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Marques, M.L.; Silva, N.P. da; Heijde, D. van der; Stal, R.; Baraliakos, X.; Braun, J.; ... ; Gaalen, F.A. van

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ORIGINAL RESEARCH

Inflammation, bone loss and 2-year bone formation at the same vertebra in axial spondyloarthritis: a multilevel MRI and low-dose CT analysis

Mary Lucy Marques ^{1,2}, Nuno Pereira da Silva,³ Desirée van der Heijde ¹, Rosalinde Stal ¹, Xenofon Baraliakos ⁴, Juergen Braun ⁴, Monique Reijnierse,⁵ Caroline Bastiaenen,⁶ Sofia Ramiro ^{1,7}, Floris A van Gaalen ¹

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For numbered affiliations see end of article.

Correspondence to
Dr Mary Lucy Marques;
mary.lucy.marques@gmail.com

ABSTRACT

Objective To investigate whether in radiographic axial spondyloarthritis (r-axSpA) inflammation is associated with lower trabecular bone density (TBD), and subsequently, if a lower TBD increases the likelihood of 2-year bone formation at the same vertebra.

Methods Whole spine (C3–L5) data from patients included in the multicentre 2-year Sensitive Imaging in Ankylosing Spondylitis cohort was used. Two readers measured baseline TBD by Hounsfield units (HU) on low-dose CT (ldCT). Baseline MRI bone marrow oedema (BME) status scores and ldCT syndesmophyte formation and/or growth change-from-baseline scores were assessed by three and two readers, respectively. Average of readers' continuous measurements or readers' agreement in binary scores generated within the same vertebra (1—present in ≥1 quadrant/0—absent in all quadrants) were used. Multilevel generalised estimating equations models were used, the unit of analysis being the vertebra.

Results In 50 patients with r-axSpA, TBD HU decreased from cranial to caudal vertebrae. Baseline MRI-BME was present in 300/985 (30%) and syndesmophytes in 588/910 (65%) vertebrae, both most prevalent at thoracolumbar region. Syndesmophyte formation or growth was observed in 18% of at-risk vertebrae (124/691). A significant confounder-adjusted association was found between inflammation and lower TBD (regression coefficient=−51; 95% CI−63 to −39). TBD was not associated with 2-year syndesmophyte formation or growth (adjusted OR 1.00; 95% CI 0.99 to 1.00).

Conclusion In r-axSpA, while vertebral inflammation was associated with lower vertebral TBD, lower vertebral TBD itself did not increase the risk for new bone formation at the same vertebra. In preventing syndesmophyte progression, targeting local inflammation seems more important than targeting vertebral trabecular bone loss.

INTRODUCTION

Radiographic axial spondyloarthritis (r-axSpA), traditionally known as ankylosing spondylitis, is a chronic inflammatory disease

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ In radiographic axial spondyloarthritis (r-axSpA), inflammation-driven vertebral trabecular bone loss has been suggested to trigger ectopic bone formation to stabilise the spinal structure.
- ⇒ Studies assessing this hypothesis at the vertebral level are scarce, assessed few vertebrae (due to imaging limitations), and have conflicting results.

WHAT THIS STUDY ADDS

- ⇒ For the first time, inflammation, trabecular bone density and bone formation were assessed at the same vertebra in the entire spine (from C3 to L5).
- ⇒ Vertebral inflammation and syndesmophytes were most prevalent in vertebrae with lower trabecular bone density (thoracolumbar vertebrae).
- ⇒ The presence of inflammation was cross-sectionally significantly associated with lower vertebral trabecular bone density.
- ⇒ Lower vertebral trabecular bone density was not significantly associated longitudinally with syndesmophyte formation and/or growth after 2 years.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ Our results suggest that inflammation-driven trabecular bone loss (by itself) is not associated with subsequent bone formation at the same vertebra in r-axSpA.
- ⇒ To prevent new bone information, targeting local inflammation seems to be more important than targeting trabecular bone loss.

affecting the spine and sacroiliac joints, typically starting in young adulthood.¹ The disease encompasses potentially reversible inflammation and irreversible structural damage, both associated with disease burden.²

Bone involvement in r-axSpA is a complex phenomenon. Paradoxically, structural damage,

in the form of ectopic bone formation (eg, syndesmophytes), coexists with bone loss.³ Bone loss may occur as local bone erosions in the sacroiliac joints and vertebrae and as systemic low bone mineral density (BMD).^{4,5} Low BMD, and particularly osteoporosis with increased vertebral fracture risk, is a common comorbidity in r-axSpA.⁵ Systemic bone loss in r-axSpA is considered a consequence of decreased physical activity and functional capacity related to pain, stiffness and ankylosis,⁵ but is also associated with inflammation, especially earlier in the disease.⁶ What leads to bone loss and bone formation and its possible relationship with inflammation is still poorly understood.

In r-axSpA, a recent theoretical model proposes local inflammation-driven trabecular bone loss not exclusively as a comorbidity, but as an active factor in the pathogenesis of structural damage.⁷ Briefly, inflammation is suggested to act as an inhibitory mechanism on the normal bone cycle, making osteoblasts in the vertebral trabecular bone incapable of compensating for the bone loss. Therefore, structural disease progression (ectopic bone formation) represents an anabolic reaction to compensate for spinal fragility. In short, the inflammation-driven bone loss may act as a trigger to bone repair occurring at distinct sites of the same vertebra (bone loss in the trabecular bone and bone repair in the periosteum).⁷ Proven right, preventing and/or treating trabecular bone loss may emerge as an additional target to enhance structural damage prevention in r-axSpA.

Previous studies reported a general association between systemic low BMD and spinal structural damage in r-axSpA.^{8–11} However, the above-mentioned theoretical association has seldom been studied at the same vertebra,^{12,13} with conflicting results, and only a few vertebrae analysed.^{12,13} Either dual-energy X-ray absorptiometry (DXA) was used to assess bone density, and therefore, lumbar spine vertebrae only were analysed,¹² or quantitative CT (QCT), implying high ionising radiation exposure, precluded the inclusion of many vertebrae.¹³ Considering r-axSpA as a disease affecting the entire spine, the comprehensive assessment of the whole spine is an unmet need.¹²

Low-dose CT (ldCT) has excelled as advantageous to assess bone changes in the entire spine in r-axSpA using acceptable levels of ionising radiation exposure.¹⁴ Syndesmophyte formation in the spine of patients with r-axSpA can be reliably assessed using CT Syndesmophyte Score (CTSS) on ldCT.^{15,16} Moreover, the measurement of trabecular bone density through vertebral Hounsfield units (HU), which correlate with DXA BMD in the general population and spinal surgery candidates,¹⁷ is feasible in ldCT scans.¹⁸ We recently adapted an innovative methodology for the assessment of vertebral bone density using ldCT HU measurements in patients with r-axSpA.^{17,18} This methodology was proven to be reliable at the same vertebra, from C3 to L5.¹⁸

Using ldCT that allows whole spine evaluation, we aimed to assess at the same vertebra whether: (1) the presence of inflammation is associated with lower trabecular

bone density (expressing trabecular bone loss) and (2) trabecular bone loss increases the likelihood for new bone formation after 2 years of follow-up, in patients with r-axSpA.

MATERIAL AND METHODS

Study design and population

We used data from the Sensitive Imaging in Ankylosing Spondylitis (SIAS) study, a multicentre 2-year prospective cohort of patients with r-axSpA recruited in two centres (Leiden, the Netherlands and Herne, Germany). This cohort was previously described in detail.¹⁵ For the present study, patients could be included if they had both ldCT and MRI at baseline, and an additional ldCT after 2 years of follow-up. Of note, the unit of analysis was the vertebra. Therefore, a maximum of 50 vertebrae per vertebral level (C3–L5), from 50 patients could be included, which was considered appropriate according to a sample size calculation focusing on inter-reader reliability (measurements of two readers, using predefined intraclass correlation coefficient of 0.80 or higher with a 95% CI ± 0.1).¹⁸

Imaging assessments and descriptions of how the respective variables were analysed

We used baseline MRI data, and baseline plus 2-year ldCT data from the whole spine (C3–L5). A standardised protocol was applied in both centres for imaging acquisitions.¹⁸ MRI images were acquired in Leiden and Herne, respectively, on a 3 Tesla MRI (Philips Medical systems, Best, The Netherlands) and 1.5 Tesla MRI (Siemens, Erlangen, Germany) using sagittal T1-weighted and short tau inversion recovery (STIR) sequences with a slice thickness of 3 mm. ldCT images were obtained on 64-section and 16-section CT scanners (Leiden: Aquilion 64, Toshiba Medical Systems, Otawara, Japan; Herne: Somatom Emotion 16, Siemens, Erlangen, Germany). Spiral CT scans were performed using automatic exposure control. The effective dose estimates for the whole spine were 3.8 (2.6) mSv and 4.7 (2.4) mSv per ldCT, respectively—further details were previously published.¹⁸

Inflammation assessment

For the present study, we used baseline MRI bone marrow oedema (BME) previously scored at the vertebral corner on STIR images according to the Spondyloarthritis Research Consortium of Canada method.¹⁹ Briefly, the anterior and posterior rim of the vertebra were scored at the vertebral unit level (from the lower half of C2 to the upper half of S1, in a total of 23 vertebral units) on a sagittal view (maximum of four quadrants per vertebra) by three trained central readers independently. For the present study, MRI-BME scores were converted to the same vertebra, that is, from the upper half of C3 to the lower half of L5 (figure 1 and online supplemental figure S1). For each quadrant, a binary consensus score was generated if MRI-BME was reported to be present by ≥ 2 out of 3 MRI readers (yes=1; no=0). Afterwards, a binary

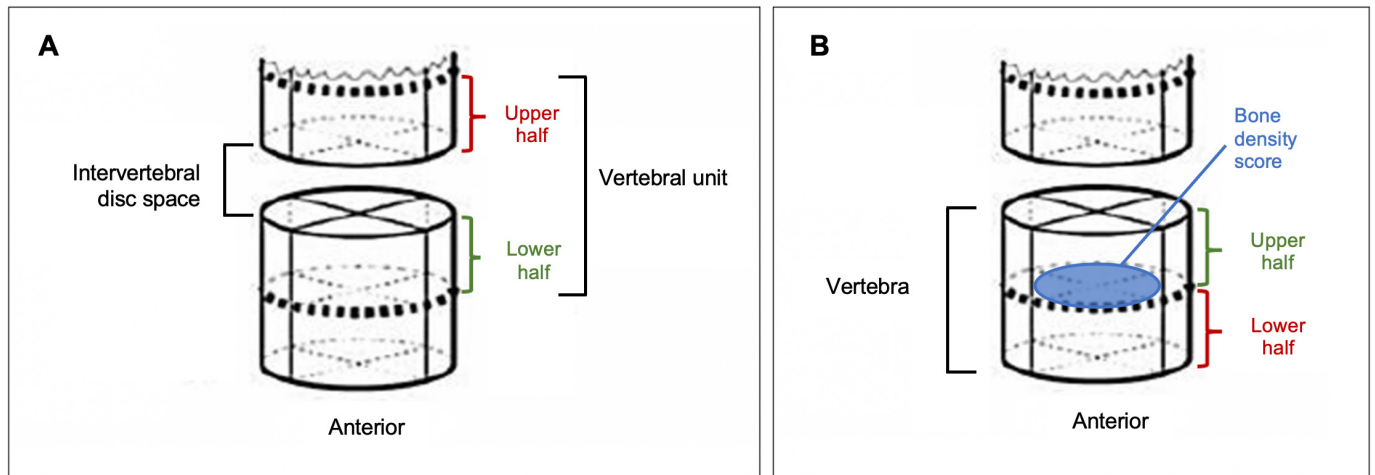


Figure 1 Transformation of scores from the vertebral unit level (A) to the same vertebra (B). MRI bone marrow oedema and CT syndesmophyte scores were performed at the vertebral unit level. A vertebral unit consists of the lower half of a vertebra, the intervertebral disc space and the upper half of the next vertebra (A). For the current study, to match the measurements of bone density Hounsfield units, the analyses were performed at the same vertebra, that is considering the sum scores of the upper half and lower half of the same vertebra (B).

score for each vertebra was generated in which the total MRI-BME sum scores of zero (MRI-BME absent in all the quadrants within the same vertebra) were coded as 0, and total MRI-BME sum scores of 1–4 (MRI-BME present in ≥ 1 of the quadrants within the same vertebra) were coded as 1.

Bone loss assessment

Bone density was assessed using baseline ldCT scans. Two trained readers independently assessed HU at each vertebra from C3 to L5. HU (continuous trabecular bone density score for the whole vertebra) were obtained from one reconstructed cross-sectional slice in a specific region of interest positioned at the centre of each vertebra using OsiriX software (V.6.5.1) (online supplemental figure S2).¹⁸ Vertebrae with incident density abnormalities or artefacts that could affect HU values (eg, sclerosis of the vertebral body, haemangiomas and photon starvation artefacts) were identified. In statistical analysis, the average of the measurements of HU by two readers at each vertebra was used.

Bone formation assessment

Baseline and 2-year ldCT scans were used for the assessment of bone formation, using the CTSS.¹⁵ The anterior/posterior rim of the vertebra and the left/right rim of the vertebra were previously scored by two trained readers independently in 23 vertebral units (from the lower half of C2 to the upper half of S1) on both sagittal and coronal views.¹⁵ New bone formation was defined based on the 2-year change-from-baseline scores and encompassed not only new syndesmophyte formation, but also growth of previously present syndesmophytes.

Syndesmophyte status scores (at baseline and 2 years) were converted to the vertebral level, that is, considering the quadrants of the upper/lower half of the same vertebra from C3 to L5 (figure 1 and online supplemental figure

S1). For each quadrant within each vertebra (maximum of eight quadrants per vertebra), the CTSS score could range from zero (absence of syndesmophytes) to three (bridging syndesmophyte). Thus, a vertebra without syndesmophytes had a total sum of status scores of 0, while a total ankylosed vertebra had a total sum of status scores of 24 (score of 3 in each of the 8 quadrants).

The sum of the status scores per vertebra was used to select vertebrae at risk for syndesmophyte formation and/or growth and to generate the change scores (online supplemental figure S3). Briefly, for each reader and vertebra, at baseline, only vertebrae without syndesmophytes were at risk for new syndesmophyte formation, while vertebrae with at least one syndesmophyte were at risk for syndesmophyte growth. For the combined outcome (syndesmophyte formation or growth), all vertebrae were selected except those with total ankylosis since no further progression could occur.

The change-from-baseline scores for the bone formation outcomes were transformed and analysed as binary (1—syndesmophyte formation/growth occurred in ≥ 1 quadrant (2-year sum score > baseline sum score); 0—absence of formation and/or growth in all quadrants (baseline sum score = 2-year sum score)) (online supplemental figure S3). After obtaining the binary change scores for each vertebra by reader, a binary consensus score for each vertebra was defined according to the absolute agreement of the two readers on syndesmophyte formation and/or growth change scores (yes=1; no=0).

Inter-reader reliability of imaging assessments at the same vertebra

Inter-reader reliability for ldCT HU status scores at each vertebra (C3–L5) in the SIAS cohort was previously shown to be good to excellent.¹⁸ In the present study, inter-reader reliability and agreement for MRI-BME and syndesmophyte

scores needed to be assessed separately after transformation to the vertebral level. Fleiss kappa (n=3 readers) was used for MRI-BME status scores, and Cohen's kappa (n=2 readers) for ldCT CTSS change scores.

Main outcome(s) and main determinant(s)

To test the association between local inflammation and low vertebral trabecular bone density, the main outcome was bone density HU (continuous status score) and the main determinant was the presence of MRI-BME (binary status score). For the association between low vertebral trabecular bone density and 2-year bone formation, the main outcome and determinant were respectively 2-year syndesmophyte formation and/or growth (binary change score) and bone density HU (continuous status score).

Potential confounders

Confounders were defined a priori based on theoretical rationale.^{5 6 20-25} The directed acyclic graphs for the two tested associations are provided in figure 2. The confounder variables were analysed as the following:

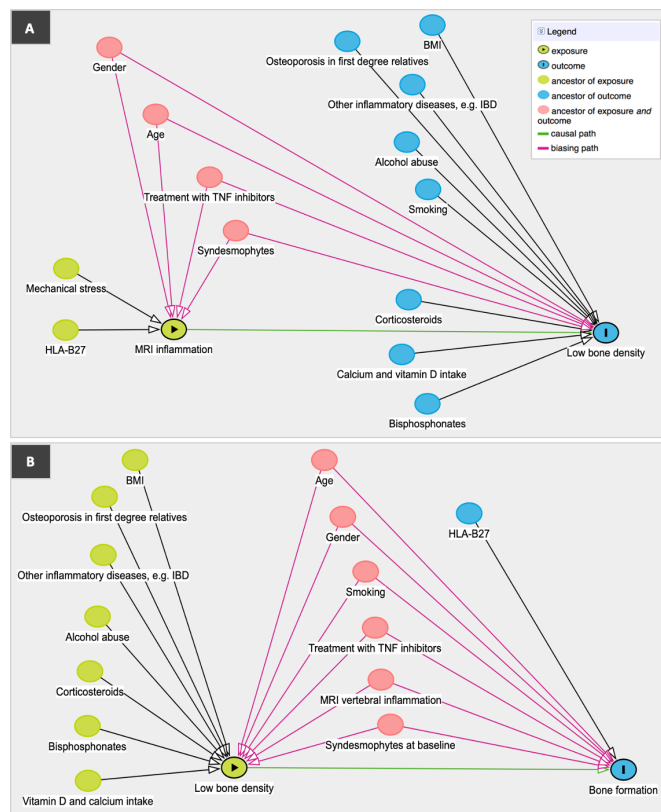


Figure 2 Directed acyclic graphs for the tested associations. Minimal sufficient adjustment sets for estimating the total effect of MRI inflammation on low bone density (A): age, gender, treatment with TNF inhibitors and syndesmophytes at baseline. Minimal sufficient adjustment sets for estimating the total effect of low bone density on 2-year bone formation: age, gender, MRI vertebral inflammation, smoking, treatment with TNF inhibitors and presence of syndesmophytes at baseline. BMD, bone mineral density; BMI, body mass index; IBD, inflammatory bowel disease; TNF, tumour necrosis factor.

age (continuous: years), gender (male/female), current smoking at baseline (yes/no), treatment with tumour necrosis factor inhibitors (TNFi) at any time point (yes/no); presence of baseline inflammation (MRI-BME) at the same vertebra (yes/no) and presence of baseline syndesmophytes (yes/no). The two latter confounders were defined privileging specificity at the vertebral level (consensus of ≥ 2 readers regarding MRI-BME presence/absence and agreement of both readers on the syndesmophyte presence/absence). Confounders were assessed, whenever possible, at the vertebral level. Therefore, we included local inflammation (MRI-BME) rather than a disease activity index reflecting systemic inflammation (eg, Ankylosing Spondylitis Disease Activity Score (ASDAS)).

Statistical analysis

Data were analysed at the same vertebra (figure 1). The entire spine was analysed from C3 to L5 (22 vertebrae per patient, 50 patients). A maximum of 1100 vertebrae could be used for each hypothesised association. Vertebrae in which artefacts or density abnormalities were reported by at least one of the readers were excluded from the main analysis. Baseline frequencies of vertebrae with MRI-BME as well as the frequencies of new syndesmophytes and/or new growth per vertebra were computed. Mean (SD) of all patients ldCT HU was provided for each spinal segment.

To test the main research questions, univariable and multivariable analyses were performed. Generalised estimating equations (GEE; linear or logistic) were applied making use of all data, across all levels, that is, patient and vertebra.²⁶ These multilevel analyses adjusted for the dependence of observations arising from measurements in multiple vertebrae within the same patient. The 'exchangeable' correlation structure, demonstrating the best fit to the data, was used, and the analyses were adjusted for potential confounders that varied according to the tested association (figure 2). For the assessment of the association between vertebral trabecular bone density and 2-year bone formation, only the vertebrae at risk for progression were included, selected according to the definitions provided in online supplemental figure S2. The predictor variables 'presence of syndesmophytes at baseline' and 'presence of MRI-BME at baseline' were colinear, and therefore, were not included simultaneously in the multivariable model. For each research question, the interaction between these two independent variables on the outcome(s) was tested. If a significant interaction was found ($p < 0.15$), the analysis should be stratified by vertebrae with (vs without) syndesmophytes at baseline.

To evaluate whether the association between vertebral trabecular bone density and inflammation would be different across different spine regions, an analysis was planned to test the interaction between MRI-BME and the spinal region (cervical, thoracic and lumbar) on bone density ldCT HU. Likewise, the interaction between

Table 1 Baseline characteristics of patients with radiographic axial spondyloarthritis

Assessment	N=50*†
Male	43 (86.0)
Age, years	49.1 (9.9)
HLA-B27 positive	42 (84.0)
BMI, kg/m ²	26.8 (3.9)
Current smoking	19 (38.8)
ASDAS	2.6 (1.2)
ASDAS inactive disease (ASDAS<1.3)	6 (12.8)
ASDAS low disease activity (1.3≤ ASDAS <2.1)	11 (23.4)
ASDAS high disease activity (2.1≤ ASDAS ≤3.5)	22 (46.8)
ASDAS very high disease activity (ASDAS >3.5)	8 (17.0)
TNFi treatment	12 (24.0)
NSAIDs treatment	32 (64.0)
Patients with syndesmophytes‡	50 (100.0)
Patients with spine MRI-BME§	47 (94.0)
Cervical spine HU¶	319 (104.6)
Thoracic spine HU¶	195 (68.8)
Lumbar spine HU¶	153 (58.2)

*Mean (SD) or no. (%).
†Missing values <10%: smoking status (n=1 patients); ASDAS variables (n=3 patients).
‡Defined as a patient with ≥1 quadrant per vertebra that received a CT Syndesmophytes Score ≥1 (absolute agreement of two readers).
§Defined as a patient with ≥1 quadrant per vertebra with MRI-BME (agreement of 2 out of 3 readers).
¶Average of the two readers' scores.
ASDAS, Ankylosing Spondylitis Disease Activity Score; BME, bone marrow oedema; BMI, body mass index; HLA, human leucocyte antigen; HU, Hounsfield units; NSAIDs, non-steroidal anti-inflammatory drugs; TNFi, Tumour necrosis factor inhibitors.

bone density ldCT HU and spinal region on bone formation was investigated.

A sensitivity analysis was performed in which vertebrae with incident density abnormalities or artefacts were also included, using otherwise the same methodology.

A significant level of 5% was considered for all the statistical analyses. Statistical analyses were performed using STATA software V.16.0.

RESULTS

Baseline characteristics

Overall, we analysed 985 vertebrae from 50 patients with r-axSpA. **Table 1** summarises the baseline characteristics. Patients had a mean (SD) age of 49 (10) years, 86% were male, 84% were HLA-B27 positive, most having high or very high disease activity (mean (SD) ASDAS: 2.6 (1.2)).

In total, 115 vertebrae (10%) were excluded. The reasons for exclusion included technical issues in the vertebral reconstruction (not possible to identify the

limits of the vertebra, n=50) and incident density abnormalities or artefacts as reported by at least one of the readers (n=65). The latter included: sclerotic changes affecting the vertebral body (n=31), photon starvation artefact (n=18), poor imaging quality/noise (n=14) and vascular changes (n=2). Seven vertebrae had negative values consistent with fat metaplasia and were not excluded because this reflects extensive bone loss. No vertebral fractures were detected.

LdCT HU values decreased from cranial to caudal vertebrae (mean (SD): 319 (105) for cervical spine; 195 (69) for thoracic spine and 153 (58) for lumbar spine). The highest mean (SD) value for HU was obtained at C3 (355 (106)), while the lowest was found at L3 (147 (60)).

The baseline frequencies of inflammation and syndesmophytes in relation to the average of bone density HU for each vertebra are presented in **table 2**. Baseline MRI-BME and syndesmophytes were present in 300/985 (30%) and 588/910 (65%) vertebrae, respectively, being more prevalent in the lower thoracic and upper lumbar spine (where ldCT HU had the lowest values).

Inter-reader reliability analysis at the same vertebra

For MRI-BME scores, Cohen's kappa was, in general, highest for the reader pair 1/3 (0.62–0.99) and lowest for the reader pair 2/3 (0.35–0.80) (online supplemental table S1). Fleiss kappa indicated an overall good agreement for all vertebrae across the three readers (0.41–0.78). The only exception was C3 (Fleiss kappa=0.11), in which there was a high agreement between the readers, but the prevalence of inflammation was virtually null (all the vertebrae scored with absence of inflammation by two of the readers).

Cohen's kappa for syndesmophyte formation or growth change scores was overall fair to good, varying from 0.39 to 0.68 in cervical spine, 0.36 to 0.74 in thoracic spine and 0.39 to 0.44 in lumbar spine (online supplemental table S2).

Association between local inflammation and bone loss

In the multilevel multivariable model, a significant confounder-adjusted association was found between inflammation and bone loss at the same vertebra (regression coefficient (95% CI) -51 (-63 to -39)) (**table 3**). By switching category from absence to presence of MRI-BME, bone density significantly decreased 51 HU. Similar results were obtained in the sensitivity analysis, that is, when adding vertebrae with artefacts or density abnormalities (online supplemental table S3). No significant interaction was found between spinal region and MRI-BME on trabecular bone density (interaction p values of 0.271 (cervical and thoracic spine) and 0.398 (cervical and lumbar spine)). Likewise, no significant interaction was observed between the presence of MRI-BME and syndesmophytes at baseline on trabecular bone density (p=0.232). Therefore, no stratification of the analyses by spinal region or by vertebrae with (vs without) syndesmophytes at baseline was performed.

Table 2 Baseline presence of inflammation and syndesmophytes and mean bone density (Hounsfield Units) for each vertebra

Vertebra	Presence of inflammation* n/N (%)	Presence of syndesmophytes† n/N (%)	Hounsfield units‡ mean (SD)
C3	0/44 (0)	17/40 (43)	355 (106)
C4	2/42 (5)	17/38 (45)	353 (111)
C5	3/43 (7)	21/34 (62)	330 (108)
C6	3/41 (7)	13/30 (43)	286 (85)
C7	4/38 (11)	8/22 (36)	261 (77)
T1	5/33 (15)	7/20 (35)	243 (81)
T2	5/48 (10)	16/39 (41)	242 (59)
T3	10/47 (22)	24/45 (53)	220 (65)
T4	14/46 (30)	30/46 (65)	212 (68)
T5	22/48 (46)	30/48 (63)	203 (61)
T6	22/48 (46)	31/48 (65)	194 (63)
T7	19/47 (40)	33/47 (70)	183 (71)
T8	24/48 (50)	39/48 (81)	175 (64)
T9	19/46 (41)	39/46 (85)	175 (62)
T10	20/45 (44)	37/43 (86)	171 (62)
T11	25/48 (52)	39/46 (85)	168 (61)
T12	20/44 (45)	38/42 (90)	163 (49)
L1	21/46 (46)	37/46 (80)	163 (56)
L2	18/46 (39)	31/44 (70)	154 (52)
L3	18/47 (38)	31/47 (66)	147 (60)
L4	12/45 (27)	27/45 (60)	149 (63)
L5	14/46 (30)	23/46 (50)	154 (61)
Total	300 (31) 985 obs	588 (65) 910 obs	211 (95) 985 obs

*According to the agreement of 2 out of 3 readers.

†Absolute agreement of both readers.

‡Average of both readers' continuous score.

BMD, bone mineral density; n, number of vertebrae in which the outcome was reported as present; N, number of vertebrae in which the outcome was assessed; obs, observations.;

Association between bone loss and 2-year bone formation

Of the 218 vertebrae at risk for syndesmophyte formation at baseline, 8 (4%) were not scored at follow-up due to poor imaging quality. Therefore, the syndesmophyte formation change scores were obtained for a total of 210 vertebrae of which 14 (7%) showed development of new syndesmophytes after 2 years of follow-up. Of the 513 vertebrae at risk for syndesmophyte growth at baseline, 481 were scored at follow-up. Syndesmophyte growth was observed in 110 (23%) vertebrae. Of the 731 vertebrae at risk for the combined outcome, 691 were scored by both readers at baseline and 2 years. Syndesmophyte formation or growth was detected in 124 (18%) vertebrae. The detailed results are provided in online supplemental tables S4-S6.

Table 3 Association between baseline MRI-detected spinal inflammation and trabecular bone density at the same vertebra

Independent variables	Bone density (Hounsfield units)	
	Univariable analysis Reg coeff. (95% CI) N=910 to 985	Multivariable analysis Reg coeff. (95% CI) N=985
MRI-BME (presence)	-51 (-63 to -39)	-51 (-63 to -39)
Age (years)	-1 (-2 to 1)	-1 (-2 to 1)
Gender (male)	21 (-20 to 63)	16 (-24 to 57)
TNFi treatment (yes)	26 (-7 to 59)	27 (-6 to 61)
Baseline syndesmophytes (presence)*	-42 (-54 to -30)	†

Significant associations were highlighted in bold (p<0.05).
*Absolute agreement of readers.
†Not included in the multivariable analysis because of collinearity with MRI-BME.
BME, bone marrow oedema; TNFi, tumour necrosis factor inhibitors.

In the multilevel models, no significant association was found between bone density and bone formation after 2 years on the same vertebra (table 4). This was strikingly similar in univariable and multivariable analysis, for all the bone formation outcomes (syndesmophyte formation and/or growth), for the absolute agreement of readers (table 4) and for each reader separately (online supplemental tables S7-S8).

The lack of association was also shown in the sensitivity analyses when including vertebrae with artefacts and density abnormalities (online supplemental tables S9). A nearly two-time higher likelihood of syndesmophyte formation or growth was observed in vertebrae with MRI-BME at baseline (regression coefficient (95% CI) 1.88 (1.06 to 3.34)) (table 4). No significant interaction was found between spinal region and bone density on bone formation (interaction p values of 0.257 (cervical and thoracic spine) and 0.211 (cervical and lumbar spine), and therefore, no stratification per spinal region was needed. Also, the interaction between MRI-BME and syndesmophytes at baseline was not statistically significant (p=0.150), and stratified analyses revealed no relevant difference between the patients with (vs without) syndesmophytes at baseline (data not shown).

DISCUSSION

In this multilevel analysis of patients with r-axSpA, a cross-sectional statistically significant association between inflammation and bone density was observed at the same vertebra in patients with r-axSpA: the higher the inflammation, the lower the bone density. However, no statistically significant association was found longitudinally

Table 4 Association between trabecular bone loss at baseline and 2 year bone formation at the same vertebra

Independent variables	Syndesmophyte formation§	
	Univariable analysis OR (95% CI) N=203 to 210	Multivariable analysis adjOR (95% CI) N=203
Bone density (HU)	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.01)
Age (years)	1.01 (0.96 to 1.06)	1.01 (0.96 to 1.06)
Gender (male)	0.60 (0.08 to 4.22)	0.50 (0.05 to 4.82)
Smoking (current)	0.68 (0.17 to 2.73)	0.56 (0.10 to 3.13)
Treatment with TNFi (yes)	0.97 (0.21 to 4.51)	0.37 (0.05 to 2.89)
MRI-BME (presence)	5.31 (1.29 to 21.87)	5.35 (0.74 to 38.96)
Baseline syndesmophytes (presence)*	-†	-†
Independent variables	Syndesmophyte growth§	
	Univariable analysis OR (95% CI) N=469 to 481	Multivariable analysis adjOR (95% CI) N=469
Bone density (HU)	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.01)
Age (years)	1.01 (0.98 to 1.05)	1.01 (0.97 to 1.05)
Gender (male)	0.43 (0.14 to 1.38)	0.49 (0.16 to 1.54)
Smoking (current)	0.86 (0.39 to 1.90)	0.90 (0.40 to 2.06)
Treatment with TNFi (yes)	1.44 (0.63 to 3.29)	1.42 (0.54 to 3.71)
MRI-BME (presence)	1.66 (0.83 to 3.32)	1.51 (0.02 to 4.58)
Baseline syndesmophytes (presence)*	-‡	-‡
Independent variables	Syndesmophyte formation or growth§	
	Univariable analysis OR (95% CI) N=672 to 691	Multivariable analysis adjOR (95% CI) N=672
Bone density (HU)	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.00)
Age (years)	1.02 (0.99 to 1.06)	1.02 (0.98 to 1.05)
Gender (male)	0.44 (0.13 to 1.52)	0.56 (0.15 to 2.06)
Smoking (current)	0.89 (0.40 to 1.97)	1.02 (0.42 to 2.44)
Treatment with TNFi (yes)	1.34 (0.56 to 3.21)	1.30 (0.43 to 3.90)
MRI-BME (presence)	2.03 (1.23 to 3.71)	1.73 (1.06 to 3.34)
Baseline syndesmophytes (presence)*	2.84 (1.83 to 4.41)	-§

Significant associations were highlighted in bold ($p < 0.05$).

*Absolute agreement of readers.

†Omitted because the outcome was only possible in vertebrae without syndesmophytes at baseline.

‡Omitted because the outcome was only possible in vertebrae with syndesmophytes at baseline and no ankylosis.

§Not included in the multivariable analysis because of collinearity with MRI-BME.

adjOR, adjusted OR; BME, bone marrow oedema; HU, Hounsfield units; TNFi, Tumour necrosis factor inhibitors.

between low bone density and syndesmophyte formation or growth after 2 years of follow-up.

To our knowledge, this is the first study comprehensively assessing both bone formation and trabecular bone density at the same vertebra including the entire spine (from C3 to L5). Previous studies assessed lumbar spine only,¹² or only a few lumbar and thoracic vertebrae,¹³ omitting most thoracic vertebrae, where syndesmophytes have been shown to be most prevalent.^{15 16} DXA allowed solely the assessment of lumbar vertebrae, and the high

ionising radiation exposure in QCT scans precluded the assessment of larger numbers of vertebrae.^{13 27}

Another important novelty of the present study was the use of IdCT scans for measuring trabecular bone density, driven by the recent development of an innovative methodology to assess vertebral HU.²⁸ This methodology was shown by us, in the same patients with r-axSpA from the SIAS cohort, to be feasible and reliable from C3 to L5.²⁸ Lower HU values denote a lower IdCT attenuation, with less-dense bones presenting lower HU values. While

avoiding the overestimation of BMD reported in DXA (ldCT HU measurements are taken from the centre of the vertebra), this methodology enabled bone density assessments in the entire spine.

In the present study, we observed a higher prevalence of active inflammation (MRI-BME) at the thoracolumbar spine, in vertebrae with the lowest HU values. The presence of inflammation was significantly associated with a mean loss of 51 HU at the same vertebra, when compared with the absence of inflammation. These results strongly suggest that bone loss in r-axSpA is indeed locally present throughout the spine (in some degree related to local inflammation) and not only in the lumbar spine, the latter more consistent with systemic bone loss.^{5,7}

Regarding bone formation, up to one-fifth of the vertebrae at risk had new or grown syndesmophytes, with a predominance of growth after 2 years of follow-up. This reflects the increased sensitivity for capturing bone formation using CTSS when compared with the 7% of bone formation shown in our previous analysis using modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) in the ASSERT trial.¹² Yet, no association between low bone density and 2-year bone formation was observed, reinforcing the previous results.¹² This was strikingly similar for all the outcomes (syndesmophyte formation, growth and the combined outcome), independent of the reader, and also in the sensitivity analysis, despite the higher number of included vertebrae and subsequent increased power.

Other studies have shown conflicting results. A retrospective study assessing L1, showed that higher bone fragility (defined as ≤ 145 QCT HU, using QCT scanographic bone attenuation coefficient) was not significantly associated with the presence of at least one syndesmophyte (mSASSS ≥ 2) at the same level.²⁹ A recent 2-year longitudinal analysis of 33 patients with r-axSpA, tested the association between bone loss/strength and bone formation bidirectionally using QCT from T11 to L3.¹³ In their study, vertebrae without bridging syndesmophytes ($n=77$) with low trabecular BMD and low strength showed increased syndesmophyte growth over time, but with very subtle effects (adjusted coefficient BMD: -0.01 , and adjusted coefficient strength: -0.0003). However, these results should be interpreted with extreme caution due to relevant pitfalls: only few vertebrae were included, trabecular bone density variation throughout the spine was not accounted for,^{28,30} and the analyses were not adjusted for local vertebral inflammation, a known relevant confounder for this relationship.³¹

Though out of the scope of our research questions, when included as a confounder, the presence of MRI-BME at baseline was associated with a nearly twofold higher probability of 2-year syndesmophyte formation or growth, with this association persisting in multivariable analysis. Similar results have already been shown at the vertebral unit level in studies using radiographs,^{24,32} but also using ldCT scans in the SIAS cohort.³¹ Since no effect of inflammation-driven bone loss on subsequent

bone formation was shown in our study, taken together, these results suggest a solid independent direct effect of inflammation on bone formation. In this scenario, trabecular bone loss and peripheral bone formation may coexist as a consequence of inflammation at the same vertebra.

The present study is not without limitations. Clustering data at the same vertebra led to a slightly higher percentage of missing values ($\sim 5\%$) than for quadrant level analysis since complete quadrant data were required to avoid misclassification of vertebrae at risk for bone formation. We additionally sensitively excluded vertebrae with density abnormalities or artefacts potentially affecting HU values, increasing the missing data up to 10%. This approach aimed to prevent spurious results due to misclassification of vertebral trabecular bone density. Nevertheless, in sensitivity analyses using all vertebrae, the direction and significance of results were similar, with no important power issues detected. For the second research question, the need to further exclude vertebrae with complete ankylosis at baseline (not at risk for the outcome) may have led to a decrease in power to show statistically significant associations. However, the reasons for concern may be limited since we captured expected associations, namely a nearly twofold higher likelihood of new bone formation in vertebrae with baseline BME (vs without), and the CIs were narrow. Whether vertebral osteopenia/osteoporosis were present is not possible to prove. Bone density was assessed as a relative concept, using a continuous variable. In fact, although the validation of HU against DXA was previously performed in trauma subjects and spine surgery candidates,^{17,33,34} it was not repeated in r-axSpA. Importantly, previously proposed HU cut-offs in relation to osteoporosis definitions have shown large interstudy heterogeneity, imposing caution in their usage.³⁴ In addition, r-axSpA poses specific challenges. A possible validation against DXA would not be accurate as DXA measurements cannot avoid ectopic bone formation, while HU do. Moreover, whole spine validation against QCT would imply unacceptable ionising radiation exposure. Therefore, HU values were used (as appropriate) to reliably compare trabecular bone density across vertebrae.

Our study has several strengths. In the present study, the enhanced methodology, namely the use of ldCT scans, overcome most of the imaging-related limitations reported in former studies.^{12,13,27,29} The levels of ionising radiation exposure per ldCT scan of the entire spine (including all thoracic vertebrae) varied from 4 to 5 mSv (half of the dose of a lumbar CT scan).^{15,18} Current estimations of more modern ldCT scanners with improved AI-powered reconstruction algorithms can yield lower exposure than the dose cited here (without noticeable imaging quality loss).¹⁸ The inter-reader reliability and agreement at the same vertebra for all imaging assessments were within acceptable levels, strengthening the validity of the results. This study was conducted using a robust and comprehensive statistical analysis, namely,

several bone formation outcomes were tested encompassing new syndesmophyte formation and/or growth; the agreement of the readers (specific definitions) were privileged; a multilevel statistical approach adjusted for the dependence of multiple observations and clustered data within the same patient/vertebra; and the analyses were adjusted for potential confounders.²⁶

In summary, these results suggest that while in r-axSpA vertebral inflammation is associated with low vertebral bone density, lower vertebral bone density itself does not increase the risk for subsequent syndesmophyte development or growth. Our results also highlight the major role of inflammation in both trabecular bone loss and bone formation at the same vertebra in r-axSpA.

Author affiliations

¹Rheumatology department, Leiden University Medical Center, Leiden, Netherlands

²Rheumatology department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal

³Radiology department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal

⁴Rheumazentrum Ruhrgebiet, Ruhr University Bochum, Herne, Germany

⁵Radiology department, Leiden University Medical Center, Leiden, Netherlands

⁶Epidemiology department, Maastricht University, Maastricht, Netherlands

⁷Rheumatology department, Zuyderland Medical Centre, Heerlen, Netherlands

Contributors XB, JB, FAvG and MR performed the data collection in SIAS cohort. MLM, FAvG, DvdH and SR developed the study design. MLM and NPdS independently performed all the Hounsfield units measurements. MLM and RS prepared the dataset. MLM and SR performed the statistical analyses and synthesised the data. MLM has drafted the first version of the manuscript, and all authors have critically reviewed and agreed with the final version of the manuscript. Guarantor: MLM.

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ORCID iDs

Mary Lucy Marques <http://orcid.org/0000-0002-3071-9425>

Desirée van der Heijde <http://orcid.org/0000-0002-5781-158X>

Rosalinde Stal <http://orcid.org/0000-0002-6706-3933>

Xenofon Baraliakos <http://orcid.org/0000-0002-9475-9362>

Juergen Braun <http://orcid.org/0000-0002-9156-5095>

Sofia Ramiro <http://orcid.org/0000-0002-8899-9087>

Floris A van Gaalen <http://orcid.org/0000-0001-8448-7407>

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