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

Rademacher, Judith; Siderius, Mark; Gellert, Laura; Wink, Freke R.; Verba, Maryna; Maas, Fiona; Tietz, Lorraine M.; Poddubnyy, Denis; Spoorenberg, Anneke; Arends, Suzanne

Published in:
SEMINARS IN ARTHRITIS AND RHEUMATISM

DOI:
10.1016/j.semarthrit.2022.151974

Publication date:
2022

Seminars in Arthritis and Rheumatism 53 (2022) 151974

Contents lists available at ScienceDirect

Seminars in Arthritis and Rheumatism

journal homepage: www.elsevier.com/locate/semarthrit

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

Judith Rademacher[a,b,c,*], Mark Siderius[d], Laura Gellert[a], Freke R Wink[e], Maryna Verba[a], Fiona Maas[d], Lorraine M Tietz[a], Denis Poddubnyy[a,c], Anneke Spoorenberg[d], Suzanne Arends[d]

[a] *Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin; Department of Gastroenterology, Infectiology and Rheumatology (including Nutrition Medicine), Hindenburgdamm 30, 12203 Berlin, Germany*
[b] *Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany*
[c] *German Rheumatism Research Centre, Berlin, Germany, Virchowweg 12, 10117 Berlin, Germany*
[d] *Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, the Netherlands*
[e] *Department of Rheumatology, Medical Center Leeuwarden, Henri Dunantweg 2, 8934 CE Leeuwarden, the Netherlands*

ARTICLE INFO

Keywords:
Axial spondyloarthritis
Biomarkers
Radiographic progression
TNF inhibitor

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-α 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 ≥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 ≥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.

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-α

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-α inhibitors; UMCG, University Medical Center Groningen; VEGF, vascular endothelial growth factor

* Corresponding author at: Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12203 Berlin.

https://doi.org/10.1016/j.semarthrit.2022.151974
0049-0172/

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 ≥50%) as well as ASDAS-clinical important improvement (ASDAS-CI, reduction of ≥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 ≥2 units in the first 2 years and ≥4 units after 4 years of follow-up. The progression of syndesmophytes was defined as the formation of ≥1 new syndesmophyte according to both readers in the first 2 years and ≥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, Biomedia 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 ± 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 (≥ 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, validatory 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±11 years and overall high disease activity at baseline, before the start of TNFi (mean ASDAS-CRP 3.9±0.7). Mean radiographic damage at baseline according to mSASSS was 10.6±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 ≥2 units and 24% developed ≥1 syndesmophyte during the first 2 years. Progression of ≥4 mSASSS units was observed in 32% of the patients and 30% developed ≥2 syndesmophytes during 4 years of treatment with TNFi (**Supplementary Tables S2, S3**).

Table 1
Baseline characteristics of the 137 included ankylosing spondylitis patients.

Age, years	42.0±10.8
Symptom duration, years	15.0 [7.0–23.0]
Male sex	98 (71.5%)
HLA-B27 positive	108 (78.8%)
BMI (kg/m2)	26.1±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 ± 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 ± 2.0
mSASSS (0–72)	3.0 [0–14.0]
≥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 ± standard deviation, or median [Interquartile range].

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

Patients with radiographic progression of ≥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**).

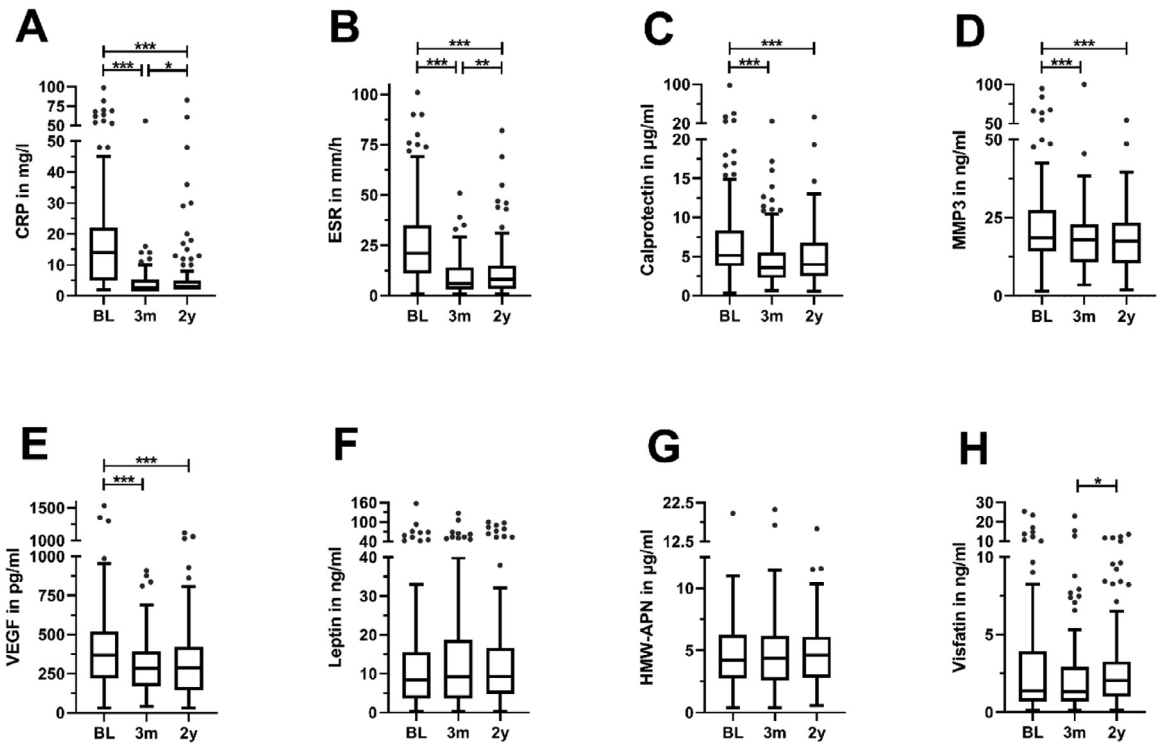

Fig. 1. Levels of markers of inflammation (A-E) and adipokines (F-H) during treatment with TNF inhibitors: serum levels at baseline (BL; before start of TNFi), after 3 months (3 m) and 2 years (2y) of treatment. Wilcoxon Signed Rank Test: * p value <0.05; ** p value <0.01; *** p value <0.001.

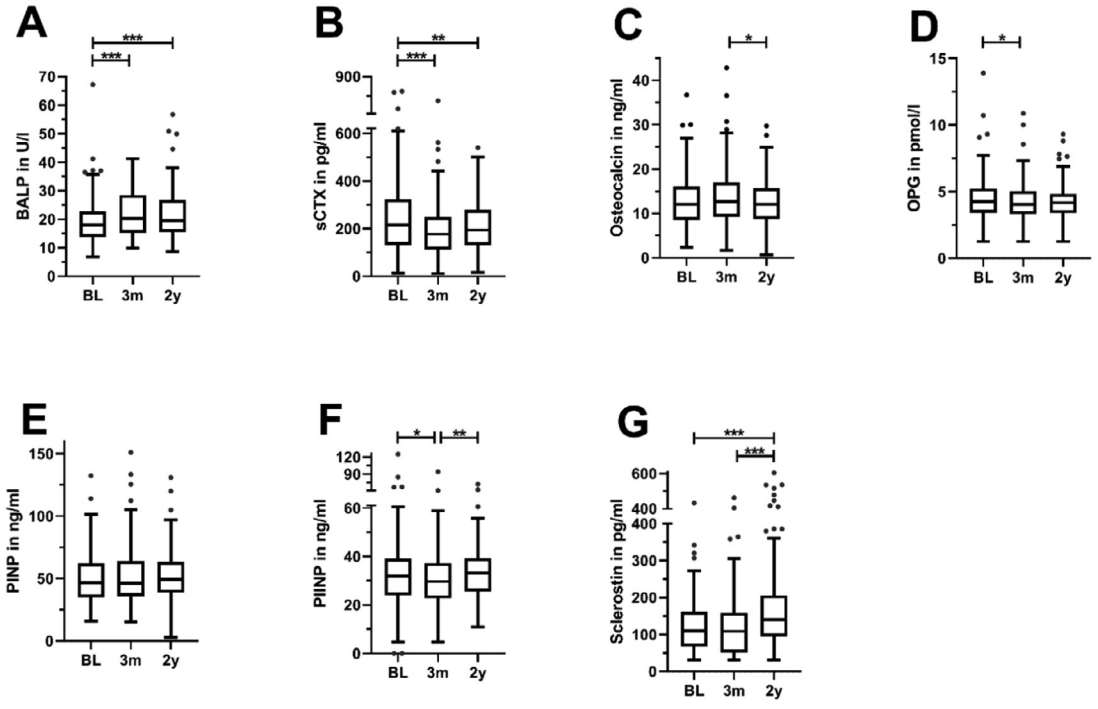

Fig. 2. Levels of bone turnover marker under treatment with TNF inhibitors: serum levels at baseline (BL; before start of TNFi), after 3 months (3 m) and 2 years (2y) of treatment. Wilcoxon Signed Rank Test: * p value <0.05; ** p value <0.01; *** p value <0.001.

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
Markers of Inflammation					
Calprotectin	**2**	1.041 (0.988–1.097)	0.129	**1.195 (1.055–1.355)**	**0.005**
	4	0.992 (0.952–1.035)	0.716	1.074 (0.970–1.189)	0.169
MMP-3	**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
VEGF	**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
Bone Turnover Markers					
BALP	**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
OC	**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
OPG	**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
PINP	**2**	1.012 (0.995–1.030)	0.156	1.020 (0.989–1.052)	0.198
	4	**1.020 (1.002–1.038)**	**0.030**	1.015 (0.986–1.046)	0.310
PIINP	**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
Sclerostin	**2**	**1.006 (1.001–1.011)**	**0.015**	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
sCTX	**2**	1.001 (0.999–1.004)	0.22	1.003 (1.000–1.007)	0.083
	4	**1.003 (1.001–1.005)**	**0.016**	1.004 (1.000–1.008)	0.052
Adipokines					
HMW-Adiponektin	**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
Leptin	**2**	0.972 (0.941–1.004)	0.087	0.896 (0.770–1.042)	0.154
	4	**0.954 (0.914–0.996)**	**0.034**	**0.614 (0.453–0.832)**	**0.002**
Visfatin	**2**	**1.106 (1.007–1.215)**	**0.036**	**1.465 (1.137–1.889)**	**0.003**
	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	Univariable model		Multivariable model	
	in years	OR (95% CI)	p value	OR (95% CI)	p value
Markers of Inflammation					
Calprotectin	**2**	1.033 (0.988–1.080)	0.156	**1.107 (1.001–1.225)**	**0.048**
	4	1.014 (0.953–1.079)	0.66	1.075 (0.973–1.188)	0.153
MMP-3	**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
VEGF	**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
Bone Turnover Markers					
BALP	**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
OC	**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
OPG	**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
PINP	**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
PIINP	**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
Sclerostin	**2**	**1.007 (1.001–1.013)**	**0.023**	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
sCTX	**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	**1.004 (1.001–1.008)**	**0.018**
Adipokines					
HMW-Adiponektin	**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
Leptin	**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
Visfatin	**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
Markers of Inflammation						
Calprotectin	**Δ 3 months**	**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
	Δ 2 years	**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
MMP-3	**Δ 3 months**	**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
	Δ 2 years	**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
VEGF	**Δ 3 months**	**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
	Δ 2 years	**2**	1.002 (1.000–1.004)	0.114	1.005 (1.000–1.010)	**0.042**
		4	1.000 (0.998–1.002)	0.887	1.005 (0.929–1.088)	0.894
Bone Turnover Markers						
BALP	**Δ 3 months**	**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
	Δ 2 years	**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
Osteocalcin	**Δ 3 months**	**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
	Δ 2 years	**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
OPG	**Δ 3 months**	**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
	Δ 2 years	**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
PINP	**Δ 3 months**	**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
	Δ 2 years	**2**	**1.027 (1.003–1.052)**	**0.030**	1.007 (0.953–1.065)	0.794
		4	**1.032 (1.007–1.058)**	**0.013**	0.981 (0.939–1.026)	0.407
PIINP	**Δ 3 months**	**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
	Δ 2 years	**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
Sclerostin	**Δ 3 months**	**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
	Δ 2 years	**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
sCTX	**Δ 3 months**	**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
	Δ 2 years	**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
Adipokines						
HMW-Adiponektin	**Δ 3 months**	**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
	Δ 2 years	**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
Leptin	**Δ 3 months**	**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
	Δ 2 years	**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	**1.489 (1.041–2.130)**	**0.029**
Visfatin	**Δ 3 months**	**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
	Δ 2 years	**2**	**1.108 (1.004–1.224)**	**0.041**	1.978 (0.790–4.948)	0.145
		4	1.035 (0.945–1.132)	0.461	**2.255 (1.108–4.591)**	**0.025**

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.

Funding

The Groningen Leeuwarden Axial Spondyloarthritis (GLAS) cohort was supported by research grants from Pfizer, AbbVie and Novartis. They had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

Declaration of Competing Interest

JR declares support for attending meetings from Novartis and UCB. FRW received consultancy fees from Janssen. DP declares grants or contract from AbbVie, Eli Lilly, MSD, Novartis and Pfizer, consultancies from AbbVie, Biocad, Eli Lilly, Gilead, GlaxoSmithKline, Janssen, MSD, Novartis, Pfizer, Samsung Bioepis, and UCB, speaking fees and honoraria from AbbVie, Bristol-Myers Squibb, Eli Lilly, MSD, Novartis, Pfizer, and UCB. AS received research grants from AbbVie, Novartis and UCB and consultancy fees from AbbVie, Novartis, UCB and Lilly, speaking fees and honoraria from Pharmacotherapy online,

Novartis and AbbVie. SA declares research grant from Pfizer. MS, LG, MV, FM, LMT declare no conflicts of interest.

Acknowledgments

Dr. Judith Rademacher is participant in the BIH-Charité Clinician Scientist Program funded by the Charité - Universitätsmedizin Berlin and the Berlin Institute of Health.

Supplementary materials

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

References

[1] Baraliakos X, Listing J, Rudwaleit M, Sieper J, Braun J. The relationship between inflammation and new bone formation in patients with ankylosing spondylitis. Arthritis Res Ther 2008;10(5):R104.
[2] Arends S, Spoorenberg A, Efde M, Bos R, Leijsma MK, Bootsma H, et al. Higher bone turnover is related to spinal radiographic damage and low bone mineral density in ankylosing spondylitis patients with active disease: a cross-sectional analysis. PLoS One 2014;9(6):e99685.
[3] Poddubnyy D, Listing J, Haibel H, Knuppel S, Rudwaleit M, Sieper J. Functional relevance of radiographic spinal progression in axial spondyloarthritis: results from the GErman SPondyloarthritis Inception Cohort. Rheumatology (Oxford) 2018.
[4] 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(6):863–7.
[5] van der Heijde D, Ramiro S. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. 2017;76(6):978−91.
[6] Sieper J, Listing J, Poddubnyy D, Song IH, Hermann KG, Callhoff J, et al. Effect of continuous versus on-demand treatment of ankylosing spondylitis with diclofenac over 2 years on radiographic progression of the spine: results from a randomised multicentre trial (ENRADAS). Ann Rheum Dis 2016;75(8):1438–43.
[7] Karmacharya P, Duarte-Garcia A. Effect of therapy on radiographic progression in axial spondyloarthritis: a systematic review and meta-analysis. 2020.
[8] Maas F, Arends S, Brouwer E, Essers I, van der Veer E, Efde M, et al. Reduction in spinal radiographic progression in ankylosing spondylitis patients receiving prolonged treatment with tumor necrosis factor inhibitors. Arthr Care Res 2017;69(7):1011–9.
[9] Ramiro S, Stolwijk C, van Tubergen A, van der Heijde D, Dougados M, van den Bosch F, et al. Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study. Ann Rheum Dis 2015;74(1):52–9.
[10] Poddubnyy D, Haibel H, Listing J, Marker-Hermann E, Zeidler H, Braun 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(5):1388–98.
[11] Min HK, Lee J, Ju JH, Park S-H, Kwok S-K. Alcohol consumption as a predictor of the progression of spinal structural damage in axial spondyloarthritis: data from the Catholic Axial Spondyloarthritis COhort (CASCO). Arthritis Res Ther 2019;21(1):187.
[12] Arends S, Brouwer E, van der Veer E, Groen H, Leijsma MK, Houtman PM, et al. Baseline predictors of response and discontinuation of tumor necrosis factor-alpha blocking therapy in ankylosing spondylitis: a prospective longitudinal observational cohort study. Arthritis Res Ther 2011;13(3):R94.
[13] Rademacher J, Tietz LM, Le L, Hartl A, Hermann KA, Sieper J, et al. Added value of biomarkers compared with clinical parameters for the prediction of radiographic spinal progression in axial spondyloarthritis. Rheumatology (Oxford) 2019;58(9):1556–64.
[14] Maksymowych WP, Landewe R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis Rheum 2007;56(6):1846–53.
[15] Jarlborg M, Courvoisier DS, Lamacchia C, Martinez Prat L, Mahler M, Bentow C, et al. Serum calprotectin: a promising biomarker in rheumatoid arthritis and axial spondyloarthritis. Arthritis Res Ther 2020;22(1):105.
[16] Poddubnyy D, Conrad K, Haibel H, Syrbe U, Appel H, Braun J, et al. Elevated serum level of the vascular endothelial growth factor predicts radiographic spinal progression in patients with axial spondyloarthritis. Ann Rheum Dis 2014;73(12):2137–43.
[17] Turina MC, Sieper J, Yeremenko N, Conrad K, Haibel H, Rudwaleit M, et al. Calprotectin serum level is an independent marker for radiographic spinal progression in axial spondyloarthritis. Ann Rheum Dis 2014;73(9):1746–8.
[18] Braun J, Baraliakos X, Hermann KG, Xu S, Hsu B. Serum vascular endothelial growth factor levels lack predictive value in patients with active ankylosing spondylitis treated with golimumab. J Rheumatol 2016;43(5):901–6.
[19] Gamez-Nava JI, de la Cerda-Trujillo LF, Vazquez-Villegas ML, Cons-Molina F, Alcaraz-Lopez MF, Zavaleta-Muñiz SA, et al. Association between bone turnover markers, clinical variables, spinal syndesmophytes and bone mineral density in Mexican patients with ankylosing spondylitis. Scand J Rheumatol 2016;45(6):480–90.
[20] Appel H, Janssen L, Listing J, Heydrich R, Rudwaleit M, Sieper J. Serum levels of biomarkers of bone and cartilage destruction and new bone formation in different cohorts of patients with axial spondyloarthritis with and without tumor necrosis factor-alpha blocker treatment. Arthritis Res Ther 2008;10(5):R125.
[21] Arends S, Spoorenberg A, Houtman PM, Leijsma MK, Bos R, Kallenberg CGM, et al. The effect of three years of TNF alpha blocking therapy on markers of bone turnover and their predictive value for treatment discontinuation in patients with ankylosing spondylitis: a prospective longitudinal observational cohort study. Arthritis Res Ther 2012;14(2):R98.
[22] Heiland GR, Appel H, Poddubnyy D, Zwerina J, Hueber A, Haibel H, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. Ann Rheum Dis 2012;71(4):572–4.
[23] Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum 2009;60(11):3257–62.
[24] Syrbe U, Callhoff J, Conrad K, Poddubnyy D, Haibel H, Junker S, et al. Serum adipokine levels in patients with ankylosing spondylitis and their relationship to clinical parameters and radiographic spinal progression. Arthrit Rheumatol 2015;67(3):678–85.
[25] Hartl A, Sieper J, Syrbe U, Listing J, Hermann KG, Rudwaleit M, et al. Serum levels of leptin and high molecular weight adiponectin are inversely associated with radiographic spinal progression in patients with ankylosing spondylitis: results from the ENRADAS trial. Arthritis Res Ther 2017;19(1):140.
[26] Maas F, Spoorenberg A, Brouwer E, Bos R, Efde M, Chaudhry RN, et al. Spinal radiographic progression in patients with ankylosing spondylitis treated with TNF-α blocking therapy: a prospective longitudinal observational cohort study. PLoS One 2015;10(4):e0122693.
[27] Spoorenberg A, de Vlam K, van der Linden S, Dougados M, Mielants H, van de Tempel H, et al. Radiological scoring methods in ankylosing spondylitis. Reliability and change over 1 and 2 years. J Rheumatol 2004;31(1):125–32.
[28] Wanders AJ, Landewe RB, Spoorenberg A, Dougados M, van der Linden S, Mielants H, et al. What is the most appropriate radiologic scoring method for ankylosing spondylitis? A comparison of the available methods based on the Outcome Measures in Rheumatology Clinical Trials filter. Arthritis Rheum 2004;50(8):2622–32.
[29] Creemers MCW, Franssen MJAM, van 't Hof MA, Gribnau FWJ, van de Putte LBA, van Riel PLCM. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. Ann Rheum Dis 2005;64(1):127–9.
[30] van der Heijde D, Landewe R. Selection of a method for scoring radiographs for ankylosing spondylitis clinical trials, by the Assessment in Ankylosing Spondylitis Working Group and OMERACT. J Rheumatol 2005;32(10):2048–9.
[31] Syrbe U, Callhoff J, Conrad K, Poddubnyy D, Haibel H, Junker S, et al. Serum adipokine levels in patients with ankylosing spondylitis and their relationship to clinical parameters and radiographic spinal progression. Arthrit Rheumatol 2015;67(3):678–85.
[32] Østgård RD, Deleuran BW, Dam MY, Hansen IT, Jurik AG, Glerup H. Faecal calprotectin detects subclinical bowel inflammation and may predict treatment response in spondyloarthritis. Scand J Rheumatol 2018;47(1):48–55.
[33] Di Ceglie I, Blom AB, Davar R, Logie C, Martens JHA, Habibi E, et al. The alarmin S100A9 hampers osteoclast differentiation from human circulating precursors by reducing the expression of RANK. FASEB J 2019;33(9):10104–15.
[34] Zreiqat H, Howlett CR, Gronthos S, Hume D, Geczy CL. S100A8/S100A9 and their association with cartilage and bone. J Mol Histol 2007;38(5):381–91.
[35] Hulejová H, Kropáčková T, Bubová K, Kryštůfková O, Filková M, Mann H, et al. Serum visfatin levels in patients with axial spondyloarthritis and their relationship to disease activity and spinal radiographic damage: a cross-sectional study. Rheumatol Int 2019;39(6):1037–43.
[36] Xie H, Tang SY, Luo XH, Huang J, Cui RR, Yuan LQ, et al. Insulin-like effects of visfatin on human osteoblasts. Calcif Tissue Int 2007;80(3):201–10.
[37] Moschen AR, Geiger S, Gerner R, Tilg H. Pre-B cell colony enhancing factor/NAMPT/visfatin and its role in inflammation-related bone disease. Mutat Res 2010;690(1−2):95–101.
[38] Ajrawat P, Touma Z, Sari I, Taheri C, Diaz Martinez JP, Haroon N. Effect of TNF-inhibitor therapy on spinal structural progression in ankylosing spondylitis patients: A systematic review and meta-analysis. Int J Rheumat Dis 2020;23(6):728–43.
[39] Kang KY, Jung JY, Hong YS, Ju JH, Park SH. Positive correlation between inflammation on sacroiliac joint MRI and serum C-terminal telopeptide of type-I collagen in ankylosing spondylitis but not in non-radiographic axial spondyloarthritis. Clin Exp Rheumatol 2017;35(3):415–22.
[40] Munk HL, Gudmann NS, Christensen AF, Ejstrup L, Sorensen GL, Loft AG, et al. Cartilage collagen type II seromarker patterns in axial spondyloarthritis and psoriatic arthritis: associations with disease activity, smoking and HLA-B27. Rheumatol Int 2016;36(4):541–9.
[41] Inman RD, Baraliakos X, Hermann KA, Braun J, Deodhar A, van der Heijde D, et al. Serum biomarkers and changes in clinical/MRI evidence of golimumab-treated patients with ankylosing spondylitis: results of the randomized, placebo-controlled GO-RAISE study. Arthritis Res Ther 2016;18(1):304.
[42] Turina MC, Yeremenko N, Paramarta JE, De Rycke L, Baeten D. Calprotectin (S100A8/9) as serum biomarker for clinical response in proof-of-concept trials in axial and peripheral spondyloarthritis. Arthritis Res Ther 2014;16(4):413.
[43] He D, Zhu Q, Zhou Q, Qi Q, Sun H, Zachariah LM, et al. Correlation of serum MMP3 and other biomarkers with clinical outcomes in patients with ankylosing spondylitis: a pilot study. Clin Rheumatol 2017;36(8):1819–26.

[44] Saad CG, Ribeiro AC, Moraes JC, Takayama L, Goncalves CR, Rodrigues MB, et al. Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? Arthritis Res Ther 2012;14(5):R216.
[45] Visvanathan S, van der Heijde D, Deodhar A, Wagner C, Baker DG, Han J, et al. Effects of infliximab on markers of inflammation and bone turnover and associations with bone mineral density in patients with ankylosing spondylitis. Ann Rheum Dis 2009;68(2):175–82.
[46] Woo JH, Lee HJ, Sung IH, Kim TH. Changes of clinical response and bone biochemical markers in patients with ankylosing spondylitis taking etanercept. J Rheumatol 2007;34(8):1753–9.
[47] Derdemezis CS, Filippatos TD, Voulgari PV, Tselepis AD, Drosos AA, Kiortsis DN. Leptin and adiponectin levels in patients with ankylosing spondylitis. The effect of infliximab treatment. Clin Exp Rheumatol 2010;28(6):880–3.
[48] Miranda-Filloy JA, López-Mejias R, Genre F, Carnero-López B, Ochoa R, Diaz de Terán T, et al. Leptin and visfatin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy. Clin Exp Rheumatol 2013;31(4):538–45.
[49] Miranda-Filloy JA, Lopez-Mejias R, Genre F, Carnero-Lopez B, Ochoa R, Diaz de Teran T, et al. Adiponectin and resistin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-alpha antagonist therapy. Clin Exp Rheumatol 2013;31(3):365–71.
[50] Maksymowych WP, Landewé R, Tak PP, Ritchlin CJ, Ostergaard M, Mease PJ, et al. Reappraisal of OMERACT 8 draft validation criteria for a soluble biomarker reflecting structural damage endpoints in rheumatoid arthritis, psoriatic arthritis, and spondyloarthritis: the OMERACT 9 v2 criteria. J Rheumatol 2009;36(8):1785–91.