



# Analysis of Bone Samples from Patients with Spondyloarthritides — Identifying Causes of New Bone Formation in Axial Spondyloarthritis

Ankylosing spondylitis (AS) is a chronic inflammatory disease predominantly affecting the axial skeleton, leading potentially to bone erosions, new bone formation, and ankylosis of the spine. The burden of disease is determined by the grade of acute inflammation causing pain and stiffness and by new bone formation causing a reduction in spinal mobility<sup>1</sup>.

Histopathological studies from intervertebral discs<sup>2</sup>, femoral heads<sup>3,4</sup>, sacroiliac joints<sup>5,6</sup>, manubriosternal junction<sup>7</sup>, and zygapophyseal joints<sup>8,9,10</sup> enabled us in the past to define characteristic histomorphological and immunohistochemical features of acute inflammation in bony samples from patients with AS: enthesitis and subchondral inflammation at the interface between bone and cartilage — subchondral osteitis — are the primary events in AS immunopathology. Cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>5</sup>, interleukin 17 (IL-17)<sup>11</sup>, and IL-23<sup>9</sup> seem to be triggering factors during inflammatory processes. The rather unexpected observation that TNF- $\alpha$  blocking therapy did not reduce new bone formation and radiographic progression in patients with AS underscored the need for a better pathophysiological understanding of osteoproliferative mechanisms in AS.

Currently, investigations are focused on 2 major hypotheses. The first is that new bone formation is an independent feature uncoupled from inflammation triggered by mechanical stress. Microdamage and/or cell stress at the enthesial site trigger both an inflammatory and an anabolic process in the bone, i.e., through bone morphogenic protein signaling, leading to typical clinical signs of AS<sup>12,13</sup>. The second is that new bone formation is closely linked to inflammation through initial inflammation causing osteo-destruction, and consequently, through a repair mechanism, including formation of fibrous tissue, triggering osteoblast stimulation and new bone formation<sup>10,14</sup>.

In this issue of *The Journal* Pacheco-Tena and colleagues describe immunohistochemical features of inflammation and new bone formation in human bony samples from patients with ankylosing spondylitis<sup>15</sup>. This is a unique set of bony samples from patients with SpA, and the questions addressed in this report are indeed of relevance. Even if most of the presented data are descriptive, precluding further quantitative and statistical evaluation, they give evidence that fibrous tissue, together with vascular proliferation and development of osteoid within cartilage matrix, as illustrated in their Figure 2, might be the source of new bone formation in SpA. Moreover, the authors describe features of both direct bone formation and of enchondral ossification. Their immunohistochemical analysis detected increased frequencies of osteoprotegerin (OPN), osteocalcin (OCN), and bone sialoprotein (BSP)-positive osteoblasts. Interestingly, OPN-positive and OCN-positive cells could also be detected within fibroblasts. The authors report a lack of inflammatory lesions in their set of tissue samples, but according to radiographic results displaying advanced ankylosing spondylitis (see their Figure 1), acute inflammatory lesions are not necessarily expected in this advanced stage. Therefore, bony samples from patients with advanced disease might not be useful in excluding a link between inflammation and new bone formation, as hypothesized by the authors in their final comment.

To study the link between inflammation and new bone formation in SpA, or to determine a possible sequence of events, or underline the absence of such a sequence, 3 different approaches are used. First, magnetic resonance imaging (MRI) and radiographs have been assessed in several analyses to study the relationship between spinal inflammation, fatty degeneration, and new bone formation. By using imaging studies, some groups reported that the sequence “inflammation to fatty degeneration to new bone

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formation” is not frequently observed (i.e.<sup>16</sup>). Others reported that inflammatory lesions of the spine on MRI predict the development of new bone formation in AS<sup>17,18</sup>. A limitation of all MRI studies is that MRI, even if performed within short intervals, will not capture all inflammatory lesions between disease onset and occurrence of bone proliferative changes as detected by radiography. Second, new bone formation has been analyzed in “spondyloarthritis-like” animal models. This subject cannot be discussed in detail at this point, but the results from DBA/1 mice that develop arthritis and enthesial ankylosis have led to new insights into molecular pathways<sup>19</sup>, which might also be involved in disease progression in patients with AS. This work and the recently published analysis of TNFΔARE mice<sup>20</sup> in which typical inflammatory lesions similar to SpA as well as new bone formation were observed — both induced through mechanical stress — give evidence that new bone formation can also be driven by mechanical stress in this animal model. Third, inflammation and new bone formation in bony tissue samples from patients with SpA have been analyzed histomorphologically and immunohistochemically. Several groups have initiated systematic histomorphological analysis in human samples to cast more light on the question about triggering factors initiating new bone formation in AS. However, Pacheco-Tena, *et al* investigated mostly samples with advanced disease progression<sup>15</sup>. Our own set of bony tissue samples from AS facet joints was also obtained from patients usually with an average disease duration of more than 20 years<sup>11</sup>. However, as shown in the recent manuscript of Bleil, *et al*<sup>10</sup>, a heterogeneous set of AS facet joints with different stages of disease progression allowed us to differentiate between different stages of disease. In addition to inflammation, we found cartilage degeneration, indicated by cartilage thinning, enhanced chondrocyte apoptosis and proteoglycan loss, and subchondral bone thinning, promoted by the invasion of fibrous tissue originating from the bone marrow through the subchondral bone endplate, as hallmarks of joint remodeling in AS<sup>10</sup>. The presence of fibrous tissue as a main histomorphological finding located at sites with less inflammation but already showing bone destruction (i.e., bone endplate) and the formation of osteoblasts within the same tissue with features of direct bone formation has also been reported extensively in historical histological analyses by Cruickshank<sup>2</sup>, Bywaters and Olsen<sup>3</sup>, Savill<sup>7</sup>, and Francois, *et al*<sup>6</sup>.

A striking histomorphological analysis published 2 years ago is, to our knowledge, one of very few studies with bony tissue samples from AS patients with a rather short mean disease duration of only 3 to 4 years<sup>21</sup>. The original objective of the study was to evaluate the usefulness of needle biopsies of the sacroiliac joints for the diagnosis of early sacroiliitis in comparison to MRI in 109 patients with axial SpA. Interestingly, besides the fact that the authors

identified a higher sensitivity for needle biopsies compared to MRI, they described a link between inflammation as detected by subchondral bone marrow edema and mononuclear cell infiltrates and proliferating connective tissue infiltrates and pannus formation (which we identified as fibrous tissue). They also reported subchondral bone endplate destruction combined with pannus invasion, cartilage degeneration, fibrosis, and ossification.

As mentioned, the work by Pacheco-Tena, *et al* mostly refers to advanced disease stages and mechanisms that might contribute to new bone formation<sup>15</sup>. This included features of direct bone formation through osteoblasts with high OPG, OCN, and BSP expression originating from fibrous tissue. Additionally, enchondral ossification was reported, described as an “intrusion of osteoid material within cartilage matrix” and that most likely resembles fibrocartilage. This is of interest because a recent study from AS facet joints did not find evidence of enchondral ossification originating within hyaline cartilage (Bleil, *et al*, submitted). However, in both studies, cartilage degradation was reported, possibly pointing to disturbed cartilage homeostasis promoting cartilage degeneration as a general feature in AS.

*In situ* analysis of human bony samples has added insight into mechanisms leading to inflammation and new bone formation in AS. The exact sequence of events is still not clear and needs further analysis. Analysis of earlier disease stages with closer followup is a necessary but difficult undertaking because bony samples from patients with AS are difficult to collect. The molecular mechanisms involved in the formation of fibrous (repair) tissue and the mechanisms of new bone formation starting from these sites will need to be analyzed.

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