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

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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 ratio (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 ratio 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

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

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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 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, χ² 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 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) (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 ratio (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 = 0.012). 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 rS was found for HGF and WBC. In addition, s-HGF was still higher in current smokers and users of prednisolone whereas no
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 ($\Delta$HGF) 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 $\Delta$HGF (Table 4).

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

| Demographic variables | Total group ($n = 163$) | Men ($n = 88$) | Women ($n = 75$) | P-value |
|-----------------------|------------------------|----------------|-----------------|---------|
| Age, median (IQR), years | 49 (40–62) | 49 (39–61) | 49 (42–63) | 0.33 |
| BMI, median (IQR), kg/m² | 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) | 20 (11–31) | 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) | 1.8 (1.0–3.2) | 2.5 (1.0–4.5) | 0.25 |
| BASDAI, median (IQR), score | 3.1 (1.7–5.1) | 2.5 (1.4–4.9) | 3.6 (1.9–5.5) | 0.065 |
| ASAS-ESR, 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), $\times 10^{9}$/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 |
| $\geq$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 | 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 | 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. *n = 161. $^b$n = 86. $^c$n = 162. $^d$n = 74. $^e$n = 87. $^f$n = 162. $^g$n = 162. 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. 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. ASAS-ESR: 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.

**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, 27].
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 2 Comparing baseline serum-HGF and the average serum-HGF in patients with and without radiographic progression

|                          | Progression ≥2 mSASSS | Development ≥1 syndesmophyte |
|--------------------------|------------------------|-------------------------------|
|                          | Yes (n = 163) | No (n = 163) | Yes (n = 163) | No (n = 163) |
| Baseline serum HGF, median (IQR), pg/ml | 1527 (1315–1711) | 1484 (1235–1693) | 0.34 | 1408 (1236–1688) | 1367 (1161–1584) | 0.18 |
| Average serum HGF, median (IQR), pg/mla | 1455 (1263–1676) | 1346 (1129–1466) | 0.025 |
|                          | Yes (n = 36) | No (n = 36) | Yes (n = 36) | No (n = 36) |
| Baseline serum HGF, median (IQR), pg/ml | 1537 (1393–1805) | 1475 (1242–1706) | 0.21 | 1465 (1302–1665) | 1358 (1163–1591) | 0.031 |
| Average serum HGF, median (IQR), pg/mla | 1478 (1381–1722) | 1289 (1147–1453) | <0.001 |

*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

**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.
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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)**      | **OR, adjusted<sup>a</sup> (95% CI)** |
| **P-value**                      | **P-value**            |
| Total group (**n = 163**)        | 1.23 (0.86, 1.78)      | 1.13 (0.81, 1.60) |
| Men (**n = 88**)                 | 2.39 (1.31, 4.36)      | 1.64 (0.98, 2.76) |
| Women (**n = 75**)               | 0.52 (0.22, 1.22)      | 0.83 (0.48, 1.45) |
| **Baseline s-HGF ≥1520 pg/ml**   |                        |                       |
| Total group (**n = 163**)        | 2.19 (1.03, 4.68)      | 1.33 (0.67, 2.62) |
| Men (**n = 88**)                 | 5.25 (1.96, 14.10)     | 1.89 (0.78, 4.56) |
| Women (**n = 75**)               | 0.47 (0.11, 2.04)      | 0.88 (0.28, 2.72) |
| **Average-HGF pg/ml, per 1 s.d.**|                        |                       |
| Total group (**n = 163**)        | 1.49 (1.04, 2.13)      | 1.30 (0.93, 1.81) |
| Men (**n = 88**)                 | 2.59 (1.44, 4.67)      | 1.78 (1.09, 2.90) |
| Women (**n = 75**)               | 0.65 (0.30, 1.43)      | 0.89 (0.50, 1.58) |

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. For men, adjusted for age, smoking and baseline syndesmophyte. For total group adjusted for age, sex, smoking, baseline syndesmophyse 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. In the analysis adjusted for BMI, ever smoker, baseline CRP and mSASSS, the variable did not remain in the final model. HGF: hepaticocyte growth factor; mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score; NA: not applicable; OR: odds ratio.

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 TNF-α 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 S95 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
explored in future research, and at present we cannot recommend the use of 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.

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analysis and interpretation of data. M.N. participated in acquisition of data. M.G. participated in acquisition and interpretation of data. H.C. participated in the conception and design of the work. L.T.H.J. participated in drafting the manuscript and interpretation of data. H.F.d’E. participated in the conception and design of the work, acquisition, analysis and interpretation of data and drafting the manuscript. All authors critically reviewed the manuscript and participated in the editing until its final version. All authors agreed to be accountable for all aspects of the work and have read and approved the final manuscript. We wish to thank all the patients and HCs who participated in this study.

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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.

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