# Do fatty lesions explain the effect of inflammation on new syndesmophytes in patients with radiographic axial spondyloarthritis? Results from the SIAS cohort and ASSERT trial

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ORIGINAL RESEARCH

# Do fatty lesions explain the effect of inflammation on new syndesmophytes in patients with radiographic axial spondyloarthritis? Results from the SIAS cohort and ASSERT trial

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## ABSTRACT

**Objectives** To determine how much of the effect of vertebral corner inflammation on development of syndesmophytes is explained by vertebral corner fat deposition.

**Methods** Patients with radiographic axial spondyloarthritis (r-axSpA) from the SIAS (Sensitive Imaging in Ankylosing Spondylitis) cohort and ASSERT (Ankylosing Spondylitis Study for the Evaluation of Recombinant Infliximab Therapy) trial were assessed at T0, T1 (SIAS: 1 year; ASSERT: 24 weeks) and T2 (2 years). Syndesmophytes assessed in each vertebral corner by whole spine low-dose CT (SIAS) or spinal radiographs (ASSERT) at T0 and T2 were considered present if seen by two of two readers. Inflammation (T0) and fat deposition (T0 and T1) on MRI were present if seen by ≥2 of 3 readers (SIAS) or 2 of 2 readers (ASSERT). Vertebral corners showing fat deposition or a syndesmophyte at baseline were ignored. Mediation analysis was applied to determine what proportion of the total effect of inflammation on syndesmophyte formation could be explained via the path of intermediate fat deposition.

**Results** Forty-nine SIAS patients (with 2667 vertebral corners) and 168 ASSERT patients (with 2918 vertebral corners) were analysed. The presence of inflammation at T0 increased the probability of a new syndesmophyte in the same vertebral corner at T2 by 9.3%. Of this total effect, 0.2% (2% (0.2 of 9.3) of the total effect) went via intermediate new fat deposition. In ASSERT, the total effect was 7.3%, of which 0.8% (10% of the total effect) went via new fat deposition.

**Conclusion** In r-axSpA, vertebral corner inflammation may lead to syndesmophyte formation but in a minority of cases via visible fat deposition.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Both MRI vertebral corner inflammation and vertebral corner fat deposition are associated with syndesmophyte formation.

### WHAT THIS STUDY ADDS

⇒ By using a causal mediation model in two studies, we showed that the largest part of the effect of inflammation on syndesmophyte formation is not explained by new fatty lesions on the same vertebral corner as an intermediate factor.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Since only a small part of the effect of inflammation on syndesmophyte formation is explained by fatty lesions, getting more insight into how inflammation leads to syndesmophytes should be the priority.

⇒ These results suggest caution in using fatty lesions as surrogate markers for structural damage.

⇒ Intervening on the suppression of inflammation seems to be a more promising route to prevent syndesmophyte formation.

## INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease involving the sacroiliac joints, the spine and several other musculoskeletal and extramusculoskeletal sites.[1] Typical abnormalities in the axial skeleton include inflammatory lesions, fatty lesions and bony lesions at entheseal sites, visible as erosions and (partial) ankylosis in the sacroiliac joints, and syndesmophytes and fusion of vertebral bodies.[1 2] Both bone proliferations and inflammation in the spine are associated with impaired function and decreased spinal mobility.[3–5] Understanding how pathogenic bone formation occurs is crucial in order to prevent or decelerate it.

Imaging plays a key role in improving our understanding of bone proliferation. Previous studies have consistently shown that vertebral corner inflammation (VCI) on MRI increases the risk of syndesmophyte formation in the same vertebral corner (VC) on conventional radiography (CR).[6–8] Other studies have shown that not only VCI but also vertebral corner fat deposition (VCFD) is associated with syndesmophyte formation visible either on CR or on whole spine low-dose CT (ldCT).[9–12] However, even though it was clearly demonstrated that VCI and VCFD are associated with bone formation in axSpA, their relative importance and the temporal sequence of their development are unclear.

Most studies assessing the effects of VCI and VCFD on syndesmophyte development reported that if these lesions occur together in the same VC, the likelihood for the subsequent formation of syndesmophytes is higher than for each individual lesion independently.[9–11] Two of these studies suggested that new bone is more likely to occur when VCI is followed by VCFD than when it is not.[10 11] Another study, however, reported that this sequence is uncommon and therefore cannot explain how most syndesmophytes form.[9]

These past studies have addressed the issue of new bone formation in the spine in terms of predictive ability: how well we can predict the formation of syndesmophytes based on the presence of preceding VCI, VCFD or both as assessed by MRI. Unfortunately, this type of analysis provides no valid insight into causality. Such studies tell us that VCI generally increases the risk of syndesmophyte development. In some VCs, VCI is followed by the formation of a new syndesmophyte, but this is not the case for all corners with VCI. The same is true for VCFD and for the combination of VCFD and VCI. But the question of the temporal sequence of VCI–VCFD–syndesmophyte formation is a question of causality rather than prediction, and requires a different analytical method.

Two of the mentioned studies which found an effect of VCI and VCFD on syndesmophyte development were executed in datasets of the Sensitive Imaging in Ankylosing Spondylitis (SIAS) cohort and Ankylosing Spondylitis Study for the Evaluation of Recombinant Infliximab Therapy (ASSERT) randomised controlled trial.[10 12] Since these associations were found in these datasets, the association between VCI and syndesmophyte formation can be further examined in these datasets to answer the causal mediation question of whether VCFD is a frequent or even necessary intermediate between VCI and subsequent syndesmophyte formation in patients with axSpA.

## PATIENTS AND METHODS

Data were used from two independent studies: the SIAS observational cohort[13] and the ASSERT randomised controlled trial.[14]

For SIAS, patients were recruited in Leiden (the Netherlands) and Herne (Germany); had a clinical diagnosis of radiographic axSpA (r-axSpA); fulfilled the modified New York criteria and had inflammation and structural damage in the spine; ≥1 spinal inflammatory lesion on MRI assessed with the SPondyloArthritis Research Consortium of Canada (SPARCC) scoring system[15]; and 1–18 syndesmophytes on CR assessed with the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS).[16]

For ASSERT, patients were recruited in 33 centres throughout the USA, Canada and Europe. Patients fulfilled the modified New York criteria and had a Bath Ankylosing Spondylitis Disease Activity Index and a Visual Analogue Scale spinal pain assessment score of ≥4.

In the ASSERT trial, patients were randomised on a 3:8 ratio to receive either infusions of placebo or infliximab until week 24.[14] After week 24, the study continued with an open extension until week 102 with all patients using infliximab. Patients were allowed to use concurrent stable doses of non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol or tramadol during the study. In the SIAS observational cohort, patients were not limited in medication use, with 66% of patients using NSAIDs, 22% of patients using biological disease-modifying antirheumatic drugs and 10% of patients using conventional synthetic disease-modifying antirheumatic drugs at baseline.

### Imaging techniques and scoring methods

For both studies, imaging was performed at baseline (T0), an intermediate visit (T1: 1 year for SIAS, 24 weeks for ASSERT) and at the end of follow-up (2 years (officially 102 weeks for ASSERT)). For the current study, MRI scores from T0 and T1 for both ASSERT and SIAS were used, assessed by two (ASSERT) and three readers (SIAS). In SIAS, VCI was assessed with the SPARCC scoring system on STIR sequences and VCFD was assessed with the CanDen scoring method on T1-weighted sequences.[15 17] For ASSERT, STIR and T1-weighted sequences were gadolinium enhanced and anterior corners were assessed for presence of VCI using the AS spinal MRI activity (ASspiMRI-a) score and for VCFD using the CanDen score.[10 17–19] In SIAS, whole spine ldCT images were assessed by two readers with the CT Syndesmophyte Score (CTSS), described in detail elsewhere.[13] In short, the CTSS assesses four quadrants per half of a vertebra with scores on a range 0–3 (0: normal, 1: syndesmophyte reaching <50% of the intervertebral disc space, 2: syndesmophyte reaching or crossing 50% of the intervertebral disc space, 3: bridging syndesmophyte). For ASSERT, lateral CR of the cervical and lumbar spine was made at T0 and T2 and assessed by two readers with the mSASSS. In short, the mSASSS assesses anterior corners of the cervical and lumbar spine on a range 0–3 (0: normal, 1: erosion, sclerosis or squaring, 2: syndesmophyte, 3: bridging syndesmophyte).[16] All readers were centrally trained. Time points of the same modality were assessed together blinded for time order. Details on imaging and scoring methods are provided in online supplemental text 1.

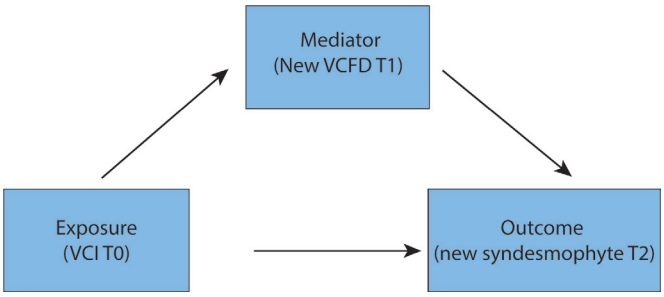


**Figure 1** Directed acyclic graph of the proposed pathways. The pathways under study: VCI at baseline can lead directly to new syndesmophyte formation (direct effect) or through the formation of VCFD at the same corner (indirect effect). T0, baseline; T1, 24 weeks (ASSERT) or 1 year (SIAS); T2, 2 years; VCFD, vertebral corner fat deposition; VCI, vertebral corner inflammation.

### VCI, VCFD and syndesmophyte combined scores

Syndesmophytes were assessed at the VC level, and considered 'present' on CR if mSASSS was 2 or 3, and on ldCT if CTSS was 1, 2 or 3. For status scores, VCI, VCFD and syndesmophytes were deemed present when seen by ≥2 of 3 readers (SIAS MRI) or 2 of 2 readers (SIAS ldCT and ASSERT). For VCI at T0, this binary agreement score was used in the analyses. For VCFD and syndesmophytes, the agreement scores at the separate time points were used to determine the presence of new VCFD at T1 and new syndesmophytes at T2. New VCFD was deemed present when readers agreed on absence of VCFD at T0 and presence of VCFD at T1. New syndesmophytes were deemed present when readers agreed on absence of a syndesmophyte at T0 and presence of a syndesmophyte at T2. Only corners with non-missing scores for all mentioned variables were included. Corners with presence of a syndesmophyte or VCFD at baseline were not at risk of these outcomes and thus excluded from the analyses.

### Mediation analysis

The directed acyclic graph (DAG) in figure 1 is the graphical representation of our research question: does VCI at T0 lead straight to a new syndesmophyte at T2 (direct effect) or through the formation of new VCFD (indirect effect) at T1? Provided that the assumption of sequential ignorability holds (online supplemental figure 1), the DAG in figure 1 is a causal structural graph.

To separate the total effect into a direct and indirect effect, we first calculated the probability of new VCFD at T1 conditional on VCI at T0 and the probability of a new syndesmophyte at T2 conditional on both VCI at T0 and new VCFD at T1, separately in SIAS and ASSERT. We then used Pearl's 'mediation formula' (online supplemental table 1) which, unlike the classic approach to mediation,[20] is robust to non-linear equations and to the presence of exposure–mediator interaction.[21] The 'mediation formula' takes the above-mentioned conditional probabilities to calculate the contrast in probabilities (absolute risk difference) of two potential outcomes, in which only one is observed and the other is the contrary of what is observed (the counterfactual outcome).

According to this so-called 'counterfactual approach', the direct effect of VCI on syndesmophyte formation is the increase in probability to develop a new syndesmophyte when there is VCI at T0 compared with when there is not, relative to some 'natural' level of VCFD at T1 (which may vary from VC to VC). This means the direct effect is calculated keeping VCFD constant at a certain value and this is done in two ways, resulting in the natural direct effect and the total direct effect. When calculating the natural direct effect, VCFD at T1 is kept constant at whatever value it would 'naturally' have obtained if there was no VCI at T0. When calculating the total direct effect, VCFD at T1 is kept constant at whatever value it would have obtained if there was VCI at T0. In essence, the natural direct effect and total direct effect are weighted averages of the effect of VCI on new syndesmophytes at each level of VCFD, with the probability of VCFD conditional on VCI as a weighting factor.

Like the direct effect, the indirect effect is calculated in two ways: as the natural indirect effect and the total indirect effect. The indirect effect is defined as the expected change in the probability of a new syndesmophyte at T2 by changing VCFD to whatever value it would have attained if there was VCI at T0, opposed to whatever value VCFD would have attained if there was no VCI at T0. For the natural indirect effect, this is calculated while keeping VCI constant at VCI=0 at T0. The total indirect effect is the same but holding VCI constant at VCI=1 at T0. Intuitively, the natural indirect effect and total indirect effect capture the effect of VCI at T0 on new syndesmophytes at T2 due to the effect of VCI at T0 on VCFD at T1.

In case the exposure–mediator interaction is significant ($p<0.15$), the natural direct effect, total direct effect, natural indirect effect and total indirect effect are reported separately. If the interaction is not significant, the average direct effect (=natural direct effect+total direct effect/2) and the average indirect effect (=natural indirect effect+total indirect effect/2) can be calculated with their sum being the total effect (=average direct effect+average indirect effect). The proportion mediated is equal to the average indirect effect divided by the total effect and represents the proportion of the total effect of VCI at T0 on new syndesmophytes at T2 that is explained by the new VCFD at T1.

Under the assumption of sequential ignorability, the natural and total direct/indirect effects can be identified and calculated non-parametrically (table 2 and online supplemental table 1). However, since VCs are nested within patients, the analysis must take the within-patient correlation into account. We used the method proposed by Imai *et al*[22 23] to implement, parametrically, Pearl's mediation formula in a two-level data structure. Parametric estimates may differ from non-parametric estimates. In addition, the exposure–mediator interaction was tested in a two-level mixed-effects model with new syndesmophyte as outcome and with VCI at T0, new

**Table 1** Baseline characteristics for the SIAS and ASSERT Studies

| Baseline characteristics | SIAS (N=49) | ASSERT (N=168) |
|---|---|---|
| Age at inclusion (mean (SD)) | 49 (9.8) | 38.1 (10.2) |
| Sex (males) | 42 (86%) | 135 (80%) |
| HLA-B27 status | 41 (84%) | 152 (91%) |
| Elevated CRP or ESR | 26 (53%) | * |
| Elevated CRP | * | 138 (82%) |
| BASDAI (mean (SD)) | 3.8 (2.2) | 6.4 (1.5) |
| ASDAS-CRP (mean (SD)) | 2.6 (1.2) | 4.0 (0.9) |

SpA features (HLA-B27 and elevated CRP or ESR) refer to both current or past presence. Numbers are presented as N (%) unless otherwise specified.
*Not measured.
ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; SpA, spondyloarthritis.

VCFD at T1 and their interaction term as covariates. The mediation analysis was performed with the 'Mediation' package in R V.3.6.3.

## RESULTS

For SIAS, 49 patients had imaging on all relevant time points contributing (49×92=) 4508 corners. Of these, 300 corners were not assessed on all time points by all readers. A further 1541 corners had a syndesmophyte and/or VCFD at T0. Therefore, a total of 2667 corners per time point was included in the analyses. The average age was 49 years (SD 9.8), 42 (86%) were male and 41 (84%) were HLA-B27+ (table 1). From ASSERT, 168 patients had imaging on all relevant time points contributing (168×24=) 4032 corners. Of these, 125 corners were not assessed on all time points by all readers and a further 989 corners had presence of a syndesmophyte and/or VCFD at T0. Therefore, a total of 2918 corners per time point was included in the analyses. The average age was 38 years (SD 10.2), 135 (80%) were male and 152 (91%) were HLA-B27+.

### Marginal and conditional probabilities of VCI, new VCFD and new syndesmophytes

VCI at T0 was present in 201 of 2667 (7%) VCs in SIAS and 147 of 2918 (5%) VCs in ASSERT. New syndesmophytes at T2 developed in 124 of 2667 (5%) VCs in SIAS and 91 of 2918 (3%) VCs in ASSERT. Slightly lower frequencies were found for the development of new VCFD (98 of 2667 (4%) in SIAS, 61 of 2918 (2%) in ASSERT).

The probability of new VCFD at T1 conditional on the VCI status at T0 and the probability of new syndesmophyte conditional on the VCI status at T0 and VCFD at T1 is shown in table 2. In both studies, new VCFD at T1 was more common in corners with VCI (12% in SIAS, 18% in ASSERT) than without VCI at T0 (3% in SIAS, 1% in ASSERT). The risk of new syndesmophytes in VCs with new VCFD comparing VC with and without VCFD was greater if VCI was present (SIAS: 21–14=6%; ASSERT: 19–7=8%) than in VC without VCI at T0 (SIAS: 5–4=1%; ASSERT: 3–3=0%).

Of note, most new syndesmophytes formed in VCs without VCFD at T1 both in absence and in presence of VCI at T0. In SIAS, 94, out of the total 124 new syndesmophytes, were formed in VCs without VCI at T0, with the majority (90 of 94=96%) forming in the absence of VCFD. Likewise, of the 30 new syndesmophytes in VCs that did have VCI at T0, the majority (25 of 30=83%) formed in the absence of preceding VCFD. These two figures were 99% and 64% in ASSERT. In fact, the sequence, VCI followed by a new VCFD and then by a new syndesmophyte, occurred in only 0.2% of all corners (5 of 2667 in SIAS; 5 of 2918 in ASSERT).

### Mediation analysis

The exposure–mediator interaction was not significant in both cohorts (p=0.88 for SIAS; p=0.82 for ASSERT). Thus, average effects (average direct effect and average indirect effect) are appropriate to be used (natural direct effects, total direct effects, natural indirect effects and total indirect effects are reported in online supplemental table 2). For SIAS, the presence of VCI at T0 increased the probability of developing a new syndesmophyte in the same vertebral corner at T2 by 9.3% (table 3). When decomposing this total effect into the two effects, the average direct effect was 9.1% and the average indirect effect was 0.2%. This means that out of the total increase in probability of 9.3% a VC had of developing a new syndesmophyte 2 years later when VCI was present at baseline, only 2% of this effect (0.2 of 9.3) was explained by new VCFD at 1 year.

Similar results were found in ASSERT. There, the total effect was 7.3% which was composed of the average direct effect of 6.5% and the average indirect effect of 0.8%. Thus, out of the 7.3% increase in probability a VC had to develop a new syndesmophyte 2 years later when VCI was present at baseline, only 10% of this effect (0.8 of 7.3) was explained by new VCFD at 24 weeks.

## DISCUSSION

Previous studies on SIAS and ASSERT data showed that both VCI and VCFD are associated with syndesmophyte formation in patients with r-axSpA.[10 12] Taking this finding as a starting point for the current study, we now used a causal mediation approach to study whether VCI often requires VCFD to develop a syndesmophyte in these two independent datasets. We found that VCFD occurred proportionally more often if this was preceded by VCI, suggesting that VCI has an effect on VCFD development. However, the mediation analysis has shown that the contribution of new VCFD as an intermediate on syndesmophyte formation was, in both studies, very small and non-significant.

**Table 2** Conditional probability of new vertebral corner fat deposition (VCFD) and new syndesmophytes in each cohort

| VCI T0 | New VCFD T1 | New SYND T2 | n | P (SYND\|VCI, VCFD) | P(VCFD\|VCI) |
|---|---|---|---|---|---|
| **SIAS (n=2667 VCs)** | | | | | |
| 0 | 0 | 0 | 2302 | P (SYND\|0, 0)=90/2392=0.038 ($g_{00}$) | P(VCFD\|0)=74/2466=0.030 ($h_0$) |
| 0 | 0 | 1 | 90 | | |
| 0 | 1 | 0 | 70 | P (SYND\|0, 1)=4/74=0.054 ($g_{01}$) | |
| 0 | 1 | 1 | 4 | | |
| 1 | 0 | 0 | 152 | P (SYND\|1, 0)=25/177=0.141 ($g_{10}$) | P(VCFD\|1)=24/201=0.119 ($h_1$) |
| 1 | 0 | 1 | 25 | | |
| 1 | 1 | 0 | 19 | P (SYND\|1, 1)=5/24=0.208 ($g_{11}$) | |
| 1 | 1 | 1 | 5 | | |
| **ASSERT (n=2918 VCs)** | | | | | |
| 0 | 0 | 0 | 2660 | P (SYND\|0, 0)=76/2736=0.028 ($g_{00}$) | P(VCFD\|0)=35/2771=0.013 ($h_0$) |
| 0 | 0 | 1 | 76 | | |
| 0 | 1 | 0 | 34 | P (SYND\|0, 1)=1/35=0.029 ($g_{01}$) | |
| 0 | 1 | 1 | 1 | | |
| 1 | 0 | 0 | 112 | P (SYND\|1, 0)=9/121=0.074 ($g_{10}$) | P(VCFD\|1)=26/147=0.177 ($h_1$) |
| 1 | 0 | 1 | 9 | | |
| 1 | 1 | 0 | 21 | P (SYND\|1, 1)=5/26=0.192 ($g_{11}$) | |
| 1 | 1 | 1 | 5 | | |

The table shows the occurrence of all possible scenarios in each cohort on the left-hand side and the conditional probabilities of new syndesmophyte and new VCFD on the right-hand side. For example, for SIAS, in the first row, there are 2302 corners without VCI, without new VCFD and without a new syndesmophyte, and in the second row, there are 90 corners without VCI and without new VCFD, but with a new syndesmophyte. Thus, in corners without VCI and new VCFD, there is a probability of 90/(90+2302)=0.038 of developing a new syndesmophyte. If we compare this with corners without new VCFD but with VCI, there is a probability of 25/(25+152)=0.141 of developing a new syndesmophyte. The last column provides proportions of the development of new VCFD for scenarios without VCI P(VCFD|0) and with VCI P(VCFD|1). The formula in online supplemental table 1 can be applied to these data to obtain the effects non-parametrically. Due to the multilevel structure of the data (vertebral corners nested within patients), a parametric approach was needed to incorporate this two-level structure; thus, the non-parametric effects are not presented.
n, number of vertebral corners; P, probability; SYND, syndesmophytes; T0, baseline; T1, intermediate visit; T2, end of follow-up; VCI, vertebral corner inflammation; VCs, vertebral corners.

Our findings only partially contradict hypotheses previously formulated in literature. We do confirm that VCI increases the probability of syndesmophyte formation, but we also find that this probability is rather low (9.3% for SIAS, 7.3% for ASSERT). This is also in line with previous reports consistently showing that most syndesmophytes are not preceded by observed MRI lesions.[6–12] Moreover, as reported previously, we also found that new VCFD occurs more often in corners with than without VCI.[9–11] However, our mediation analyses do not support the hypothesis that new VCFD detected after demonstrated VCI has a specifically strong contribution to syndesmophyte development.

Even though the proportion of the effect of VCI on syndesmophyte formation that was mediated by VCFD was slightly larger in ASSERT than in SIAS (10% vs 2%),

**Table 3** Mediation analysis performed at the vertebral corner level

| Effect | SIAS | ASSERT |
|---|---|---|
| Total effect | **9.3% (4.5% to 15.0%)** | **7.3% (2.0% to 16.0%)** |
| Average direct effect | **9.1% (4.3% to 15.0%)** | **6.5% (1.3% to 14.0%)** |
| Average indirect effect (AIE) | 0.2% (–0.4% to 1.0%) | 0.8% (–0.2% to 3.0%) |
| Proportion mediated (AIE/total effect) | 2.0% (–4.0% to 13.0%) | 10.2% (–3.1% to 44.0%) |

Values are the average increase in probability (95% CI) of a new syndesmophyte at the end of follow-up driven by the presence of VCI at baseline (total effect), the increase in this probability that is unexplained (direct effect) and explained (indirect effect) by the formation of fat deposition in the intermediate visit and the proportion mediated (AIE/total effect). Values in bold are statistically significant. All estimates are derived parametrically according to the method of Imai *et al*.[22 23]
VCI, vertebral corner inflammation.

the conclusions from both studies are the same. Nevertheless, there are several differences between the two studies, including the design (trial vs cohort), imaging methods for syndesmophyte detection (gold standard (CR assessed with mSASSS) vs the more novel ldCT assessed with CTSS) and detection of VCI (gadolinium-enhanced STIR and T1 assessed with ASspiMRI-a vs STIR and T1 assessed with SPARCC) and the geographical areas in which patients had been recruited for the studies. As a result of the different study designs, medication use differed significantly over the two studies. However, despite all differences, both studies showed a direct effect (with similar magnitudes) of VCI on syndesmophyte formation and demonstrated similar average indirect effects, which were low.

The most important strength of this study is the use of a causal mediation model. Due to the structure of our data, the mediation formula by Pearl was the best choice for the analyses.[21] Apart from the ability to disentangle the direct and indirect effects from the total effect, this approach enabled us to intuitively view the data through the use of a conditional probability table and allowed, in case of one-level data, to make the calculations non-parametrically. Previous studies showed a positive association between both VCI and VCFD on the development of syndesmophytes. Here we show, in two independent studies, that this effect mostly 'travels' either directly from VCI to syndesmophyte development or through unknown pathways, rather than via a new VCFD. For the detection of VCFD, only one intermediate time point was used, which begs the question whether VCFD was sufficiently captured. Given our research question, this analysis is sensitive to the number of time points and the spacing between them. However, by using two studies with two different time points of the intermediate visit (SIAS 1 year, ASSERT 24 weeks), we could assess the sequence with different time intervals.

In our analyses, we did not control for patient characteristics such as HLA-B27 or C reactive protein (CRP). These could be considered potential confounders of the association between inflammation and new bone formation. However, with our methods, we analysed the data at the individual corner level. And importantly, by using change scores (ie, new fatty lesions and new syndesmophytes), we are effectively looking into the so-called 'within-patient' effects. Since the patient characteristics (eg, CRP) do not vary across quadrants within the same patient, they cannot explain the variability of the outcome within each patient and therefore cannot confound the association of interest. In other words, the value of CRP is 'fixed' for all quadrants within the same patient. These patient-level characteristics can however vary and therefore explain the variability of the outcome across patients. However, as mentioned, we have isolated 'within-patient effects'; thus, if present, such an effect would not have influenced to a great extent our mediation estimates. This is what we refer to as the sequential ignorability assumption.

Our findings can have several implications for future studies and clinical practice. Under the hypothesis that VCI is often followed by VCFD before the formation of a syndesmophyte, VCFD would have been a good proxy for syndesmophyte formation and could be a target for future intervention studies. However, since our results do not support this hypothesis, future studies should focus on the biological pathways through which VCI directly leads to syndesmophyte formation. For clinical practice, our results suggest that the observation of resolving VCI not followed by VCFD does not imply that in this vertebral corner no syndesmophyte will develop.

In summary, the current study showed in two independent studies of patients with r-axSpA that by far the largest part of the effect of VCI on syndesmophyte formation is not explained by new VCFD as an intermediate factor.

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**Contributors** Study coordination—DvdH, FAvG, AS, SR, RL, XB and JB. Data collection—PMM, RvdB, XB and JB. Radiology protocol preparation and execution—MR. Image readings—RvdB and MdH. Preparation of database, manuscript draft, tables and figures—RS. Analyses execution—RS and AS. Analyses supervision and advising—SR, DvdH, AS, RL and FAvG. All authors contributed to the writing by reviewing and editing. Guarantor—RS.

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**Competing interests** AS—honoraria from Novartis, support for meetings from Lilly and UCB. DvdH—grant from Dutch Rheumatism Association; consulting fees from AbbVie, Gilead, Glaxo-Smith-Kline, Lilly, Novartis and UCB Pharma; other interest as director of Imaging Rheumatology. FAvG—grants from Stichting vrienden van Sole Mio, Stichting ASAS, Jacobus stichting, Novartis and UCB; consulting fees from Novartis, MSD, AbbVie, Bristol Myers Squibb and Eli Lilly. MdH—grants from FWRO/FRSR; participation in data safety monitoring board for UCB; member of EULAR Advocacy Committee, Young ASAS leader and ASAS-EULAR taskforce. MR—fees for ASAS CLASSIC Study. PMM—consulting fees from AbbVie, BMS, Celgene, Eli Lilly, Galapagos, Janssen, MSD, Novartis, Orphazyme, Pfizer, Roche and UCB. RL—consulting fees from AbbVie, Amgen, BMS, GSK, Janssen, Eli Lilly, Novartis, Pfizer and UCB; honoraria from AbbVie, Amgen, BMS, GSK, Janssen, Eli Lilly, Novartis, Pfizer and UCB; participation in data safety monitoring board for UCB; chair of quality of care for EULAR. SR—grants from AbbVie, Galapagos, MSD, Novartis, Pfizer and UCB; consulting fees from AbbVie, Eli Lilly, MSD, Novartis, Pfizer, UCB and Sanofi; honoraria from Eli Lilly, MSD, Novartis and UCB. JB, RS, RvdB, XB—nothing to declare.

**Patient consent for publication** Not required.

**Ethics approval** This study involves human participants. The SIAS Study was approved by the medical ethical committees from both centres (Leiden: Medisch Ethische Toetsings Commissie, P10.021; Herne: Ethikkommission der Ruhr Universität Bochum, 4366-12). For ASSERT, patients were recruited in 33 centres throughout the USA, Canada and Europe. Each centre's institutional review board or ethical committee reviewed and approved the study protocol. The names of each research centre and IDs are not known to the authors of the current paper. Participants gave informed consent to participate in the study before taking part.

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**Data availability statement** Data are made available upon reasonable request to the corresponding author.

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