Published in final edited form as:

Clin Rheumatol. 2015 June ; 34(6): 1009–1018. doi:10.1007/s10067-015-2949-3.

Biomarkers for diagnosis, monitoring of progression, and treatment responses in ankylosing spondylitis and axial spondyloarthritis

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Abstract

With the growing awareness of the impact of chronic back pain and axial spondyloarthritis and recent breakthroughs in genetics and the development of novel treatments which may impact best on early disease, the need for markers that can facilitate early diagnosis and profiling those individuals at the highest risk for a bad outcome has never been greater. The genetic basis of ankylosing spondylitis has been considerably advanced, and HLA-B27 testing has a role in the diagnosis. Knowledge is still incomplete of the rest of the genetic contribution to disease susceptibility, and it is likely premature to use extensive genetic testing (other than HLA-B27) for diagnosis. Serum and plasma biomarkers have been examined extensively in assessing disease activity, treatment response, and as predictors of radiographic severity. For assessing disease activity, other than C-reactive protein and erythrocyte sedimentation rate, the most work has been in examining cytokines (particularly interleukin 17 and 23), matrix metalloproteinase (MMP) markers (particularly MMP3). For assessing those at the highest risk for radiographic progression, biomarkers of bony metabolism, cartilage and connective tissue degradation products, and adipokines have been most extensively assessed. The problem is that no individual biomarkers has been reproducibly shown to assess disease activity or predict outcome, and this area still remains an unmet need, of relevance to industry stakeholders, to regulatory bodies, to the healthcare system, to academic investigators, and finally to patients and providers.

Keywords

Biomarkers; Diagnostic tools; Genetics; Radiographic outcome; Spondyloarthritis; Treatment response

Introduction

Most recent data from NHANES 2009–2010 report a frequency of chronic back pain of nearly 20 % in those in the USA between the ages of 20 and 70 years [1]. Of these, up to one third have inflammatory back pain (IBP) [1, 2]. IBP is a hallmark of axial spondyloarthritis

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Disclosures None.

(AxSpA), which occurs in up to 25 % of those with IBP [3]. Other than by imaging, there is no direct test to diagnose AS. There is no serologic or blood marker specific for the disease.

Disease activity in AS has been measured by the Bath Ankylosing Spondylitis Disease Activity Index [4], which includes only patient-reported measures. Although used extensively since first described in 1994, it has noted limitations. It does not include the physician's assessment of the disease and does not assess the impact of specific clinical factors. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) contains inherent redundancy in the questions and is not specific for true inflammation (as opposed to pain from other sources). The Assessment of SpondyloArthritis International Society (ASAS) developed a new AS disease activity score (ASDAS) that combines patient-reported assessments with acute phase reactants (C-reactive protein [CRP] or erythrocyte sedimentation rate [ESR]) [5]. ASDAS discriminates patients with different levels of disease activity and is sensitive over time, thus potentially useful in clinical trials. However, it too is based largely on subjective measures, and what is lacking is a comprehensive, objective biomarker of disease activity.

Disease severity is characteristically measured by metrology, functional impairment (the Bath Ankylosing Spondylitis Functional Index (BASFI)) [6], and radiographic severity, most commonly measured by the modified Stoke Ankylosing Spondylitis Severity Score (mSASSS) [7]. The presence of sacroiliitis on plain X-rays is required for the classification of ankylosing spondylitis [8]. However, inflammation and pain precede radiographic sacroiliitis features on plain X-rays by several years [9]. In order to capture the disease earlier, the concept of AxSpA has been formulated, based on the evidence of active inflammation in the sacroiliac joints on magnetic resonance imaging (MRI) and/or a combination of other findings. Classification criteria have been put forth by the Assessment of SpondyloArthritis International Society (ASAS) to facilitate early diagnosis of AxSpA [10]. However, it would be especially useful if a biomarker could be found to identify those at the highest risk for progressing from AxSpA to AS or those at the highest risk for rapid radiographic progression to spinal fusion. AxSpA can progress to AS in a large proportion of patients and in some cause fusion and complete rigidity of the spine, which is irreversible. It may take up to 10 years before sacroiliitis can be clearly detected by conventional radiography [9]—critical delays before beginning novel and effective therapies. With observational data suggesting that early intervention in AS is most effective in slowing radiographic progression [11], early identification is important to prevent the incapacity caused by this disease. Moreover, novel therapies are expensive and fraught with side effects. The identification of those patients most likely to respond to these therapies would be a valuable addition to the current treatment armamentarium.

This review will focus on the role of markers, genetic, blood and synovial, in the management of AS and AxSpA, both in diagnosis, determining disease activity, and predicting those at risk for a worse outcome.

Genetic markers

HLA-B27

While HLA-B27 was not included in the modified New York classification criteria for AS [4], this HLA allele is an integral part of the new ASAS criteria for axial SpA [11]. The prevalence of HLA-B27 varies among different ethnicities. In a recent US national survey, its prevalence was 7.5 % among non-Hispanic whites while it was lower at 4.6 % in Mexican Americans and approximately 1.1 % among non-Hispanic blacks [12]. While 85–95 % of white AS patients have HLA-B27, only 7–8 % of HLA-B27 carriers in the general population develop AS. Thus, a positive test for HLA-B27 is not sufficient for the diagnosis of AS. Furthermore, the prevalence of HLA-B27 is substantially lower among African American AS patients at 50 % [5]. In terms of disease features, HLA-B27 is associated with younger age at disease onset, development of anterior uveitis, and positive family history of SpA among AS patients but is not associated with increased structural damage on radiographs.

HLA-B27 is particularly helpful in diagnosing non-radiographic AxSpA. In a longitudinal study with a mean follow-up time of 7.7 years, the combination of severe sacroiliitis on MRI with HLA-B27 positivity was an excellent predictor of future AS development [13]. The usefulness of HLA-B27 in determining who would benefit from rheumatologic evaluation in primary care setting was shown in a recent international study. Patients with chronic back pain (>3 months) and age of onset before 45 years who had either HLA-B27 positivity, inflammatory back pain, or sacroiliitis on MRI were referred to rheumatologists for further evaluation. Following this simple referral strategy, 35 % of patients were diagnosed with SpA [14]. Outside of HLA-B27, other MHC genes that have been implicated in disease susceptibility include other HLA-B alleles (B*40:01) and MICA.

Non-MHC genes

To date, over 41 genes have been identified in predisposition to AS. The overwhelming majority of these have genes are outside the MHC and include genes involved in Th17 mediated immunity (IL23R, TYK2, IL6R, IL7R?, IL7?, IL1R2/IL1R1, IL12B), peptide presentation (MHC class I proteins, ERAP1, UBE2E3, UBE2L3, NPEPPS), CD8 T cell function (RUNX3, EOMES, IL7R), microbial sensing (CARD9, NOS2) as well as genes involved in other or unclear immunologic function (ZMIZ1, FCGR2A, KIF21B, SH2B3, TNFRSF1A, GPR65, SULT1A1, GPR35, BACH2, ICOSLG, NKX2-3) [15]. These data beg the question: Can genetic testing be used to diagnose AS? Over 41 genes have been implicated in SpA risk; but other than HLA-B27 and other MHC genes (MICA, HLA-B40), the amount of risk imposed by individual non-MHC genes is very small [15] (Table 1). In fact, it is estimated that only about 25 % of the genetic contribution to overall disease susceptibility is currently known, the rest coming from such things, yet unknown, as rare genetic variants (that would not be detected from the standard GWAS approach), copy number variation of genes (including insertions and deletions), epigenetic factors, and the added variance provided by gene:gene interaction not currently recognized. This incomplete knowledge about the impact of genetic factors on disease susceptibility, common to most complex diseases, was one of the factors that led the US Food and Drug Administration to

send genetic testing company “23andMe” a warning letter in November 2013 ordering it to “immediately discontinue marketing the PGS [Saliva Collection Kit and Personal Genome Service]” [16]. In addition, genetic factors have been identified that are associated with complications of AS such as acute anterior uveitis [17] and radiographic severity [18] (Table 2).

Markers of disease activity

The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)

CRP and ESR are the two markers of disease activity most commonly utilized in clinical practice and in clinical trials. However, they have both low sensitivity and specificity [19] and do not comprehensively represent the disease process in AS [20]. An elevated CRP is included in the ASAS AxSpA classification criteria as well as in the ASDAS, a measure of disease activity [10]. However, an elevated CRP or ESR is present in only about 40–50 % of patients with AS [21]. Thus, a normal ESR or CRP does not rule out AS or comprehensively capture active disease. Levels of both acute phase reactants are higher in patients with AS than in non-radiographic axial SpA [22]. Elevated CRP is also associated with increased radiographic changes on spine X-rays [21] and signs of inflammation on sacroiliac MRI [23]. Furthermore, an elevation of CRP and ESR predict future radiographic progression in sacroiliac joints and spine in AS patients. A recent paper found hsCRP to correlate better than routine CRP with clinical disease activity parameters in patients with axial SpA [24].

Cytokines

Studies of cytokine levels have been highly variable. Interleukin 6 (IL-6) has been the one most extensively studied [25–28]. IL-6 is a classic proinflammatory cytokine produced by a variety of immune cells that induces production of a number of positive acute phase proteins such as serum amyloid A and C-reactive protein. IL-6 is involved in initiation and maintenance of inflammation by facilitating neutrophil trafficking to the inflammation site and regulates T lymphocytes activation and differentiation. Some studies have demonstrated associations of IL-6 levels with either disease activity or other inflammatory markers [25–27] whereas others have not [28, 29]. Interleukin 17 (IL-17) and IL-23 are key cytokines in the TH17 pathway. A number of studies have reported elevated IL-17 and IL-23 levels in the plasma and sera of AS patients [27, 29–35] and associated them with disease activity [31, 33], though the latter was not seen in all [30, 32]. Interleukin 33 (IL-33) is a member of the IL-1 family and has been recently implicated in several inflammatory and autoimmune diseases. IL-33 can be produced by various types of tissues and cells and induce gene expression of Th2-associated cytokines via binding to the orphan receptor ST2. Three independent centers in China [33–35] have reported elevated serum levels of IL-33 (and in one, also its receptor ST2 [33]) in patients with AS and correlated them with disease activity or other inflammatory markers.

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which are involved in the degradation of extracellular matrix proteins, including the cleavage of cell surface receptors, the release of apoptotic ligands and chemokine/cytokine inactivation. MMPs are

also thought to play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense. Several studies have examined serum levels of MMPs in AS, especially MMP3 [28, 36–44], MMP8 [37], MMP9 [38], TIMP-1 [36, 37] and –2 [36]. Higher MMP3 levels have been shown to reflect disease activity and treatment response, though this has not been universally seen [38, 43, 44]. MMP3 has also been shown to be an independent predictor of structural damage progression in patients with ankylosing spondylitis [45]. Another recent study examined 30 potential biomarkers on a multiplex, bead-based assay on a suspension array system and examined by both principal component and hierarchical clustering analysis and found MMP8 and MMP9 (and not MMP3) to be better associated with disease activity [38].

Osteoprotegerin (OPG)

Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily. Activated T cells can directly trigger osteoclastogenesis through OPG ligand (RANKL). OPG levels have been described as lower in AS patients in some studies [46, 47] and higher in others [29, 48–51] and have been correlated with the BASDAI but not with treatment response [48, 51].

Human cartilage glycoprotein-39 (HC gp-39, otherwise known as YKL-40)

YKL-40 is one of the major secreted proteins of human articular chondrocytes and has been associated with disease activity in AS. However, longitudinal studies have not shown it to perform as well as other biomarkers (i.e., CRP, MMP3) [28].

Cartilage oligomeric matrix protein (COMP)

COMP is a pentameric extracellular matrix protein that catalyzes the assembly of collagens and promotes formation of well-defined fibrils. Elevated levels of COMP have been reported in a variety of inflammatory joint diseases, although association with other clinical markers and other biomarkers in AS is inconsistent [28].

Aggrecan

Aggrecan is a major component of cartilage extra-cellular matrix. The extent of glycosaminoglycan side chain substitution in aggrecan and resulting fixed charge density attracts counter-ions and water, resulting in swelling pressure that is crucial for the biomechanical properties of cartilage. Patients with AxSpA have compared with healthy subjects depressed levels of total aggrecan [28]. After anti-TNF treatment, the levels changed towards normal in clinical responders.

Calprotectin

Calprotectin is a heterodimeric protein complex composed of S100A8 and S100A9 subunits and is a major calcium- and zinc-binding protein in the cytosol of neutrophils, monocytes, and keratinocytes. It has become established as a marker of whole gut inflammation [52] and is one of the most upregulated proteins specifically expressed in lesional psoriatic skin [53]. The concept of calprotectin as a biomarker in SpA is not new. Over 20 years ago, Hammer et al. found peripheral SpA it to be a plasma marker of inflammation and treatment response

in reactive arthritis [54]. Subsequently, it was found to be elevated in the feces of 41 % of patients with AS [51]. Serum levels of calprotectin are also highly significantly increased in AxSpA and that treatment with anti-TNF agent significantly decrease calprotectin levels [44]. In another study, baseline calprotectin serum levels were significantly higher in patients with mSASSS worsening versus those without [55].

Markers of radiographic severity and progression (Table 3)

Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor is a heparin-binding growth factor specific for vascular endothelial cells that is able to induce angiogenesis in vivo. VEGF levels have been shown to be elevated in patients with AS and AxSpA [28, 33, 56–58], including in those with active disease [56] and to be higher in those patients with greater radiographic progression (increasing mSASSS scores) [56] compared to those with no worsening.

Circulating protein fragments of cartilage and connective tissue degradation

One study showed that the variation in radiological damage and progression correlated and was explained by type II collagen (CTX-II) levels but not with Type I (CTX-I) [58, 59]. CTX-II also correlated with serological markers of inflammation. Another found that levels of degraded citrullinated vimentin fragments (VICM) were significantly higher in AS patients than in healthy controls and that AS patients with the highest levels of VICM and a high baseline mSASSS had the highest risk of radiographic progression with progression occurring in 67 % of these patients [60]. In yet another examination looking at MRI scans in AxSpA patients [61], higher pretreatment MRI inflammation scores for SI joints and/or lumbar spine were associated with higher baseline CTX-II levels, but not with higher levels of other biomarkers of inflammation and bone turnover. Two neo-epitope biomarkers, C2M, which measures a matrix metalloproteinase (MMP)-generated neo-epitope of type II collagen and C3M, a marker of soft tissue turnover associated with inflammation which directly measure tissue remodeling, have been shown to be elevated in AS patients [62], and in one report, a combination of C2M and C3M was dichotomized according to the best cut-offs for individual markers to predict 80 % of the radiographic progressors and 61 % of the non-progressors.

Sclerostin

Sclerostin and noggin (NOG) are bone morphogenic protein (BMP) antagonists that modulate mitogenic activity through sequestering BMPs. Studies of sclerostin levels in patients with AS have been inconsistent. One report [63] showed that sclerostin expression by immunohistochemical analysis of osteocytes from German patients with AS was markedly reduced (compared to osteocytes of healthy individuals and RA patients) and that serum levels of sclerostin were significantly lower in patients with AS than in healthy individuals. Of particular note, low serum sclerostin levels in patients with AS in this study were significantly associated with the formation of new syndesmophytes. Similar findings were seen in two other cohorts of Swedish [50] and Brazilian AS patients [64]. Yet, other studies showed no differences or even demonstrated *elevated* levels of sclerostin comparing between AS patients and controls [46, 65, 66].

Wnt proteins

Wnt proteins are extracellular signaling molecules involved in the control of embryonic development. They may also contribute to neoplastic processes. There are at least two families of secreted inhibitors of Wnt signaling: the secreted frizzled-related protein family, all of which have an N-terminal cysteine-rich domain and are transmembrane receptors, and the Dickkopf (German for “big head” or “stubborn”) family, which includes Dkk1. In general, Dkk1 levels have been described as being lower in most [50, 67–69] but not all studies [46, 51]. Furthermore, AS patients with no syndesmophyte formation have been shown to have significantly higher functional Dkk1 levels, suggesting that blunted Wnt signaling suppresses new bone formation and consequently syndesmophyte growth and spinal ankylosis [66, 67], although they were not affected by 3-month anti-TNF- α therapy [68]. In yet another study [50], patients with AS had significantly higher serum levels of Wnt-3a compared with the controls.

Osteocalcin

Bone gamma-carboxyglutamic acid (Gla) protein (BGLAP, or BGP, otherwise known as osteocalcin) is a small, highly conserved molecule associated with the mineralized matrix of bone. Initial studies showed osteocalcin levels to be lower [70], but in more recent reports higher in AS patients than in controls, especially in those who would develop new syndesmophytes [61, 69, 71, 72] and increased bone mineral density [71]. Osteocalcin level increased after the 3-month anti-TNF- α therapy.

Bone-specific alkaline phosphatase (BAP)

Bone-specific alkaline phosphatase (bone ALP) is a marker of active bone formation. Higher levels of bone-specific alkaline phosphatase have been reported, albeit inconsistently, in patients with AS and AxSpA, although no clear-cut changes with treatment have been observed [51, 59, 63, 66].

Fetuin-A

The fetuin family is part of the cystatin superfamily and encompasses a series of tightly related proteins that have been implicated in several diverse functions, including osteogenesis and bone resorption, regulation of the insulin and hepatocyte growth factor receptors, and response to systemic inflammation. AS patients with syndesmophytes have been shown to have significantly higher levels of fetuin-A compared with patients without syndesmophytes and controls [65]. However, fetuin-A levels were not different between patients without syndesmophytes and controls, and on regression analysis, fetuin-A was an independent, significant predictor of syndesmophyte formation.

Adipokines

Adipokines are cytokines (cell signaling proteins) secreted by adipose tissue that mediate energy homeostasis, immune responses, and regulate bone mass. In relevance to AS, they include adiponectin, resistin, leptin, visfatin, and apelin. The result is an increase in cortical bone growth through either inhibition of osteoclasts or stimulation of osteoblasts. Studies of leptin levels in AS have been inconsistent, with some showing association with disease

activity and others not [73]. Likewise, no consistent association has been identified with treatment response. Serum levels of leptin have been associated with syndesmophyte formation in male AS patients [74]. Serum resistin and visfatin levels were recently found to be elevated in AS patients and elevated visfatin levels at baseline were in a recent study associated with subsequent progression of radiographic damage in AS patients [75].

To whom are these questions of potential interest?

These questions are of no little importance. To patients and providers, this offers the possibility to improve diagnosis, treatments, and predictions of prognosis of AS. To the healthcare system, this can inform judicious use of resource-intensive preventive care in the highest risk patients and minimize cost. To academic investigators, valuable resource for biomarker research into pathogenesis of AS and related SpA diseases can emerge. To regulatory bodies, such as the FDA, this can result in the development of biomarkers for prognostic, predictive, pharmacodynamic, and safety markers in trials of AS. And finally, for industry stakeholders, especially those who are manufacturers of AS or SpA-related therapeutics, this can lead to smaller and shorter trials, better patient selection and stratification. In other words, for all, this can reduce costs.

Acknowledgments

We would like to express our appreciation to Dr. Lianne Gensler for her suggestions and help in preparing this manuscript.

References

1. Weisman MH, Witter JP, Reveille JD. The prevalence of inflammatory back pain: population-based estimates from the US National Health and Nutrition Examination Survey, 2009–10. *Ann Rheum Dis.* 2013; 72(3):369–373. [PubMed: 22791746]
2. Rudwaleit M, Metter A, Listing J, Sieper J, Braun J. Inflammatory back pain in ankylosing spondylitis: a reassessment of the clinical history for application as classification and diagnostic criteria. *Arthritis Rheum.* 2006; 54(2):569–578. [PubMed: 16447233]
3. Reveille JD, Witter JP, Weisman MH. Prevalence of axial spondyloarthritis in the United States: estimates from a cross-sectional survey. *Arthritis Care Res (Hoboken).* 2012; 64(6):905–910. [PubMed: 22275150]
4. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol.* 1994; 21(12):2286–2291. [PubMed: 7699630]
5. Van der Heijde D, Lie E, Kvien TK, Sieper J, Van den Bosch F, Listing J, Braun J, Landewé R. ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. *Ann Rheum Dis.* 2009; 68(12):1811–1818. [PubMed: 19060001]
6. Calin A, Garrett S, Whitelock H, Kennedy LG, O’Hea J, Mallorie P, Jenkinson T. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol.* 1994; 21(12):2281–2285. [PubMed: 7699629]
7. van der Heijde D, Landewé R. Selection of a method for scoring radiographs for ankylosing spondylitis clinical trials, by the Assessment in Ankylosing Spondylitis Working Group and OMERACT. *J Rheumatol.* 2005; 32(10):2048–2049. [PubMed: 16206368]
8. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum.* 1984; 27(4):361–368. [PubMed: 6231933]

9. Rudwaleit M, Khan MA, Sieper J. The challenge of diagnosis and classification in early ankylosing spondylitis: do we need new criteria? *Arthritis Rheum.* 2005; 52(4):1000–1008. [PubMed: 15818678]
10. Rudwaleit M, Landewe R, van der Heijde D, Listing J, Brandt J, Braun J, et al. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann Rheum Dis.* 2009; 68(6):770–776. [PubMed: 19297345]
11. Haroon N, Inman RD, Learch TJ, Weisman MH, Lee M, Rahbar MH, et al. The impact of TNF-inhibitors on radiographic progression in Ankylosing Spondylitis. *Arthritis Rheum.* 2013; 65(10): 2645–2654. [PubMed: 23818109]
12. Reveille JD, Hirsch R, Dillon CF, Carroll MD, Weisman MH. The prevalence of HLA-B27 in the US: data from the US National Health and Nutrition Examination Survey, 2009. *Arthritis Rheum.* 2012; 64(5):1407–1411. [PubMed: 22139851]
13. Bennett AN, McGonagle D, O'Connor P, Hensor EM, Sivera F, Coates LC, et al. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-B27 status in early inflammatory back pain predict radiographically evident ankylosing spondylitis at eight years. *Arthritis Rheum.* 2008; 58(11):3413–3418. [PubMed: 18975311]
14. Sieper J, Srinivasan S, Zamani O, Mielants H, Choquette D, Pavelka K, et al. Comparison of two referral strategies for diagnosis of axial spondyloarthritis: the Recognising and Diagnosing Ankylosing Spondylitis Reliably (RADAR) study. *Ann Rheum Dis.* 2013; 72(10):1621–1627. [PubMed: 23065731]
15. Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet.* 2013; 45(7):730–738. [PubMed: 23749187]
16. Annas GJ, Elias S. 23andMe and the FDA. *N Engl J Med.* 2014; 370:985–988. [PubMed: 24520936]
17. Robinson PC, Claushuis TA, Cortes A, Martin TM, Evans DM, Leo P, et al. Genetic dissection of acute anterior uveitis reveals similarities and differences in associations observed with ankylosing spondylitis. *Arthritis Rheumatol.* 2015; 67(1):140–151. [PubMed: 25200001]
18. Cortes A, Maksymowych WP, Wordsworth BP, Inman RD, Danoy P, Rahman P, et al. Association study of genes related to bone formation and resorption and the extent of radiographic change in ankylosing spondylitis. *Ann Rheum Dis.* 2014 Epub ahead of print. 10.1136/annrheumdis-2013-204835
19. Spoorenberg A, van der Heijde D, de Klerk E, Dougados M, de Vlam K, Mielants H, van der Tempel H, van der Linden S. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. *J Rheumatol.* 1999; 26(4):980–984. [PubMed: 10229432]
20. Ruof J, Stucki G. Validity aspects of erythrocyte sedimentation rate and C-reactive protein in ankylosing spondylitis: a literature review. *J Rheumatol.* 1999; 26(4):966–970. [PubMed: 10229429]
21. Rudwaleit M, Haibel H, Baraliakos X, Listing J, Marker-Hermann E, Zeidler H, et al. The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheum.* 2009; 60(3):717–727. [PubMed: 19248087]
22. Wallis D, Haroon N, Ayearst R, Carty A, Inman RD. Ankylosing spondylitis and nonradiographic axial spondyloarthritis: part of a common spectrum or distinct diseases? *J Rheumatol.* 2013; 40(12):2038–2041. [PubMed: 24187102]
23. Bredella MA, Steinbach LS, Morgan S, Ward M, Davis JC. MRI of the sacroiliac joints in patients with moderate to severe ankylosing spondylitis. *AJR Am J Roentgenol.* 2006; 187(6):1420–1426. [PubMed: 17114530]
24. Poddubnyy DA, Rudwaleit M, Listing J, Braun J, Sieper J. Comparison of a high sensitivity and standard C reactive protein measurement in patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis. *Ann Rheum Dis.* 2010; 69(7):1338–1341. [PubMed: 20498207]

25. Gratacós J, Collado A, Filella X, Sanmartí R, Cañete J, Llana J, Molina R, Ballesta A, Muñoz-Gómez J. Serum cytokines (IL-6, TNF-alpha, IL-1 beta and IFN-gamma) in ankylosing spondylitis: a close correlation between serum IL-6 and disease activity and severity. *Br J Rheumatol.* 1994; 33(10):927–931. [PubMed: 7921752]
26. Bal A, Unlu E, Bahar G, Aydog E, Eksioglu E, Yorgancioglu R. Comparison of serum IL-1 beta, sIL-2R, IL-6, and TNF-alpha levels with disease activity parameters in ankylosing spondylitis. *Clin Rheumatol.* 2007; 26(2):211–215. [PubMed: 16583185]
27. Romero-Sanchez C, Jaimes DA, Londoño J, De Avila J, Castellanos JE, Bello JM, Bautista W, Valle-Oñate R. Association between Th-17 cytokine profile and clinical features in patients with spondyloarthritis. *Clin Exp Rheumatol.* 2011; 29(5):828–834. [PubMed: 22041179]
28. Pedersen SJ, Sørensen IJ, Garnero P, Johansen JS, Madsen OR, Tvede N, et al. ASDAS, BASDAI and different treatment responses and their relation to biomarkers of inflammation, cartilage and bone turnover in patients with axial spondyloarthritis treated with TNF α inhibitors. *Ann Rheum Dis.* 2011; 70(8):1375–1381. [PubMed: 21551511]
29. Sveaas SH, Berg IJ, Provan SA, Semb AG, Olsen IC, Ueland T, et al. Circulating levels of inflammatory cytokines and cytokine receptors in patients with ankylosing spondylitis: a cross-sectional comparative study. *Scand J Rheumatol.* 2015; 44(2):118–124. [PubMed: 25756521]
30. Mei Y, Pan F, Gao J, Ge R, Duan Z, Zeng Z, et al. Increased serum IL-17 and IL-23 in the patient with ankylosing spondylitis. *Clin Rheumatol.* 2011; 30(2):269–273. [PubMed: 21161669]
31. Chen WS, Chang YS, Lin KC, Lai CC, Wang SH, Hsiao KH, et al. Association of serum interleukin-17 and interleukin-23 levels with disease activity in Chinese patients with ankylosing spondylitis. *J Chin Med Assoc.* 2012; 75(7):303–308. [PubMed: 22824043]
32. Andersen T, Rasmussen TK, Hvid M, Holm CK, Madsen KJ, Jurik AG, et al. Increased plasma levels of IL-21 and IL-23 in spondyloarthritis are not associated with clinical and MRI findings. *Rheumatol Int.* 2012; 32(2):387–393. [PubMed: 21116820]
33. Li XL, Lin TT, Qi CY, Yuan L, Xia LP, Shen H, Lu J. Elevated serum level of IL-33 and sST2 in patients with ankylosing spondylitis: associated with disease activity and vascular endothelial growth factor. *J Investig Med.* 2013; 61(5):848–851.
34. Li GX, Wang S, Duan ZH, Zeng Z, Pan FM. Serum levels of IL-33 and its receptor ST2 are elevated in patients with ankylosing spondylitis. *Scand J Rheumatol.* 2013; 42(3):226–231. [PubMed: 23409750]
35. Han GW, Zeng LW, Liang CX, Cheng BL, Yu BS, Li HM, Zeng FF, Liu SY. Serum levels of IL-33 is increased in patients with ankylosing spondylitis. *Clin Rheumatol.* 2011; 30(12):1583–1588. [PubMed: 21968692]
36. Keyszer G, Lambiri I, Nagel R, Keysser C, Keysser M, GromnicaIhle E, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. *J Rheumatol.* 1999; 26(2):251–258. [PubMed: 9972954]
37. Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen HA, et al. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. *Rheumatology (Oxford).* 2006; 45(4):414–420. [PubMed: 16287916]
38. Matthey DL, Packham JC, Nixon NB, Coates L, Creamer P, Hailwood S, Taylor GJ, Bhalla AK. Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis. *Arthritis Res Ther.* 2012; 14(3):R127. [PubMed: 22640827]
39. Yang C, Gu J, Rihl M, Baeten D, Huang F, Zhao M, et al. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. *Arthritis Rheum.* 2004; 51(5):691–699. [PubMed: 15478146]
40. Wendling D, Cedoz JP, Racadot E. Serum levels of MMP-3 and cathepsin K in patients with ankylosing spondylitis: effect of TNF alpha antagonist therapy. *Joint Bone Spine.* 2008; 75(5):559–562. [PubMed: 18674944]
41. Sun S, Bay-Jensen AC, Karsdal MA, Siebuhr AS, Zheng Q, Maksymowych WP, Christiansen TG, Henriksen K. The active form of MMP-3 is a marker of synovial inflammation and cartilage

- turnover in inflammatory joint diseases. *BMC Musculoskelet Disord.* 2014; 19(15): 93.10.1186/1471-2474-15-93 [PubMed: 24641725]
42. Woo JH, Lee HJ, Sung IH, Kim TH. Changes of clinical response and bone biochemical markers in patients with ankylosing spondylitis taking etanercept. *J Rheumatol.* 2007; 34(8):1753–1759. [PubMed: 17610317]
 43. Arends S, van der Veer E, Groen H, Houtman PM, Jansen TL, Leijnsma MK, et al. Serum MMP-3 level as a biomarker for monitoring and predicting response to etanercept treatment in ankylosing spondylitis. *J Rheumatol.* 2011; 38(8):1644–1650. [PubMed: 21632677]
 44. Turina MC, Yeremenko N, Paramarta JE, De Rycke L, Baeten D. Calprotectin (S100A8/9) as serum biomarker for clinical response in proof-of-concept trials in axial and peripheral spondyloarthritis. *Arthritis Res Ther.* 2014; 16(4):413.10.1186/s13075-014-0413-4 [PubMed: 25135077]
 45. Maksymowych WP, Landewé R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, Poole AR, Wang N, van der Heijde D. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum.* 2007; 56(6):1846–1853. [PubMed: 17530713]
 46. Taylan A, Sari I, Akinci B, Bilge S, Kozaci D, Akar S, et al. Biomarkers and cytokines of bone turnover: extensive evaluation in a cohort of patients with ankylosing spondylitis. *BMC Musculoskelet Disord.* 2012; 13:191.10.1186/1471-2474-13-191 [PubMed: 23025387]
 47. Franck H, Meurer T, Hofbauer LC. Evaluation of bone mineral density, hormones, biochemical markers of bone metabolism, and osteoprotegerin serum levels in patients with ankylosing spondylitis. *J Rheumatol.* 2004; 31(11):2236–2241. [PubMed: 15517638]
 48. Genre F, López-Mejías R, Miranda-Fillooy JA, Ubilla B, Carnero-López B, Palmou-Fontana N, et al. Osteoprotegerin correlates with disease activity and endothelial activation in non-diabetic ankylosing spondylitis patients undergoing TNF- α antagonist therapy. *Clin Exp Rheumatol.* 2014; 32(5):640–646. [PubMed: 25190453]
 49. Chen CH, Chen HA, Liao HT, Liu CH, Tsai CY, Chou CT. Soluble receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoprotegerin in ankylosing spondylitis: OPG is associated with poor physical mobility and reflects systemic inflammation. *Clin Rheumatol.* 2010; 29(10): 1155–1161. [PubMed: 20690034]
 50. Klingberg E, Nurkkala M, Carlsten H, Forsblad-d'Elia H. Biomarkers of bone metabolism in ankylosing spondylitis in relation to osteoproliferation and osteoporosis. *J Rheumatol.* 2014; 41(7):1349–1356. [PubMed: 24931960]
 51. de Andrade KR, de Castro GR, Vicente G, da Rosa JS, Nader M, Pereira IA, Fröde TS. Evaluation of circulating levels of inflammatory and bone formation markers in axial spondyloarthritis. *Int Immunopharmacol.* 2014; 21(2):481–486. [PubMed: 24925756]
 52. Matzkies FG, Targan SR, Berel D, Landers CJ, Reveille JD, McGovern DP, Weisman MH. Markers of intestinal inflammation in patients with ankylosing spondylitis: a pilot study. *Arthritis Res Ther.* 2012; 14(6):R261.10.1186/ar4106 [PubMed: 23194008]
 53. Schonhaler HB, Guinea-Viniegra J, Wculek SK, Ruppen I, Ximenez-Embun P, Guio-Carrion A, Navarro R, Hogg N, Ashman K, Wagner EF. S100A8-S100A9 protein complex mediates psoriasis by regulating the expression of complement factor C3. *Immunity.* 2013; 39:1171–1181. [PubMed: 24332034]
 54. Hammer HB, Kvien TK, Glennäs A, Melby K. A longitudinal study of calprotectin as an inflammatory marker in patients with reactive arthritis. *Clin Exp Rheumatol.* 1995; 13(1):59–64. [PubMed: 7774104]
 55. Turina MC, Sieper J, Yeremenko N, Conrad K, Haibel H, Rudwaleit M, Baeten D, Poddubnyy D. Calprotectin serum level is an independent marker for radiographic spinal progression in axial spondyloarthritis. *Ann Rheum Dis.* 2014; 73(9):1746–1748. [PubMed: 24845387]
 56. Drouart M, Saas P, Billot M, Cedoz JP, Tiberghien P, Wendling D, Toussiroit E. High serum vascular endothelial growth factor correlates with disease activity of spondylarthropathies. *Clin Exp Immunol.* 2003; 132(1):158–162. [PubMed: 12653851]
 57. Poddubnyy D, Conrad K, Haibel H, Syrbe U, Appel H, Braun J, Rudwaleit M, Sieper J. Elevated serum level of the vascular endothelial growth factor predicts radiographic spinal progression in

patients with axial spondyloarthritis. *Ann Rheum Dis.* 2014; 73(12):2137–2143. [PubMed: 23956246]

58. Vosse D, Landewé R, Garnero P, van der Heijde D, van der Linden S, Geusens P. Association of markers of bone- and cartilage-degradation with radiological changes at baseline and after 2 years follow-up in patients with ankylosing spondylitis. *Rheumatology (Oxford).* 2008; 47(8):1219–1222. [PubMed: 18539620]
59. Arends S, Spoorenberg A, Efde M, Bos R, Leijnsma MK, Bootsma H, et al. Higher bone turnover is related to spinal radiographic damage and low bone mineral density in ankylosing spondylitis patients with active disease: a cross-sectional analysis. *PLoS ONE.* 2014; 9(6):e99685.10.1371/journal.pone.0099685 [PubMed: 24918782]
60. Bay-Jensen AC, Karsdal MA, Vassiliadis E, Wichuk S, Marcher-Mikkelsen K, Lories R, Christiansen C, Maksymowych WP. Circulating citrullinated vimentin fragments reflect disease burden in ankylosing spondylitis and have prognostic capacity for radiographic progression. *Arthritis Rheum.* 2013; 65(4):972–980. [PubMed: 23280360]
61. Pedersen SJ, Sørensen IJ, Lambert RG, Hermann KG, Garnero P, Johansen JS, et al. Radiographic progression is associated with resolution of systemic inflammation in patients with axial spondylarthritis treated with tumor necrosis factor α inhibitors: a study of radiographic progression, inflammation on magnetic resonance imaging, and circulating biomarkers of inflammation, angiogenesis, and cartilage and bone turnover. *Arthritis Rheum.* 2011; 63(12):3789–3800. [PubMed: 22127697]
62. Bay-Jensen AC, Wichuk S, Byrjalsen I, Leeming DJ, Morency N, Christiansen C, Karsdal MA, Maksymowych WP. Circulating protein fragments of cartilage and connective tissue degradation are diagnostic and prognostic markers of rheumatoid arthritis and ankylosing spondylitis. *PLoS ONE.* 2013; 8(1):e54504.10.1371/journal.pone.0054504 [PubMed: 23365672]
63. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* 2009; 60(11):3257–3262. [PubMed: 19877044]
64. Saad CG, Ribeiro AC, Moraes JC, Takayama L, Goncalves CR, Rodrigues MB, et al. Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? *Arthritis Res Ther.* 2012; 14(5):R216.10.1186/ar4055 [PubMed: 23062122]
65. Tuylu T, Sari I, Solmaz D, Kozaci DL, Akar S, Gunay N, Onen F, Akkoc N. Fetuin-A is related to syndesmophytes in patients with ankylosing spondylitis: a case control study. *Clinics (Sao Paulo).* 2014; 69(10):688–693. [PubMed: 25518021]
66. Korkosz M, G sowski J, Leszczy ski P, Pawlak-Bu K, Jeka S, Kucharska E, Grodzicki T. High disease activity in ankylosing spondylitis is associated with increased serum sclerostin level and decreased wingless protein-3a signaling but is not linked with greater structural damage. *BMC Musculoskelet Disord.* 2013; 14:99.10.1186/1471-2474-14-99 [PubMed: 23509994]
67. Heiland GR, Appel H, Poddubnyy D, Zwerina J, Hueber A, Haibel H, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. *Ann Rheum Dis.* 2012; 71(4):572–574. [PubMed: 22186710]
68. Yucong Z, Lu L, Shengfa L, Yongliang Y, Ruguo S, Yikai L. Serum functional dickkopf-1 levels are inversely correlated with radiographic severity of ankylosing spondylitis. *Clin Lab.* 2014; 60(9):1527–1531. [PubMed: 25291949]
69. Kwon SR, Lim MJ, Suh CH, Park SG, Hong YS, Yoon BY, et al. Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. *Rheumatol Int.* 2012; 32(8):2523–2527. [PubMed: 21833531]
70. Franck H, Keck E. Serum osteocalcin and vitamin D metabolites in patients with ankylosing spondylitis. *Ann Rheum Dis.* 1993; 52(5):343–346. [PubMed: 8323382]
71. Yilmaz N, Ozaslan J. Biochemical bone turnover markers in patients with ankylosing spondylitis. *Clin Rheumatol.* 2000; 19(2):92–98. [PubMed: 10791618]
72. Visvanathan S, van der Heijde D, Deodhar A, Wagner C, Baker DG, Han J, et al. Effects of infliximab on markers of inflammation and bone turnover and associations with bone mineral density in patients with ankylosing spondylitis. *Ann Rheum Dis.* 2009; 68:175–182. [PubMed: 18495735]

73. Genre F, López-Mejías R, Miranda-Fillooy JA, Ubilla B, Carnero-López B, Blanco R, Pina T, González-Juanatey C, Llorca J, González-Gay MA. Adipokines, biomarkers of endothelial activation, and metabolic syndrome in patients with ankylosing spondylitis. *Biomed Res Int*. 2014; 2014:860651.10.1155/2014/860651 [PubMed: 24757680]
74. Kim KJ, Kim JY, Park SJ, Yoon H, Yoon CH, Kim WU, Cho CS. Serum leptin levels are associated with the presence of syndesmophytes in male patients with ankylosing spondylitis. *Clin Rheumatol*. 2012; 31(8):1231–1238. [PubMed: 22644087]
75. Syrbe U, Callhoff J, Conrad K, Poddubnyy D, Haibel H, Junker S, et al. Serum adipokine levels in patients with ankylosing spondylitis and their relationship to clinical parameters and radiographic spinal progression. *Arthritis Rheumatol*. 2015; 67(3):678–685. [PubMed: 25417763]

Table 1

The relative impacts of genes associated with AS identified by genome-wide association studies on disease susceptibility*

Locus	Gene	SNP	Odds ratio	Heritability explained (%)
1p36	<i>RUNX3</i>	rs6600247	1.164	0.138
1p31	<i>IL23R</i>	rs11209026	1.650	0.352
1p31	<i>IL23R</i>	rs12141575	1.147	0.100
1q21	<i>IL6R</i>	rs4129267	1.176	0.151
1q23	<i>FCGR2A</i>	rs1801274	1.123	0.080
1q23	<i>FCGR2A</i>	rs2039415	1.088	0.037
1q32	<i>GPR25-KIF21B</i>	rs41299637	1.201	0.162
1q32	<i>HHAT</i>	rs12758027	1.094	0.048
2p15	Intergenic	rs6759298	1.308	0.407
2q11	<i>IL1R2-IL1R1</i>	rs4851529	1.103	0.055
2q12	<i>IL1R2-IL1R1</i>	rs2192752	1.112	0.047
2q31	<i>UBE2E3</i>	rs12615545	1.109	0.062
2q37	<i>GPR35</i>	rs4676410	1.131	0.060
3p24	<i>EOMES</i>	rs13093489	1.119	0.067
5p13	<i>PTGER4</i>	rs12186979	1.093	0.047
5p13	<i>IL7R</i>	rs11742270	1.113	0.054
5q15	<i>ERAP1</i>	rs30187	1.318	0.411
5q15	<i>ERAP1</i>	rs1065407	1.171	0.136
5q15	<i>ERAP2</i>	rs2910686	1.171	0.147
5q33	<i>IL12B</i>	rs6871626	1.117	0.066
5q33	<i>IL12B</i>	rs6556416	1.107	0.054
6p21	<i>HLA-B*27/MICA</i>	rs116488202	60	20.089
6p21	<i>HLA-A*02</i>	rs2394250	1.214	0.220
6q15	<i>BACH2</i>	rs17765610	1.172	0.064
6q15	<i>BACH2</i>	rs639575	1.081	0.034
7q31	<i>GPR37</i>	rs2402752	1.108	0.052
9q34	<i>CARD9</i>	rs1128905	1.124	0.082
10q22	<i>ZMIZ1</i>	rs1250550	1.110	0.059
10q24	<i>NKX2-3</i>	rs11190133	1.181	0.136
12p13	<i>LTBR-TNFRSF1A</i>	rs1860545	1.131	0.086
12p13	<i>LTBR-TNFRSF1A</i>	rs7954567	1.113	0.062
12q24	<i>SH2B3</i>	rs11065898	1.129	0.060
14q31	<i>GPR65</i>	rs11624293	1.234	0.086
16p11	<i>IL27-SULT1A1</i>	imm_16_28525386	1.112	0.064
16p11	<i>IL27-SULT1A1</i>	rs35448675	1.236	0.007
17q11	<i>NOS2</i>	rs2531875	1.122	0.074
17q11	<i>NOS2</i>	rs2297518	1.129	0.055
17q21	<i>NPEPPS-TBKBPI-TBX21</i>	rs9901869	1.146	0.111

Locus	Gene	SNP	Odds ratio	Heritability explained (%)
19p13	<i>TYK2</i>	rs35164067	1.155	0.080
19p13	<i>TYK2</i>	rs6511701	1.098	0.036
21q22	Intergenic	rs2836883	1.190	0.140
21q22	<i>ICOSLG</i>	rs7282490	1.100	0.052
22q11	<i>UBE2L3</i>	rs2283790	1.124	0.052

* Modified from reference [15]

Table 2**Markers of disease activity and treatment response**

Marker	Comment	Ref.
C-reactive protein (CRP)	Perhaps, it is the most widely used biomarker in clinical trials and practice. An elevated CRP is present in about 40–50 % of patients with AS.	[19–23]
Erythrocyte sedimentation rate (ESR)	It is also widely used in clinical trials and practice. An elevated ESR is present in only about 40–50 % of patients with AS.	[19–23]
Interleukin 6 (IL-6)	IL-6 levels elevated in the serum of SpA patients compared to controls and correlated with CRP and ESR levels in some but not all studies.	[25–29]
Interleukin 17 (IL-17)	Serum IL-17 levels elevated in SpA patients and, in one study, associated with enthesitis and peripheral joint involvement.	[27–34]
Interleukin 23 (IL-23)	Several studies reported significantly higher serum IL-23 levels in SpA patients compared with healthy subjects, with highest levels in one in synovial fluid—not seen in all studies.	[27–34]
Interleukin 33 (IL-33)	It was elevated in Chinese AS patients and correlated with clinical and serologic markers of disease activity.	[33–35]
Matrix metalloproteinase 3 (MMP3)	MMP3 levels were significantly higher in AS patients than in healthy controls and were associated with higher disease activity in most (but not all) studies, and they correlated significantly with greater structural damage.	[28, 36–45]
Matrix metalloproteinase 8 (MMP8)	It was associated with disease activity (BASDAI) and functional impairment (BASFI) in one study, but this needs further confirmation.	[38]
Matrix metalloproteinase 9 (MMP9)	It was associated with disease activity in one study from the UK, not replicated in a second from Taiwan.	[37, 38]
Chemokine, CXC motif, ligand 8 (CXCL8)	This correlated with disease activity in one study, but this needs further confirmation.	[38]
Osteoprotegerin (OPG)	OPG levels higher in some and lower in other in AS patients, although patients with active disease had significantly higher concentration of OPG compared with the inactive group. Other study showed that OPG correlated with markers of disease activity such as BASDAI and VAS spinal pain. Anti-TNF treatment did not lead to a reduction in OPG levels in three studies.	[29, 47–51]
Bone alkaline phosphatase (BAP)	In higher levels reported in patients with AS and SpA compared to controls, there were no clear-cut and consistent changes with biologic treatment.	[51, 59, 63, 66]
Aggrecan	Patients with AxSpA have compared with healthy subjects' depressed levels total aggrecan. After anti-TNF treatment, levels changed towards normal in clinical responders.	[28]
Nitric oxide metabolites (NOx)	Higher levels seen in AxSpA patients versus controls, with levels lower in those on anti-TNF agents versus NSAIDs alone.	[51]
Cartilage oligomeric matrix protein (COMP)	Elevated levels of COMP have been reported in a variety of inflammatory joint diseases, although association with other clinical markers and other biomarkers is inconsistent.	[28]
YKL-40	AS patients had elevated YKL-40 levels compared with controls, and after treatment with anti-TNF agents, levels changed towards normal levels in clinical responders, with persistent reductions over 3 years.	[28]
Calprotectin	Serum levels of calprotectin significantly increased in AxSpA, especially in those with worsening radiographic severity. Treatment with anti-TNF agents decreased calprotectin levels.	[44, 52–55]

Table 3**Markers of radiographic severity and progression**

Marker	Comment	Ref.
C-reactive protein, ESR	Elevated CRP associated with radiographic severity on spine X-rays and elevation of CRP and ESR predict future radiographic progression in the sacroiliac joints and spine in AS patients.	[61]
Vascular endothelial growth factor (VEGF)	Mean baseline VEGF values were significantly higher in patients with mSASSS worsening by 2 units after 2 years than in those without progression and in patients with syndesmophyte formation as compared with those without new bone formation.	[56–58]
Matrix metalloproteinase 3 MMP3	Serum MMP is an independent predictor of structural damage progression in patients with ankylosing spondylitis.	[45]
C-terminal cross-linking telopeptide of type I (CTX-I) and type II (CTX-II) collagen	Baseline radiological damage correlated with CTX-II but not with CTX-I levels. There was a negative correlation between CTX-I and BMD of the trochanter. In multivariate analyses, CTX-II significantly and independently contributed to explaining variation in radiological damage and progression.	[59–61]
Circulating protein fragments of cartilage and connective tissue degradation (C2M and C3M)	Circulating protein fragments of cartilage and connective tissue degradation (C2M and C3M) were significantly higher in serum samples from AS patient than from healthy controls. A combination of C2M and C3M, dichotomized according to best cut-offs for individual markers, could correctly identify 80 % of the progressors and 61 % of the non-progressors. It needs further confirmation.	[62]
Degraded citrullinated vimentin fragments (VICM)	VICM levels were higher in AS patients than in healthy controls—those with highest levels of VICM levels had the highest mSASSS score. VICM levels associated with radiographic progression after 2 years.	[60]
Sclerostin	Serum levels of sclerostin were significantly lower in patients with AS than in healthy individuals. Of particular note, low serum sclerostin level in patients with AS was significantly associated with the formation of new syndesmophytes. It was not seen in other studies	[46, 50, 63–66]
Dickkopf-1 (Dkk-1)	AS patients with no syndesmophyte formation show significantly higher functional Dkk1 levels suggesting that blunted Wnt signaling suppresses new bone formation and consequently syndesmophyte growth and spinal ankylosis.	[46, 50, 51, 66–69]
Osteoprotegerin (OPG)	OPG levels were higher in AS patients than in the controls, decreasing significantly after the 3-month anti-TNF- α therapy.	[50, 69]
Osteocalcin	Osteocalcin levels were higher in AS patients than in controls. Osteocalcin level increased after the 3-month anti-TNF- α therapy.	[61, 70–72]
Fetuin-A	Patients with syndesmophytes had significantly higher levels of fetuin-A compared with patients without syndesmophytes and controls. However, fetuin-A was not different between the patients without syndesmophytes and controls.	[65]
Adipokines	Serum levels of adipokines were inconsistently associated with disease activity. One study showed an association of leptins with syndesmophyte formation and another elevated levels of visfatin with radiographic progression in AS.	[73–75]