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# Loss and gain of bone in spondyloarthritis: what drives these opposing clinical features?

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***Abstract*:** The breadth of bone lesion types seen in spondyloarthritis is unprecedented in medicine and includes increased bone turnover, bone loss and fragility, osteitis, osteolysis and erosion, osteosclerosis, osteoproliferation of soft tissues adjacent to bone and spinal skeletal structure weakness. Remarkably, these effects can be present simultaneously in the same patient. The search for a potential unifying cause of effects on the skeleton necessarily focuses on inflammation arising from the dysregulation of immune response to microorganisms, particularly dysregulation of $T_H17$ lymphocytes, and the dysbiosis of established gut and other microbiota. The compelling notion that a common antecedent pathological mechanism affects existing bone and tissues with bone-forming potential (entheses), simultaneously with variable effect in the former but bone-forming in the latter, drives basic research forward and focuses our awareness on the effects on these bone mechanisms of the increasing portfolio of targeted immunotherapies used in the clinic.

***Keywords:*** ankylosing spondylitis (AS), axial spondyloarthritis (axSpA), bone pathophysiology, enthesophyte, osteoimmunology, osteomicrobiology, osteoporosis, osteoproliferation, spondyloarthritis (SpA), syndesmophyte

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## Introduction

Bone pathophysiology is an integral part of all the spondyloarthritis (SpA) conditions and is intriguing given the complexity of mechanisms that result in either net loss of bone mass, increased general bone turnover, or bone gain.[1] The range of skeletal effects that occur in SpAs, including ankylosing spondylitis (AS; Table 1), include: generalised bone loss (osteoporosis), fragility fractures, focal and sub-entheseal osteitis and erosion, osteoproliferation, either at peripheral (enthesophytes) or axial (syndesmophytes) skeletal ligament or tendon entheses and osteosclerosis (Figure 1). This range of skeletal effects reflects the potential of disease pathophysiology to affect different bone and non-bone (potentially 'bone-forming') cells, at either a focal or more general scale. Remarkably, these effects can exist in the same patient over time or even simultaneously. SpA is one of a few conditions where osteoproliferation at entheses is a key part of the pathophysiology, a feature which has been recognised for over 300 years[2] and highlighted in the medical imaging literature for many years.[3,4] Both diffuse idiopathic skeletal hyperostosis (DISH)[5] and X-linked hypophosphatemic rickets (XLH)[6] are also conditions where osteoproliferation at entheses occur and may offer insights into the general and specific causes of entheseal osteoproliferation in SpA. Elucidating causative mechanisms of the various bone changes in SpA will be an important contribution to the design and use of effective long-term therapies. Here we review the clinical features, causes and consequences of bone pathology in SpA, and discuss advances in the osteoimmunology of these disease features.

## The clinical relevance of bone pathophysiology in SpA

### *Osteoporosis and fragility fracture*

*Ankylosing spondylitis.* Most data on fragility fracture risk and osteoporosis relate to a diagnosis

**Table 1.** The spectrum of bone effects ('lesions') in spondyloarthritis.

| Bone lesion | Example |
|---|---|
| Generalised low bone mass | Vertebral body osteoporosis |
| Osteitis | Sub-enthesial and isolated 'MRI defined bone edema' (MRE) lesions; bone erosions |
| Osteoproliferation (new bone forming in bone-adjacent soft tissues) | Periosteal irregularities/whiskering at fibrous entheses, typically pelvi-ileal or ischial |
| | Syndesmophytes |
| | Enthesophytes at ligament and tendon insertions (e.g. plantar fascia origin/Achilles' tendon insertion, at greater and lesser trochanters) |
| Osteosclerosis | Vertebral corner Romanus lesions subsequent to osteitis; or periosteal proliferation at the interface of anterior vertebral body margin and anterior longitudinal ligament |

MRI: magnetic resonance imaging.

of AS rather than the broader SpA or axial-SpA (axSpA) definition.[7] Osteoporosis in AS was recognised over 50 years ago[8] but in AS, osteoporosis, conventionally defined as 'low bone mass, structural weakness and increased fracture risk', requires some clarification given that there are some unique AS-specific effects on the skeleton. The likelihood of a vertebral fracture occurring in AS is up to four times the risk compared with control groups.[9,10] However, evidence on the risk of hip fractures in patients with AS is inconsistent[9] and fragility fracture incidence at other sites is not well known.

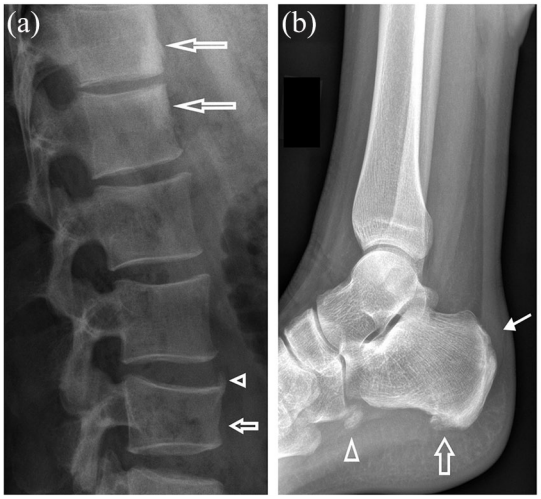


**Figure 1.** Osteoproliferative lesions in spondyloarthritis.
a. Romanus lesions (long arrows): osteosclerosis at the vertebral enthesis attachment of both the anterior longitudinal ligament and anterior intervertebral disc annulus. There is a syndesmophyte (arrowhead) arising from a previously fractured vertebra (short arrow). b. Erosion and osteosclerosis at the Achilles' tendon insertion (thin arrow), osteoproliferation (enthesophyte) at the plantar fascia origin (wide arrow) and osteoproliferation (periosteal irregularity) of the os peroneum (arrowhead), which is a sesamoid bone in the peroneus longus tendon attached to the tendon on all its sides by entheses. We gratefully acknowledge Professor Andrew Grainger for the images.

Major risk factors for vertebral fractures in AS include low bone mineral density (BMD) at the femoral neck and total hip (but not lumbar spine), male sex, longer disease duration, higher disease symptoms scores, inflammatory bowel disease,[9] and the duration and structural severity of the disease.[11] Two notable issues arise from these data. Firstly, there is a need to understand the relative effects of vertebral body bone loss and of disease-specific-related changes in spinal structure in contributing risk to fracture; and secondly it is important to understand that dual X-ray absorptiometry (DXA) derived lumbar spine BMD does not predict vertebral fractures.

*Consequences of vertebral body and spinal fractures in AS.* The surgical literature is rich with case reports (summarised elsewhere[12]) highlighting the consequences of sustaining spinal, not just vertebral body, fragility fractures in AS. Serious complication risk is high, and effects can be catastrophic (67% of patients with neurological complications; 3% mortality within 3 months). Such consequences probably relate to the mechanical effects of fracture through a rigid, or semi-rigid, spine where extra-skeletal new bone formation

(e.g. syndesmophytes, posterior vertebral element ankylosis) results in reduced dissipation of loading forces at the time of fracture and displacement of large, rather than small, segments of bone tissue. A large number of the 345 patients with AS in the literature have had cervical spine fractures, not an area in the spine typically associated with vertebral osteoporosis in the general population.[12] This suggests that cervical spine fractures, and by logical extension all spinal fractures in AS, may relate critically to skeletal fragility from compromised vertebral structure and strength as well as low vertebral body bone mass.

*Patients defined as having axSpA.* In patients diagnosed with axSpA (including nonradiographical SpA) data on osteoporosis, risk of fractures and fracture incidence are less well understood compared with data from earlier AS-defined cohorts. However, between, 2006 and 2016 there were 21 studies comparing either osteoporosis or fracture rates in 'axSpA' patients with control groups.[13] Osteoporosis prevalence varied from 12% to 34% whilst fracture prevalence was between 11% and 24%. However, as the continuing debate regarding the definition of axSpA has evolved over the last 10 years, so the data on fracture incidence and risk will need to be refined. Notably, recent reviews have focussed on an AS definition rather than a wider axSpA disease definition.[14,15]

*Predicting osteoporosis and fracture risk in SpA.* Osteoporosis and fracture risk will be a function of both nonspecific and SpA disease-specific factors. There are some data showing that general fracture risk assessment tools that compute fracture risk using data like age, body mass index and history of previous fracture, smoking and parental hip fracture (e.g. FRAX® or Q-Fracture) can be legitimately applied for SpA patients. FRAX® predicts a higher 10-year risk of fracture in axSpA compared with controls,[16] but FRAX® fracture prediction has not been widely examined across different SpA populations, nor either in SpA patients stratified for spinal structural changes, with or without DXA-derived bone mass data in the algorithm.

Hip BMD measurement assessed by DXA predicts vertebral fracture in AS but anteroposterior (AP) lumbar spine BMD measurement does not;[9] a likely result of 'nonqualifying' calcified tissue (e.g. syndesmophytes, calcification of ligaments and posterior element entheseal osteo-proliferation) being captured within the AP lumbar spine scanning field of view. Accordingly, measuring lateral and volumetric vertebral body BMD is more sensitive than AP BMD in detecting osteoporosis and is less affected by syndesmophyte formation.[17] In addition, using a (DXA-derived) trabecular bone score (TBS) that assesses mean thickness and volume fraction of trabecular bone microarchitecture, can complement vertebral body BMD evaluation of osteoporosis.[18] TBS is not influenced by syndesmophyte formation,[19] negatively correlates with systemic inflammatory markers,[20] and is a promising technique for monitoring vertebral body osteoporosis, specifically in axSpA. Quantitative computerised tomography (QCT)[21] can also estimate BMD in vertebral bodies avoiding bone-adjacent osteoproliferative changes. QCT can detect early vertebral bone loss in AS and shows deterioration of vertebral body bone loss with progressive spinal disease,[22] where AP lumbar spine BMD, assessed by DXA, shows increased bone mass.[23] Korkosz's study[23] neatly illustrates the osteolytic effect of progressive axSpA on trabecular rich vertebral body bone and simultaneously the osteoproliferative effect at the periosteal envelope and at entheses, in the same patients.[23] Such an inverse relationship between osteoproliferation and osteopenia had been predicted by earlier studies.[24,25] Both processes, osteoproliferation and bone loss, are likely to have a common association with disease severity, with bone loss being evident chiefly at trabecular bone rich sites throughout the skeleton.[17]

Early studies suggested that peripheral BMD may be normal in AS.[26] However, using high-resolution peripheral QCT and careful comparative analysis, there can be significant and unexplained decreases in peripheral BMD in patients with AS compared with controls; more marked in human leukocyte antigen (HLA) B27-negative patients. Whether this relationship is predictive of the degree of enteropathic pathophysiology, local osteoproliferation or an effect of metabolic or hormonal comorbidity is unknown.

### Osteitis

*Osteitis and magnetic resonance imaging bone marrow 'edema'..* The earliest references of 'osteitis' in SpA were in relation to either radiographically described osteitis pubis,[27] erosion,[3] or as a bone scintigraphy abnormality[28] perhaps best referenced to SAPHO syndrome,[29,30] with abnormally increased localisation of technetium-99m-labelled

diphosphonate. This radionuclide locates abnormally according to increased blood delivery, accessing bone tissue through changes in the vascular endothelium and binding to hydroxyapatite, which in turn correlates with bone turnover and reflects the rate of new bone formation.[29] More direct evidence of the nature of osteitis in axSpA was disclosed by peri-sacroiliac bone biopsy[31], which correlated with abnormally increased signal on fat-suppressed magnetic resonance imaging (MRI) sequences, a feature originally described in 2004.[32] The features of $CD45^+/CD68^+$ macrophages, $CD68^+$ osteoclast staining and bone tissue replacement suggested bone inflammation and alluded to increased bone resorption.[31]

Osteitis is broadly accepted to be synonymous with bone erosion in SpA; however, latterly the term osteitis in SpA has been associated with MRI high signal on fat-suppressed sequences, termed bone marrow edema (BME). However, there are some constraints in using these terms interchangeably that are worth noting in interpreting studies. First, BME is not specific to axSpA and exists in many diverse clinical situations with potentially different pathophysiology.[33] Second, there is a lack of correlative histological data for BME both in axSpA spine lesions and for BME in other conditions; and thirdly, using MRI BME to define axSpA disease has limitations of sensitivity and specificity, which is partly a consequence of iterative analyses applying MRI diagnostically in filtered groups based on clinical likelihood of disease.[34]

*Osteitis, MRI BME and pain.* The contributors to pain and pain experience in SpA are not well understood. In general, peripheral triggering of pain is through stimulation of sensory Aδ and unmyelinated C nerve fibres (nociceptors), from tissue inflammation and damage.[35] In axSpA candidate tissues include entheses, perisotea and bone marrow, in sacroiliac joints and synovium (facet or sacroiliac joints). In support of a direct link between MRI BME and pain, symptomatic indices of pain appear to correlate well MRI BME in axSpA.[36,37] However, MRI BME in axSpA[38] and enthesitis, can occur without any symptoms.[39] Indeed, clinicians will likely recognise (both ways) a disconnection between imaging and symptoms, but whether there is poor sensitivity of MRI BME lesion detection, 'unimageable' pain-generating lesions, or contributory effects of central abnormal pain processing, or all three factors, remains to be shown.

*Osteitis, MRI BME, bone turnover and bone loss or gain.* In bone, where there is coupled bone turover,[40] abnormally increased or decreased bone turnover does not necessarily lead to bone loss (erosion/osteolysis) or gain (osteosclerosis), though it can under conditions where the dominant pathophysiology drives oscteoclastic resorption (e.g. pre-osteoclast migration to bone) or osteoblastic bone formation (e.g. switching pluripotential MSC cells to osteoblast lineage) respectively. In axSpA, osteitis/MRI BME may indicate increased bone turnover, though direct evidence is weak underscored by few bone biopsy or serial site-specific bone mass data. Inflammation (see later) and ischaemia (noted as a cause of osteitis in other conditions)[33] are plausible candidate triggers of increased bone turnover. In typical AS bone lesions, classical analysis of radiographs and *post mortem* material[3] broadly suggests a sequence of osteitis/erosion (caused by inflammation) followed by osteosclerosis. However, to what degree osteosclerosis arises from (or follows cessation of) inflammation-driven bone resorption, or is triggered independently, is not clear (Figure 2).

Finally, when using blood-derived bone biomarkers as surrogate measures of bone turnover, or indeed bone phenotype, caution is needed. Biomarkers will be poorly specific given the metabolic implications of potentially synchronously occurring osteitis, osteolytic, osteosclerotic and osteoproliferative bone lesions of unknown interdependence in any given patient with SpA.[40] Biomarkers potentially affected will include bone alkaline phosphatase, procollagen peptides (e.g. P1NP), 1,25 dihydroxy vitamin D3 (and its effect on parathyroid hormone; PTH), Dickkopf (DKK)-1, sclerostin (SOST), and fibroblast growth factor (FGF)23. Assuming that a single over-arching bone phenotype for an individual patient from any given biomarker profile will be difficult.

### Osteoproliferation

Osteoproliferation at entheses is a defined consequence of progressive axSpA (i.e. AS), but is not an ubiquitous finding in axSpA. It would seem likely that bone formation at entheses in axSpA is not a pain-triggering process, similarly thought to be the case in DISH enthesopathy.[1] Enthesopathy pain may relate more to inflammation and neuropeptide elaboration in surrounding 'enthesis organ' tissues.[36,41] Notably, in XLH as far as we know, and in mice lacking equilibrative nucleoside

transporter 1 (ENT-1; a murine phenotype resembling human DISH) inflammation at entheses neither precedes nor associates with osteoproliferation,[42,43] but with all three conditions there is undoubtedly a lot to learn in regard to how pain is generated from enthesis pathology. Of significant consequence clinically is how osteoproliferation in entheses, which are soft tissues designed to respond and adapt to mechanical stress, will affect the mechanical properties of entheses and their attached tendons and ligaments. Progressive entheseal osteoproliferation is well recognised to be a profound indicator of long-term disability in SpA,[44] partly due to morbidity arising from the biomechanics of skeletal stiffness[45] and of course fracture.[10] Accordingly, prevention of osteoproliferation at entheses in SpA is an extremely worthwhile goal of disease treatment.

## Mechanisms of bone pathophysiology in SpA

### *Bone turnover determines net gain or loss of bone: general considerations*

In SpA, competing inflammatory and mechanical effects on regular bone physiology contribute to alterations causing site-specific net gains or loss of bone. Bone loss, within the vertebrae is perhaps most easily explained. The inappropriate new bone formation seen in axSpA though remains a puzzle. Is it an exacerbated repair process, an adaptation to altered mechanical load, a response to inflammatory cells and the factors they produce, or an alteration in Wnt signalling (for example), or some or all of the above? There are of course a number of candidate effects to consider. For example, experimentally, gp-130 receptor family members such as oncostatin M; transforming growth factor (TGF)-β family members; bone morphogenetic proteins (BMPs); Ephs/Ephrins; and PTH (1–34) have all been shown to enhance bone formation.[46,47] Also, Wnt family members play an integral role in the formation and activity of osteoblasts as evidenced by mutations in low-density lipoprotein receptor-related protein (LRP)5, resulting in either high or low bone mass depending on the mutation.[48,49] LRP5/6 was subsequently shown to be the receptor for SOST, an inhibitor of Wnt signalling produced by osteocytes that limits osteoblast formation to tether the bone formation process.[50,51]

### *Genetic determinants of bone pathophysiology*

In SpA, genetic factors play an important role in defining disease susceptibility and have been examined extensively in genome-wide association studies (GWAS). A genetic component of AS is seen in ~90% of patients who have specific variants of the major histocompatibility complex gene HLA-B27. In terms of heritability though, only ~20% is explained by HLA-B27 with 113 identified AS-associated single-nucleotide polymorphisms (SNPs) accounting for an additional ~7.4% heritability.[52] Despite being identified as a risk factor several decades ago, the precise role of HLA-B27 remains unclear.[53] GWAS revealed common genes, including IL-23R, IL-12B, STAT3, and CARD9, to be associated with AS, psoriasis, and inflammatory bowel disease (IBD), but not rheumatoid arthritis.[54,55] Endoplasmic reticulum aminopeptidase (ERAP)-1 and ERAP-2 trim endogenous peptides for HLA-mediated presentation to the immune system. SNPs in these genes are strongly associated with AS.[56] ERAP-1 deficient mice exhibit spinal ankylosis, osteoporosis, spinal inflammation by micro CT and

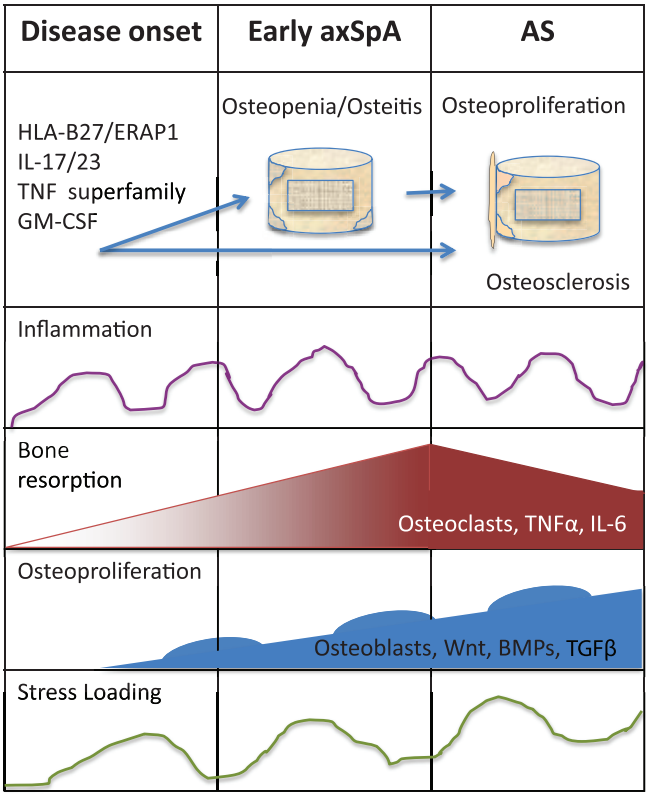


**Figure 2.** Effects of inflammation and stress loading on bone and enthesis tissue in spondyloarthritis.
Direct effects of inflammation lead to bone loss (osteopaenia/osteoporosis and bone erosion) due to increased osteoclast activity. Inflammation further influences bone sclerosis and osteoproliferation both directly and indirectly. The stress-loading component influences entheseal pathophysiology, which can amplify the effects of inflammation in enthesial and ligament tissue to cause osteoproliferation.

spontaneous intestinal dysbiosis.[57] However, these genetic determinants are primarily linked to inflammation, as opposed to a direct effect on bone formation per se.

New bone formation is a delicate balance between activating Wnt signalling and inhibitors such as secreted frizzled related protein (sFRP)1, DKK-1 and SOST, as well as contributions from factors such as BMPs.[58] However, in a mouse model of AS, SOST was unable to prevent peripheral or axial disease development, or affect bone density or disease severity.[59] In humans, there are no reported genetic links from GWAS studies to suggest a role for the Wnt family members in SpA. So, although SOST levels have been suggested as a biomarker in SpA, there is considerable controversy in this area and changes in SOST and DKK1 appear to be consequential, rather than causative, of bone changes.

BMPs are growth and differentiation factors that are part of the TGF-β superfamily. At the periosteal surface BMPs are able to upregulate the expression of Id genes in surrounding muscles leading to endochondral bone formation spreading from the bone surface into the medullary canal. They also stimulate the differentiation of periosteum progenitor cells into osteoblasts.[60] We, and others, have previously demonstrated the importance of muscle precursor cells,[61,62] and the periosteum in fracture repair.[63,64] Polymorphisms in BMP6 have been linked to the severity of radiological progression in AS. Two SNPs in BMP6 (rs270378 and rs1235192) have been associated with increased risk of syndesmophyte formation with a stronger effect in patients with both SNPs suggesting that they confer the risk for syndesmophytes independently.[65]

### *Alterations in mechanical load*

Osteocytes are the main mechanosensitive cells in bone. The ability of osteocytes to sense and respond to mechanical stimuli depends on many factors, such as the shape of the osteocyte cell bodies, number and length of the cell processes, structure of the cytoskeleton, and presence of primary cilia.[66] Osteocytes reduce their release of sclerostin in response to mechanical stimuli acting on bone, and thus promote the activation of osteogenic pathway Wnt/β-catenin in osteoblasts.[67,68]

The most prominent osteoproliferative feature in axSpA is syndesmophyte formation.[69] In a recent study, it was shown that syndesmophytes were non-randomly distributed around the vertebral rim. Posterolateral regions of the rim were more commonly affected by the tallest syndesmophytes and had most bridging, followed by the anterolateral regions. The anterior and posterior rims were least affected by syndesmophytes.[70] As the posterior half of vertebrae along with the pedicles and facet joints bear a large amount of mechanical stress, then the localisation of syndesmophytes fits the persuasive explanation of how local tissue mechanical stress influences new bone formation in SpA.[71,72] Such site-specific mechanical stress probably also directs the site of inflammation (at least as defined above by osteitis/BME on MRI) in the spine, as stress can direct osteitis elsewhere, both in SpA and generally in people prone to skeletal trauma.[73] Indeed, in SpA, prospective analyses suggest syndesmophytes can form at sites of previous adjacent osteitis though it is difficult to know precisely whether MRI studies are telling us there is a necessary progression of osteitis/BME to intraosseous fat metaplasia/degeneration and then to adjacent syndesmophyte formation in all lesions and in all patients.[74,75]

Earlier studies showed that AS patients had reduced SOST expression linked to radiological progression[76] however the weight of literature over the following decade makes a definitive conclusion elusive. Osteoblasts are known to react to mechanical forces resulting in increased bone formation. Furthermore studies have shown that cells derived from the facet joints of AS patients, as opposed to cells from spinal injury due to trauma, have an increased osteogenic capacity.[77] Thus, the combination of reduced Wnt inhibition, altered mechanical strain, and an increased propensity to form osteoblasts could all contribute to syndesmophytes formation in axSpA.

### *Osteoimmunology of SpA*

The complex relationship between bone and the immune system is never more apparent than when studying the underlying causes of bone changes in SpA/AS and arthritis associated with IBD. In SpA/AS, in understanding how both systemic bone loss and localised osteitis occurs with significant abnormal bone formation (syndesmophytes and enthesophytes) in areas associated with prior inflammation,[78] some important questions arise. For example, are the mechanisms that direct bone erosion at entheses and around

peripheral joints, and those that direct bone formation leading to syndesmophytes in the spine, occurring due to the same type of inflammation? Does the duration and magnitude of inflammation affect the final outcome? And what are the cytokines and growth factors driving these changes?

*Interleukin-17/23.* The interleukin (IL)-17–IL-23 axis is central to the pathogenesis with the anti-IL-17 monoclonal antibody, secukinumab, proving to be a highly efficacious therapeutic option.[79,80] In patients with AS there is a skewing of the helper T ($T_H$) cell profile towards $T_H17$ cells in the peripheral blood compared with healthy controls. IL-17 released by $T_H17$ and other cells is highly proinflammatory. In the context of bone, IL-17 was thought to primarily induce osteoclastogenesis;[81] however, recent reports show that IL-17 has direct bone-promoting effects on osteoblasts and their mesenchymal precursors.[82,83] Inhibition of IL-17A reduced inflammation and bone formation in the HLA-B27 rat model of AS *in vivo* providing further proof of the importance of this cytokine in the bony manifestations.[84]

Type 3 immunity is characterized by the production of IL-17A, IL-17F, IL-22, and IL-26 by neutrophils, mast cells, group 3 innate lymphoid cells [ILC3; RAR-related orphan receptor γt (ROR-γt)$^+$], γδ T cells, invariant natural killer (NK) T cells, and $T_H17$ and $T_H22$ cells.[80,85,86] Gut-derived IL-17$^+$, IL-22$^+$ ILC3 are expanded in the peripheral blood, synovial fluid and bone marrow of AS patients, suggesting the presence of an active homing axis between the gut and inflamed sacroiliac joints.[87] Using overexpression of IL-23, Sherlock *et al.* showed that IL-23 was essential in enthesitis by inducing entheseal resident T cells [IL-23R$^+$, ROR-γt$^+$, CD3$^+$, CD4$^-$, CD8$^-$, stem cell antigen 1 (Sca1$^+$)] to produce IL-22.[88] The IL-22 expression then activates signal transducer and activator of transcription 3 (STAT3), a known mediator of osteoblastic bone formation,[46] resulting in aberrant bone changes at the enthesis. *In vitro* investigation of the effects of IL-17, IL-22 and IL-23 suggested that IL-17 inhibits osteoblast differentiation by blocking BMP2 signalling;[89] however, this work was primarily done in cell lines. Other investigators have described a role for γδ T cells that produce IL-17A, in bone formation and fracture repair due to the cytokines ability to stimulate proliferation and differentiation of mesenchymal progenitor cells.[90]

More recently, the dependence of bone changes on IL-23 has come into question. Whilst IL-23 clearly induces IL-17 production and many of the murine models show a strong IL-23-depenence,[91] clinical trials evidence shows that IL-23 blockade is less effective that IL-17A inhibition on disease progression in the spine suggesting potential differences between the role of IL-23 in spinal *versus* peripheral skeleton enthesitis.[92] The weight of evidence is strongly in favour of the IL-17–IL-23 axis as a central component affecting bone in SpA but as evidence evolves it may become clear that effects on bone loss or formation may vary at different bone sites.

*Tumour necrosis factor superfamily.* Following its success in rheumatoid arthritis,[93] tumour necrosis factor (TNF)-α blockade was one of the first biological therapies tested in axSpA. There is extensive experimental and clinical evidence linking TNF-α to osteoclast development however a direct role on osteoblast formation has remained somewhat controversial;[61,94,95] on balance most studies report that TNF-α inhibits osteoblast differentiation. Thus, initial clinical observations that anti-TNF-α was effective on inflammation but less so on radiological changes may be attributed, in part, to different effects on osteoclasts and osteoblasts.[96]

The TNF superfamily includes the osteoclast differentiation factor, receptor activator of NF-κB ligand (RANKL), and its decoy receptor, osteoprotegerin (OPG). RANKL was initially shown to be expressed by osteoblasts, but its expression was then also shown on T cells, NK cells, and fibroblasts to name but a few. Consequently, general inflammatory cell infiltration makes a significant contribution to osteoclast formation and bone turnover.[94,97] The RANKL:OPG ratio determines the extent of osteoclastogenesis and is subject to a myriad of external influences such as osteotropic agents, inflammation and ageing.[97–99] There are a number of clinical interventions to prevent osteoclastic bone loss, from oestrogens to bisphosphonates to denosumab (anti-RANKL monoclonal antibodies). However, it has been noted that few cells in vertebrae affected by AS express RANKL,[100] suggesting these cells may not augment osteoclast differentiation or function. Small numbers of patients with SpA have been noted with circulating OPG antibodies.[101] OPG has been shown to prevent osteoclast apoptosis by blocking another TNF superfamily member, TNF-related apoptosis-inducing ligand (TRAIL).[102] A recent study

showed elevated serum TRAIL receptor 1 concentrations in AS however this did not correlate with disease activity scores.[103]

*Interleukin-6.* IL-6 promotes both osteoclastogenesis (by inducing RANKL expression) and bone formation. Increased bone formation occurs *via* the release of 'osteotransmitters' from osteoclasts that act through the cortical osteocyte network to stimulate periosteal bone formation.[104,105] Furthermore, it has been reported that IL-6 shows an inverse correlation to the Wnt inhibitor, DKK1, in the synovial fluid of patients with SpA and that IL-6 can suppress TNF-induced expression of DKK1.[106] As such, the involvement of IL-6 in inflammation and in bone changes would intuitively make it an ideal target for the treatment of SpA. However, although biological therapies targeting IL-6 have proven efficacious in rheumatoid arthritis, the same cannot be said for SpA. Randomised placebo-controlled clinical trials using tocilizumab (anti-human IL-6R) or sarilumab (anti-human IL-6Rα) in patients with AS showed a reduction in C-reactive protein levels yet failed to demonstrate any difference in Ankylosing Spondylitis Response Criteria (ASAS20) at week 12 between the biological therapy and placebo control arms of the study leading to the early termination of these trials.[107,108]

*Other cytokines and growth factors modifying bone in SpA.* It appears that inflammation intensity-dependent expression of osteoinductive Wnt proteins may be a key link between inflammation and ectopic new bone formation in AS. Activation of both the canonical Wnt/β-catenin and noncanonical Wnt/PKCδ pathways is required for inflammation-induced new bone formation in murine models and in patient tissues.[109] Experimentally, constitutive low intensity TNF-α expression, as opposed to short bursts or high TNF-α levels, resulted in bone formation *via* persistent expression of osteoinductive Wnt proteins and subsequent bone formation through NF-κB and JNK/activator protein 1 (c-Jun) signalling pathways.[109]

Granulocyte–macrophage colony-stimulating factor (GM-CSF) may play an important role. Increased numbers of GM-CSF-producing $CD4^+$ and $CD8^+$ lymphocytes and increased numbers of IL-$17A^+$, GM-$CSF^+$ double-producing $CD4^+$, $CD8^+$, γδ T cells and NK cells[110] have been demonstrated in the blood and joints of patients with SpA. Experimentally, blocking GM-CSF in the SKG model of AS resulted in complete ablation of bone lesions, both erosions at peripheral joints and periosteal bone formation.[111]

There has also been close scrutiny of the role of BMPs. Meta-analysis of serum BMP-2, but not SOST, showed a positive correlation with the development of AS.[112] Serum BMP-7 levels and the BMP-7/DKK-1 ratio have been reported to correlate significantly with sacroiliitis severity, 'osteoproliferation-weighted' radiographic indices and disease duration in AS.[113] The authors also found a significant correlation between BMP-2, BMP-4 and BMP-6 and BASRI-total and disease duration. Thus, there are both genetic and correlative serum data for a role of BMPs in the formation of bone in AS. Furthermore, in support of a general osteoproliferative role of BMPs, enhanced BMP and Indian Hedgehog Homolog signalling in the development of enthesopathy has been described in XLH.[114]

### Osteomicrobiology of axSpA

AS has been linked to IBD (then termed regional enteritis) for 60 years.[115,116] In a landmark study over 30 years ago, inflammatory gut lesions were found in a majority of patients with AS regardless of gut symptomology.[117] The role of the ileum and loss of ileocecal integrity in predicting SpA phenotype in patients with Crohn's disease then brought greater focus on the need to study the role of gut microbiota (GM) and local gut wall T-cell dysregulation in AS and SpA aetiopathogenesis.[86,118,119]

Alterations in the human microbiome are associated with various disease states; but are there direct roles on bone loss and/or formation? Osteomicrobiology refers to the role of microbiota in bone health and how the microbiota regulate postnatal skeletal development, bone ageing, and pathologic bone loss.[120,121] In rodent models of AS, namely curdlan-treated SKG mice or HLA-B27 transgenic rats, treatment with broad spectrum antibiotics or rearing under germ-free conditions prevents inflammation and associated bone changes.[122,123] In patients with SpA, it is unclear whether enteral dysbiosis and gut immunopathobiology are direct contributors to bone changes but a growing body of literature shows that there are links between the gut and bone that may go beyond inflammation alone.[120,124] Addressing dysbiosis may be fruitful: the probiotic *Lactobacillus reuteri* reduces intestinal dysbiosis, prevents intestinal barrier dysfunction and

suppresses osteoclast differentiation;[125,126] and we await the results of how the SpA inflammasome and AS pathogenesis might be influenced by faecal microbiota transplantation, with interest [ClinicalTrials.gov identifier: NCT03726645].

## Therapeutic measures to address bone pathophysiology in SpA

Therapeutically addressing bone pathophysiology in SpA is a challenge. Therapies will need scrutiny for their success at reducing and not worsening: osteitis/BME, bone erosion, osteosclerosis, osteoproliferation and importantly, fracture risk (osteoporosis).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the initial treatment in SpA, and clearly work well in reducing symptoms; however, whether NSAIDs reduce osteitis/BME is unknown but is the focus of an ongoing study (https://w3.abdn.ac.uk/hsru/DyNAMISM). Whether, and somewhat implausibly, NSAIDs might reduce osteosclerosis, osteoproliferation or fracture risk, is unknown. Sulfasalazine, a conventional synthetic disease-modifying anti-rheumatic drug (csDMARD), has modest effects on reducing axial skeletal pain and stiffness in axSpA at clinically safe doses.[127] There are no other data showing efficacy of other csDMARDs (reviewed elsewhere[128]). Accordingly, csDMARDs have not been studied for their effect on vertebral osteitis/BME, vertebral or spinal fracture risk or osteoproliferation otherwise.

Directly inhibiting osteoclast function with intravenous bisphosphonate reduces osteitis/BME in SpA including AS,[129,130] and increases lumbar spine bone mass in the short term.[131] Pamidronate specifically may also reduce AS spinal pain.[132] However, the effect of bisphosphonates on progressive osteoproliferation, osteosclerosis and vertebral or spinal fracture risk, is unknown. The need to know how bisphosphonates (and by extension, denosumab, a RANK ligand inhibitor) might affect all axSpA-related bone lesions in the spine over the short and long term, has been emphasised by data suggesting that by reducing bone turnover, bisphosphonates might promote osteoproliferation;[133] a worry given that progressive ankylosis may be the most important change in the spine dictating spinal fracture risk.[134] An additional theoretical concern would be if bisphosphonates were to be given at the time of development of osteoproliferation. We know nothing of the structural integrity of ossified spinal entheses and syndesmophytes that have incorporated bisphosphonate into their structure. Would bisphosphonate incorporation lead to even less strength than might be present otherwise in the spinal structure overall?

Inhibiting TNF-α reduces vertebral osteitis/BME in axSpA including AS,[135] and is associated with increases in spinal bone mass in the short term.[133,136] There was concern that the increased bone mass may have been - at least partly - due to increased syndesmophyte formation, which, as outlined above, might be counterproductive if aiming to reduce fracture risk overall with anti-TNF-α treatment. Notably however, two recent comprehensive overviews of published studies (~20 studies each) suggest that inhibiting TNF-α probably slows 'progressive structural change' in AS.[137,138] The definition of 'progressive structural change' in most of the reviewed studies is dependent on scoring radiographical changes heavily weighted towards syndesmophyte development. However, as TNF-α inhibition does not abolish new bone formation and other structural changes in spinal bone,[137,138] it will be important to know where exactly and how bone is gained at a tissue level and how that affects fracture risk. For example, there may be in theory: regain of previously lost bone within existing bone (primarily the vertebral body), osteosclerosis within existing bone (e.g. in the vertebral body), or as was originally considered, facilitation of osteoproliferation (at one or more sites such as the longitudinal ligament entheses [syndesmophytes], at the anterior vertebral body border, or posterior elements of vertebral segments such as at facet joints and spinal processes). Osteoproliferation at each of these sites may have different effects on fracture risk ultimately once bone mass, skeletal strength and force dissipation, is considered.

By extension then, it is of additional importance to understand how our clinical measurement tools (anteroposterior or lateral DXA, DXA-TBS, QCT, composite radiographical structural progression analysis) might capture some but not all of the effects of inhibiting TNF-α on bone pathophysiology.[139] It may be that no one-single measure will be predictive of the fracture risk affected by inhibiting TNF-α.

Inhibiting IL-17A resolves osteitis/BME in AS[140] and appears to slow osteoproliferation in AS, as measured by *in vivo* composite radiographical damage indices heavily weighted for syndesmophyte

formation,[141,142] (an effect which may surpass the anti-osteoproliferative effect of inhibiting TNF-α).[143] Inhibiting IL-23, a potential trigger of entheseal resident $\gamma\delta^+$/IL-23R$^+$ T cells has modest anti-symptom activity in axSpA, a disease in which its use has therefore been limited; notably however ustekinumab (IL-12/IL-23 p40 inhibitory) reduces osteoproliferation in psoriatic arthritis.[144]

## Future directions

Despite extensive research and decades of clinical data elucidating the contributions from genetics, mechanical forces, inflammation, and the microbiome, in explaining fully the bone disease of SpA, there are still many unanswered questions.

### *Genetic Influences*

If and how genotype predicts relevant, and ultimately modifiable, therapy goals in SpA is of key interest. For example, can we predict bone phenotypes from relevant gene haplotype profiles? Direct effects of HLA-B27 on bone in SpA remain unclear. Recent work suggests that HLA-B27 antagonises the inhibitory effect of ALK2 on TGF-β/BMP signalling thus releasing the brakes holding the action of these bone-forming factors in check.[145] Insight may also come from interrogation of genetic influences on osteoproliferation elsewhere, for example from DISH and in XLH.[114] Future genotype–phenotype correlative studies will be useful.

### *Spinal and vertebral fractures*

Evidence on vertebral and spinal fracture incidence and their predictors might suggest that applying conventional anti-resorption bone therapies (e.g. bisphosphonates, anti-RANKL) without addressing the evolving osteoproliferation may not meaningfully reduce the risk of fracture. As such therapeutic strategies to reduce osteoproliferation need evaluation for effects on fracture incidence both with and without simultaneous anti-resorption therapy. Monitoring patient cohorts and treatment effects will need to be cognisant of age and SpA disease duration and thus able to capture the relative effects of non-SpA comorbidities and osteoporosis risks, and stratified for the burden of baseline SpA-related vertebral body bone loss and osteoproliferation. Key to this is defining how to make accurate, well-tolerated and precise serial measurements of site-specific spinal osteoproliferation and vertebral body bone loss, independently.

A key question pertinent to understanding the potential of oral and intravenous bisphosphonate either in early axSpA or (the 'osteoproliferation-established') AS is whether syndesmophytes incorporate bisphosphonate and if so, how that affects the structural properties of formed bone and spinal resilience to force. There is evidence from studies in male DBA1 mice prone to arthritis and entheseal bone formation that zoledronic acid does not affect ankylosis originating from entheses.[146] However, under normal bone homeostatic conditions, therapies that prevent osteoclast activity ultimately lead to a reduction in bone formation due to 'coupling'.[47] Further modelling of syndesmophyte and enthesophyte formation in rodents would be warranted to explore these questions.

### *Osteitis and bone loss*

Key to understanding the potential of SpA immunotherapies and bisphosphonates, and optimum timing of different therapeutics, will be knowledge of the presence and nature of inflammation within bone *versus* systemic triggers for general bone turnover that are derived systemically. We are still relatively ignorant of the prevalence and influence of high bone turnover in SpA.

Alterations in GM composition and host responses to the microbiota contribute to pathological bone loss for a variety of reasons including the disruption of metabolites, such as short chain fatty acids, that diffuse from the gut into the systemic circulation, altered inflammatory status, and hormonal changes.[147,148] We would anticipate that delineating the details of enteral dysbiosis and associated changes in bowel wall regulatory mechanisms are key to understanding the pathogenesis of bone changes in SpA.

### *Osteoproliferation*

Mechanistic studies in animals together with tissue biopsy analysis in treatment-naive and (various) biologic-treated patients should help our understanding of how tissue-resident T cells are relevant to osteoproliferation at entheses, and telling what links $T_H17$ cells, innate lymphoid cells and their activating cytokines with BMP activation and bone formation at entheses - and how this process might interplay with mechanical stress. We think there are many other key questions though, for example, including:

- Is the inhibition of osteoproliferation mediated by TNF-α inhibition contingent on other signals affecting bone formation at entheses?
- Are there measurable bone formation biomarkers that predict osteoproliferation?
- Does ischaemia or mast cell (another enthesis resident cell) activation play a role?
- Are the triggers for intra-bone osteosclerosis the same as those for osteoproliferation or is osteosclerosis just a consequence of an outcome of osteitis at the same site?
- How does HLA-B27 specifically influence or signpost osteoproliferation? Is it amplified bacterial antigen presentation as part of enteral dysbiosis or something more nuanced in causing immune cell activation and/or BMP effects in enthesis tissue?

## Conclusion

In SpA, loss of existing bone and osteoproliferation (specifically, new bone formation in entheseal tissues) are highly relevant to clinical symptomology, disability and long-term outcome, including spinal fracture. In addressing bone pathophysiology, we need robust clinical measures of both bone loss in vertebrae (including knowing predictors of osteitis and bone turnover) and of the drivers of osteoproliferation. Ultimately, we need therapies that reduce osteitis/erosion, bone turnover and osteoproliferation to fully enable improved long-term skeletal outcomes.

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