylosing spondylitis categorized according to the cut-off point baseline s-HGF of 1520 pg/ml. HGF: hepatocyte growth factor; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score.

24]. However, whether HGF promotes or inhibits osteogenesis is still under debate. There are studies indicating that HGF promotes osteogenic differentiation [25, 26], that blocking HGF signalling disrupts mineralization *in vitro* [25] and that treatment with HGF improves fracture healing in rabbits [27]. In contrast, other studies have found that HGF inhibits differentiation of osteoblasts *in vitro* [28], and that inhibition of cMET has a

regenerative effect on bone defects in mice [29]. Whether different effects on osteogenesis could be due to HGF levels or timing of HGF administration in studies is still under debate [30].

Another possible link between new bone formation in AS and HGF could be related to the correlation between inflammatory parameters and HGF demonstrated for CRP, ESR and WBC considering that elevated CRP and high disease activity are associated to spinal radiographic progression [5, 7, 8, 31]. In a study on a large

TABLE 3 Logistic regression analyses for spinal radiographic progression over 5 years in patients with ankylosing spondylitis

	Development ≥1 new syndesmophyte				Progression >2 mSASSS	
	OR, unadjusted (95% CI)	P-value	OR, adjusted[a] (95% CI)	P-value	OR, unadjusted (95% CI)	P-value
Baseline s-HGF pg/ml, per 1 s.d.						
Total group (n = 163)	1.23 (0.86, 1.78)	0.26	NA		1.13 (0.81, 1.60)	0.47
Men (n = 88)	**2.39 (1.31, 4.36)**	**0.004**	**1.90 (1.01, 3.59)**	**0.048**	1.64 (0.98, 2.76)	0.061
Women (n = 75)	0.52 (0.22, 1.22)	0.13	NA		0.83 (0.48, 1.45)	0.51
Baseline s-HGF ≥1520 pg/ml						
Total group (n = 163)	**2.19 (1.03, 4.68)**	**0.042**	Not in final model		1.33 (0.67, 2.62)	0.41
Men (n = 88)	**5.25 (1.96, 14.10)**	**0.001**	**3.97 (1.36, 11.60)**	**0.012**	1.89 (0.78, 4.56)	0.16
Women (n = 75)	0.47 (0.11, 2.04)	0.31	NA		0.88 (0.28, 2.72)	0.82
Average-HGF pg/ml, per 1 s.d.						
Total group (n = 163)	**1.49 (1.04, 2.13)**	**0.031**	Not in final model		1.30 (0.93, 1.81)	0.12
Men (n = 88)	**2.59 (1.44, 4.67)**	**0.002**	**2.07 (1.11, 3.85)**	**0.022**	**1.78 (1.09, 2.90)**	**0.020**[b]
Women (n = 75)	0.65 (0.30, 1.43)	0.28	NA		0.89 (0.50, 1.58)	0.69

Significant associations are shown in bold typeface. Development ≥1 syndesmophyte, in total group n = 36/163, men n = 27/88, women n = 9/75. Progression ≥2 mSASSS, in total group n = 47/163, men n = 32/88, women n = 15/75., [a]For men, adjusted for age, smoking and baseline syndesmophyte. For total group adjusted for age, sex, smoking, baseline syndesmophyte and exposure to bisphosphonates. If baseline CRP or type of occupation was included in the models, the ORs and P-values for s-HGF per 1 s.d., s-HGF ≥1520 and the average s-HGF per 1 s.d. remained the same. [b]In the analysis adjusted for BMI, ever smoker, baseline CRP and mSASSS the variable did not remain in the final model. HGF: hepatocyte growth factor; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score; NA: not applicable; OR: odds radio.

panel of biomarkers and disease activity in AS, HGF clustered with MMP-8 and -9 and CXCL8 and was associated with higher CRP and BASDAI [32]. In our cohort, patients with AS had higher levels of HGF than had HCs, which has also been found in patients with rheumatoid arthritis (RA) [33, 34], IBD [35] and SLE [36]. Also, HGF in synovial fluid from patients with RA was higher than in peripheral blood and one prospective study on RA found plasma HGF to predict joint destruction, affecting both bone erosions and cartilage with joint space narrowing [37–39]. Cytokines like IL-1 and -6 and TNFα upregulate HGF and cMET during tissue repair [40]. Whether HGF has a mechanistic role in inflammatory diseases or if levels are increased as an unspecific response to inflammation is not clear. However, in mouse models of collagen-induced arthritis and experimental colitis, HGF treatment led to suppression of inflammation [11, 41, 42]. In vitro studies suggest that HGF has multiple effects on the immune system, but are inconclusive regarding the effect, whereas animal studies suggest an anti-inflammatory effect [11].

Higher baseline s-HGF was correlated with smoking, older age, elevated CRP and higher BMI, factors previously found to be associated with spinal radiographic progression, which can indicate that HGF is involved in this process [5, 7, 18, 31]. However, these associations are not specific for AS. In 5395 individuals without cardiovascular disease, higher s-HGF was also associated with smoking, older age, higher CRP and higher BMI, but also with female sex [43]. We found no sex difference in s-HGF levels in this cohort of AS patients, but

HGF was associated with development of new syndesmophytes in men only, whereas the effect in women was non-significantly protective. Whether this is due to differences between sexes in mechanisms behind bone formation or has other explanations remains to be examined in future studies. Data for women in this study are difficult to interpret since few women had radiographic progression.

The cut-off point for s-HGF of 1520 pg/ml had a sensitivity of 70%, a specificity of 69% and an area under the curve of 0.70 for development of ≥1 syndesmophyte in men with AS. Other studies of biomarkers as predictors for spinal radiographic progression in AS have reported similar levels of sensitivity and specificity for the total group of patients. The optimal cut-off point for MMP-3 showed a sensitivity and a specificity of 70% for any progression in AS over 2 years [44]. For s-calprotectin, sensitivity was 72% and specificity 60% for development of new syndesmophytes over 2 years in patients with early axSpA [45]. A recent publication examined combinations of 10 biomarkers, and for the combination of vascular endothelial growth factor, leptin and high molecular weight adiponectin the area under the curve was 0.73 (95% CI: 0.61, 0.85) for prediction of ≥2 mSASSS over 2 years. When these biomarkers were added to clinical parameters, the prediction of radiographic progression improved compared with clinical parameters only, but the added value for the biomarkers was small [46]. Whether HGF in combination with other biomarkers or clinical parameters can improve the prediction of radiographic progression further is yet to be

TABLE 4 Correlations between changes in serum-HGF and characteristics and medications for 163 patients with ankylosing spondylitis

	Change in serum-HGF pg/ml between baseline and the 5-year follow-up	
	Correlation coefficient, r_s	*P*-value
Baseline characteristics		
Age, years	0.13	0.11
BMI, kg/m^2	−0.79	0.32
Smoking, Y/N	−0.13	0.11
HLA-B27$^+$	0.073	0.35
Changes in BMI and disease related variables between baseline and the 5-year follow-up		
ΔBMI, kg/m^2	−0.033	0.67
ΔESR, mm/h	**0.24**	**0.002**
ΔCRP, mg/l	**0.19**	**0.014**
ΔWBC x 10^9/l	**0.40**	**<0.001**
ΔASDAS_CRP, score	0.039	0.62
ΔBASDAI, score	−0.14	0.080
ΔBASMI, score	−0.046	0.56
ΔBASFI, score	−0.076	0.34
Medications at baseline		
NSAID, Y/N	0.14	0.077
TNFi and/or csDMARD, Y/N	0.032	0.68
Bisphosphonates	0.098	0.21
Prednisolone	−0.12	0.13
Medications during follow-up		
NSAID index, 0–100	0.11	0.18
Exposure TNFi and/or csDMARD, Y/N	−0.007	0.93
Exposure bisphosphonates, Y/N	−0.029	0.72
Exposure Prednisolone, Y/N	−0.11	0.16

Significant correlations are shown in bold typeface. ASDAS_CRP: Ankylosing Spondylitis Disease Activity Score based on CRP; BASMI: Bath Ankylosing Spondylitis Metrology Index; csDMARD: conventional synthetic DMARD; HGF: hepatocyte growth factor; TNFi: TNF inhibitor.

explored in future research, and at present we cannot recommend the of use s-HGF in clinical practice.

Over 5 years, s-HGF decreased in the AS patients with no association found for medications. However, this observational study was not designed to evaluate treatment effects. Changes in HGF correlated positively with changes in CRP and ESR. To our knowledge, changes in HGF and factors associated therewith have not been studied in other rheumatic diseases previously.

Limitations of this study are the relatively few patients with radiographic progression, limiting the statistical power and the observational nature of the study with patients having different treatments with different starting points that could affect both HGF levels and radiographic progression. Also, the NSAID index was based on the recollection of NSAID use during follow-up time. Another limitation is having only one reader for scoring the radiographs. Further, serum samples were stored in the freezer for different lengths of time and the measurement interval of 5 years for HGF is too long to draw definite conclusions about HGF levels over time and factors associated with changes. We also lack follow-up values and information about smoking status in the controls and we lack a control group with mechanical back pain for comparisons of s-HGF. The strength of the study is the longitudinal design with a well-characterized cohort of patients with AS. This is the first longitudinal study evaluating s-HGF in patients with AS and our results needs to be confirmed, preferably in combination with other biomarkers or clinical parameters in order to improve prediction. It would also be of interest to know if HGF responds to treatment and to study possible mechanisms of HGF in affected tissues.

In conclusion, elevated s-HGF was found to be associated with development of new syndesmophytes in men with AS also when adjusted for variables associated with spinal radiographic progression previously reported for this cohort. There are several possible mechanisms for HGF to influence spinal radiographic damage in AS, which include a direct effect on osteoblasts or osteoclasts, an immunomodulatory effect of HGF, or alternatively HGF levels could be elevated in response to inflammatory cytokines with no direct effect on structural progression. Thus, the role of HGF in AS needs to be further investigated.

Acknowledgements

Data availability statement

The data sets generated and/or analysed during the current study are not publicly available due to the General Data Protection Regulation (GDPR). Researchers with a specific question regarding the study are encouraged to contact the corresponding author (A.D.).

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Landewe R, Dougados M, Mielants H, van der Tempel H, van der Heijde D. Physical function in ankylosing spondylitis is independently determined by both disease activity and radiographic damage of the spine. Ann Rheum Dis 2009;68:863–7.

2 Machado P, Landewé R, Braun J *et al.* Both structural damage and inflammation of the spine contribute to impairment of spinal mobility in patients with ankylosing spondylitis. Ann Rheum Dis 2010;69:1465–70.

3 Baraliakos X, Listing J, Rudwaleit M *et al.* Progression of radiographic damage in patients with ankylosing spondylitis: defining the central role of syndesmophytes. Ann Rheum Dis 2007;66:910–5.

4 van Tubergen A, Ramiro S, van der Heijde D *et al.* Development of new syndesmophytes and bridges in ankylosing spondylitis and their predictors: a longitudinal study. Ann Rheum Dis 2012;71:518–23.

5 Poddubnyy D, Haibel H, Listing J *et al.* Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. Arthritis Rheum 2012;64:1388–98.

6 Baraliakos X, Listing J, von der Recke A, Braun J. The natural course of radiographic progression in ankylosing spondylitis—evidence for major individual variations in a large proportion of patients. J Rheumatol 2009;36:997–1002.

7 Haroon N, Inman RD, Learch TJ *et al.* The impact of tumor necrosis factor α inhibitors on radiographic progression in ankylosing spondylitis. Arthritis Rheum 2013;65:2645–54.

8 Ramiro S, van der Heijde D, van Tubergen A *et al.* Higher disease activity leads to more structural damage in the spine in ankylosing spondylitis: 12-year longitudinal data from the OASIS cohort. Ann Rheum Dis 2014;73:1455–61.

9 Maksymowych WP. Biomarkers for diagnosis of axial spondyloarthritis, disease activity, prognosis, and prediction of response to therapy. Front Immunol 2019;10:305.

10 Nakamura T, Mizuno S. The discovery of hepatocyte growth factor (HGF) and its significance for cell biology, life sciences and clinical medicine. Proc Jpn Acad Ser B Phys Biol Sci 2010;86:588–610.

11 Molnarfi N, Benkhoucha M, Funakoshi H, Nakamura T, Lalive PH. Hepatocyte growth factor: a regulator of inflammation and autoimmunity. Autoimmun Rev 2015;14:293–303.

12 Bussolino F, Di Renzo MF, Ziche M *et al.* Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol 1992;119:629–41.

13 Petrini I. Biology of MET: a double life between normal tissue repair and tumor progression. Ann Transl Med 2015;3:82.

14 Grano M, Galimi F, Zambonin G *et al.* Hepatocyte growth factor is a coupling factor for osteoclasts and osteoblasts in vitro. Proc Natl Acad Sci USA 1996;93:7644–8.

15 Torres L, Klingberg E, Nurkkala M, Carlsten H, Forsblad D. H. Hepatocyte growth factor is a potential biomarker

for osteoproliferation and osteoporosis in ankylosing spondylitis. Osteoporos Int 2019;30:441–9.

16 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.

17 Klingberg E, Lorentzon M, Mellstrom D *et al.* Osteoporosis in ankylosing spondylitis – prevalence, risk factors and methods of assessment. Arthritis Res Ther 2012;14:R108.

18 Deminger A, Klingberg E, Geijer M *et al.* A five-year pro-spective study of spinal radiographic progression and its predictors in men and women with ankylosing spondyl-itis. Arthritis Res Ther 2018;20:162.

19 Sieper J, Rudwaleit M, Baraliakos X *et al.* The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. Ann Rheum Dis 2009;68: ii1–44.

20 Niknian M, LL A, Lasater TM, Carleton RA. Use of population-based data to assess risk factor profiles of blue and white collar workers. J Occup Environ Med 1991;33:29–36.

21 Dougados M, Simon P, Braun J *et al.* ASAS recommendations for collecting, analysing and reporting NSAID intake in clinical trials/epidemiological studies in axial spondyloarthritis. Ann Rheum Dis 2011; 70:249–51.

22 Creemers MC, Franssen MJ, van't Hof MA *et al.* Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. Ann Rheum Dis 2005;64:127–9.

23 Blanquaert F, Delany AM, Canalis E. Fibroblast growth factor-2 induces hepatocyte growth factor/scatter factor expression in osteoblasts. Endocrinology 1999;140: 1069–74.

24 Skrtic S, Ohlsson C. Cortisol decreases hepatocyte growth factor levels in human osteoblast-like cells. Calcif Tissue Int 2000;66:108–12.

25 Aenlle KK, Curtis KM, Roos BA, Howard GA. Hepatocyte growth factor and p38 promote osteogenic differentiation of human mesenchymal stem cells. Mol Endocrinol 2014;28:722–30.

26 D'ippolito G, Schiller PC, Perez-stable C *et al.* Cooperative actions of hepatocyte growth factor and 1,25-dihydroxyvitamin D3 in osteoblastic differentiation of human vertebral bone marrow stromal cells. Bone 2002;31:269–75.

27 Matsubara H, Tsuchiya H, Watanabe K, Takeuchi A, Tomita K. Percutaneous nonviral delivery of hepatocyte growth factor in an osteotomy gap promotes bone repair in rabbits: a preliminary study. Clin Orthop Relat Res 2008;466:2962–72.

28 Standal T, Abildgaard N, Fagerli UM *et al.* HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. Blood 2007; 109:3024–30.

29 Kim J-W, Lee MN, Jeong B-C *et al.* Chemical inhibitors of c-Met receptor tyrosine kinase stimulate osteoblast differentiation and bone regeneration. Eur J Pharmacol 2017;806:10–7.

30 Frisch RN, Curtis KM, Aenlle KK, Howard GA. Hepatocyte growth factor and alternative splice variants—expression, regulation and implications in osteogenesis and bone health and repair. Expert Opin Ther Targets 2016;20:1087–98.

31 Park JW, Kim MJ, Lee JS *et al.* Impact of tumor necrosis factor inhibitor versus nonsteroidal antiinflammatory drug treatment on radiographic progression in early ankylosing spondylitis: its relationship to inflammation control during treatment. Arthritis Rheumatol 2019;71:82–90.

32 Mattey DL, Packham JC, Nixon NB *et al.* Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis. Arthritis Res Ther 2012;14:R127.

33 Feuerherm AJ, Borset M, Seidel C *et al.* Elevated levels of osteoprotegerin (OPG) and hepatocyte growth factor (HGF) in rheumatoid arthritis. Scand J Rheumatol 2001; 30:229–34.

34 Kara F, Yildirim A, Gumusdere M *et al.* Association between hepatocyte growth factor (HGF) gene polymorphisms and serum HGF levels in patients with rheumatoid arthritis. Eurasian J Med 2014;46: 176–81.

35 Srivastava M, Zurakowski D, Cheifetz P, Leichtner A, Bousvaros A. Elevated serum hepatocyte growth factor in children and young adults with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2001;33:548–53.

36 Robak E, Wozniacka A, Sysa-Jedrzejowska A, Stepien H, Robak T. Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity. Eur Cytokine Netw 2001;12: 445–52.

37 Grandaunet B, Syversen SW, Hoff M *et al.* Association between high plasma levels of hepatocyte growth factor and progression of radiographic damage in the joints of patients with rheumatoid arthritis. Arthritis Rheum 2011; 63:662–9.

38 Koch AE, Halloran MM, Hosaka S *et al.* Hepatocyte growth factor. A cytokine mediating endothelial migration in inflammatory arthritis. Arthritis Rheum 1996;39: 1566–75.

39 Yukioka K, Inaba M, Furumitsu Y *et al.* Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. J Rheumatol 1994;21:2184–9.

40 Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010;11: 834–48.

41 Okunishi K, Dohi M, Fujio K *et al.* Hepatocyte growth factor significantly suppresses collagen-induced arthritis in mice. J Immunol 2007;179:5504–13.

42 Yuge K, Takahashi T, Khai NC *et al.* Intramuscular injection of adenoviral hepatocyte growth factor at a distal site ameliorates dextran sodium sulfate-induced colitis in mice. Int J Mol Med 2014;33:1064–74.

43 Bancks MP, Bielinski SJ, Decker PA *et al.* Circulating level of hepatocyte growth factor predicts

incidence of type 2 diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis (MESA). Metabolism 2016; 65:64–72.

44 Maksymowych WP, Landewe R, Conner-Spady B *et al.* Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis Rheum 2007;56: 1846–53.

45 Turina MC, Sieper J, Yeremenko N *et al.* Calprotectin serum level is an independent marker for radiographic spinal progression in axial spondyloarthritis. Ann Rheum Dis 2014;73:1746–8.

46 Rademacher J, Tietz LM, Le L *et al.* Added value of biomarkers compared with clinical parameters for the prediction of radiographic spinal progression in axial spondyloarthritis. Rheumatology 2019;58:1556–64.