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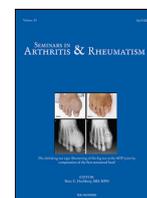
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## Baseline serum biomarkers of inflammation, bone turnover and adipokines predict spinal radiographic progression in ankylosing spondylitis patients on TNF inhibitor therapy

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### ABSTRACT

**Objective:** To analyze whether biomarker levels at baseline or their change after 3 months or 2 years predict radiographic spinal progression in ankylosing spondylitis (AS) patients treated with TNF- $\alpha$  inhibitors (TNFi).

**Methods:** 137 AS patients from the Groningen Leeuwarden Axial Spondyloarthritis (GLAS) cohort were included before starting TNFi. Serum biomarkers were measured at baseline, 3 months and 2 years: Markers of inflammation (calprotectin, matrix metalloproteinase-3, vascular endothelial growth factor), bone turnover markers (bone-specific alkaline phosphatase, serum C-terminal telopeptide fragments of type I collagen (sCTX), osteocalcin, osteoprotegerin, procollagen type I and II N-terminal propeptide, sclerostin) and adipokines (high-molecular-weight adiponectin, leptin, visfatin). Spinal radiographs were scored at baseline, 2 and 4 years. Logistic regression was performed to examine the association between biomarker values and radiographic spinal progression, adjusting for known risk factors for radiographic progression.

**Results:** Baseline calprotectin and visfatin levels were associated with mSASSS progression  $\geq 2$  points (OR 1.195 [95%CI 1.055–1.355] and 1.465 [1.137–1.889], respectively), while calprotectin was also associated with new syndesmophyte formation after 2 years (OR 1.107 [1.001–1.225]). Baseline leptin level was associated with mSASSS progression  $\geq 4$  points after 4 years (OR 0.614 [0.453–0.832]), and baseline sCTX level with syndesmophyte formation after 4 years (OR 1.004 [1.001–1.008]). Furthermore, change of visfatin and leptin levels over the first 2 years showed significant association with radiographic progression after 4 years.

**Conclusion:** Independent of known risk factors, serum levels of biomarkers at baseline are able to predict radiographic spinal progression over 2 and 4 years in AS patients on TNFi therapy.

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**Abbreviations:** AS, ankylosing spondylitis; axSpA, axial spondyloarthritis; bDMARD, biological disease-modifying antirheumatic drugs; BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; BTM, bone turnover markers; CRP, C-reactive protein; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; GESPIC, German Spondyloarthritis Inception Cohort; GLAS, Groningen Leeuwarden Axial Spondyloarthritis; HMW-APN, high molecular weight adiponectin; IQR, interquartile range; LLOQ, lower limit of quantification; MCL, Medical Center Leeuwarden; MMP-3, Metalloproteinase-3; mSASSS, modified Stoke AS Spine Score; NSAID, non-steroidal anti-inflammatory drugs; OASIS, Outcome Assessments in Ankylosing Spondylitis International Study; OC, osteocalcin; OPG, osteoprotegerin; OR, odds ratio; PINP, procollagen type I N-terminal peptide; ROC, receiver operating characteristic; sCTX, serum C-terminal telopeptide fragments of type I collagen; SD, standard deviation; SI, sacroiliac; TNFi, TNF- $\alpha$  inhibitors; UMCG, University Medical Center Groningen; VEGF, vascular endothelial growth factor

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### Introduction

Axial spondyloarthritis (axSpA) is a heterogeneous chronic rheumatic disease characterized by inflammation of especially the sacroiliac (SI) joints and spinal column. Its pathology is characterized by disruption of normal bone homeostasis, which may result in new bone formation and also bone loss [1,2]. Structural damage caused by excessive bone formation is characterized by the development of syndesmophytes which may result in ankylosing of the spine. Structural damage influences the functional status and spinal mobility in both early axSpA and longstanding disease [3,4]. Therefore, one of the primary goals of treatment, according to current recommendations, is the prevention of radiographic progression [5]. TNF- $\alpha$

inhibitors (TNFi) were the first biological disease-modifying antirheumatic drugs (bDMARD) introduced for axSpA and revolutionized treatment [6]. A recent meta-analysis showed a protective effect of TNFi on the progression of structural damage on radiographs of the spine after at least 4 years of treatment [7]. At group level, in the first 4 years of treatment with TNFi, radiographic progression follows a linear course of approximately one mSASSS unit increase per year, whereas it was shown to retard afterwards [8]. At individual patient level, the course of this spinal radiographic progression is highly variable.

Former research showed that different parameters are associated with radiographic progression: demographic characteristics (male gender, older age), genetics (HLA-B27 positivity), disease features (longer symptom duration), life style factors (cigarette smoking, alcohol consumption, higher BMI), laboratory parameters (elevated acute-phase reactants) and radiographic damage already present at baseline [9–12]. Among these risk factors, the presence of baseline syndesmophytes is the strongest predictor for radiographic progression. Besides clinical parameters, also different biomarkers were found to predict radiographic progression in ankylosing spondylitis (AS) patients treated with non-steroidal anti-inflammatory drugs (NSAIDs), among them are markers of inflammation, bone turnover and adipokines [13].

As inflammation is thought to precede structural damage, different markers related to inflammation were evaluated as prognostic biomarkers in axSpA: C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) remain the most validated biomarkers that are routinely used in daily clinical practice and were shown to be associated not only with disease activity, but also with radiographic progression in early axSpA patients of the German Spondyloarthritis Inception Cohort (GESPIC) [10]. Metalloproteinase-3 (MMP-3), reflecting synovial inflammation, was independently associated with radiographic progression in bDMARD naïve AS patients of the Outcome Assessments in Ankylosing Spondylitis International Study (OASIS) cohort [14]. Calprotectin, secreted by neutrophils, promotes inflammation [15], while vascular endothelial growth factor (VEGF) – a protein relevant for angiogenesis – links inflammation and bone formation, especially enchondral ossification [16]. Both, elevated calprotectin and VEGF levels were shown to be predictive for radiographic progression in GESPIC [16,17], a cohort in which only a minority (3%) of axSpA patients were treated with TNFi. Contrarily, VEGF levels were not associated with radiographic progression in AS patients treated with golimumab [18].

Bone turnover markers (BTM) may also reflect the processes leading to excessive bone formation in axSpA: serum C-terminal telopeptide fragments of type I collagen (sCTX), a bone resorption marker, as well as bone formation markers procollagen type I N-terminal peptide (PINP) and osteocalcin (OC) were elevated in AS patients with bridging syndesmophytes [2,19]. Another marker of bone formation, bone-specific alkaline phosphatase (BALP), which is increased during TNFi treatment, may be associated with a simultaneous increase of bone mineral density (BMD) [20,21]. On the other hand, higher serum levels of the Wnt pathway inhibitors sclerostin and dickkopf-1 were associated with no new syndesmophyte formation in AS [22,23].

Adipokines have besides metabolic, also immunomodulatory effects and alter bone metabolism: elevated visfatin serum levels were associated with radiographic progression [24], while elevated leptin and high molecular weight (HMW) adiponectin serum levels predicted protection from structural damage in AS [25].

If biomarkers including BTM could be used to predict radiographic progression, this would help to evaluate the individual patient's risk for progression and personalize treatment decisions accordingly. However, only scarce information exists about the predictive value of biomarkers for radiographic progression in patients starting treatment with TNFi. Therefore, the objective of this study was to analyze whether biomarker levels reflecting inflammation, bone turnover

and adipokines at baseline, their change after 3 months and/or 2 years, can predict spinal radiographic progression up to 4 years of follow-up in AS patients starting and continuing long-term treatment with TNFi.

## Material and methods

### Patients

Patients included in this study were consecutive AS outpatients from the University Medical Center Groningen (UMCG) and Medical Center Leeuwarden (MCL) attending the Groningen Leeuwarden Axial Spondyloarthritis (GLAS) cohort. The design and available data of the GLAS cohort have been described previously [26]. Briefly, the GLAS cohort is an ongoing prospective longitudinal observational cohort study with standardized follow-up visits including clinical assessments, biobanking and imaging. Initially, included axSpA participants were 18 years or older, fulfilled the modified New York criteria for AS and the ASAS criteria to start treatment with TNFi [12]. For the present study, GLAS patients included between November 2004 and April 2012 with radiographic data available before start of TNFi (baseline) and at 2 and/or 4 years of TNFi therapy were included [8]. Ten patients were excluded because they had no available serum samples at baseline. Clinical response was defined as BASDAI50 response (reduction of  $\geq 50\%$ ) as well as ASDAS-clinical important improvement (ASDAS-CI, reduction of  $\geq 1.1$  unit).

The GLAS cohort was approved by the local ethics committees of the MCL and the UMCG. All patients gave written informed consent according to the Declaration of Helsinki.

### Radiographic assessment

The procedure for scoring spinal radiographic damage and progression has also been published before [8,31]. In short, lumbar and cervical spine radiographs were scored biannually by two trained readers in chronological time order using the modified Stoke AS Spine Score (mSASSS; range 0–72) [27,28]. Readers were not familiar with the patients and all patient characteristics were removed from the radiographs. To avoid potential reader bias due to the knowledge of the applied therapy, radiographs of included patients were randomized and scored with longitudinal radiographs of AS patients from a historical cohort, not treated with TNFi. The average mSASSS total score of the two readers was used in the analysis. In case of missing scores at intermediate follow-up visits, data was imputed using single linear imputation as previously reported [8].

Since former analyses in this cohort showed an average increase of 0.9 mSASSS units per year at group level [8], radiographic progression was defined as mSASSS progression of  $\geq 2$  units in the first 2 years and  $\geq 4$  units after 4 years of follow-up. The progression of syndesmophytes was defined as the formation of  $\geq 1$  new syndesmophyte according to both readers in the first 2 years and  $\geq 2$  new syndesmophytes during 4 years of follow-up [29,30].

### Biomarkers

Biomarkers were chosen based on former published data according to their association with radiographic progression or excessive bone formation in AS: markers of inflammation [14,16,17], bone turnover [2,19,23] and adipokines [25,31] were included. Serum levels of the following biomarker panel were quantified at baseline, 3 months and 2 years by sandwich enzyme linked immunosorbent assay (ELISA) by persons blinded to patients' characteristics and time points:

- 1 **Markers of inflammation:** Calprotectin (BioLegend, US), MMP3 (R&D Systems, US), VEGF (R&D Systems, US).

2 **Markers of BoneTurnover:** BALP (Metra Biosystems, Mountain View, CA, USA), sCTX (ECLIA; Elecsys 2010 Roche Mannheim, Germany), OC (IRMA; BioSource Europe South Africa), Osteoprotegerin (OPG, Biomedica Medizinprodukte GmbH & Co KG, Austria), Procollagen typ I N-terminal propeptide (PINP, RIA; Orion Diagnostica, Espoo, Finland), Procollagen type II N-terminal propeptide (PIINP, Wuhan USCN Business Co., China), Sclerostin (R&D Systems, US).

3 **Adipokines:** HMW-adiponectin (HMW-APN, R&D Systems, US), Leptin (Thermo Fisher Scientific, US), Visfatin (Adipogen AG, Switzerland).

Serum levels were measured in duplicates by ELISA according to the manufacturers' protocols, performance data are given as **Supplementary Table S1**. The highest dilution of the respective standard was defined as the lower limit of quantification (LLOQ). Serum levels below LLOQ were substituted by the concentration of this lowest standard. Mean values were used for subsequent analyses. CRP and ESR were measured during routine diagnostic patient care. Serum was acquired of non-fasting patients during study visits of the GLAS cohort taking place at fixed hours (same half-day).

### Statistics

Statistical analyses were performed with IBM SPSS Statistics version 23 and 26; as well as Graph Pad Prism Version 8.4.3. Results were expressed as mean  $\pm$  standard deviation (SD) or median [interquartile range (IQR): first – third quartile] for normally distributed and non-normally distributed data, respectively. Biomarker values of radiographic progressors and non-progressors as well as clinical responders and non-responders were compared using Mann-Whitney *U* test. Wilcoxon signed rank test was used to analyze biomarker values over time.

Logistic regression was performed to examine the association of biomarker values at baseline and their change after 3 months or 2 years with radiographic spinal progression. Odds Ratio (OR) and 95% confidence intervals (CI) are given for one unit concentration of the respective biomarker. Multivariable models for each biomarker were adjusted for confounders known to be associated with radiographic progression: radiographic damage at baseline (mSASSS or syndesmophytes), elevated CRP level ( $\geq 5$  mg/l), smoking status, male gender, symptom duration and BMI. Furthermore, we also corrected for the biomarker level at baseline in the models with biomarker change.

Receiver operating characteristic (ROC) analysis was performed to determine the accuracy of baseline biomarker serum levels to predict radiographic spinal progression.

For all analysis *p* values  $<0.05$  were considered statistically significant. Due to the hypothesis-driven, validity character of this study, *p* values were not adjusted for multiple testing.

## Results

### Patients' characteristics and radiographic progression

Patients were predominantly male (72%) and HLA-B27 positive (79%), had a mean age of  $42 \pm 11$  years and overall high disease activity at baseline, before the start of TNFi (mean ASDAS-CRP  $3.9 \pm 0.7$ ). Mean radiographic damage at baseline according to mSASSS was  $10.6 \pm 16.1$  units and 74% of patients had at least one syndesmophyte. Detailed baseline characteristics are shown in **Table 1**.

Patients started TNFi after their baseline visit and retained TNFi treatment for  $>97\%$  of the follow-up time. One third (33%) of the patients showed mSASSS progression of  $\geq 2$  units and 24% developed  $\geq 1$  syndesmophyte during the first 2 years. Progression of  $\geq 4$  mSASSS units was observed in 32% of the patients and 30% developed

**Table 1**

Baseline characteristics of the 137 included ankylosing spondylitis patients.

Age, years	42.0 $\pm$ 10.8
Symptom duration, years	15.0 [7.0–23.0]
Male sex	98 (71.5%)
HLA-B27 positive	108 (78.8%)
BMI (kg/m <sup>2</sup> )	26.1 $\pm$ 3.9
Current smoking	42 of 118 (35.6%)
History of uveitis	42 of 136 (30.9%)
History of IBD	15 of 135 (11.1%)
History of psoriasis	10 of 136 (7.4%)
Peripheral arthritis	24 (17.5%)
BASDAI (0–10)	6.0 [5.1–7.2]
ASDAS-CRP	3.9 $\pm$ 0.7
CRP (mg/l)	14.0 [5.0–22.0]
ESR (mm/hour)	21.0 [11.0–35.0]
BASFI (0–10)	5.5 $\pm$ 2.0
mSASSS (0–72)	3.0 [0–14.0]
$\geq 1$ syndesmophyte	74 (54.0%)
NSAID use	111 (81.0%)
ASAS-NSAID index (0–100)	50 (20–100)
TNFi (start after baseline)	
- Etanercept	84 (61.3%)
- Infliximab	21 (15.3%)
- Adalimumab	32 (23.4%)

Values are presented as number of patients (percentage), mean  $\pm$  standard deviation, or median [Interquartile range].

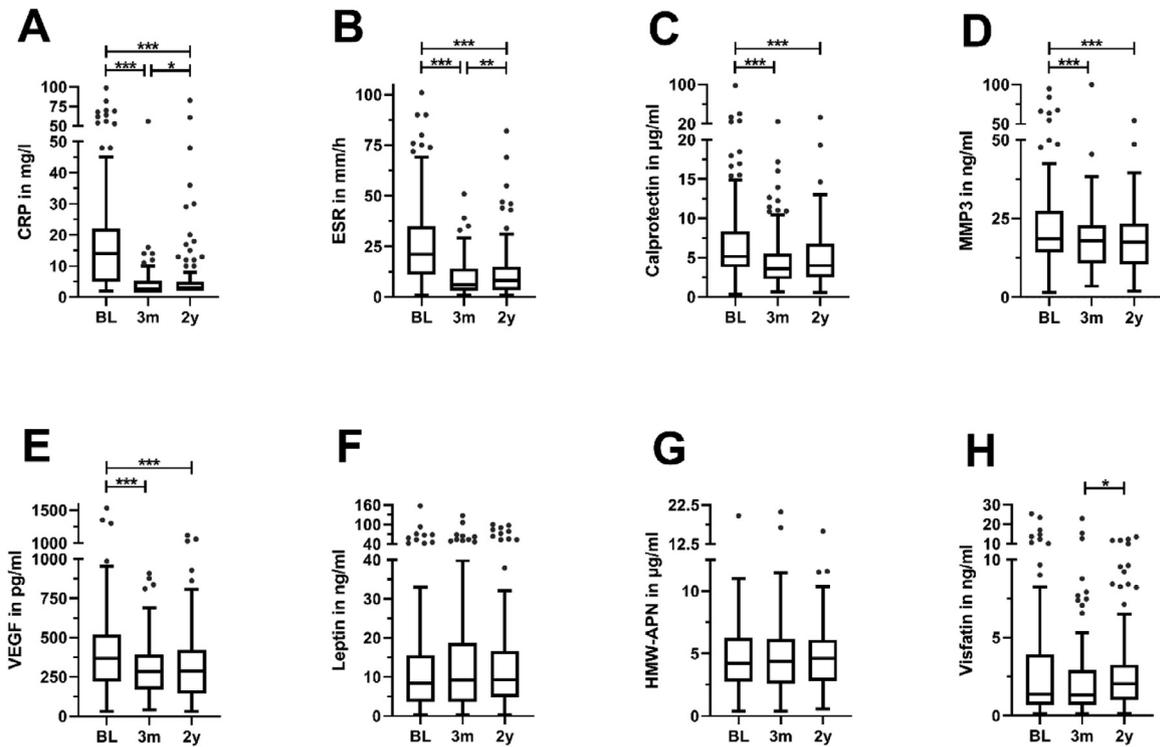
$\geq 2$  syndesmophytes during 4 years of treatment with TNFi (**Supplementary Tables S2, S3**).

### Differences in baseline serum biomarker values between patients with and without radiographic progression

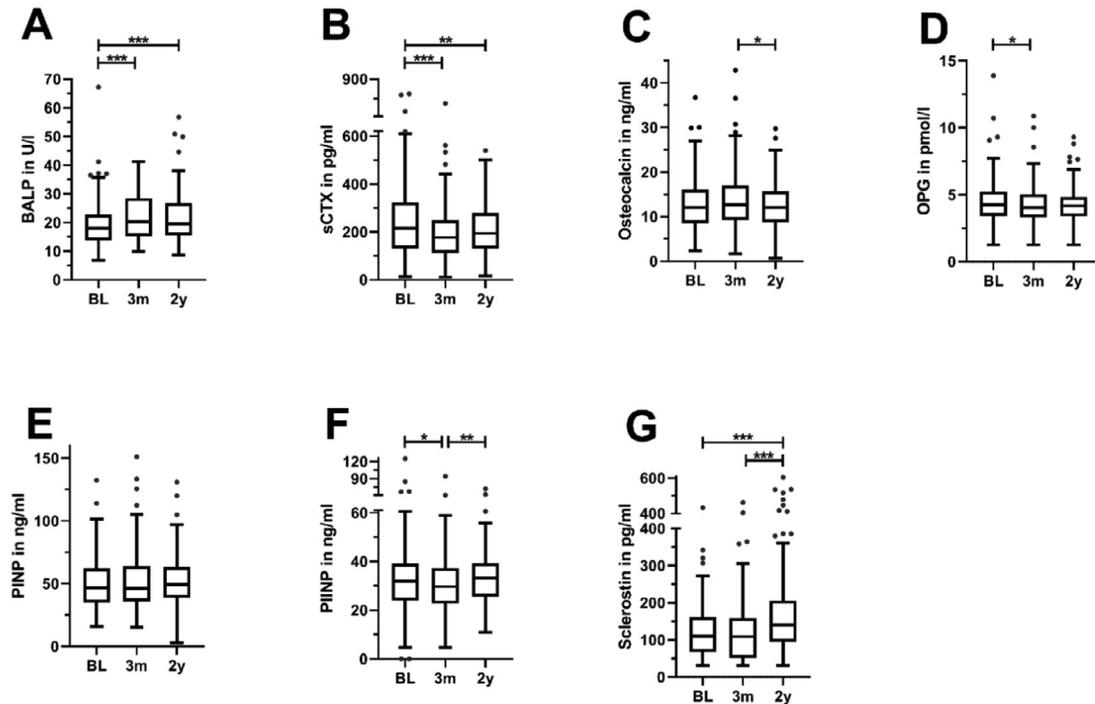
Patients with radiographic progression of  $\geq 2$  mSASSS points in the first 2 years showed significantly higher serum levels of markers of inflammation (calprotectin, CRP) and bone turnover (PINP, sclerostin) at baseline compared to non-progressors. Of these biomarkers, baseline levels of CRP and PINP were also significantly higher in mSASSS progressors at 4 years (**Supplementary Table S3**). Furthermore, baseline levels of sclerostin showed significant differences between patients with and without new syndesmophytes at 2 years (**Supplementary Table S2**). Baseline OC was significantly higher in patients with mSASSS progression at 4 years. Baseline sCTX levels were significantly higher in patients showing mSASSS and syndesmophyte progression at 4 years. No significant differences were found for baseline levels of adipokines between patients with and without radiographic progression (**Supplementary Table S3**).

### Change in serum biomarker values during TNFi treatment

Serum levels of biomarkers changed during the first 2 years of treatment with TNFi. Besides CRP and ESR, also other markers of inflammation (calprotectin, MMP3, VEGF) decreased significantly over time, which was already evident after 3 months of TNFi treatment (**Fig. 1**). Regarding BTM, levels of BALP and sclerostin showed a significant increase over time and sCTX levels a significant reduction (**Fig. 2**). OPG and PIINP decreased significantly in the first 3 months of TNFi treatment, but no difference was found between baseline and after 2 years of treatment. HMW-adiponectin and leptin levels remained stable during TNFi treatment. Although serum levels of visfatin increased significantly between 3 months and 2 years of TNFi treatment, no significant difference in visfatin serum levels were found compared to baseline (**Fig. 1**).



**Fig. 1.** Levels of markers of inflammation (A-E) and adipokines (F-H) during treatment with TNF inhibitors: serum levels at baseline (BL; before start of TNFi), after 3 months (3 m) and 2 years (2y) of treatment. Wilcoxon Signed Rank Test: \* p value <0.05; \*\* p value <0.01; \*\*\* p value <0.001.



**Fig. 2.** Levels of bone turnover marker under treatment with TNF inhibitors: serum levels at baseline (BL; before start of TNFi), after 3 months (3 m) and 2 years (2y) of treatment. Wilcoxon Signed Rank Test: \* p value <0.05; \*\* p value <0.01; \*\*\* p value <0.001.

#### Association between clinical response to TNFi and change in serum biomarkers

Patients who clinically responded after 3 months of TNFi, according to both BASDAI50 and ASDAS-CI, showed a greater reduction of serum level of markers of inflammation (CRP, ESR and VEGF) and sCTX. Furthermore, BASDAI50 response was

associated with a greater reduction of calprotectin, HMW-adiponectin and visfatin; and ASDAS-CI with PIINP (**Supplementary Table S4**). Patients with BASDAI50 reduction after 2 years had a significant greater reduction of ESR, whilst patients with ASDAS-CI after 2 years showed a greater change in serum markers of inflammation (CRP, ESR and VEGF) and PIINP (**Supplementary Table S5**).

**Table 2**  
Biomarker values predicting radiographic progression defined as mSASSS progression.

Biomarker	Timepoint for Progression in years	Univariable model OR (95% CI)	p value	Multivariable model OR (95% CI)	p value
<b>Markers of Inflammation</b>					
<b>Calprotectin</b>	2	1.041 (0.988–1.097)	0.129	<b>1.195 (1.055–1.355)</b>	<b>0.005</b>
	4	0.992 (0.952–1.035)	0.716	1.074 (0.970–1.189)	0.169
<b>MMP-3</b>	2	1.008 (0.981–1.035)	0.565	0.987 (0.948–1.027)	0.527
	4	1.003 (0.979–1.027)	0.825	0.977 (0.940–1.016)	0.24
<b>VEGF</b>	2	1.001 (0.999–1.002)	0.363	1.000 (0.998–1.002)	0.853
	4	1.000 (0.998–1.001)	0.857	1.000 (0.997–1.002)	0.807
<b>Bone Turnover Markers</b>					
<b>BALP</b>	2	1.031 (0.984–1.080)	0.196	1.074 (0.981–1.175)	0.123
	4	1.032 (0.986–1.082)	0.178	1.078 (0.981–1.185)	0.117
<b>OC</b>	2	1.032 (0.973–1.095)	0.297	1.027 (0.929–1.136)	0.598
	4	1.051 (0.991–1.115)	0.099	0.987 (0.895–1.089)	0.801
<b>OPG</b>	2	0.924 (0.746–1.144)	0.468	1.177 (0.835–1.659)	0.353
	4	0.965 (0.786–1.185)	0.733	1.325 (0.851–2.063)	0.213
<b>PINP</b>	2	1.012 (0.995–1.030)	0.156	1.020 (0.989–1.052)	0.198
	4	<b>1.020 (1.002–1.038)</b>	<b>0.030</b>	1.015 (0.986–1.046)	0.310
<b>PIINP</b>	2	1.000 (0.974–1.026)	0.989	0.987 (0.951–1.025)	0.496
	4	0.996 (0.970–1.022)	0.742	0.979 (0.940–1.018)	0.286
<b>Sclerostin</b>	2	<b>1.006 (1.001–1.011)</b>	<b>0.015</b>	1.007 (0.996–1.017)	0.213
	4	1.003 (0.998–1.007)	0.288	1.000 (0.990–1.010)	0.988
<b>sCTX</b>	2	1.001 (0.999–1.004)	0.22	1.003 (1.000–1.007)	0.083
	4	<b>1.003 (1.001–1.005)</b>	<b>0.016</b>	1.004 (1.000–1.008)	0.052
<b>Adipokines</b>					
<b>HMW-Adiponektin</b>	2	0.989 (0.868–1.127)	0.871	1.138 (0.902–1.435)	0.277
	4	0.988 (0.867–1.127)	0.859	1.034 (0.842–1.270)	0.749
<b>Leptin</b>	2	0.972 (0.941–1.004)	0.087	0.896 (0.770–1.042)	0.154
	4	<b>0.954 (0.914–0.996)</b>	<b>0.034</b>	<b>0.614 (0.453–0.832)</b>	<b>0.002</b>
<b>Visfatin</b>	2	<b>1.106 (1.007–1.215)</b>	<b>0.036</b>	<b>1.465 (1.137–1.889)</b>	<b>0.003</b>
	4	0.979 (0.893–1.074)	0.659	1.041 (0.845–1.283)	0.704

Logistic regression performed for biomarker values at baseline. Odds Ratio OR and 95% Confidence Interval (CI) as well as p values are shown. Multivariable models for each biomarker were adjusted for mSASSS, elevated CRP level at baseline, smoking status, male gender, symptom duration, BMI. Radiographic progression was defined as mSASSS progression of  $\geq 2$  units in the first 2 years and  $\geq 4$  units after 4 years. BALP Bone alkaline phosphatase; HMW high molecular weight; MMP-3 matrix metalloproteinase-3; OC osteocalcin; OPG osteoprotegerin; PINP procollagen type I N-terminal propeptide; PIINP procollagen type II N-terminal propeptide; sCTX serum C-terminal telopeptide; VEGF vascular endothelial growth factor.

### Can serum biomarker levels predict radiographic progression?

Univariable and multivariable logistic regression revealed several significant associations of baseline biomarker levels and their change after 3 months and 2 years of TNFi treatment with radiographic spinal progression.

In the univariable analysis, none of the analyzed biomarkers reflecting inflammation showed a significant association with radiographic progression. However, multivariable logistic analyses (with correction for parameters known to be associated with radiographic progression) revealed an association between baseline calprotectin levels and both mSASSS (odds ratio (OR) [95% confidence interval], OR 1.195 [1.055–1.355]; **Table 2**) and syndesmophyte progression at 2 years (OR 1.107 [1.001–1.225]; **Table 3**). The change in VEGF serum level after 2 years was associated with mSASSS progression (**Table 4**), though only in the multivariable analysis.

Univariable logistic regression on baseline levels of BTM and the progression of radiographic damage revealed the following associations: PINP with mSASSS progression at 4 years (OR 1.020 [1.002–1.038]), sclerostin with both mSASSS progression (OR 1.006 [1.001–1.011]) and syndesmophyte formation (OR 1.007 [1.001–1.013]) at 2 years; and sCTX with mSASSS progression at 4 years (OR 1.003 [1.001–1.005]). In multivariable logistic analysis, only baseline sCTX levels were independently associated with syndesmophyte progression after 4 years (OR 1.004 [1.001–1.008]). Moreover, in univariable analysis, the change of PINP levels after 2 years predicted mSASSS progression at all time points (**Table 4**). The change of sclerostin level after 3 months of TNFi therapy was positively associated with syndesmophyte progression at 2 years (OR 1.007 [1.000–1.015]) and sclerostin change after 2 years of therapy with syndesmophyte progression at 4 years (OR 1.005

[1.000–1.009]); **Supplementary Table S6**). However, all associations with sclerostin levels and radiographic progression were lost when adjusted for other known risk factors in multivariable analyses.

Regarding adipokines, univariable logistic regression revealed a significant association of baseline visfatin with mSASSS progression at 2 years (OR 1.106 [1.007–1.215]), which remained significantly associated when adjusted for confounders (OR 1.465 [1.137–1.889], **Table 2**). Furthermore, the change in visfatin serum levels after 2 years was associated with both mSASSS progression (OR 1.108 [1.004–1.224]) and syndesmophyte formation (OR 1.115 [1.002–1.24]) at 2 years. However, these associations were lost in multivariable analyses, whereas the change of visfatin level after 2 years showed a significant association with mSASSS progression at 4 years only when adjusting for other risk factors (OR 2.255 [1.108–4.591]). Baseline leptin levels showed an inverse association with mSASSS progression at 4 years in both, univariable and multivariable models (OR 0.954 [0.914–0.996] and OR 0.614 [0.453–0.832] respectively). Furthermore, the change of leptin levels after the first 2 years predicted mSASSS progression at 4 years when adjusted for confounders (OR 1.489 [1.041–2.130]).

When combining baseline levels of biomarkers with CRP in logistic regression, sclerostin ( $p=0.012$  and  $p=0.026$  for mSASSS and syndesmophyte progression after 2 years, respectively), PINP ( $p=0.038$  for mSASSS progression after 4 years), sCTX ( $p=0.059$  for mSASSS progression after 4 years), leptin ( $p=0.045$  for mSASSS progression after 4 years) and visfatin ( $p=0.050$  for mSASSS progression after 2 years) seem to contribute independently from CRP to the prediction of radiographic spinal progression.

Finally, we performed ROC analyses for baseline serum levels of acute phase reactants (CRP and ESR) and those biomarkers showing a significant association with radiographic progression in the logistic

**Table 3**  
Biomarker values predicting radiographic progression defined as syndesmophyte formation.

Biomarker	Timepoint for Progression in years	Univariable model		Multivariable model	
		OR (95% CI)	p value	OR (95% CI)	p value
<b>Markers of Inflammation</b>					
<b>Calprotectin</b>	2	1.033 (0.988–1.080)	0.156	<b>1.107 (1.001–1.225)</b>	<b>0.048</b>
	4	1.014 (0.953–1.079)	0.66	1.075 (0.973–1.188)	0.153
<b>MMP-3</b>	2	1.001 (0.970–1.032)	0.959	0.986 (0.946–1.029)	0.525
	4	0.991 (0.963–1.019)	0.519	0.970 (0.928–1.013)	0.17
<b>VEGF</b>	2	1.000 (0.998–1.002)	0.916	0.998 (0.996–1.001)	0.258
	4	0.999 (0.998–1.001)	0.347	0.999 (0.996–1.002)	0.46
<b>Bone Turnover Markers</b>					
<b>BALP</b>	2	0.984 (0.928–1.044)	0.592	0.997 (0.929–1.069)	0.924
	4	1.032 (0.983–1.082)	0.202	1.043 (0.967–1.124)	0.278
<b>OC</b>	2	0.993 (0.925–1.065)	0.837	0.957 (0.856–1.069)	0.433
	4	1.036 (0.975–1.102)	0.254	1.045 (0.948–1.153)	0.374
<b>OPG</b>	2	0.971 (0.757–1.245)	0.815	1.100 (0.805–1.504)	0.549
	4	0.983 (0.790–1.223)	0.877	1.174 (0.862–1.599)	0.308
<b>PINP</b>	2	0.992 (0.969–1.015)	0.472	0.989 (0.954–1.025)	0.539
	4	1.007 (0.989–1.025)	0.433	1.007 (0.979–1.036)	0.642
<b>PIINP</b>	2	1.009 (0.980–1.039)	0.539	1.002 (0.967–1.038)	0.901
	4	0.993 (0.965–1.021)	0.603	0.988 (0.950–1.028)	0.558
<b>Sclerostin</b>	2	<b>1.007 (1.001–1.013)</b>	<b>0.023</b>	1.001 (0.991–1.011)	0.842
	4	1.003 (0.998–1.009)	0.181	1.001 (0.992–1.010)	0.828
<b>sCTX</b>	2	1.000 (0.998–1.003)	0.829	1.000 (0.996–1.004)	0.872
	4	1.002 (1.000–1.005)	0.063	<b>1.004 (1.001–1.008)</b>	<b>0.018</b>
<b>Adipokines</b>					
<b>HMW-Adiponektin</b>	2	0.996 (0.859–1.154)	0.953	1.071 (0.875–1.311)	0.503
	4	1.039 (0.910–1.186)	0.572	1.112 (0.900–1.374)	0.327
<b>Leptin</b>	2	0.996 (0.968–1.024)	0.763	0.998 (0.946–1.053)	0.943
	4	0.983 (0.956–1.011)	0.231	0.947 (0.874–1.025)	0.177
<b>Visfatin</b>	2	1.074 (0.979–1.178)	0.128	1.091 (0.906–1.314)	0.360
	4	1.010 (0.915–1.116)	0.838	0.975 (0.796–1.195)	0.810

Logistic regression performed for biomarker values at baseline. Odds Ratio OR and 95% Confidence Interval (CI) as well as p values are shown. Multivariable models for each biomarker were adjusted for syndesmophytes at baseline, elevated CRP level, smoking status, male gender, symptom duration and BMI. BALP Bone alkaline phosphatase; HMW high molecular weight; MMP-3 matrix metalloproteinase-3; OC osteocalcin; OPG osteoprotegerin; PINP procollagen type I N-terminal propeptide; PIINP procollagen type II N-terminal propeptide; sCTX serum C-terminal telopeptide; VEGF vascular endothelial growth factor.

regression. Sclerostin levels at baseline showed somewhat larger AUC for both, mSASSS and syndesmophyte progression over 2 years compared to CRP and ESR. sCTX showed somewhat larger AUC for syndesmophyte progression over 4 years compared to CRP and ESR. However, overall, accuracy of the biomarkers to predict radiographic spinal progression was not very good (<0.7), which limits their clinical value in daily practice (**Supplementary Table S7**).

## Discussion

The present study offers the possibility of evaluating a wide range of biomarkers reflecting inflammation, bone turnover and adipokines during treatment with TNFi including their value predicting radiographic progression over a prospective long-term follow-up period of 4 years. Independent of known risk factors, baseline serum levels of inflammation marker calprotectin and adipokine visfatin were associated with radiographic spinal progression after 2 years of TNFi.

We could thereby replicate the finding of previous studies, which described serum calprotectin as independent marker for 2-year radiographic progression in axSpA patients naïve to bDMARD treatment, in our prospective observational GLAS cohort [17]. Furthermore, higher fecal calprotectin levels at baseline were reported to predict treatment response to adalimumab [32]. It is known that calprotectin subunits s100A8 and A9 have an effect on osteoclasts and osteoblasts, both seem to be involved during differentiation of osteocytes: Whilst s100A8 is suggested to play a stimulating role in osteoblast differentiation, S100A9 seems to hamper differentiation of osteoclasts [33,34]. Interestingly, calprotectin serum levels were not associated with the clinical presence or activity of concomitant inflammatory bowel disease in our cohort.

In earlier studies, visfatin levels correlated with patient reported disease activity (BASDAI) and radiographic damage (mSASSS) [35]. Furthermore, visfatin levels were found to be higher in patients with radiographic progression after 2 years [31], which is comparable to our results. Visfatin is known to promote matrix mineralization of osteoblasts as well as suppression of osteoclast development at both formation and differentiation stages [36,37], which might explain the observed association with radiographic progression in AS.

Both calprotectin and visfatin are known to promote inflammation, which is effectively inhibited by TNFi within the first 3 months of treatment. Also in our study, serum levels of both biomarkers showed an early decrease within 3 months of TNFi therapy. Interestingly, both biomarkers lost their predictive value of radiographic progression after prolonged treatment (4 years) with TNFi, which matches our earlier published data that in our axSpA cohort radiographic progression diminishes after 4 years of TNFi therapy [38].

In multivariable analyses, baseline levels of sCTX, a collagen degradation product, were associated with radiographic progression after 4 years. In cross-sectional studies, sCTX serum levels were found to be correlated with both inflammation on MRI [39] and advanced radiographic damage defined as the presence of bridging syndesmophytes in AS patients [2]. A greater decrease in serum level of sCTX was observed in patients that clinically responded to TNFi over 3 months compared to non-responders. Interestingly, the change of the serum level of PIINP, a marker for cartilage synthesis known to be correlated with CRP in axSpA [40], was associated with ASDAS-CI after 3 months and 2 years: PIINP levels were decreasing in responders and stable in non-responders.

Only limited data exist about the prediction of spinal radiographic progression by biomarkers during bDMARD treatment in axSpA,

**Table 4**  
Change of biomarker values predicting mSASSS progression.

Biomarker	Biomarker Change	Timepoint Progression	Univariable model		Multivariable model	
			OR (95% CI)	p value	OR (95% CI)	p value
<b>Markers of Inflammation</b>						
<b>Calprotectin</b>	<b>Δ 3 months</b>	2	1.039 (0.984–1.097)	0.165	1.022 (0.814–1.283)	0.851
		4	0.997 (0.956–1.040)	0.907	1.201 (0.922–1.565)	0.174
	<b>Δ 2 years</b>	2	1.030 (0.986–1.076)	0.189	1.209 (0.915–1.597)	0.183
		4	0.989 (0.948–1.032)	0.607	1.140 (0.887–1.465)	0.306
<b>MMP-3</b>	<b>Δ 3 months</b>	2	0.996 (0.966–1.027)	0.793	0.994 (0.949–1.042)	0.808
		4	0.989 (0.96–1.0180)	0.444	0.991 (0.945–1.039)	0.698
	<b>Δ 2 years</b>	2	0.994 (0.962–1.027)	0.716	1.008 (0.927–1.097)	0.847
		4	0.976 (0.942–1.011)	0.176	0.989 (0.907–1.079)	0.804
<b>VEGF</b>	<b>Δ 3 months</b>	2	1.002 (0.999–1.005)	0.168	1.000 (0.995–1.005)	0.983
		4	1.000 (0.998–1.003)	0.755	0.999 (0.994–1.004)	0.689
	<b>Δ 2 years</b>	2	1.002 (1.000–1.004)	0.114	1.005 (1.000–1.010)	<b>0.042</b>
		4	1.000 (0.998–1.002)	0.887	1.005 (0.929–1.088)	0.894
<b>Bone Turnover Markers</b>						
<b>BALP</b>	<b>Δ 3 months</b>	2	0.989 (0.923–1.060)	0.759	1.029 (0.895–1.183)	0.687
		4	1.004 (0.937–1.075)	0.912	1.134 (0.981–1.311)	0.088
	<b>Δ 2 years</b>	2	1.002 (0.948–1.060)	0.945	0.975 (0.881–1.078)	0.618
		4	1.024 (0.966–1.085)	0.429	1.032 (0.942–1.130)	0.499
<b>Osteocalcin</b>	<b>Δ 3 months</b>	2	1.085 (0.994–1.186)	0.069	1.070 (0.932–1.229)	0.338
		4	1.043 (0.962–1.131)	0.305	1.025 (0.904–1.163)	0.698
	<b>Δ 2 years</b>	2	1.056 (0.971–1.148)	0.205	1.159 (0.908–1.480)	0.236
		4	1.072 (0.984–1.168)	0.113	1.039 (0.859–1.256)	0.695
<b>OPG</b>	<b>Δ 3 months</b>	2	0.962 (0.674–1.372)	0.829	1.739 (0.673–4.490)	0.253
		4	1.066 (0.757–1.501)	0.713	1.191 (0.516–2.749)	0.681
	<b>Δ 2 years</b>	2	0.921 (0.668–1.268)	0.613	2.160 (0.895–5.215)	0.087
		4	0.933 (0.676–1.287)	0.672	2.141 (0.902–5.083)	0.084
<b>PINP</b>	<b>Δ 3 months</b>	2	1.001 (0.975–1.028)	0.943	1.013 (0.977–1.051)	0.485
		4	1.005 (0.979–1.033)	0.699	1.017 (0.979–1.056)	0.392
	<b>Δ 2 years</b>	2	<b>1.027 (1.003–1.052)</b>	<b>0.030</b>	1.007 (0.953–1.065)	0.794
		4	<b>1.032 (1.007–1.058)</b>	<b>0.013</b>	0.981 (0.939–1.026)	0.407
<b>PIINP</b>	<b>Δ 3 months</b>	2	1.003 (0.979–1.028)	0.801	1.008 (0.962–1.056)	0.740
		4	0.999 (0.974–1.024)	0.936	0.992 (0.947–1.040)	0.743
	<b>Δ 2 years</b>	2	0.999 (0.972–1.026)	0.926	0.997 (0.940–1.058)	0.924
		4	0.992 (0.964–1.020)	0.565	0.928 (0.859–1.002)	0.058
<b>Sclerostin</b>	<b>Δ 3 months</b>	2	1.003 (0.998–1.009)	0.265	0.998 (0.990–1.006)	0.622
		4	1.001 (0.996–1.007)	0.678	1.002 (0.994–1.011)	0.608
	<b>Δ 2 years</b>	2	1.001 (0.998–1.005)	0.558	0.997 (0.991–1.003)	0.294
		4	1.001 (0.998–1.005)	0.433	0.998 (0.992–1.004)	0.575
<b>sCTX</b>	<b>Δ 3 months</b>	2	1.001 (0.998–1.004)	0.726	0.999 (0.992–1.006)	0.750
		4	1.001 (0.998–1.004)	0.455	0.999 (0.993–1.006)	0.839
	<b>Δ 2 years</b>	2	1.000 (0.997–1.003)	0.964	0.999 (0.991–1.007)	0.808
		4	1.001 (0.998–1.004)	0.382	1.000 (0.993–1.008)	0.947
<b>Adipokines</b>						
<b>HMW-Adiponektin</b>	<b>Δ 3 months</b>	2	1.104 (0.877–1.391)	0.400	1.086 (0.716–1.646)	0.699
		4	1.053 (0.843–1.317)	0.649	0.957 (0.645–1.421)	0.828
	<b>Δ 2 years</b>	2	1.089 (0.904–1.312)	0.369	1.229 (0.818–1.847)	0.321
		4	0.999 (0.829–1.205)	0.993	1.022 (0.703–1.486)	0.910
<b>Leptin</b>	<b>Δ 3 months</b>	2	0.999 (0.962–1.037)	0.960	1.098 (0.906–1.330)	0.339
		4	1.006 (0.968–1.045)	0.772	1.114 (0.899–1.381)	0.323
	<b>Δ 2 years</b>	2	1.005 (0.980–1.031)	0.689	1.188 (0.956–1.477)	0.120
		4	1.001 (0.976–1.027)	0.940	<b>1.489 (1.041–2.130)</b>	<b>0.029</b>
<b>Visfatin</b>	<b>Δ 3 months</b>	2	1.072 (0.976–1.177)	0.145	1.113 (0.718–1.726)	0.631
		4	1.049 (0.960–1.147)	0.287	1.400 (0.795–2.466)	0.243
	<b>Δ 2 years</b>	2	<b>1.108 (1.004–1.224)</b>	<b>0.041</b>	1.978 (0.790–4.948)	0.145
		4	1.035 (0.945–1.132)	0.461	<b>2.255 (1.108–4.591)</b>	<b>0.025</b>

Logistic regression performed for the change of biomarker values between levels after 3 months (Δ 3 months) or 2 years (Δ 2 years) and at baseline. Multivariable models were adjusted for mSASSS, elevated CRP level, smoking status, male gender, symptom duration, BMI and biomarker level at baseline.

which might impact the predictive value of prognostic biomarkers as it was shown for VEGF [16,18]. In an analysis of 73 biomarkers in patients of the GO-RAISE study treated with golimumab, only baseline interleukin-6 was associated with mSASSS change after 104 weeks [41]. The impact of the respective treatment might explain why previously described associations of biomarker levels (e.g. MMP-3 [14] and VEGF [16]) with radiographic progression could not be replicated in our cohort of patients on TNFi therapy.

While serum levels of all biomarkers of inflammation (calprotectin, CRP, ESR, MMP-3, VEGF) and some biomarkers reflecting bone turnover (BALP, sclerostin, sCTX) showed significant changes during the first 2 years of TNFi therapy, adipokine levels did not alter from

baseline. In line with our finding, biomarkers reflecting inflammation were described to decrease during TNFi – besides the routine clinical parameters CRP [6] and ESR also calprotectin [42], MMP-3 [20,43] and VEGF [20]. This decrease might mirror the resolution of systemic inflammation and as a result the effective treatment of disease activity. In line with this, a greater decrease of the serum levels of calprotectin and VEGF as well as CRP and ESR was associated with clinical response over the first 3 months of TNFi therapy.

The Wnt pathway, one of the main regulators of bone metabolism, is suggested to play a role in the pathogenesis of axSpA. Sclerostin produced by mature osteocytes inhibits bone formation via Wnt signaling and is reduced in SpA patients compared to healthy controls

[23]. After 12 months of TNFi, an increase of sclerostin was reported [44], and contrary lower sclerostin levels were associated with persistent inflammation. In our present study, sclerostin also increased after the first 2 years of TNFi therapy. An increase was found for BALP, necessary for the start of bone mineralization, which has also been described previously and was discussed to reflect both, new bone formation repairing inflammatory lesions and an increase of BMD during TNFi therapy [20,45,46]. Serum levels of all three adipokines analyzed in our study have been reported to remain stable during treatment with infliximab [47–49], which is in line with our finding. Although visfatin showed a significant increase between month 3 and year 2 of TNFi, levels did not change between baseline and year 2.

According to the ASAS-EULAR treatment recommendations, one of the primary goals of treatment is the prevention of progressive structural damage [5]. Following the Outcome Measures in Rheumatology (OMERACT) Soluble Biomarker International Working group recommendations to analyze the predictive validity of an early change in biomarker level for the long-term structural damage [50], we also analyzed whether changes in biomarker serum levels in the first three months or two years of TNFi treatment predict structural damage in axSpA. We found that only change of sclerostin during the first 3 months of TNFi treatment was significantly associated with radiographic progression. However, this association was lost in multivariable analyses. In uni- and multivariable analyses, change in the serum levels of visfatin and leptin within the first 2 years of TNFi therapy showed an association with an mSASSS progression at 4 years. The change in VEGF levels over 2 years was associated with mSASSS progression at 2 years only in multivariable analysis. All other significant associations of biomarker change with radiographic progression were lost in multivariable analyses.

A major strength of our study is the longer follow-up period of 4 years to determine radiographic progression compared to previous studies who investigated the association of biomarkers with radiographic damage in a cross-sectional design or with radiographic progression after 2 years of follow-up [38]. This study allows to analyze not only biomarker levels in the first 2 years of TNFi treatment, but also their value as prognostic markers for radiographic progression during bDMARD therapy. The analyzed biomarker panel encompasses biomarkers reflecting inflammation, bone turnover and metabolic changes and therefore mirroring multiple aspects of the disease process of axSpA.

The limitations of this study include the lack of a control group without TNFi treatment, which would enable to analyze the impact of the bDMARD treatment on the evaluation of the respective biomarker and its value for radiographic progression. Furthermore, our study is limited in the number of patients, especially the number of female patients, which hinders the correction for gender in some multivariable analyses. Moreover, no adjusting for multiple testing was performed.

Our data indicate that even though some biomarkers reflecting inflammation, bone turnover and adipokines show potential to predict subsequent radiographic progression, the strength of these associations is limited. Furthermore, measurement of the respective biomarkers is not routinely established so far, and thus often expensive. For the clinical practice, biomarker testing to identify patients with higher risk of radiographic progression for more intensive treatment, cannot be recommended as of today. However, in research, the situation might be different, as there even a small added value may be of relevance, for example to obtain more knowledge on the pathophysiology or mechanism of more severe disease outcome.

## Conclusion

To conclude, independent of known risk factors, baseline calprotectin and visfatin levels were associated with radiographic spinal

progression after 2 years of TNFi treatment in AS patients. Baseline sCTX levels showed a positive association and baseline leptin levels an inverse association with radiographic progression at 4 years. These biomarkers of inflammation, bone turnover and adipokines have been shown before to reflect processes of radiographic progression or excessive bone formation. Our results demonstrate that serum biomarkers measured before the start of TNFi therapy might help to identify AS patients at risk of radiographic progression. However, further studies including external validation in long-term prospective cohorts with still a considerable amount of axSpA patients showing radiographic progression and variation in disease activity over time with different treatment strategies are needed to establish the true value of these biomarkers in a prediction model for radiographic progression. Furthermore, it would be interesting to investigate in future research whether the combination of biomarkers have better clinical predictive value.

Biomarkers of inflammation and bone formation showed significant changes during TNFi therapy, but these changes were not significantly related to radiographic spinal progression in our cohort of AS patients. Though adipokine levels remained stable, the change of both visfatin and leptin serum levels after 2 years showed a significant association with radiographic progression at 4 years. For all investigated biomarkers, early change during the first 3 months of treatment was not significantly associated with radiographic progression. Therefore, for daily clinical practice, it seems more helpful to look at biomarker levels at baseline (before start of TNFi) instead of evaluating change in biomarker levels in the context of early prediction of subsequent radiographic progression in AS patients.

## Author contributions

JR: conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft. MS: conceptualization, methodology, investigation, data curation, formal analysis, visualization, roles/writing – review & editing. LG: methodology, data curation, formal analysis, writing – review & editing. FRW: resources, investigation, data curation, roles/writing – review & editing. MV: formal analysis and writing – review & editing. FM: resources, investigation, data curation, roles/writing – review & editing. LMT: methodology, investigation, writing – review & editing. DP: conceptualization, methodology, resources, data curation, formal analysis, visualization and writing – review & editing. AS: conceptualization, methodology, resources, data curation, formal analysis, visualization and writing – review & editing. SA: conceptualization, methodology, investigation, resources, data curation, formal analysis, visualization and writing – review & editing.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.semarthrit.2022.151974.

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