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THESIS TITLE

SPINE AND SACROILIAC JOINTS ON NUCLEAR MAGNETIC RESONANCE IMAGING AND RESEARCH OF SEROLOGICAL BIOMARKERS PREDICTIVE OF SEVERITY AND DISEASE ACTIVITY IN EARLY AXIAL SPONDYLOARTHRITIS

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ABSTRACT

Background: Recently several studies have focused on the use of magnetic resonance imaging (MRI) and biomarkers that might facilitate early diagnosis of axial spondyloarthritis (axSpA) and identify individuals at higher risk of developing the disease. New biomarkers of inflammation, neo-apposition and bone remodeling might be helpful for early axSpA diagnosis and could be used as prognostic factors, measures of outcome and indicators of effectiveness of treatment.

Objectives: The study aimed to identify: 1) potentially useful biomarkers for early diagnosis of axSpA and their correlations with disease activity and imaging indices; 2) the prevalence of spine and pelvis MRI lesions in patients (pts) with low back pain (LBP); 3) the correlation between the site of axial pain and of MRI-lesions.

Material and Methods: Seventy-two pts with LBP (≥3 months, ≤2 years, onset ≤45 years) underwent a physical examination, questionnaires, laboratory tests, X-rays and MRI of the spine and sacroiliac joints (SIJ) at baseline and during a followup period of 24 months. Two expert rheumatologists formulated axSpA diagnosis and assessed fulfilment of Assessment of SpondyloArthritis International Society ASAS criteria. Disease activity and physical functioning were assessed using imaging, clinical and serological indices: Bath Ankylosing Spondylitis Metrology Index (BASMI);Maastricht Ankylosing enthesitis Spondilities Score (MASES);Bath Ankylosing Spondylitis Disease Activity Index (BASDAI);Bath Ankylosing Spondylitis Functional Index (BASFI);Ankylosing Spondylitis disease activity score (ASDAS);Visual Analogue Scale (VAS pain);VAS night pain;VAS disease activity;Bath Ankylosing Spondylitis Patient Global Score (BASG1);BASG2;Health Assessment Questionnaire (HAQ);ESR;serum ultrasensitive CRP (hs-CRP);matrix metalloproteinase (MMP3);interleukin (IL) IL-22, IL-17, IL-23. Spine and SIJ MRI and X-rays were scored independently by 2 readers following the SPARCC, mSASSS and NY-criteria. The axial pain and localization of MRI-lesions were classified into 4 sites: cervical/thoracic/lumbar spine and buttocks. The association between the site of pain and MRI-lesions was

evaluated through Odds Ratio (OR). The Spearman test and Kruskal Wallis test were used to compare all indices in these cohorts.

Results: Patients were diagnosed with axSpA and classified in accordance ASAS criteria as: 1) 21 pts classified according to axSpA imaging arm+;2) 29 pts classified according to axSpA clinical arm+;3) 25 pts not fulfilling ASAS criteria. The median LBP onset was 28.51±8.05 years, 45.3% were male, HLA-B27 was positive in 38.7% pts. 37.3%, 56%, 64% pts, respectively, complained about cervical/thoracic/buttock pain; 56% pts showed bone marrow edema (BME) in spine-MRI (19%, 39%, 33% in cervical/thoracic/lumbar region) and 61.3% pts in SIJ-MRI (respectively 58% on the right SIJ and 50% on the left SIJ). Signs of enthesitis were found in 47 (62.7%) pts (respectively 8%, 58%, 11% in the cervical/thoracic/lumbar spine). Eigtheen (24%) pts presented a negative BME SIJ-MRI with a positive BME spine-MRI. OR between site of pain and site of BME lesions was 20.78 (p=NS), 163.93 (p=0.0006); 0.34 (p=NS) for cervical/thoracic/lumbar spine and 304.88 (p=0.0203) for buttocks. A significant correlation between thoracic pain/ enthesitis on thoracic district (OR=32.69;p= 0.0336) was also found. There was a significant difference between the three cohorts with regard to the prevalence of radiographic sacroiliitis, active sacroiliitis on MRI and the SPARCC SIJ score. There were no differences in these groups regarding IL-17, IL-22, IL-23, MMP-3 and hsCRP. There was found a correlation between IL-22 and some clinical indices (BASFI, BASG1,HAQ,VAS pain). The correlation between mSASSS, MMP3 and hsCRP was an interesting finding.

Conclusions: The site of pain correlated in a statistically significant manner with BME lesions in thoracic and buttock districts. Since positive spine-MRI images were observed in absence of sacroiliitis, we can be therefore hyphothezise that this finding could have a diagnostic significance in suspected axSpA. Although not significantly higher in any of the three groups, IL-22, MMP3 and hsCRP values correlated with some disease activity indices and with mSASSS. Larger observational studies are warranted to confirm these preliminary findings.

INTRODUCTION

1.1. SPONDYLOARTHRITIS

1.1.1 GENERAL ASPECTS

Spondyloarthropathies (SpA) are a heterogeneous group of chronic, inflammatory, rheumatic disorders characterized by overlapping clinical signs and symptoms and a common genetic background [**1-3**]. The prevalence is about 2% in the general population. The concept of SpA, introduced in 1976 by Wright and Moll [**4-5**], was born with the intention of bringing together in the same group different eterogeneous diseases, united by some similar clinical and radiological aspects. The SpA have in common the frequent association with the "Human Leucocyte Antigen" HLA-B27, the absence of the rheumatoid factor, the peripheral inflammatory arthritis, usually asymmetric and more frequent in the lower limbs, the sacroiliitis, the enthesitis (inflammation of the particular anatomic area of insertion of tendons, ligaments and capsules on the bone), in addition to a frequent family aggregation [**6-8**]. Apart from common articular and spinal symptoms, there is also an overlap of skin, eye, and gut manifestations, such as some cutaneous lesions (psoriasis, pustulosis, keratodermia blenorragica, erythema nodosum, pyoderma gangrenose), mucositis (oral, intestinal, genital ulcers), uveitis and inflammatory bowel disease. SpA patients present with pain, morning stiffness and disability. Depending on the predominant pattern of clinical symptoms, SpA can be divided into SpA with a prevalent axial involvement (axSpA) or prevalently peripheral involvement (pSpA). In the first decade of the new millennium significant progress has been achieved, especially in the treatment of axSpA, thanks to the discovery and diffusion of the use of biotechnological anti-tumor necrosis factor α (TNFα) drugs in clinical practice. The need to assess the degree of disease activity in these forms is now becoming increasingly important and of common interest among rheumatologists, not only in terms of initial diagnosis, but also to monitorize response to therapy. Traditional radiography, able to detect bone structural alterations such as sacroiliitis, typical manifestation of radiological

axSpA or ankylosing spondylitis (AS), -according to the modified criteria of New York of 1984 [**9**]-, is a method alone no longer sufficient for an appropriate classification of the patient with suspicion of axSpA. In patients with early-onset axSpA with no evidence of radiographic damage, nuclear magnetic resonance imaging (MRI) may show acute inflammatory lesions before bone structural change is established. This category of patients may present a very active disease with axial pain symptoms, similarly to patients with AS. In addition, these patients respond quickly and effectively to the therapy with anti-TNFα drugs, therefore the identification of these forms in the early stage of the disease becomes a priority in order to establish the most appropriate treatment, right from the very beginning.

1.1.2 CLASSIFICATION OF SPONDILOARTHRITIS

SpA are divided into: ankylosing spondylitis (AS), psoriatic arthritis (PsA), arthritis associated with inflammatory intestinal diseases (Crohn's disease and ulcerative colitis), reactive arthritis (ARe) and undifferentiated forms (uSpA) [**10-12**] (**Table I**).

Table I. Classification spondylo-entheso-artritis of the adult

Spondylo-entheso-artritis of the adult:
- Ankylosing spondylitis (AS)
- Psoriatic arthritis (PsA)
- Arthritis associated with chronic inflammatory enteritis (enteropathic arthritis)
    - Arthritis of ulcerative colitis
    - Arthritis of Crohn's disease
- Reactive arthritis
    - Enteroarthritis
    - Uroarthritis
    - Other forms
- Undifferentiated Spondylo-entheso-artritis (uSpA)

In some cases, however, patients do not fulfill all the necessary criteria to be included in one of these forms, and are therefore classified as affected by uSpA. The classification according to Amor 1990 criteria (**Table II**) allows to have a more complete list of the main characteristics of these diseases and the subsequent

classification of the European Spondyloarthropathy Study Group (ESSG) (**Table III**) allowed a better definition of SpA [**13-20**]. Because of the insidious onset of the complaints, a considerable proportion of patients is not recognized using these classification criteria at an early stage of disease. With the development of new treatment strategies such as the possibly disease modifying therapy with TNF-α blocking drugs, the need to identify a patient in an earlier stage of disease increases [**21-26**]. Because of the disease burden, costs involved in searching for a diagnosis and the potential therapeutic consequences, it is important to identify these patients early in the disease course. Chronic low back pain is the main musculoskeletal complaint of these forms, however only a small proportion of these patients have low back pain as part of the spectrum of SpA (**Table IV**).

Table II. Amor's classification criteria for spondyloarthritis, 1990*

A.	**Clinical symptoms or past history of**	**score**
1.	Lumbar or dorsal pain at night or morning stiffness of lumbar or dorsal pain	1
2.	Asymmetrical oligoarthritis	2
3.	Buttock pain If alternate buttock pain	1 2
4.	Sausage-like toe or digit	2
5.	Heel pain or other well-defined enthesopathy	2
6.	Iritis	1
7.	Non-gonococcal urethritis or cervicitis within one month before the onset of arthritis	1
8.	Acute diarrhea within one month before the onset of arthritis	1
9.	Psoriasis, balanitis, or inflammatory bowel disease (ulcerative colitis or Crohn's disease)	2
B.	**Radiological findings**	
10.	Sacroiliitis (bilateral grade 2 or unilateral grade 3)	3
C.	**Genetic background**	
11.	Presence of HLA-B27 and/or family history of ankylosing spondylitis, reactive arthritis, uveitis, psoriasis or inflammatory bowel disease	2
D.	**Response to treatment**	
12.	Clear-cut improvement within 48 hours after NSAIDS intake or rapid relapse of the pain after their discontinuation	2

A patient is considered as suffering from spondyloarthritis if the sum is ≥ 6
**Critères de classification des spondyloarthropathies. Rev Rheum 57:85-9 (1990)*

Table III: ESSG criteria for SpA*

Inflammatory spinal pain

OR

Synovitis (asymmetrical, predominantly in the lower extremities)

AND

one of the following:

- Family history: first- or second-degree relative with AS, spondylitis, psoriasis, acute uveitis, reactive arthritis or IBD);
- Past or present psoriasis diagnosed by a physician;
- Past or present ulcerative colitis (or Crohn's disease) diagnosed by a physician and confirmed by radiography and/or endoscopy;
- Past or present pain alternating between the buttocks;
- Past or present spontaneous pain or tenderness at examination of the site of insertion of the Achilles tendon or plantar fascia (enthesitis);
- Episode of diarrhea occurring ≤1 month before onset of symptoms;
- Non-gonococcal urethritis or cervicitis ≤1 month before onset of symptoms;
- Bilateral grade 2-4 sacroiliitis or unilateral grade 3 or 4 sacroiliitis

* *European Spondyloarthropathy Study Group (1991)*

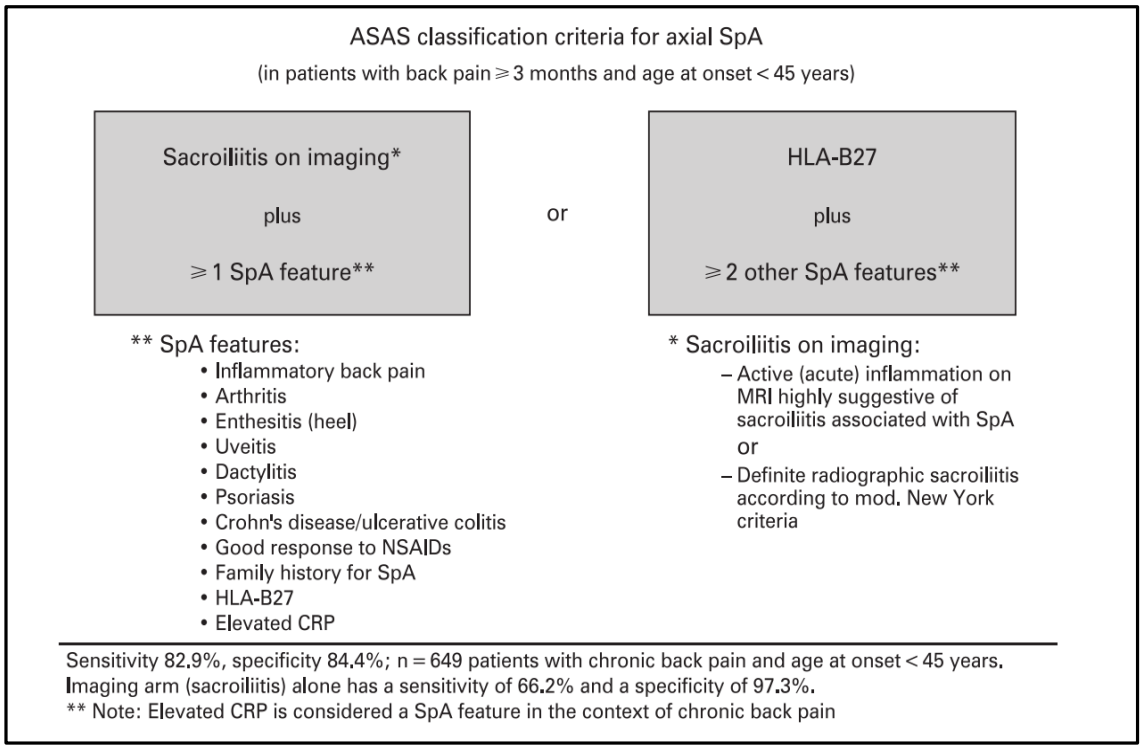
Table IV. Inflammatory low back pain:

Factors which differentiate the back pain produced by spondylitis from the back pain due to other causes:

1. Onset of back pain before the age of 40 years
2. Insidious onset
3. Persistence of at least 3 months
4. Associate with morning stiffness
5. Improvement with exercise
6. Good response to anti-inflammatory drugs
7. Night pain

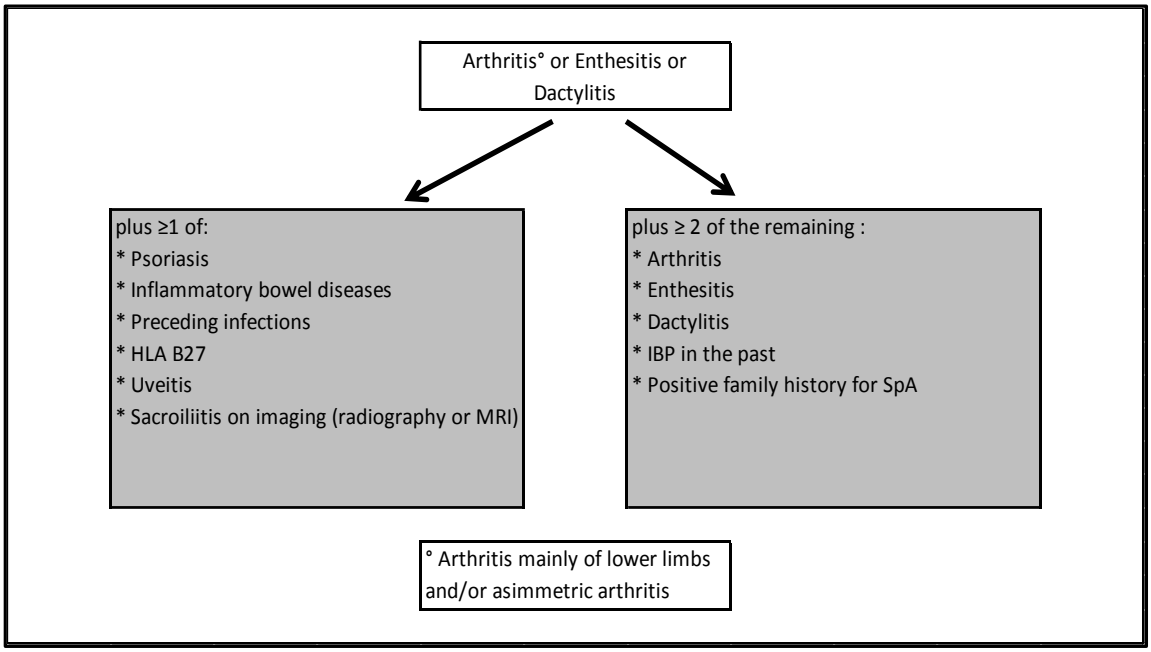
Moreover, recently the concept of axSpA has been proposed as an entity including both AS and its potential pre-stage. Patients with axSpA who still have normal radiographs of the sacroiliac joints (SIJ) are therefore defined as non-radiographic axial SpA (nr-axSpA) patients. Some of these patients might develop AS with radiographic sacroiliitis. However, some patients will be diagnosed as axSpA forever, never resulting in AS. It is now well established that a significant amount of time can pass from low back pain onset to the presence of radiologically

detectable sacroiliitis [**27,28**]. In this regard, the *Assessment of SpondyloArthritis International Society* (ASAS) has developed classification criteria, which summarize the results of two decades of studies [**29-34**]. The innovative role of these criteria derives from the fact that they take into consideration many aspects of SpA, from familiarity to anamnesis, clinical manifestations and diagnostic and laboratory investigations (**Figure 1a and 1b**). These criteria have been also developed to identify patients with early stages of axSpA [**31**] and the forms that potentially present the greatest risk of evolving to AS. For this purpose, it was established the inclusion in these criteria of the presence of sacroiliitis on magnetic resonance imaging (MRI) with bone marrow oedema (BME) as an expression of inflammatory lesion, in addition to one SpA feature for patients with chronic low back pain with onset at age ≤45 years [**35-46**] (**Figure 2**). In early stages of AS inflammatory changes may be seen only on MRI. So, these patients belong to the group of nr-axSpA. It is at present unknown if MRI inflammation should be seen as an early stage of structural damage that only becomes visible at the radiographs at a later stage, or if it does not necessarily lead to structural damage. the role of MRI seems important also in measuring disease activity and has to be further investigated. The ASAS criteria, published in 2009, are based on the distinction between axial or peripheral form, distinguishing the (–prevalently) axSpA and (prevalently) pSpA forms, among which PsA, enteropathic arthritis, ARe and uSpA are included (**table V**).



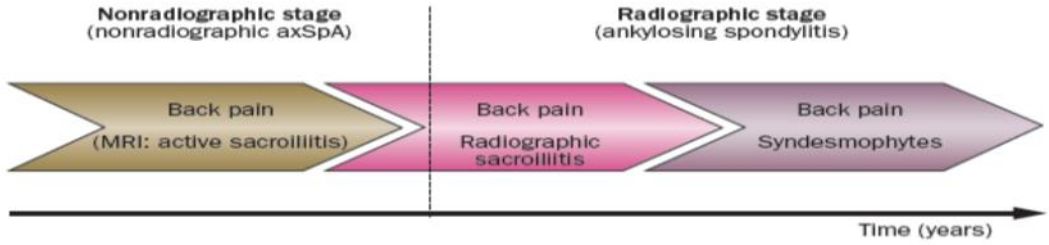
Sieper J et al. Ann Rheum Dis.2009

Figure 1a. ASAS classification criteria for axial spondyloarthritis



Sieper J et al. Ann Rheum Dis.2009

Figure 1b. ASAS classification criteria for peripheral spondyloarthritis



axSpA: Axial Spondyloarthritis.
Rudwaleit, M., Khan, M. A. &Sieper, J. *Arthritis Rheum.*52, 1000–1008 (2005); Rudwaleit, M. &Sieper, J. *Nat. Rev. Rheumatol*. 8, 262–268 (2012)

Figure 2. The concept of axSpA: transition from the early phase of the axSpA to AS on the basis of the evolution from the non-radiological to the radiological stage.

Not all patients present the evolution from the non-radiological stage of the axSpA to the radiological stage. The predictors of progression include the duration of illness, the severity and intensity of the inflammation detected by the MRI, the male gender and other possible indicators, not yet identified. AxSpA usually begins without signs of sacroiliitis that can be detected on conventional radiology, but over a period of years, the signs of sacroiliitis and spondylitis become radiologically evident. Inflammatory back pain is the main symptom, which may persist throughout the course of the disease. During the early stages of the disease, MRI can highlight acute inflammatory lesions in the absence of radiographic signs of sacroiliitis. The vertical dotted line is used, according to the defined criteria, to separate the non-radiological axSpA, from AS.

The ASAS criteria for axSpA are divided into two arms: the "*imaging*" arm or the "*clinical*" arm, both requiring the onset of inflammatory back pain before the age of 45 years, with its persistence for at least 3 months (**Figure 1a**).

To satisfy the "*imaging*" arm, the patient must present signs of sacroiliitis, at X-rays or MRI, in addition to one of the SpA manifestations. To satisfy the "*clinical*" arm, the patient must present HLA-B27 positivity, in addition to at least two manifestations of SpA, while the presence of sacroiliitis is not mandatory.

AxSpA can also be classified, according to the radiological stage in: non-radiographic stage I, for the absence of lesions; Stage II, with structural lesions in the SIJ and the absence of spinal injuries; Stage III, with SIJ and spinal lesions.

Previously, early-stage axial SpA forms, without signs of structural radiographic lesions, were often included in the uSpA. In the recent classification criteria, the term uSpA is abandoned and, due to the absence of structurally evident lesions to conventional radiology, this form is referred to as nr-axSpA. Male sex is recognized as a risk factor for the evolution of nr-axSpA to AS. The classification of these forms of SpA is still in evolution, in particular due to the need to obtain a more adequate definition of the diagnostic relevance of the different lesions on imaging and due to the recent important role played by MRI in the diagnostic process.

Table V) Nomenclature of SpA according to the ASAS criteria:

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a. (predominant) **axial spondyloarthritis (axSpA)**
1. non-radiographic axSpA (nr-axSpA)
2. ankylosing spondylitis (SA) = radiographic axSpA
both associated with:
a. psoriasis
b. inflammatory bowel disease (IBD)

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b. (predominant) **peripheral spondyloarthritis (pSpA)**
1. psoriatic arthritis (AP) and / or pSpA associated with psoriasis
2. pSpA associated with (IBD)
a. type I
b. type II
3. reactive arthritis (ARe)
4. undifferentiated peripheral SpA (uSpA)

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c. (in case of no real predominance) **spondyloarthritis associated with:**
a. psoriasis
b. inflammatory bowel disease (IBD)
c. previous infection
d. uSpA undifferentiated

1.2 THE PATHOGENESIS, THE ROLE OF THE GENETIC MECHANISMS AND BIOMARKERS IN THE AXIAL SPONDYLOARTHRITIS

1.2.1 GENETIC MECHANISMS AND THE PATHOGENESIS

The etiopathology of AS, which has not yet been fully understood, is based on an interaction between some genetic, environmental, physical and infective risk factors.The genetic basis of AS and the role of Human Leukocyte Antigen (HLA)-B27 in these patients has been established through several studies [**47**]. Some have shown, in fact, that AS has a strong genetic basis and that a strong genetic association exists with major histocompatibility complex (MHC) class I allele human leukocyte antigen (HLA)-B27 [**48**]. It has been estimated that no more than 5% of HLA-B27-positive Caucasian individuals develop AS, whereas approximately 90% of AS patients carry this allele [**49**]. Knowledge is still incomplete with regard to the role of genetic predisposition in the disease's pathogenesis and in the development of other axSpA forms. In fact, HLA-B27 is positive in up to 80-90% of AS patients, while the prevalence of this allele is less common in nr-axSpA patients (73-75%), although it continues to be helpful in diagnosing that form of the disease [**50**]. A longitudinal study with a mean follow-up of 7.7 years showed, in fact, that the combination of severe sacroiliitis on MRI and HLA-B27 positivity was a predictor of future AS development [**51**]. The exact role of HLA-B27 in axSpA is unclear. The HLA-B27 includes a family of 31 different alleles; the subtypes HLA-B *2702, B *2704 and B *2705 are closely associated with the disease. Therefore several pathogenic mechanisms are hypothesized and evaluated in the literature by now [**52**] (**Table VI**). Firstly, a genetic interaction has been identified between HLA-B27 and a gene encoding an enzyme called endoplasmic reticulum aminopeptidase (ERAP1) which processes peptides so they can be loaded onto the MHC class I molecules. ERAP1 variants may increase the risk of development of AS and nr-axSpA when HLA-B27 is

present. These interactions strongly suggest that the mechanism by which HLA-B27 contributes to the pathogenesis of axSpA is through its antigen presentation function forming a complex with β-2 microglobulin (arthritogenic peptide hypothesis). Another shared hypothesis is that the specific cytotoxic T lymphocytes (CTLs) for a bacterial peptide cross-react with an arthritogenic self-peptide, which shares a sequence homology, due to molecular mimicry [**47**]. Another theory suggests that HLA-B27 molecules can be mounted on the cell surface as a pair of homologous heavy chains (homodimers) in the absence of β2-microglobulins [**53**]; the newly formed receptor is able to bind a class of molecules present on the cell membrane (KIRs, including KIR3DL1, KIR3DL2) determining a hyperactivation of the innate immunity cells and inducing the disease [**54**]. Another mechanism proposed to explain the reduced capacity for elimination of pathogens is due to misfolding HLA-B27 (abnormal folding of the HLA-B27 heavy chain) with subsequent impaired recognition by the effector cells of the immune system [**55,56**]. According to the latter hypothesis, components of the HLA-B27 molecules, incompletely assembled in the endoplasmic reticulum (ER), are able to induce an unfolded protein response [**55**] and to trigger an immune response, with IL-23 and TNFα production and Th17 cells induction [**56,57**]. Misfolding can derive from the excess of unpaired HLA-B27 molecules, due to an imbalance with respect to the expression of those of β2-microglobulins. Additionally, misfolded HLA-B27 could also induce other autophagy-associated processes [**58**]. Beyond the different functions of HLA-B27, some investigators have suggested that its expression may predispose to developing AS. These patients demonstrate, in fact, a higher expression of HLA-B27 in peripheral blood mononuclear cells with respect to that in HLAB27-positive healthy individuals [**59**]. Since it has been estimated that HLA-B27 is responsible for 40% of the genetic risk of all forms of axSpA [**48,60**], some studies have examined the contribution of additional genes and some single nucleotide polymorphisms (SNPs) to axSpA disease susceptibility identified by genome-wide association studies [**61,62]**.

Table VI: Theories of possible disease-inducing mechanisms by HLA-B27

Theory	Mechanism
Arthritogenic peptide hypothesis	HLA-B27 presents a peptide to CD8 T cells through the classical antigen presentation function on MCH I contributing to the induction of the disease
Endoplasmic reticulum stress hypothesis	Fractions of HLA-B27 proteins incompletely assembled (misfolding) in the endoplasmic reticulum cause stress, consequently they induce disease through the production of IL-23 and consequently the modulation of oxidative pathways
Cell surface homodimer hypothesis	HLA-B27 forms homo-dimers on the cell surface that interact with innate immunity cells inducing the disease
Immunodeficiency hypothesis	HLA-B27 presents an altered number of peptides or with anormal morphology determining an inadequate or altered degradation and elimination process of heterologous microbes peptides

HLA, human leucocyte antigen; IL, interleukin.
Robinson PC et al. Internal Medicine Journal 2014

In the HLA-B27 negative axSpA forms, a high prevalence of other HLA-class I alleles of the B7-CREG group ("cross-reacting-epitope-group": HLA-B7, -B22, -B40 and -Bw42) and Bw62 / Bw35 CREG antigens was detected [**60-62**]. These axSpA forms appear to have milder clinical characteristics, with a later onset age, associated with lower frequency of uveitis, less family aggregation and less development; they are often associated with psoriasis and enteropathy.

A recent study investigated whether polymorphisms (SNPs) in the promoter region of TNFA or in the autoinflammatory TNFRSF1A and MEFV genes, concur with HLA-B27 in enhancing the risk of SpA and/or in predicting the response to anti-TNFα treatment [**63**]. The results of this study indicate the relevant role of TNF-TNFR pathway genetics in the complex network inducing SpA and conditioning response to therapy. TNFA was shown to be a predisposing factor for SpA, but

mainly for AS, while the autoinflammatory gene MEFV appears of no impact in this setting. The haplotype resulting from TNFA-1031C/-308G, seems to exert a protective role in AS, while the TNFRSF1Ac.625+10A>G polymorphism appears as a potential predictor of response to anti TNF-α drugs. Other genes involving in the axSpA pathogenesis are, also, linked to the IL-17/IL-23 pathway, the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signal, the antigen presentation and the immune T cells system activation (***Figure 3***). In literature are also been hypotized the role of genes encoding the IL-23 receptor (IL-23R), prostaglandin E receptor 4 (PTGER4), Runt-related transcription factor 3 (RUNX3) or Scr homology 2 adaptor protein 3 (SH2B3) [**64**]. While some specific haplotypes are strongly associated with an increased risk of AS, other variants appear to be AS protective [**65**]. Some data also suggest that ERAP1 inhibition could potentially be effective for clinical applications [**66**].

Unlike other inflammatory rheumatic diseases such as rheumatoid arthritis (RA), the inflammatory process of axSpA predominantly involves the enthesis. Numerous recent studies have provided evidence regarding local inflammation of enthesis. From the last data in the pathogenesis of the SpA it was discovered how the primitive triggering event is considered the "stress of the enthesis". Biomechanical stress initiates enthesitis by triggering mechanoreceptors which induce an inflammatory response via the mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway [**67**] and the prostaglandin release. These prostaglandins seems to induce osteoblast activation through inhibition of sclerostin via the prostaglandin E4 receptor and consecutive ERK signaling [**68**]. It is been also indentified the significantly presence of IL-23R-positive T cell in the enthesis and in the subchondral bone marrow of neighboring joints parallel to the discovery of elevated concentrations of systemic IL-17 and IL-23 [**69,70**].

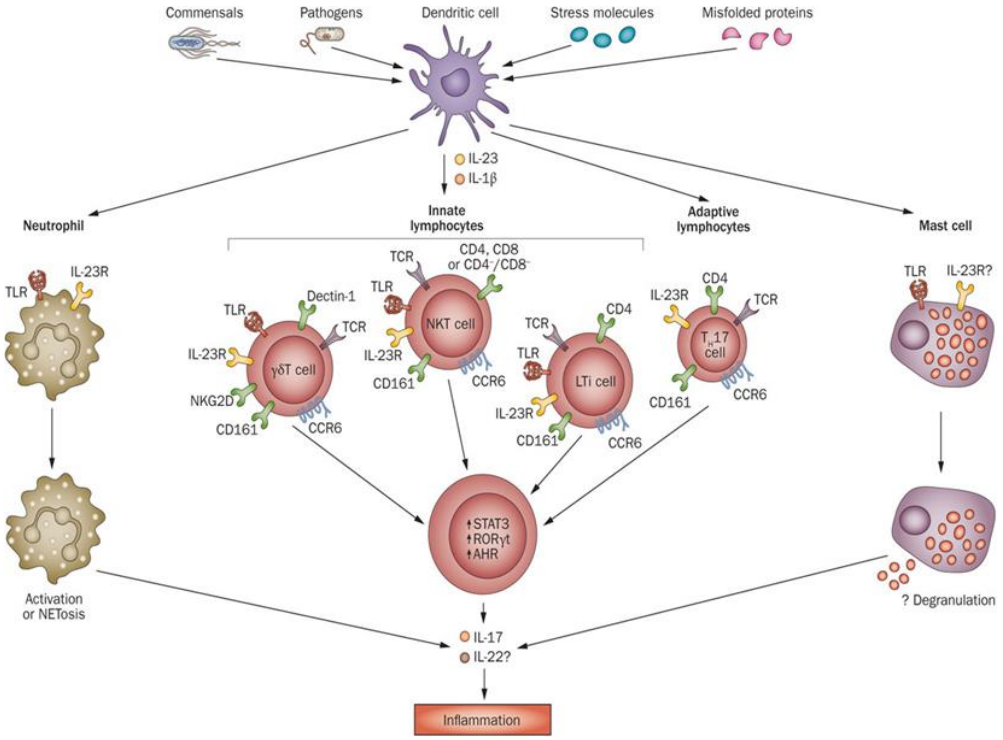

Figure 3. IL-17 secreting cells in inflammatory diseases and in axSpA.

AHR, *aryl hydrocarbon receptor*; CCR6, CC *chemokine receptor type 6*; IL-23R, *IL-23 receptor;* Lti, *lymphoid tissue inducer*; NKG2D, *natural killer cell receptor D*; NKT, *natural killer T (cell)*; NETosi (*neutrophil extracellular traps*), processo di formazione delle NET; RORγT, *thymus-restricted RAR-related orphan receptor-γ*; STAT-3, *signal transducer and activator of transcription 3*; TCR, *T-cell receptor*; Th17, *type 17 T helper*; TLR, *Toll-like receptor.*
(Da Kenna TJ, Brown MA, Nat Rev Rheumatol, 2013; 9:375-79)

Different cell types produce IL-17 in inflammatory diseases by induction by IL-23 and IL-1β. Lymphocytes of innate immunity, such as Tγδ cells, NKT cells and LTi lymphocytes, and TCD4 + Th17 lymphocytes, constitute a relevant source of IL-17, and sometimes IL-22, in the inflammatory process, as well as mast cells and polymorphonuclear cells. The stimulus capable of inducing IL-17 production by different cell types is still unclear. Sensitization to exogenous or commensal bacteria, activation of innate immunity receptors and reticular-endothelial stress caused by protein misfolding have been implicated in the pathogenetic mechanisms that can lead to the activation of these cells.

Bone homeostasis in axSpA is unbalanced due to an excessive bone formation determining the squaring of the vertebral bodies possibly evolving toankylosis of the vertebral column on one side, due to an excessive bone resorption on the other side, the latter accompanied by a reduction in bone mineral density in the spinal

vertebrae of axSpA patients and osteoporosis [**71**]. Wnt signaling seems to play an important role in osteogenesis with an increased differentiation and proliferation of osteoblasts, with the role of Dickkopf-1 (Dkk1) [**72**], the bone morphogenetic protein (BMP) [**73**] or other inflammatory molecules. Generally speaking then, entheseal and gut inflammation further enhances chronic inflammation, resulting in excessive bone formation and bone resorption of the spine, although the exact interplay between genetic predisposition, inflammation and disturbed bone homeostasis remains undefined.

Because of the absence of rheumatoid factor in axSpA, the disease is thought to be seronegative and antibodies are not considered one of its hallmarks. There is nevertheless increasing evidence that B cells and the humoral immune response play a role in axSpA [**74**]. After encountering an antigen, some of the B cells differentiate into antibody-producing plasma cells and plasmablasts found elevated in blood and joints of axSpA; the antibodies that bind to their antigen triggers the formation of immune complexes, which neutralizes foreign substances, inducing the macrophages to the phagocytosis or activing the complement pathway [**75,76**]. The exact mechanisms by which antibodies contribute to axSpA pathogenesis remain unelucidated, nevertheless it is seems antibody complexes could induce to osteoclast activity [**77**]. Besides antibodies, also cytokines such as the receptor activator of nuclear factor kappa-B ligand (RANKL), which has been found to be increased in AS patients, could mediate osteoclastogenesis [**70,78,79**]. Just as RANKL, serum levels of Interleukin 6 (IL-6) are also increased in AS patients [**80**]. Recently, anti-CD74 antibodies with specificity to a class II-associated invariant chain peptide (anti-CLIP-ABs) were found in axSpA patients [**81**]. Binding of CLIP antibodies to CD74 may lead to activation of cells and production of proinflammatory cytokines such as TNFα. Baraliakos et al. [**82**] found anti-CLIP-Abs in 85.1% in axSpA but in only 7.8% in non-SpA patients, analyzing respectively 145 sera from 94 axSpA and 51 non-SpA patients. This result seems to substain the strong association of anti-CLIP-Abs with axSpA. Nevertheless, more studies are needed to establish its usefulness in clinical practice. A discordant

result was obtained in other study conducted in early axSpA patients, participating in the SpondyloArthritis-Caught-Early (SPACE) prospective cohort study [**83**]: although anti-CD74 IgA levels have been found elevated in patients with early axSpA in previous investigations, in this study this finding appears not sufficiently specific to yield significant diagnostic value in patients suffering with inflammatory low back pain (LBP) (≥3 months, ≤2 years, onset < 45 years). An other interesting article examining the antibodies involved in axSpA and mainly in AS pathogenesis, instead demonstrated that these antibodies were detected in only a small number of axSpA patients and that they were also found in the sera of RA and other inflammatory arthropathies, thus limiting their specificity and their distinctive character as biomarkers for axSpA diagnosis [**84**].

1.2.2 THE ROLE OF SEROLOGICAL INFLAMMATORY AND BONE REMODELLING BIOMARKERS IN AXSPA

The National Institute of Health Biomarkers and Surrogate Endpoint Working Group have elaborated the definition of biomarker as a "*characteristic that can be objectively measured and evaluated as an indicator of normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention*" [**85**]. The importance of identifying new predictive biohumoral markers of disease activity and severity in order to detect and treat early axSpA has been highlighted. Unlike other inflammatory arthropathies, there are no specific biomarkers of disease activity in axSpA that are presently being used in clinical practice. Recent studies have focused on the role of some new markers to diagnose early axSpA, to assess disease activity, and to identify patients at higher risk for a worse outcome [**86,87,88-90] (Figure 4).** Serum and plasma biomarkers have recently undergone extensive examination since HLA-B27, the biomarker commonly used in spondyloarthropathies, and C-reactive protein (CRP) and the eritrocyte sedimentation rate (ESR), the inflammatory markers generally used in clinical

practice to monitor systemic inflammation, are often unable to assess disease activity [**91-94**].

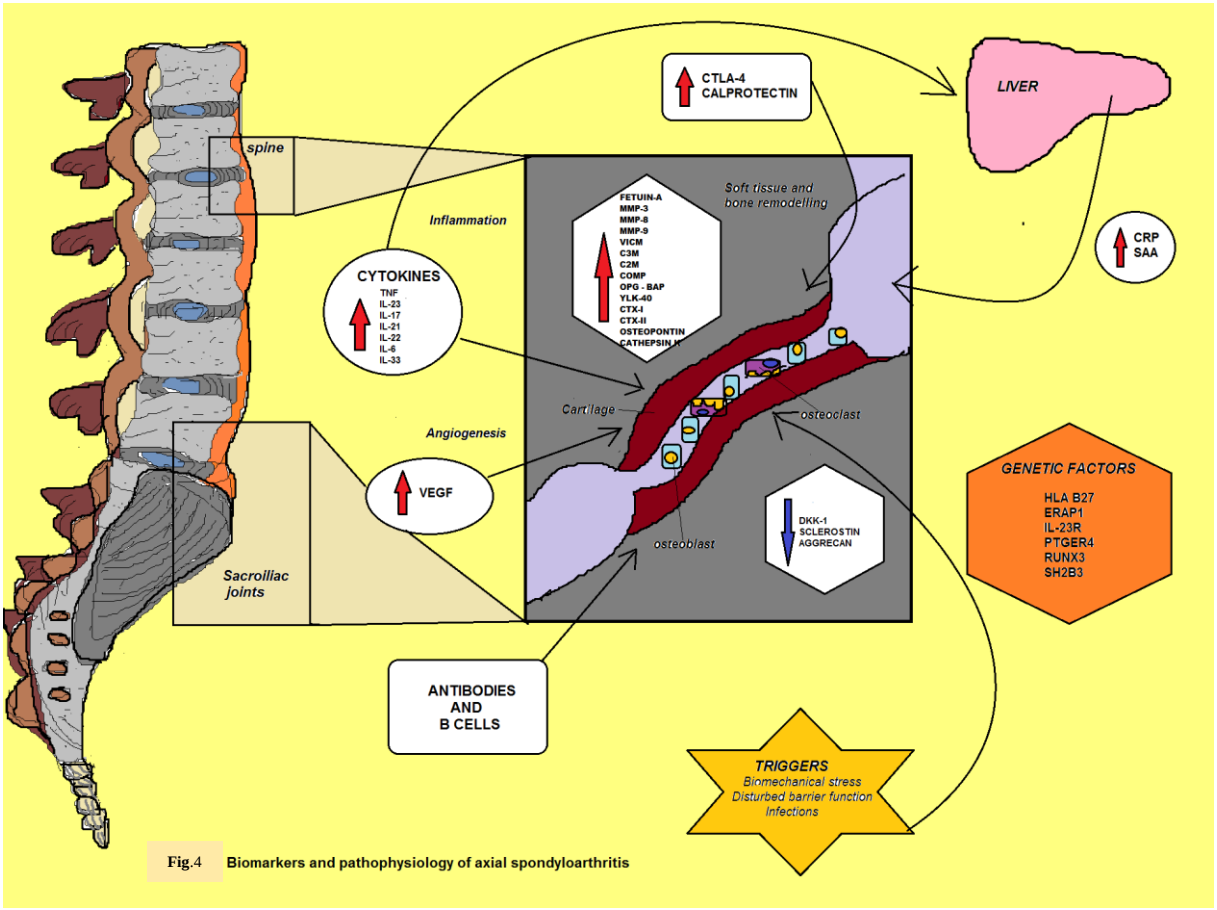

Figure 4. Biomarkers and pathophysiology of axSpA.

<u>Serum (Inflammation) markers:</u> CRP, VEGF, TNF, IL-6, IL-12, IL-21, IL-22, IL-23, IL-33, MMP-3, MMP-8, MMP-9, VICM, OPG, Aggregan, COMP, YKL-40, Calprotectin, SAA, CTLA-4;
<u>Bone remodelling markers:</u> CRP, VEGF, MMP-3, VICM, C2M and C3M, Dkk-1, OPG, BAP, Sclerostin, Osteocalcin, Fetuin-A, Cathepsin K.

Legend: *C-reactive protein (CRP); Serum amyloids A (SAA); Vascular endothelial growth factor (VEGF); Tumor necrosis factor (TNF); Interleukin 6 (IL-6); Interleukin 17 (IL-17); Interleukin 21 (IL-21); Interleukin 22 (IL-22); Interleukin 23 (IL-23); Interleukin 33 (IL-33); Cytotoxic T lymphocyte associated molecule (CTLA-4); Matrix metalloproteinase 3 (MMP-3); Matrix metalloproteinase 8 (MMP-8); Matrix metalloproteinase 9 (MMP-9); Citrullinated fragments of vimentin (VICM); Circulating protein fragments of cartilage and connective tissue degradation*

(C2M and C3M); Cartilage oligomeric matrix protein (COMP); Osteoprotegerin (OPG); Bone alkaline phosphatase (BAP); Serum human cartilage glycoprotein-39 (YKL-40); C-terminal cross-linking telopeptide of type I (CTX-I) and type II (CTX-II) collagen; Wnt proteins and Dickkop-1 (Dkk-1); Human leukocyte antigen (HLA)-B27; IL-23 receptor (IL-23R); prostaglandin E receptor 4 (PTGER4); endoplasmic reticulum aminopeptidase 1 (ERAP1); Runt-related transcription factor 3 (RUNX3); Scr homology 2 adaptor protein 3 (SH2B3).

The ***CRP*** and ***ESR***, recognized as acute-phase proteins reflecting systemic inflammation, are commonly utilized in clinical practice [**95**], although they do not reflect completely the inflammation process in axSpA due to the low sensitivity and specificity [**96**]. An elevated CRP is one of the classification criteria of the Assessment of Spondyloarthritis International Society (ASAS) for axSpA, while CRP level is taken into consideration in the *Ankylosing Spondylitis Disease Activity Score* (ASDAS) and correlates with the *Bath Ankylosing Spondylitis Disease Activity Index* (BASDAI) [**97**]. However, an elevated CRP or ESR is detected in only about 40–50 % of AS patients [**98**]. The degree of observed inflammation, in fact, fluctuates during the course of axSpA. In general, CRP level is higher in patients with radiographic axSpA with respect to nr-axSpA [**99**]. Although CRP is within the normal range in a large proportion of patients with active axSpA, it is still widely considered a reliable parameter of disease activity. In fact, CRP levels are moderately correlated with MRI inflammation [**88,89**,**93,99**]. In addition, CRP has been found to be a reliable biomarker for monitoring treatment response and predicting further radiographic progression. Several studies have demonstrated that CRP levels fall significantly during anti-TNFα treatment [**89,91,93**]. Modifications in CRP levels are correlated with changes in BASDAI and MRI scores, while elevated baseline CRP levels are associated with a good treatment response [**91,93**] and represent a strong positive predictor of radiographic sacroiliitis progression, in particular for the progression from nr-axSpA to AS [**93**]. Several prospective studies have also demonstrated that elevated CRP levels are independently associated with radiographic spinal progression in axSpA patients [**100**]. In summary, CRP currently appears to be a reliable biomarker for assessing disease

activity and predicting structural progression and treatment response. ESR, instead, appears to be a non-specific measure of inflammation that may be influenced by a variety of other non-rheumatic conditions and comorbidities. However, some studies reported that higher ESR levels such as increased CRP levels are independently associated with structural disease progression in nr-axSpA patients [**100**].

Studies on ***cytokines'*** levels have produced variable results. The discovery of the involvement of ***TNFα*** in disease pathology led to the development of therapeutic antibodies targeting this cytokine. Considered a hallmark of the disease, TNFα has been for a long time the only cytokine known to be involved in axSpA pathogenesis. TNFα plays a key role in the pathogenesis of axSpA, providing a possible link between the inflammatory response and disturbed bone homeostasis [**101**], exerting several effector and biological functions via the pro-inflammatory cytokines and chemokine release, the activation of endothelial cells with up-regulation of adhesive molecules, the leucocyte accumulation, the angiogenesis, the lymphocyte activation, the fibroblast proliferation and the chondrocyte and osteoclast activation. TNFα levels are significantly higher in AS patients with respect to subjects with non-inflammatory back pain or healthy controls [**101**], and TNFα expression is strongly up-regulated in the SIJ biopsies of AS patients [**102]** Although TNFα is not a reliable biomarker of disease activity, the discovery of anti-TNF α agents has been demonstrated an important pharmacological result in the axSpA treatment [**103**], and it has significantly improved the outcome of these patients. According to the updated 2016 ASAS recommendations, axSpA patients who meet the ASAS classification criteria can be treated with anti-TNF α agents if a minimum of four weeks of first-line treatment with two non steroidal anti-inflammatory drugs (NSAIDs) prove ineffective in reducing disease activity [**103**]. These biological agents are considered efficacious in preventing irreversible joint damage, ankylosis, impaired functionality, and work disability when prescribed at an early disease stage [**104**]. One of the most extensively studied cytokines, ***IL-6***, is a classic proinflammatory cytokine produced by a variety of immune cells that

stimulates the production of several acute phase proteins [**101]** and it is involved in the initial phases of multiple immune-mediate diseases. The IL-6 is involved in the first phases of the inflammation disease by inducing neutrophils'accumulation in the inflammation site and modulating T cell activation and differentiation. Some researchers have reported higher IL-6 serum levels in AS patients with respect to healthy controls [**101,105**]. Elevated IL-6 levels have been found in SIJ biopsies of AS patients, particularly in early stage [**106**]. While some studies have demonstrated associations between IL-6 levels and either disease activity or inflammatory markers [**101**,**107,108**], others did not confirm these results [**88,109**]. Normalization of IL-6 levels has also been reported in association with lower MRI inflammation scores [**88**]. ***Interleukin 17 (IL-17)*** and ***IL-23*** are key cytokines in the Th17 pathway, and the IL-23/IL-17 immune axis appears to be a very important pathway in the pathogenesis of axSpA **[110-112**]. A number of studies reported elevated IL-17 and IL-23 levels in the plasma and serum of AS patients and associated them with disease activity [**70,113,114**]. The IL-17/IL-23 axis is also crucially involved in other inflammation and autoimmune diseases [**115**]. IL-17 is mainly produced by Th17+ T-helper cells under the stimul of the IL-23. The number of IL-17+T cells and the IL-17 serum levels in circulation is higher in AS patients with respect to healthy individuals and to subjects with degenerative changes [**70,110,116-120**]. Interestingly, the number of IL-17+T cells is increased in patients with early active axSpA [**110**]. However, in some studies, systemic IL-17 levels correlated neither with inflammatory indices nor with clinical disease activity parameters [**70,118**]. The fact that TNFα agents could reduce the number of Th17 cells and serum IL-17 levels confirms the role of IL-17 axis in axSpA pathogenesis [**118**]. Just as IL-23 induces IL-17 production, even IL-23 serum levels [**110,114,119**] and IL-23 expression in the subchondral bone marrow from the facet joints [**70**] are up-regulated in AS patients, but only a few studies have found a positive correlation between IL-23 serum levels and CRP values, MRI inflammation or clinical disease activity [**114,118**]. Both IL-17 and IL-23 levels significantly fall in responders to anti-TNF α therapy while they rise in non-

responders **[118**]. Nowadays, both cytokines (IL-17, IL-23) have become important therapeutic targets [**121-125**]: clinical trials showed that ustekinumab, a monoclonal antibody against the p40 subunit shared by IL-12 and IL-23, and secukinumab, a monoclonal antibody against IL-17, produced clinical improvements in axSpA patients [**121-125**]. In addition to the above discussed cytokines, ***IL-21*** and ***IL-22***, both interconnected with IL-17 and Th17 cells, are elevated in AS patients [**70, 126-128**]. ***Interleukin 33*** (***IL-33***), a member of the IL-1 family, has recently been recognized its role in several inflammatory and autoimmune diseases. Three independent centers in China [**129-131**] have reported elevated IL-33 serum levels in AS patients and found correlations with disease activity or other inflammatory markers.

Matrix metalloproteinases (MMPs) (and in particular ***MMP-3***) are zinc-dependent endopeptidases which are involved in the degradation of extracellular matrix proteins. These molecules, which also play a role in cell proliferation, migration, differentiation, angiogenesis, apoptosis and host defence, have been considered possible biomarkers of disease activity. MMP-3 serum levels are increased in AS patients with respect to healthy controls [**132-134**]. In fact, some investigators have reported that MMP-3 was correlated with CRP levels, disease activity and functional status, as assessed by the BASDAI and BASFI [**89, 132, 135-138**]. Another study reported a correlation between serum MMP-3 levels and BASDAI and ESR **[88**]. That study found that MMP-3 levels were significantly higher in 42 AS patients with respect to 20 controls and were correlated with BASDAI and BASFI, supporting the hypothesis that MMP-3 is more reliable than ESR or CRP in assessing disease activity [**88**]. MMP-3 levels have even been found to be correlated with disease activity in PsA patients [**138-140**]. Maksymowych et al. reported that anti-TNFα treatment induced a significant decrease in serum MMP-3 levels together with a reduction in conventional variables (ESR and BASDAI) [**137**]. Although serum MMP-3 represents a reliable parameter of disease activity, this has not always been reflected in MRI-documented studies examining inflammation of the SIJ [**135**]. According to a study by Van Kuijk et al. [**141**]

evaluating 24 SpA patients who were randomized to receive adalimumab 40 mg once every 14 days or placebo, a reduction in serum MMP-3 levels was noted after only 4 weeks of anti-TNFα therapy, but no change was detected in serum levels in the placebo group. As this molecule is increased in active forms of SpA or in patients with important clinical involvement, this would support the hypothesis that MMP-3 reliably assesses disease activity. Maksymowych et al. showed that MMP-3 was a significant independent predictor of radiographic progression over a two year observation period in 97 axSpA patients [**136**], suggesting that MMP-3 could be considered a parameter of bone metabolism. Although fewer studies have been carried out on ***MMP-8*** and ***MMP-9***, they have been shown to be associated with disease activity, as assessed by the BASDAI score [**142**].

Citrullinated fragments of vimentin (VICM) are MMP-degraded fragments modified by citrullination, an unspecific modification during the inflammatory phase [**143**]. VICM levels are significantly higher in AS patients with respect to healthy controls. Bay-Jensen et al. [**143**] found that higher VICM levels and high baseline mSASSS values were associated to a worse risk of radiographic progression over a two year period.

Osteoprotegerin (OPG), a member of the TNF receptor superfamily, has been shown to be involved in osteoclastogenesis by activating T cells through the OPG ligand (RANKL). According to some studies, OPG levels were lower in patients with AS, [**144,145**], while according to others they are higher [**146,147**]. OPG seems to correlate with BASDAI scores, but not with treatment response [**146,147**].

Serum human cartilage glycoprotein-39 (YKL-40), a secretory protein of human articular chondrocytes and synoviocytes, has been considered a marker of cartilage remodelling and associated with disease activity in AS [**88**]. YKL-40 levels are significantly higher in SpA patients with respect to healthy controls [**105**].

The ***cartilage oligomeric matrix (COMP) protein*** catalyzes the assembly of collagen in the extracellular matrix. Although findings on its association with other clinical and disease activity markers in AS have been inconsistent, elevated COMP levels have been reported in a variety of inflammatory joint diseases [**88**]. One study

nevertheless reported an inverse relationship between COMP levels and MRI inflammation in axSpA patients [**89**].

Aggrecan is the central component of cartilage extra-cellular matrix. The replacement of the glycosaminoglycan side chain with aggrecan with charge density modification determines osmotic processes, fundamental for the biomechanical properties of cartilage. AxSpA patients have been found to have depressed levels of total aggrecan with respect to healthy subjects [**88**].

Calprotectin is a heterodimeric calcium- and zinc-binding protein complex, composed of S100A8 and S100A9 subunits, expressed in the cytosol of keratinocytes, neutrophils and monocytes. Hammer et al. found it to be a plasma marker of inflammation and treatment response in reactive arthritis and axSpA [**148**]. Higher calprotectin levels have been found in the SIJ of AS patients **[147**]. Some studies have reported that serum calprotectin levels are significantly higher in axSpA and that anti-TNFα treatment significantly reduces calprotectin levels [**149,150**]. Baseline calprotectin serum levels have been found significantly increased in patients with higher modified Stoke Ankylosing Spondylitis Spinal Scores (mSASSS) versus those without [**151**]. According to another study, a significant decrease in serum calprotectin was noted after intensive physiotherapy in both nr-axSpA and AS patients [**152**].

Serum amyloids A (SAA) proteins are a family of apolipoproteins associated with high-density (HDL) lipoproteins that are produced as a response to an inflammatory trigger. AS patients have higher serum SAA levels with respect to healthy controls; those levels were also correlated with other inflammatory and disease activity parameters [**153,154**].

Cytotoxic T lymphocyte associated molecule (CTLA-4) is an inhibitor molecule of immune response. One study demonstrated higher CTLA-4 serum levels in axSpA patients with respect to healthy controls. It also appears a marker of disease activity due to correlation with CRP and BASDAI [**155**].

Vascular endothelial growth factor (VEGF) is a molecule inducing angiogenesis, an inflammatoryinitial phase in the remodelling bone process with the new bone

formation. VEGF serum levels appeared higher in AS and axSpA patients [**88,156,157**], expecially in those with active disease [**156**] and with a worse radiographic progression according to mSASSS.

Type I and type II collagen are proteins of cartilage and components of connective tissue. Some studies reported that variations in radiographic progression were correlated to both type I and II C-terminal telopeptides (***CTX-I*** and ***CTX-II***), other studies demonstrated the role of only CTX-II levels [**157,158**]. It has been shown that urinary CTX-II levels are higher in AS patients with respect to those in healthy controls [**159**]. CTX-II was also found to be correlated with other serological inflammation markers [**157,160**]. According to another study, it has been found a correlation between higher baseline CTX-II levels and higher pre-treatment MRI inflammation scores for SIJ and/or lumbar spine in axSpA patients. [**89**]. Anti TNFα treatment contributed to significantly reduce urinary CTX-II levels, that correlated with improvement in disease activity [**160**]. CTX-II also appears to be a biomarker of radiographic progression in axSpA. Higher CTX-II levels have been found in AS patients with worse radiographic spinal progression respect to those without [**157,159**]. In some studies in AS patientes higher levels of two neo-epitope biomarkers have been demonstrated: ***C2M***, a serum biomarker that measures a matrix metalloproteinase (MMP)-generated neo-epitope of type II collagen, and ***C3M***, a biomarker of soft tissue turnover associated with inflammation [**143,161**]. One study used therefore a combination of C2M and C3M, dichotomized according to the best cut offs for individual markers, as predictor index of radiographic progression. CTX-I is a marker of osteoclast activity reflecting bone degradation [**162**]. Both urinary and serum CTX-I levels are higher in AS patients with respect to those in healthy controls [**159,163**].

Sclerostin (SOST) and ***noggin (NOG)*** are bone morphogenic protein (BMP) antagonists. Findings reported by studies focusing on sclerostin levels in AS patients have not always been consistent. One German study reported that both sclerostin expression in osteocytes from AS patients measured using immunohistochemical techniques and both sclerostin serum levels were

significantly lower (with respect to that in osteocytes and blood of healthy individuals and RA patients)[**164**]. Importantly, that study reported that low serum sclerostin levels in AS patients were significantly associated with new syndesmophytes formation. Similar findings were reported in axSpA [**147**] and in a Brazilian cohort of AS patients [**165**].

Wnt proteins are extracellular signaling molecules. There are at least two families of secreted inhibitors of Wnt signaling: the secreted frizzled-related transmembrane receptors with N-terminal cysteine-rich domain and the Dickkopf proteins, which includes Dkk1. In several studies AS patients with no syndesmophyte formation have been shown to have significantly higher Dkk1 levels. This result seem to suggest that blunted Wnt signaling suppresses new bone formation and consequently syndesmophyte growth and spinal ankylosis [**166,167**], although no modifications were found after anti-TNF-α treatment [**168**]. In contrast with other studies [**169,170**], lower levels were reported in the AS patients with respect to those in the healthy subjects.

Osteocalcin is a small molecule of the mineralized bone matrix. Initial studies have shown that osteocalcin levels are lower [**171**], but more recent studies have reported higher levels in AS patients with respect to those in controls, especially in those individuals who later developed new syndesmophytes [**93, 170**].

Bone-specific alkaline phosphatase (BAP) is a marker of active bone formation. Higher levels of BAP have been reported in patients with axSpA [**158, 164, 166**].

The ***fetuin*** family encompasses a series of proteins that have been implicated in several biological functions involving in both inflammatory stage (regulation of insulin and hepatocyte growth factor receptors and acute phase proteins) and bone remodelling phase (osteogenesis and bone resorption). AS patients with syndesmophytes have been shown to have significantly higher levels of fetuin-A with respect to patients without syndesmophytes and to healthy controls [**172**].

The ***cathepsin K***, which is a highly potent collagenase predominantly expressed in osteoclasts, is involved in bone remodelling and resorption; its expression is stimulated by inflammatory cytokines that are released after tissue injury. There are

actually discordant results about the cathepsin K levels in AS: some studies reported higher expression of cathepsin K in mononuclear cells, fibroblast-like cells, and cells attached to bone but similar systemic levels to those in healthy controls [**173, 174**]. Some studies reported higher ***adipokines*** levels, in particular leptin values, in some rhematological diseases [**175,176**]. In AS patients with structural bone damage and syndesmophytes were found higher adiponectin levels [**176**]. The results so far obtained are discordant; other studies have demonstrated lower or similar leptin levels in AS patients respect to healthy subjects [**177**].

1.3 THE EVALUATION WITH IMAGING OF THE AXIAL SPONDYLOARTHRITIS

1.3.1 THE ROLE OF THE STANDARD RADIOGRAPHIC EXAM

In the diagnosis of axSpA a relevant role is played by the radiographic exam, which is also useful for monitoring the progression of bone lesions. We can distinguish initial, late and advanced signs. On pelvis X-rays a symmetrical or asymmetric sacroiliitis, which is classified in grades (I-II-III-IV) according to the severity of the radiological damage, can be recognized. The earliest detectable lesion in the SIJ at the standard radiograph is an initial disruption of the cortical margin of the subchondral bone, with initial involvement of the third lower segment of the joint, followed secondarly by erosions and sclerosis. The evolution of erosion produces a false enlargement of the joint spaces. When fibrosis and ankylosis occur, the SIJ appear to be obliterated until complete fusion (***Images 3 a-d, see iconography***). The spinal lesions are initially characterized by a straightening of the column (***Figure 4, see iconography***). There may also be an osteitis in the vertebral bodies which causes a "square" alteration of the vertebral bodies ("squaring") which may result in erosion (sign of "Romanus") (***Images 5-6, see iconography***). The progressive ossification of the fibrous ring of the intervertebral disc induces the formation of marginal syndesmophytes, detectable radiographically in the anterior and lateral

views, which connect the vertebral bodies to each other; the syndesmphytes are typical osteoproductive aspects that may be found in the axSpA (***Images 7-9, see iconography***). With the passage of time, the calcifications and ossifications involve the entire spine, sometimes leading to bone flows, involving the anterior longitudinal ligament (sign of the "bamboo cane") or of the inter-apophyseal joints (sign of the "track") or inter-spinous ligaments (sign of the "dagger") or all these structures together (sign of the "cog railway") (***Images 10 a-b, see iconography***). Sometimes, the osteoproductive process derived from the inflammatory involvement of the spinal enthesis determines the formation of non-marginal calcific bridges (pseudo-syndesmophytes) which are generally monolateral and asymmetric (especially found in the form of axial psoriatic spondyloarthritis). The osteoproductive processes with the formation of calcifications can also be observed in the periphery, especially in shoulders, hips and heels. In the established disease phase, traditional radiographs are largely sufficient to clarify the clinical picture. The three main radiological scoring methods used for the spine are BASRI (*Bath Ankylosing Spondylitis Radiology Index*), SASSS (*Stoke Ankylosing Spondylitis Spine Score*), mSASSS (*modified Stoke Ankylosing Spondylitis Spine Score*) [**178-181** ***(Table VII***). The mSASSS method appeared to be the most appropriate method for assessing and monitoring the development of syndesmophytes and the radiographic progression. In a recent study the SASSS and BASRI methods were found to have good reproducibility, but both showed a low sensitivity to detect change [**182**]. Furthermore, the mSASSS method was more reliable than the remaining two methods in quantifying the score of structural spinal lesions [**183**]. These methods have been performed and adopted in clinical trials in order to evaluate the therapeutic response of anti-TNFα agents and non-steroidal antinflammatory drugs (NSAIDS) also in terms of slowing of radiological progression [**184-190**].

Table VII. Radiological scoring methods for radiographic axSpA or AS

BASRI-spine (range 2-12) (for the lumbar spine, AP and lateral views are analyzed, and the projection with the highest score is considered; for the cervical spine, the lateral view is analyzed)
0 = normal (no alteration)
1 = suspicious or mild (initial alteration not well defined)
2 = average (some erosions, squaring, or sclerosis, with or without syndesmophytes, visible on ≤ 2 vertebrae)
3 = moderate (syndesmophytes on ≥3 vertebrae, with or without fusion involving ≥2 vertebrae)
4 = severe (fusion involving ≥3 vertebrae)

Modified SASSS (range 0-72) (anterior corners of the lumbar spine and anterior corners of the cervical spine from the inferior margin of C2 to the upper margin of T1 analyzed on the lateral view)
0 = normal
1 = erosion, sclerosis, or squaring
2 = syndesmophytes
3 = bone bridges with fusion

New York criteria for sacroiliitis (range 0-4) (mean score of both SIJ is used in BASRI)
0 = normal
1 = suspicious or mild (initial alteration not well defined)
2 = minimum (minimum sacroiliitis, defined as the loss of definition of the SIJ rhyme, mild juxta-articular sclerosis, minimal erosions and the presence of a slight reduction of joint space)
3 = moderate (moderate sacroiliitis, defined as marked sclerosis on both sites, thickened and indistinct margins of SIJ and erosive lesions, with loss of joint space)
4 = severe (complete fusion or ankylosis of SIJ)

SIJ: sacroiliac joint; BASES: Bath Ankylosing Spondylitis Radiology Index; AP: anterior-posterior; SASSS: Stoke Ankylosing Spondylitis Spine Score

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1.3.2 THE ROLE OF THE MAGNETIC RESONANCE IMAGING

The MRI has revolutionized the diagnosis and the concept of the SpA. Some recent studies [**36-38, 191-193**] have shown the superiority of MRI compared to other methods in the search of recent onset lesions. This method appears to be able to define inflammatory lesions in the active phase. It is both sensitive and very specific in demonstrating lesions in the articular cartilage, the synovium and the subchondral bone. Its ability to detect such alterations is of considerable help in the diagnosis of early disease forms, when no alterations or erosions or other structural lesions are detected, and allows early therapy to be undertaken **[42, 194-197**]. The diagnosis of axSpA is currently based on the presence of inflammatory and / or structural lesions in the SIJ and in the spine. The appearance of inflammatory signs in progress or previous is crucial for the possibility of early recognition of the transition from non-radiological forms of axSpA to the AS.

Two sequences in MRI are used, both of which can detect both inflammatory and structural lesions. The weighted spin-echo T1 (T1SE), with relaxation time in which the water shows an hypointense signal, is mainly used for the identification of structural alterations, such as chronic osteitis, erosions, ossification and sclerosis; the STIR (Short-Tau-Inversion-Recovery), a sequence able to differentiate the water signal from that of fat and to obtain the saturation of fat (FS) in T1, is preferable for the study and visualization of inflammatory lesions, characterized by an important liquid and vascular component, such as BME and synovitis. BME, characterized by the increase in the STIR signal, represents the main alteration induced by inflammatory activity in the axSpA and is highlighted early on the SIJ by the MRI (***Images 11-12, see iconography***). Among the structural lesions, erosions are the most specific for axSpA, and are well highlighted by the sequences T1SE as interruptions of the cortical bone, with altered signal in the subchondral bone (***Images 13-14, see iconography***). At spine MRI the BME of the vertebral anterior corners (anterior osteitis) and the posterior corners (posterior spondylitis) appears as hyperintense area in the STIR sequence; the spondylodiscitis appears as hyperintense region in the STIR and hypo-intense sequence in T1SE (***Images 15-***

21, see iconography). Adipose metaplasia appears as a hyperintense area in the same sequences. The ossification of vertebral enthesies (syndesmophytes) appears as hypointense areas near the cortical bone, and when they are not associated with adipose metaplasia, they are more difficult to identify in MRI because they could be confused with the peri and para-vertebral ligaments that appear as ipo-intense areas. A set of MRI-ASAS criteria is currently underway for the definition of the MRI imaging pattern specific for the classification of axSpA forms [**191, 192, 36-38, 46**], where both inflammatory and structural alterations are considered, expression of inflammatory activity or of chronic pathological process, different phases of the natural course of SpA (**Table VIII**). Based on the definition of the ASAS / OMERACT criteria, SIJ MRI is considered positive if ≥1 highly suggestive inflammatory lesion (BME) per SpA is present on ≥2 consecutive slices or if ≥2 inflammatory bone lesions are visible on a single slice [**191, 37**]. The presence of synovitis, enthesitis or capsulitis alone, unaccompanied by BME, is not sufficient to consider a SIJ MRI as positive. According to the ASAS criteria, the spondylitis is confirmed by the signs of BME, anterior or posterior, in ≥3 vertebrae, or of adipose metaplasia located in several vertebral bodies [**198-201**]. Less specific to SpA are spondylodiscitis or flogistic involvement of the inter-apophyseal joints [**202**]. This definition is still very preliminary and only the application on large series will allow to classify and distinguish more precisely the aspects of degenerative nature and those derived from the inflammatory process that can be seen at the MRI. As for conventional radiology, also different methods of evaluation of acute and chronic lesions have been proposed both for the spinal and SIJ MRI [**203-205**]. Recently it has been proposed a scoring method for the evaluation of acute and chronic spinal lesions, with score between 0-6 (activity score: ASspiMRI-a and chronicity score: ASspiMRI-c), and for SIJ lesions, with score ranging 0-3 for acute lesions and 0-5 levels for chronic lesions [**206,207**]. The second method for the evaluation of acute lesions (BME) detectable in spine and pelvis MRI, is the one recently proposed and validated by the Canadian research

group for SpA, defined as Spondyloarthritis Research Consortium Canada (SPARCC) magnetic resonance imaging index [**43,44**].

Table VIII) Definition of MRI imaging according to ASAS

- **<u>for the positivity of sacroiliitis</u>:**
  - **presence of active inflammatory lesions::**
    - **bone oedema** in STIR* or **osteitis** inT1-FS-Gd*****, with typical appearance and localized in specific anatomical sites (i.e. subchondral or periarticular bone marrow).
    - **synovitis, enthesitis or active capsulitis,** without the previous ones is not considered sufficient
  - **presence of structural lesions:**
    - **adipose metaplasia, sclerosis, erosions or ankylosis** reflect previous inflammation, but are not sufficient in the absence of inflammatory lesions

The presence of a single area is required in two consecutive slides, or more areas in a single slide.

- **<u>for the positivity of spondylitis:</u>**
  - **presence of active inflammatory lesions:**
    - **anterior and posterior spondylitis** (inflammatory lesions at vertebral **corners**) are typical lesions of the axSpA, when present in at least three sites
    - **spondylodiscitis** (inflammatory lesions of the vertebral plates) are considered less specific
    - lesions of the **interapophyseal** and **costovertebral joints** are specific, but less standardized
  - **presence of structural lesions:**
    - **adipose metaplasia, sclerosis, erosions or ankylosis**, with localization in multiple sites, particularly in younger subjects with axSpA.
    - **erosions, syndesmophytes and ankylosis**, are not yet standardized

*Short-Tau-Inversion-Recovery sequence
***** T1-wighted fat saturated post-Gadolinium sequence

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PURPOSE OF THE THESIS

AxSpA which mainly affects the spine and the SIJ, has an early onset at a young age and can be further subdivided between nr-axSpA and radiographic axSpA, the latter also known as AS [**31**]. If undiagnosed and untreated, axSpA may lead to permanent damage and lifelong disability [**31,32,103**]. Significant steps forward have been taken in the management of axSpA in clinical practice after the discovery and spread of the use of anti-tumor necrosis factor α (TNFα) drugs. It has been demonstrated that axSpA patients respond quickly to anti-TNFα drugs, which appear to be very effective. Therefore, identifying early stages of these disorders could be a benefit for patients, whom could be prescribed appropriate treatment [**2,3**]. The Assessment of SpondyloArthritis International Society (ASAS) has established classification criteria to identify patients with early stages of axSpA [**31**]; the imaging arm of the criteria requires the presence of sacroiliitis on magnetic resonance imaging (MRI) or radiographs in addition to one SpA feature for patients with chronic low back pain with onset at age ≤45 years. Conventional radiographs of SIJ, which are still frequently used to detect sacroiliitis, do not appear to provide adequate information to classify patients with suspected early axSpA [**9**]. This is due to the fact that they are able to detect only structural damage, which is indicative of a more advanced disease stage [**9,31**]. Hence, MRI constitutes an important additional screening option since it can detect inflammatory lesions of SIJ in patients with early-onset axSpA without evidence of radiographic sacroiliitis, and thus before bone damage begins to be visible [**35**]; in spite of this, MRI is often prescribed only to confirm a rheumatologist's suspicion of axSpA as it is an expensive, relatively time-consuming test, which is not readily available at all health care centers [**35**]. Positive SIJ MRI scans were defined by the ASAS/Outcome Measures in Rheumatology MRI working group (ASAS/OMERACT) as the presence of inflammatory lesions such as BME which is highly suggestive of SpA [**35**]. Whether structural SIJ lesions should be added to this definition and if structural and inflammatory spinal lesions could contribute to

detecting axSpA remains to be established [**36**]. Inflammatory spinal lesions on MRIs may nevertheless occur in the absence of SIJ involvement [**192**]. Spinal MRIs in AS patients have uncovered abnormalities in this district even before they are noted on plain radiographs [**45**]. The introduction of fat-suppression sequences has allowed the visualization of lesions within bone marrow that may be obscured on MRI by marrow fat. These lesions include BME adjacent to vertebral endplates at the attachment of the annulus fibrosus to the vertebral rim and at the insertion of anterior and posterior longitudinal ligaments, both within the facet joints. Since there is evidence that spondylitis may also occur prior to or even without sacroiliitis, it was considered important to define the characteristics of a spinal MRI considered positive for inflammation. The ASAS/OMERACT working group thus set out to define spinal MRIs positive for inflammatory lesions (spondylitis) and structural changes (fat deposition) [**36**]. It is also unknown whether the localization of lesions is correlated to the site of axial pain. Imaging of the thoracic spine, which is often involved in axSpA, has not yet been taken into consideration when structural damage is being evaluated [**180-182**]. Since patients usually display a wide variety of clinical features and there is no standardized laboratory test protocol, a diagnosis of axSpA is rarely straightforward. As a result of the growing awareness of the impact of chronic back pain in axSpA patients, and in view of recent breakthroughs in genetics research and the development of novel treatments with a potentially positive impact on early disease stages, recent studies have been focusing on the use of MRI and the role of new biomarkers that could facilitate early diagnosis and identify those individuals at higher risk for poor prognosis [**86-89**]. As early treatment can reduce the disease burden of axSpA patients and disease-related costs, uncovering biomarkers that can facilitate early diagnosis of axSpA has become an urgent undertaking.

The our aims are the following:

1) To evaluate patients with early low back pain (> 3 months and ≤ 2 years) and to study whether patients presenting with back pain of short duration can be diagnosed as early axial SpA according to ASAS classification

criteria;

2) Evaluate the predictive parameters for the diagnosis of early axSpA and the role of imaging methods (standard plain radiography and MRI of the spine and of the pelvis) in the diagnostic process in the forms with or without SIJ involvement;
3) To study the additional value information of imaging (plain radiography and MRI) to the other parameters in making a diagnosis of SpA;
4) To define the characteristics and prevalence of inflammatory and structural on MRI of the spine and of the pelvis in patients with early axSpA;
5) To investigate how MRI features evolve over time and how they relate to radiographic damage;
6) To evaluate if the localization of axial pain correlates with the site of the inflammatory and structural lesions on MRI;
7) To analyze the correlation of radiographic and MRI lesions with the clinical, serological, functional and disease activity indices;
8) To evaluate the expression of some serological biomarkers (hsCRP, ESR, MMP3, IL-17, IL-22, IL-23) as possible predictive indicators of severity and / or disease activity or as possible indicators of bone remodeling in patients with axSpA;
9) To evaluate the biomarkers in relation to the different axial involvement (presence or absence of sacroiliitis) and to other clinical, functional and disease activity indices;
10) To identify the subset of patients with a more active and severe disease pattern and with a higher probability of radiological progression with severe structural bone damage;
11) To evaluate the response to early treatment in terms of change of clinical, serological, functional, disease activity indices in patients with low back pain for a follow-up of 24 months.

MATERIALS AND METHODS

Patients who were at least 16 years old, suffering by inflammatory low back pain (LBP) (≥3 months, ≤2 years, onset < 45 years) of unknown origin and referred to a rheumatologist were included in this on-going observational cohort study. Eligible patients underwent physical examinations, laboratory tests, following a standardized protocol at baseline (T0), at 6 months (T6), at 12 months (T12) and 24 months (T24). The patients also completed questionnaires on disease activity, physical functioning, pain, and disease-related impairment. SIJ and spinal plain radiographs and MRIs were performed at T0 and once year. Axial pain and MRI lesions were localized in 4 sites: in the cervical/thoracic/lumbar spine and the SIJ. Two experienced rheumatologist formed the diagnosis of axSpA according ASAS 2009 criteria [**31**]. In order to meet the ASAS criteria [**31**], it was necessary to verify if the patients had MRI evidence of active inflammatory lesions of the SIJ with definite BME which is highly suggestive of sacroiliitis. After the X-rays and MRI images were read, the patients were divided into three cohorts: those fulfilled the imaging arm of ASAS axSpA criteria (**axSpA imaging arm+**), those fulfilled the clinical arm of ASAS axSpA criteria (**axSpA clinical arm+**) and those not fulfilled completely ASAS axSpA criteria (**not full ASAS axSpA**).

The local medical ethical committee approved the study and informed consent was obtained from all patients at study inclusion.

PATIENTS

1. Subject selection

1.1. Inclusion criteria

- ✓ Male and female patients of at least 18 years
- ✓ Chronic back pain of at least 3 months with a maximum duration of 2 years with onset before the age of 45 years
- ✓ An increased risk for axSpA. This can be achieved by the following combination of features in addition to the chronic back pain.

At least one of the following features:

- HLA-B7 pos
- positive family history
- acute anterior uveitis
- inflammatory and or structural changes on the MRI of SIJ
- radiographic sacroiliitis according to the modified New York criteria

OR at least two of the following features:

- Inflammatory back pain (defined as at least 3 out of the following 4: age at onset< 40 years, insidious onset, morning stiffness and improving with exercise)
- Heel pain typical for enthesitis
- Peripheral arthritis
- Dactylitis
- Psoriasis
- IBD
- Good response to NSAIDs
- Elevated ESR and/or CRP

✓ Written informed consent

1.2 Exclusion criteria

- ✓ Patients with back pain for more than 2 years
- ✓ Patients with a painful condition not related to axSpA, which could interfere with the evaluation of disease activity i.e. Paget disease.
- ✓ Intake of conventional synthetic Disease-Modifying Anti-Rheumatic Drugs (csDMARDS) and biological Disease-Modifying Anti-Rheumatic Drugs (bDMARDS) already at the recruitment visit
- ✓ A history of alcoholism, drug abuse, psychological or other emotional problems, severe co-morbidity that are likely to invalidate informed consent or limit the ability of the subject to comply with the protocol requirements.

2. Visits

Follow-up visits

Baseline, after T6, T12, and T24 for all patients, followed by annual visits for patients fulfilling classification criteria of SpA.

METHODS

1. Physician clinical assessments:

a) ***collection of anamnestic information***, related to age, sex, characteristics of chronic inflammatory low back pain, duration and onset of axial pain, type of joint involvement (axial and / or peripheral), presence of other articular and extra-articular manifestations, presence or familiarity for arthritis and / or psoriasis, association with other rheumatological and / or non-rheumatological diseases, previous or current pharmacological therapy at the time of the visit;

b) ***Overall assessment of disease activity by the physician on an 10-point numerical rating scale (NRS)*** (0=inactive disease with no symptoms and 11=extremely active disease). The physician should consider all clinical information in assessing the patient's disease activity on the day of the visit;

c) ***Number of tender joints*** of a total 61 joints be examined. The following joints are examined by pressure for tenderness: temporo-mandibular joint (R+L), sternoclavicular joint (R+L), acromioclavicular joint (R+L), shoulder (R+L), elbow (R+L), wrist (R+L), 10 MCPs, 8 PIPs, 2 IPs of the thumb, 8 DIPs, knee (R+L), ankle (R+L), subtalar joint (R+L), midtarsal (R+L), 10 MTPs. Pain on motion will be examined for cervical spine and hip (R+L);

d) ***Number of swollen joints*** of a total 52 joints be examined. The following joints are examined for presence of swelling: sternoclavicular joint (R+L), acromioclavicular joint (R+L), shoulder (R+L), elbow (R+L), wrist (R+L), 10 MCPs, 8 PIPs, 2 IP of the thumb, 8 DIPs, knee (R+L), ankle (R+L), 10 MTPs;

e) ***Chest expansion****:* it should be measured as the difference in centimetres to the nearest 0.1 cm between full expiration and full inspiration, measured at the fourth intercostals space. The better of two tries should be recorded;

f) ***The Bath Ankylosing Spondylitis Metrology Index*** **(*BASMI*)****:** composite index formed by the set of results of cervical rotation, occipital-to-wall distance, modified Schober test, lateral flexion, intermalleolar distance (score 0-10). *Occiput-to-wall test:* heels and, if possible, the back against the wall, with the distance measured in

centimetres to the nearest 0.1 cm from the occiput to the wall during maximal effort to touch the head to the wall, without raising the chin above its usually carrying level. The better of two tries should be recorded. *Modified Schober test*: it is performed by marking a point over the spinous process of L5 (found as the first process below the projected line across the back at the level of the top of the iliac crest, although the exact point is not absolutely critical). The second point is measured 10 cm directly above the first while the patient is extending his lumbar spine in a neutral position. The patient then flexes forward as far as possible and the (now longer) distance between the two points is measured. Normally the initial 10 cm distance increases to 16 cm or more. The actual distance in centimetres measured in full flexion should be recorded. *Cervical rotation*: it is measured with a gravity action goniometer. The patient lies supine in the neutral position and the goniometer is placed centrally on the forehead. The patient is then asked to turn the head as far as possible to the right and then to the left. The better of two tries is recorded for left and right. The mean of left and right gives the final result for cervical rotation (in degrees). *Lateral spinal flexion*: it is measured by fingertip to floor distance in full lateral flexion without flexing forward or bending the knees. The patient should stand as close to the wall as possible with shoulders level. The distance between patient's middle fingertip and the floor is measured with a tape measure. The patient is asked to bend sideways without bending his knees or lifting his heels and attempting to keep his shoulders in the same place. A second reading is taken and the difference between the two is recorded. The better of two tries is recorded for left and right. The mean of left and right gives the final result for lateral spinal flexion (in cm). *Intermalleolar distance*: it is measured with the patient supine, the knees straight and the feet pointing straight up. The patient is asked to separate the legs as far as possible and the distance between the medial malleoli is measured (in cm to the nearest cm). The better of two tries should be recorded;

g) The Maastricht Ankylosing enthesitis Spondilities Score (MASES): Enthesis index score that measures the number of painful enthesis to pressure (0-13).

2. Patient assessments:

a) Pain of the spine (Visual Analogical Scale Back Pain- VAS back pain): this will be assessed by the patient on a 11-point numerical rating scale (NRS) where 0 at the left end of the scale is no pain and 10 is unbearable pain. The following question will be asked: "How severe was the pain of your spine on average during the last week?";

b) Patient assessment of disease activity (Visual Analogical Scale Disease Activity- VAS disease activity): this will be assessed by the patient by on a 11-point numerical rating scale (NRS) where 0 at the left end is inactive disease with no symptoms and 10 is extremely active disease. The following question will be asked: "How active was your disease on average last week?";

c) Severity of night pain (Visual Analogical Scale Night Pain- VAS night pain): this will be assessed by the patient by on a 11-point numerical rating scale (NRS) where 0 at the left end is inactive disease with no pain and 10 is unbearable pain. The following question will be asked: "How much pain due to your disease did you have at night on average last week?";

d) the Bath Ankylosing Spondylitis Patient Global Score (BASG1), as an assessment of the influence of disease activity on the state of health in the last week on a numerical scale from 0 to 10;

e) BASG2 (evaluation of the influence of disease activity on the state of health in the last six months on a numerical scale from 0 to 10);

f) BASFI (Bath Ankylosing Spondylitis Functional Index): validated composite questionnaire composed of 10 questions concerning the functional abilities of the patient, whose answers will be evaluated using the numerical scale NRS;

g) HAQ (Health Assessment Quality): questionnaire comprising 20 questions concerning activities of daily life and subdivided into 8 categories (the sum of the scores between 0-24 and divided by 8 gives the final score).

3. Disease activity assessments: ***a) the Ankylosing Spondylitis Disease Activity Score (ASDAS***), a composite index comprising both subjective measures and

laboratory parameters (back pain-pain of the peripheral joints-duration of morning stiffness-VAS disease activity-CRP); ***b) BASDAI (Bath Ankylosing Spondylitis Disease Activity Index):*** validated composite questionnaire composed of 6 questions concerning the activity of the disease, the answers of which will be evaluated using the numerical scale NRS.

4.Biochemical assessments:

Biochemical parameters include the erythrocyte sedimentation rate (ESR) (Westergren method in mm after 1 hour; normal range 0-15 mm/h) and C reactive protein (CRP) [normal range 0-6 mg/L] and Serum ultrasensitive C reactive protein (hsCRP, ELISA in mg/l; Research & Diagnostic Systems, Inc., expressed in mg/L, with a lower limit of detection of 0 mg/L). The following serological markers were assessed: matrix metalloproteinase MMP3, (Quantikine MMP3 R&D Systems Europe, expressed in ng/mL, with a lower limit of detection of 0 ng/mL); interleukin IL-22, IL-17 and IL-23 (R&D Systems Europe, expressed in pg/mL, with lower limit of detection respectively of 5 pg / mL, 17 pg / ml, of 20 pg/mL) using an enzyme-linked immunosorbent assay (ELISA). Bank serum, plasma, urine will be stored at -70C. DNA will also be collected. Serum for the assays was separated by centrifugation at 3000 rpm for 10 minute. All blood samples were analyzed twice using the same method.

5. Radiological assessments:

MRI assessments.

SIJ and spinal MRIs were performed at baseline using a 1.5 T scanner Magnetom Harmony, Siemens AG Medical Solutions, Munich, with phased-array surphace coil, acquiring T1-weighted turbo spin echo (T1TSE; TR 550/TE 10) and short-tau inversion recovery (STIR; TR 2500/TE60) sequences. The coronal oblique and sagittal views of the SIJ and spine were taken, with a slice thicknessof 4 mm. The images were independently analyzed by two expert radiologists trained in MRI

scoring according to the ASAS/OMERACT [**36**] definition and the SPARCC scoring system [**43,44**] for both SIJ and the spine. If the two readers scored positive, the image was scored accordingly. All readers were blinded for clinical and laboratory data, and for the results of the other imaging methods. The mean scores were calculated using those of both of the readers. Intra and inter-observer reliability was calculated.

SIJ MRIs were graded using the SPARCC scoring system for the inflammatory lesions typical of SpA: MRI is graded positive if ≥1 BME lesion highly suggestive of SpA is visible on ≥2 consecutive slices or if several BME lesions highly suggestive of SpA are visible on a single slice [**35**]. The presence of only synovitis, enthesitis, or capsulitis without BME is not sufficient for a positive reading. According to the SPARCC scoring method, the presence of an increased signal corresponding to BME lesions on SIJ-MRIs should be assessed on 6 consecutive coronal slices selected as representing the synovial compartment of the SIJ. The left and right SIJ MRIs were divided into quadrants for a total of 8 per coronal slice. Each quadrant was assessed and evaluated for the presence (scored 1) or absence (scored 0) of BME. Each coronal slice per SIJ was given an additional score of 1 for the presence of an "intense" signal and an additional score of 1 for a "deep" lesion, defined as a homogeneous, unequivocal increase in a signal 1 cm from the articular surface. The maximum possible score across 6 slices was 48 for the presence of BME, 12 for intense edema, and 12 for deep edema, for a maximum possible total score of 72 [**43**]. The presence of structural lesions on SIJ-MRIs were also evaluated.

With regard to MRI of the spine, according to the ASAS/OMERACT MRI group, BME and fatty lesions on spinal MRIs are present when they are visible on ≥2 consecutive slices, while the presence of ≥1 slice is enough for structural lesions (erosions, sindesmophytes) [**36**]. For the spine, the 6 most severely affected disco-vertebral units (DVUs) were selected and each was divided into 4 quadrants, with each quadrant assessed for the presence (scored 1) or absence (scored 0) of BME. Each quadrant was scored on 3 consecutive sagittal slices per disco-vertebral unit

(DVU), yielding a maximum possible score of 12 per DVU for BME. Each sagittal slice per DVU was given an additional score of 1 for the presence of an "intense" signal and an additional score of 1 for a "deep" lesion, defined as a homogeneous, unequivocal increase in STIR signal > 1 cm from the vertebral end plate. The maximum possible score for all 6 DVUs was 72 for the presence of BME, 18 for intense edema, and 18 for deep edema, for a maximum possible total score of 108 [**44**]. Structural lesions on spinal MRIs were also evaluated.

Radiographs assessments.

Lateral view radiographs of the cervical and lumbar spine and anterior-posterior view radiographs of the pelvis were taken. The images were obtained with a Philips vertical bucky, with a focus-film distance of 140 cm, film size of 18x43 cm. The images were read independently by two expert trained musculoskeletal radiologists, blinded for patients characteristics, clinical outcome and for the results of the other imaging methods. The mean scores were calculated using those of both of the readers. It was performed intra and inter-observer reliability. The mSASSS scoring method modified by Creemers was used [**180**]. According to this method, lateral views of the anterior vertebral corners (VCs) of the cervical (lower border of C2 to upper border of T1) and lumbar (lower border of T12 to upper border of S1) segments (a total of 24 VCs) are scored for the presence of erosions and/or sclerosis and/or squaring (1 point), syndemophyte (2 points) and bridging syndesmophytes (3 points). The total score ranges from 0 to 72 [**180**]. Evaluation of the SIJ was based on the New York criteria [**9**], with scores ranging from 0 to 4 (0=no change, 1=look slightly faded edge joint, pseudo-widening or narrowing of the rhyme, mild subchondral sclerosis, 2=irregular margin joint with images of erosion, shrinkage of rhyme, subchondral sclerosis evident, 3=erosions and subchondral sclerosis evident with initial synostosis, 4=complete ankylosis).

Treatments:

At T0 all the patients were being treated with non-steroidal anti-inflammatory drugs. All patients will be treated according to the physician assessments and the severity of the disease if the latter is diagnosed. There is no limitation on pharmacological treatments, physical therapies or other treatments.

STATISTICAL ANALYSIS

The statistics used mainly descriptive methods. Odds ratio was used to evaluate the correlation between localization of the axial pain and the position of inflammatory and structural lesions on the spinal and SIJ MRIs at the baseline evaluation (T0). The Cohen's Kappa test was used to assess the intra and inter-observational reliability of the two radiologists' evaluations. Since the variables examined are not normally distributed, the differences between the values of the T0, T6, T12 and T24 indices were evaluated using the Kruskal-Wallis nonparametric test for repeated measurements followed by the Dunn's multiple Comparison test. These tests were therefore used to compare the clinical (BASMI, MASES), bioumoral,serological (ESR, CRP, hsCRP, MMP3, IL-22, IL-17, IL-23), functional (BASFI, HAQ, BASG1, BASG2 , VAS back pain, VAS night pain, VAS disease activity) anddisease activity indices (BASDAI, ASDAS) at T0, T6, T12 and T24. The same method was used to compare the imaging scores (mSASSS, score SI mNY, SPARCC-SIJ and SPARCC-spine) at T0 and T24 in all patients and among the three cohorts (axSpA imaging arm +, axSpA clinical arm + and not full ASAS axSpA). A T0 regression analysis was performed to identify the predictors of inflammationand radiological progression of the disease evaluated using mSASSS, score SI mNY, SPARCC- SIJ and SPARCC-spine indices. The following independent variables were considered in the univariate analysis: female sex, age of CBP onset, duration of CBP, presence of HLA-B27, elevated inflammation indices, BASDAI>4, use of NSAIDs. The significant independent variables ($p<0.1$) in the univariable analysis were tested using multivariable regression models. Interactions and correlations between the variables were analyzed using the

Spearman correlation coefficient. The radiographic progression of SIJ from T0 to T24 was evaluated (defined according to the criteria formulated by DESIR study [**208**]: 1- passage from the non-radiographic form to the radiographic stage of sacroiliitis according to the mNY criteria; 2- change of> 1 degree of sacroiliitis but ignoring the passage from degree 0 to 1 of sacroiliitis), using the non-parametric Kruskal-Wallis test. The radiographic progression of the spine from T0 to T24 was evaluated according to the method provided by Poddubnyy tested in the GESPIC cohort [**209**] (increase in mSASSS score> 2 over the course of 2 years of observation). All statistical analyzes were performed using the SPSS 13.0 program (SSPS Inc, IL, USA). The values of $p < 0.05$ were considered statistically significant.

RESULTS

In this study, **75** patients diagnosed with axSpA were enrolled. According to the ASAS criteria for axSpA; **21** (28%) patients were classified as axSpA imaging arm+, **29** (38.7%) patients as axSpA clinical arm+ and **25** (33.3%) patients did not full ASAS criteria for axSpA. The average age at back pain onset was 28.51 ± 8.05 years, 45.3% were male, 38.7% had HLA-B27+. 52% (39) of patients presented an exclusive axial involvement, while 48% (36) of patients also had peripheral involvement. A high prevalence of psoriasis and heel enthesitis was observed (33.3% and 72%, respectively). Other characteristics of the patients, including the typical aspects for SpA, have been reported in **Table IX**. Out of all 75 patients, 37.3%, 56%, 100% and 64%, respectively, complained of cervical / thoracic / lumbar / buttock pain. The clinical (MASES and BASMI), disease activity (BASDAI, ASDAS), functional (HAQ, BASFI, VAS back pain, VAS pain night, VAS disease activity, BASG1, BASG2) and serological indices (ESR, CRP, hsCRP, MMP3, IL17, IL22, IL23) were analyzed and measured over time at T0, T6, T12 and T24. **Out of all 75 patients, 68 (90.7%) were evaluated at T6, of these 59 (78.7%) patients at T12, of these 54 (72%) at T24.**

Table IX: Baseline characteristics of patients with LBP (n=75)	
Age of onset LBP, mean (±SD)	28.51 (±8.05)
Male, n (%)	34 (45.3%)
Duration (months) di LBP, mean (±SD)	13.37 (±6.14)
Only axial involvement, n (%)	39 (52%)
Axial and peripheral involvment, n(%)	36 (48%)
HLA-B27 positive, n (%)	29 (38.7%)
Positive family history of SpA, n (%)	35 (46.7%)
IBP, n (%)	75 (100%)
Peripheral arthritis, n (%)	34 (45.3%)
Psoriasis, n (%)	25 (33.3%)
Dactylitis, n (%)	15 (20%)
Heel enthesitis, n (%)	54 (72%)
Uveitis, n (%)	7 (9.3%)
IBD, n (%)	9 (12%)
Preceding infections, n (%)†	4 (5.3%)
Good response to NSAIDs, n (%)	73 (97.3%)
Elevated CRP/ESR, n (%)	42 (56%)
Cervical pain, n (%)	28 (37.3%)
Thoracic pain, n(%)	42 (56%)
Buttock pain, n (%)	48 (64%)
Alternating buttock pain, n (%)	37 (49.3%)
Morning stiffness, n (%)	57 (76%)
Night pain, n(%)	71 (94.7%)
Sacroiliitis MRI *, n (%)	46 (61.3%)
Sacroiliitis x-ray **, n (%)	25 (33.3%)
Weight (kg), mean (±SD)	70.22 (16.15)
Height (cm), mean (±SD)	170.6 (8.67)

HLA-B27, Human Leukocyte Antigen; LBP, low back pain; IBP, Inflammatory Back Pain; IBD Inflammatory Bowel Disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MRI, Magnetic Resonance Imaging; SpA, Spondyloarthritis; NSAID, nonsteroidal antiinflammatory drugs

* Sacroiliitis on MRI according ASAS/EULAR criteria

** Sacroiliitis on X-Rays according modified New York criteria (0-4)

† Balanitis, Urethritis or Cervicitis

a) The prevalence of the inflammatory and structural lesions on pelvis and spine MRI in patients with LBP at T0.

All spine and SIJ on MRI images were evaluated by two expert readers and the inter-observer reliability was good to moderate (kappa 0.73 for inflammatory lesions and 0.60 for structural lesions on spine MRI;kappa 0.78 for inflammatory lesions and 0.61 for structural lesions on SIJ MRI respectively). The inter-observer reliability for all X rays images was good (kappa 0.79 for spine radiological lesions and kappa 0.77 for SIJ radiological lesions). The intra-observer reliability was good for all spine and SIJ images on X-rays and MRI (respectively kappa 0.79 for spine and 0.83 for SIJ). Fifty-two (69.3%) patients presented structural and/or inflammatory lesions on SIJ-MRIs at T0 (on the right SIJ in 63% of the patients and on left one in 59% of the patients). BME lesions were present in 46 (61.3%) patients (58% on the right SIJ and 50% on the left one). Structural lesions on SIJ-MRI were present in 33 (44%) patients (37% on the right SIJ and 30% on the left one). Fifty (66.7%) patients presented inflammatory and/or structural lesions on the spinal MRI at T0. BME lesions at the anterior corner of the spine were present in 42 (56%) patients (19%, 39% and 33%, respectively, in the cervical/thoracic/lumbar regions). Structural spine lesions were present in 28 (37.3%) patients (18%, 14%, 19%, in the cervical/thoracic/lumbar regions respectively,) (**see Figures 5a-b**). Signs of enthesitis were found in 47 (62.7%) patients: at the level of the cervical spine in 8% of patients, of the thoracic spine in 58% of patients, at the lumbar spine in 11% of patients. At T0 18 (8 patients of axSpA clinical arm+ cohort and 10 patients of not full ASAS axSpA cohort) patients with inflammatory lesions on spinal MRIs showed no abnormalities in SIJ, while 11 (14.7%) patients without active sacroiliitis on SIJ MRIs did not present spine MRIs lesions (**Table X**).

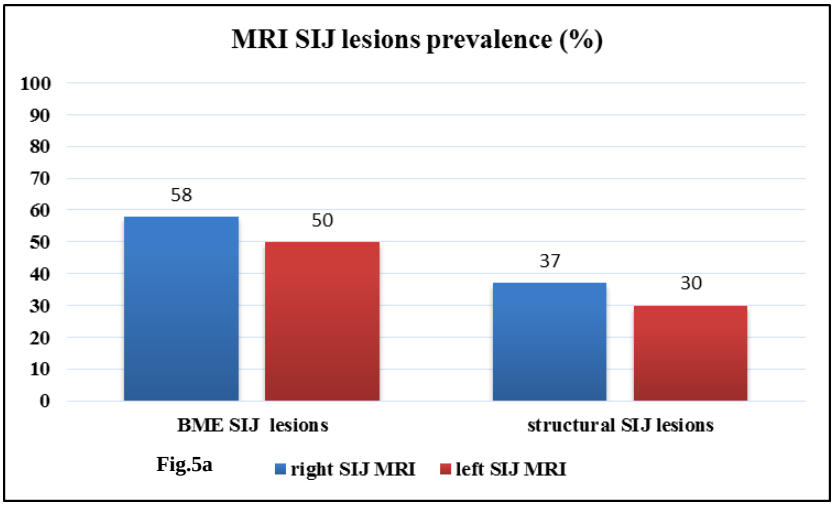


Fig.5a

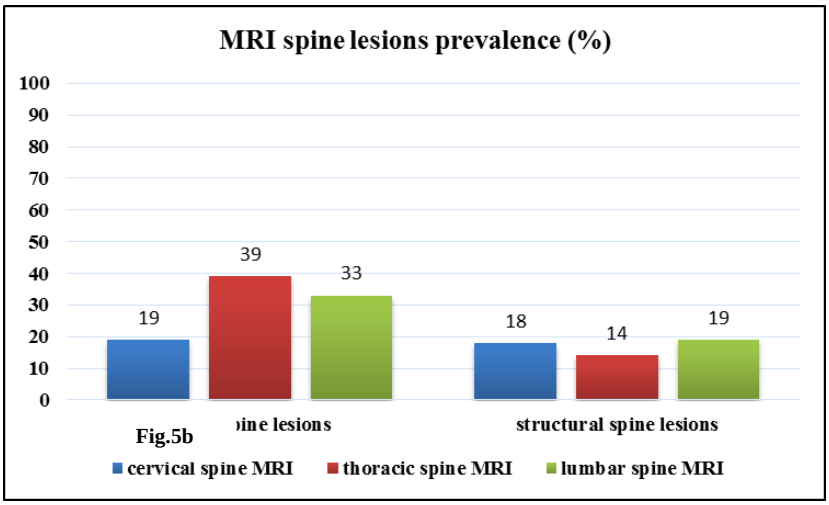


Fig.5b

Figures 5 a-b: Prevalence in percentage (%) of MRI lesions for single articular district at T0.

Table X: Prevalence of inflammatory (BME) lesions on MRI at T0, T12 and T24.

INFLAMMATORY LESIONS (BME)	T0	T12	T24
MRI SPINE +/ MRI SIJ +	29 (38.7%)	24 (40.7%)	26 (48.1%)
MRI SPINE +/ MRI SIJ-	18 (24%)	13 (22.1%)	5 (9.3%)
MRI SPINE -/ MRI SIJ +	17 (22.7%)	12 (20.3%)	9 (16.7%)
MRI SPINE -/ MRI SIJ -	11 (14.7%)	10 (16.9%)	14 (25.9%)
total patients (=n / %)	**75**	**59**	**54**

b) Association between localization of axial pain and the site of BME and structural lesions at MRI at T0.

The OR between the pain site and the corresponding localization of BME lesions were 20.78 (CI: 0.39-11.05; p=NS), 163.93 (CI: 3.31-81.28; p=0.0006); 0.34 (CI: 0.01-17.91; p=NS) for cervical / thoracic / lumbar spine and 304.88 (CI: 1.71-546.56; p=0.0203) for SIJ. Pain was not significantly associated with structural lesions on MRI at the same site, except for the correlation between buttock pain and structural lesions on SIJ MRI (OR=70.1, CI: 0.84-58.40; p=0.0122). Moreover, the association between the axial pain site and the localization of enthesitis evaluated by OR showed a significant association between thoracic pain and enthesitis of the thoracic district (OR=32.69; CI: 1.096-9.748; p=0.0336).

c) The prevalence and the charateristics of MRI lesions in the three cohorts at T0 and T24.

The prevalence of inflammatory and structural MRI lesions at T0 in the three cohorts (**axSpA imaging arm+, axSpA clinical arm+ and not full ASAS axSpA**) is outlined in **Table XI**. As it could be expected, an increased prevalence of structural lesions on SIJ MRI was found in the axSpA imaging arm+ patients with respect to the other two cohorts in accordance with a greater presence of sacroiliitis involvement on standard X-rays in this cohort. AxSpA imaging arm+ and axSpA clinical arm+ patients had more inflammatory and structural spinal lesions with respect to the not full ASAS axSpA patients.

Table XI. The prevalence of inflammatory and structural lesions at T0 and T24 in three cohorts (axSpA imaging arm +, axSpA clinical arm +, not full ASAS axSpA).

	T0 MRI evaluation		
	axSpA imaging arm+	**axSpA clinical arm+**	**Not full ASAS axSpA**
Total number of patients	**21 (28%)**	**29 (38.7%)**	**25 (33.3%)**
SIJ total lesions	21 (100%)	27 (93.1%)	4 (16%)
BMO lesions	21 (100%)	25 (86.2%)	0 (0%)
sclerosis lesions	10 (47.6%)	14 (48.28%)	0 (0%)
fatty lesions	7 (33.3%)	2 (6.9%)	4 (16%)
erosions lesions	8 (38.1%)	3 (10.3%)	0 (0%)
Spine total lesions	20 (95.2%)	19 (65.5%)	11 (44%)
BMO lesions	18 (85.7%)	15 (51.7%)	9 (36%)
enthesitis lesions	17 (80.9%)	19 (65.5%)	11 (44%)
fatty lesions	6 (28.6%)	7 (24.1%)	4 (16%)
sclerosis/syndesmophytes lesions	6 (28.6%)	6 (20.7%)	3 (12%)
erosions lesions	2 (9.5%)	2 (6.9%)	1 (4%)
	T24 MRI evaluation		
	axSpA imaging arm+	**axSpA clinical arm+**	**Not full ASAS axSpA**
Total number of patients	**16 (29.6%)**	**22 (40.7%)**	**16 (29.6%)**
SIJ total lesions	16 (100%)	15 (68.2%)	1 (6.3%)
BMO lesions	9 (56.3%)	13 (59.1%)	0 (0%)
sclerosis lesions	7 (43.8%)	10 (45.5%)	0 (0%)
fatty lesions	6 (37.5%)	4 (18.2%)	1 (6.3%)
erosions lesions	3 (18.8%)	2 (9.1%)	0 (0%)
Spine total lesions	11 (68.8%)	4 (18.2%)	7 (43.8%)
BMO lesions	7 (43.8%)	5 (22.7%)	5 (31.3%)
enthesitis lesions	7 (43.8%)	6 (27.3%)	7 (43.8%)
fatty lesions	6 (37.5%)	4 (18.2%)	4 (25%)
sclerosis/syndesmophytes lesions	7 (43.8%)	3 (13.6%)	2 (12.5%)
erosions lesions	1 (6.3%)	2 (9.1%)	0 (0%)

d) Correlation analysis using Spearman tests between clinical, serological, disease activity indices and radiological scores in 75 patients with LBP at T0.

Spearman analysis was conducted at T0 across all parameters and as expected, significant correlations were found among the clinical indices (BASFI, BASG1, BASG2, VAS) and between clinical and disease activity indices (BASDAI, ASDAS) (data not shown). The correlations between the clinical indices, MMP3 and IL-22 are reported in **Table XII**, while in **Table XIII** correlations between ESR and hsCRP and clinical indices are shown. Of these, the most significant are also represented in the figures **(Figures 6 a-f).**

Table XII: Correlations between MMP-3, IL-22 and clinical indices and imaging scores.

	MMP3	**IL-22**
ESR	0.047	ns
hsCRP	0.026	ns
mSASSS	0.005	ns
SPARCC-SIJ	ns	0.046
BASMI	ns	0.038
BASFI	ns	0.008
BASG1	ns	0.008
HAQ	ns	0.041
MMP3	ns	0.017

P was calculated according Spearman's correlation test. The coefficient is positive unless otherwise stated.

Table XIII. Correlations between hsCRP, ESR and clinical indices and imaging scores.

	hsCRP	**ESR**
BASG1	ns	0.027
BASG2	ns	0.044
ASDAS	ns	0.025
SPARCC-SIJ	0.043	ns
mSASSS	ns	0.033

P was calculated according Spearman's correlation test. The coefficient is positive unless otherwise stated.

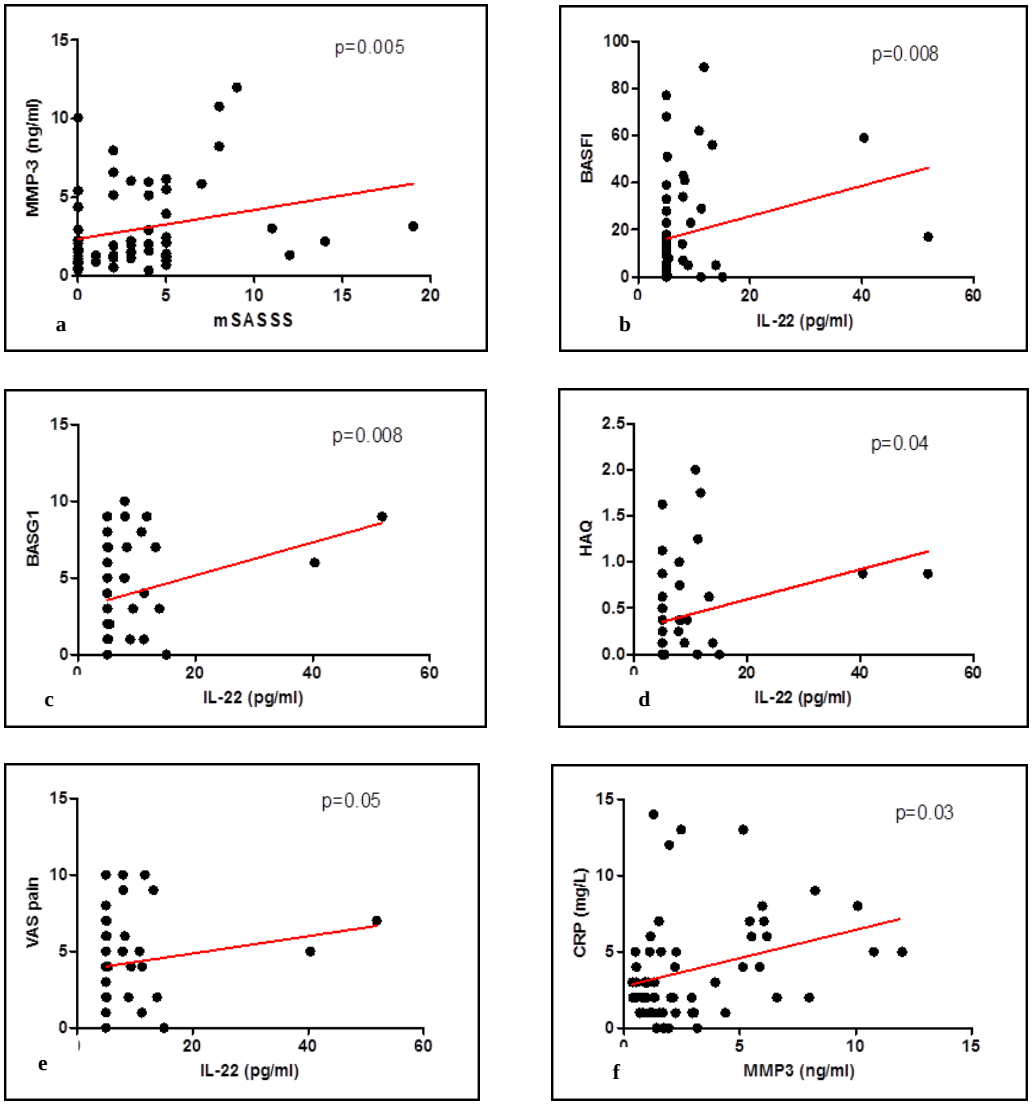


Figure 6 a-f: Spearman test: $p<0.05$

Legend

Correlations between serological, clinical and disease activity indices in patients with early axSpA at baseline according to the Spearman correlation test ($p<0.05$)

(a) mSASSS and MMP3; (b) IL-22 and BASFI; (c) IL-22 and BASG1; (d) IL-22 and HAQ; (e) IL-22 and VAS pain; (f) MMP3 and CRP.

ESR, erytrocyte sedimentation rate; CRP, C-reactive protein; MMP3 matrix-metallo-proteinase 3; IL, Interleukine; BASFI: Bath Ankylosing Spondylitis Functional Index, HAQ: Health Assessment Questionnaire; BASG1,Bath Ankylosing Spondylitis Patient Global Score 1; VAS, Visual Analogue Scale.

e) Analysis using Kruskal-Wallis test of clinical, serological, disease activity, imaging indices in total patients and in the following 3 cohorts (axSpA imaging arm+, axSpA clinical arm+, not full ASAS axSpA) at T0, T6, T12 and T24.

Table XIV shows all the disease activity indices and imaging scores of the all patients and the three cohorts at T0. Using the Kruskal-Wallis test, a significant difference was observed between the three cohorts with regard to the percentage of radiographic sacroiliitis, of sacroiliitis at MRI and of radiographic SIJ scores according to the mNY and SPARCC-SI score criteria, but not as for the remaining clinical, functional, disease activity and serological parameters. In **Tables XV and XVI** the indices (values expressed as mean and standard deviation-SD) are reported for both total patients and the 3 cohorts (at T0, T6, T12 and T24), analyzed by Kruskal-Wallis test. Considering the total 75 patients, the comparison between the various timepoint highlighted a significant decrease of the following parameters values from T0 to T24: MASES ($p=0.008$), BASG1 ($p=0.02$), BASG2 ($p<0.0001$), HAQ ($p=0.0002$), VASpain ($p=0.01$), VAS pain night ($p=0.04$), VAS disease activity ($p=0.05$), BASFI ($p=0.02$), ESR ($p=0.04$), BASDAI ($p<0.0001$), ASDAS ($p<0.0001$). On the other hand, BASMI and CRP did not decrease significantly. There were no differences in the serological markers (ILs, MMP3 and hsCRP) values during the 2 years follow up period in the three groups. The values of disease activity parameters (ASDAS and BASDAI) are also shown in **Figures 7 a, b**. Consideringthe patients subdivided in the 3 cohortsa downward trend for all functional indices (HAQ, BASFI, BASG1, BASG2, VAS back pain, VAS night pain and VAS disease activity) and disease activity indices (BASDAI, ASDAS) could be observed, which in some cases was statistically significant (see **Table XV and Table XVI**), but there was no more markedly significant decrease of these indices in one cohort with respect to the others.

Table XIV: Serological, clinical, disease activity and imaging score indices at T0 in the whole group of patients (n=75) and in the three cohorts (axSpA Imaging Arm+, axSpA Clinical Arm+, not full ASAS axSpA).

Serological, clinical, disease activity and imaging score indices	Cohort 1 axSpA Imaging Arm +, n=21	Cohort 2 axSpA Clinical Arm +, n=29	Cohort 3 not full ASAS axSpA, n=25	p§	total=75 pts
ESR (mm/h), mean (SD)	17.52 (12.98)	15.14 (11.76)	21.68 (21.19)	ns	17.99 (15.85)
CRP (mg/L), mean (SD)	4.81 (3.61)	3.17 (3.32)	4.24 (3.28)	ns	3.98 (3.42)
hs-CRP (mg/L), mean (SD)	2.54 (2.79)	1.56 (1.71)	2.76 (3.76)	ns	2.11 (2.50)
MMP3 (ng/L), mean (SD)	3.04 (2.69)	3.51 (3.12)	2.47 (2.68)	ns	3.01 (2.80)
IL-22 (pg/mL), mean (SD)	6.44 (3.52)	5.91 (2.45)	9.83 (8.54)	ns	12.58 (11.69)
IL-17 (pg/mL), mean (SD)	3 (3.12)	3 (3.11)	3 (3.97)	ns	3 (4.11)
IL-23 (pg/mL), mean (SD)	29.3 (31.2)	21.87 (5.21)	20.69 (21.56)	ns	71.53 (60.56)
HLA-B27, n (%)	0 (0)	29 (38.7%)	0 (0)	ns	29 (38.7)
BASMI, mean (SD)	1 (1.18)	0.76 (1.02)	0.92 (1.08)	ns	0.88 (1.08)
MASES, mean (SD)	3.33 (2.65)	3.41 (2.35)	3.56 (2.47)	ns	3.44 (2.45)
BASFI, mean (SD)	17.43 (20.21)	13.68 (15.14)	22.44 (25.75)	ns	17.65 (20.64)
HAQ, media (DS)	0.34 (0.53)	0.30 (0.38)	0.52 (0.55)	ns	0.39 (0.49)
BASG1, media (DS)	3.43 (2.79)	3.52 (2.77)	4.64 (3.35)	ns	3.87 (2.99)
BASG2, media (DS)	5.71 (3.02)	5.10 (2.77)	5.28 (2.94)	ns	5.33 (2.87)
VAS pain, mean (SD)	3.48 (2.82)	4.10 (2.97)	5.08 (3.23)	ns	4.25 (3.05)
VAS disease activity, mean (SD)	3.81 (3.03)	4.07 (3.15)	5.04 (3.35)	ns	4.32 (2.18)
VAS pain night, mean (SD)	3.24 (3.33)	4.17 (3.57)	4.20 (3.33)	ns	3.92 (3.40)
BASDAI, mean (SD)	39.50 (25.99)	44.27 (25.03)	53.48 (24.97)	ns	46.01 (25.07)
ASDAS, mean (SD)	2.29 (0.86)	2.61 (0.56)	2.66 (0.88)	ns	2.54 (0.77)
Sacroiliitis x-ray **, n(%)	11 (52.4)	14 (48.3)	0 (0)	<0.001	25 (33.3)
Sacroiilitis MRI *, n(%)	21 (100)	25 (86.2%)	0 (0)	<0.001	46 (61.3)
mSASSS, mean (SD)	3.57 (4.25)	2.14 (2.84)	2.48 (3.82)	ns	2.65 (3.61)
score SIJ mNY*, mean (SD)	0.61 (0.67)	0.79 (0.98)	0 (0)	<0.01	0.55 (0.78)
SPARCC-spine, mean (SD)	6.52 (14.28)	6.45 (10.98)	2.32 (4.31)	ns	5.09 (10.52)
SPARCC-SIJ, mean (SD)	12.95 (8.23)	14.45 (15.11)	0 (0)	<0.001	9.21 (12.28)

ESR, erytrocyte sedimentation rate; CRP, C-reactive protein; hs-CRP, high sensitive C-reactive protein; MMP3 matrix-metallo-proteinase 3; IL, Interleukine; HLA-B27, Human Leukocyte Antigen; BASMI, Bath Ankylosing Score Metrology Index; MASES, Maastricht Ankylosing Spondilities Enthesitis Score; BASFI: Bath Ankylosing Spondylitis Functional Index, HAQ: Health Assessment Questionnaire; BASG1,Bath Ankylosing Spondylitis Patient Global Score 1; BASG2, Bath Ankylosing Spondylitis Patient Global Score 2;BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, VAS, Visual Analogue Scale; ASDAS, Ankylosing Spondylitis Disease Activity Score; SPARCC, Spondyloarthritis Research Consortium of Canada; mSASSS, modifiedStokeAnkylosingSpondylitis Spine Score, SIJ, sacroiliac joints.
* Sacroiilitis MRI according ASAS/EULAR; ** Sacroiliitis x-ray according New York criteria; p§ Anova (Kruskal Wallis) a t0: $p<0.05$; SD=deviation standard.

Table XV: Clinical and functional indices values from T0 to T24 in the whole group of patients (n=75) and in three cohorts (axSpA Imaging Arm+, axSpA Clinical Arm+, not full ASAS axSpA).

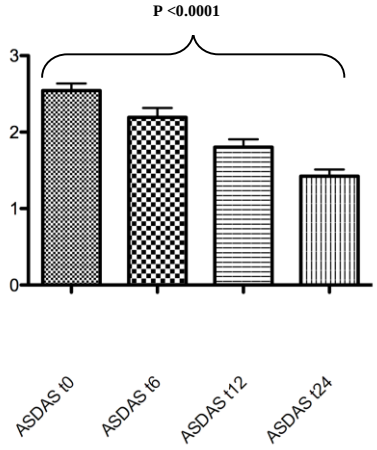
		BASMI	MASES	BASFI	HAQ	BASG1	BASG2	VAS pain	VAS dis act	VAS pain N
axSpA imaging arm+	T0	1 (1.18)	3.33 (2.65)	17.43 (20.21)	0.34 (0.53)	3.43 (2.79)	5.71 (3.02)	3.48 (2.82)	3.81 (3.03)	3.24 (3.33)
	T6	0.80 (1.15)	3.30 (2.85)	17.95 (22.52)	0.21 (036)	3.15 (2.87)	4.80 (2.48)	3.40 (2.72)	3.35(2.91)	2.90 (2.92)
	T12	0.39 (0.78)	3.06 (2.98)	12.67 (12.82)	0.15 (0.31)	2.01(2.03)	3.71 (2.44)	3.47 (2.61)	3.18 (2.74)	2.06 (2.34)
	T24	0.44 (0.63)	2.06 (2.11)*	9.25 (8.12)**	0.14 (0.38)**	2.29 (2.23)*	2.71 (2.64)**	1.75 (1.77)*	1.81 (1.71)*	1.56 (1.93)*
axSpA clinical arm +	T0	0.76 (1.02)	3.41 (2.35)	13.680 (15.14)	0.30 (0.38)	3.52 (2.77)	5.10 (2.77)	4.10 (2.97)	4.07 (3.15)	4.17 (3,57)
	T6	0.54 (0.86)	2.58 (2.39)	15.85 (20.65)	0.21 (0.37)	2.73(2.54)	3.84 (2.31)	3.08 (2.64)	3.19 (2.80)	2.62 (2.70)
	T12	0.43 (0.79)	2.39 (2.90)	11.04 (15.63)	0.10 (0.25)	2.67 (2.33)	3.33 (2.12)	2.41 (2.04)*	2.92 (2.22)	2.25 (2.26)*
	T24	0.50 (0.86)	2.09 (2.37)*	10.27 (14.29)*	0.10 (0.23)*	1.50 (1.87)**	1.91 (1.98)**	2.23 (2.20)*	2.18 (2.32)*	2.36 (2.32)*
not full ASAS axSpA	T0	0.92 (1.08)	3.56 (2.47)	22.44 (25.75)	0.52 (0.55)	4.64 (3.35)	5.28 (2.94)	5.08 (3.23)	5.04 (3.35)	4.20 (3.33)
	T6	0.96 (1.22)	3.43 (2.59)	17.52 (21.71)	0.34 (0.41)	4.26 (3.01)	4.82 (3.52)	4.52 (3.36)	4.30 (3.21)	3.48 (3.17)
	T12	0.65 (0.81)	2.40 (2.14)	12.38 (14.63)*	0.20 (0.29)	3.35 (2.69)	3.18 (2.46)	3.58 (3.17)	4.70 (3.16)	3.10 (3.01)
	T24	0.50 (0.73)	2.40 (2.14)*	12.84 (12.63)**	0.15 (0.22)**	3.33 (2.19)*	3.67 (2,32)*	2.81 (2.32)**	2.81 (2.26)*	3.00 (2.59)*
Total patients with IBP, n=75	T0	0.88 (1.08)	3.44 (2.45)	17.65 (20.64)	0.39 (0.49)	3.87 (2.99)	5.33 (2.87)	4.25 (3.05)	4.32 (2.18)	3.92 (3.40)
	T6	0.75 (1.08)	3.07 (2.59)	17.01 (21.26)	0,25 (0,38)	3.36 (2.83)	4.45 (2.82)	3.65 (2.95)	3.61 (2.97)	2.98 (2.91)
	T12	0.49 (0.79)	2.59 (2.67)	11.95 (14.30)*	0,16 (0,30)**	2.67 (2.38) *	3.40 (2.29)**	3.08 (2.61)	3.57 (2.77)	2.47 (2.54)
	T24	0.48 (0.75)	2.12 (2.13)**	10.73 (12.12)**	0,14 (0,29)***	2.25 (2.17) **	2.63 (2.24)***	2.26 (2.12)**	2.26 (2.14)**	2.28 (2.30)**

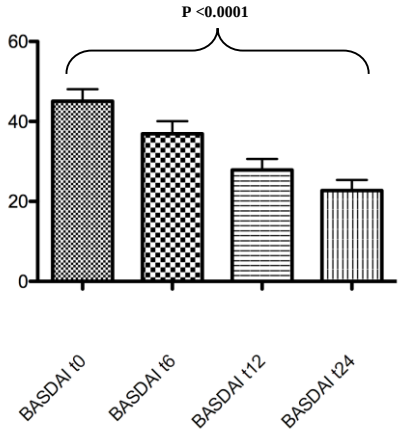
Data are expressed as mean ± SD. The test Kruskal Wallis repeated measures test and Dunn's multiple comparison test were used * $p<0.0001$ vs t0, ** $p<0.001$ vs T0, *** $p<0.01$ vs t0.
BASMI, Bath Ankylosing Score Metrology Index; MASES, Maastricht Ankylosing Spondilities Enthesitis Score; BASFI, Bath Ankylosing Spondylitis Functional Index; HAQ: Health Assessment Questionnaire; BASG1,Bath Ankylosing Spondylitis Patient Global Score 1; BASG2, Bath Ankylosing Spondylitis Patient Global Score 2; VAS pain, Visual Analogue Scale pain; VAS dis act, Visual Analogue Scale Disease Activity; VAS pain N, Visual Analogue Scale pain night.

Table XVI: Serological and disease activity indices values from T0 to T24 in the whole group of patients (n=75) and in three cohorts (axSpA Imaging Arm+, axSpA Clinical Arm+, not fullASAS axSpA).

		ESR	CRP	hs CRP	MMP3	IL-17	IL-22	IL-23	BASDAI	ASDAS
axSpA imaging arm+	T0	17.52 (12.98)	4.81 (3.61)	2.54 (2.79)	3.04 (2.69)	3 (3.12)	6.44 (3.52)	29.3 (31.2)	39.50 (25.99)	2.29 (0.86)
	T6	13.55(11.59)	3.04 (2.01)	2.34 (2.23)	3.54 (2.11)	3 (3.08)	6.12 (3.92)	28.5 (38.6)	29.17 (26.19)	2.14 (0.98)
	T12	15.06 (10.23)	4.06 (2.98)	2.26(3.21)	3.43 (2.80)	3 (2.89)	5.57 (5.23)	20(21.7)	29.06 (26.71)*	1.78 (0.87)*
	T24	11.13(5.73)**	3.13 (1.31)	1.44 (1.28)	1.89(1.02) *	3 (2.77)	5.1 (5.08)	20 (20.5)*	18.72 (18.25)**	1.36 (0.56)**
axSpA clinical arm +	T0	15.14 (11.76)	3.17 (3.32)	1.96 (1.61)	3.41 (3.35)	3 (3.19)	5.34 (2.71)	21.87 (5.21)	44.27 (25.03)	2.61 (0.56)
	T6	14.88 (9.27)	3.12 (2.19)	1.56 (1.71)	3.51 (3.12)	3 (3.11)	5.91 (2.45)	26.93 (6.46)	35.11 (24.70)	1.95 (0.79)
	T12	12.73 (9.39)	4.05 (6.31)	2.92 (3.79)	2.12(1.82)	3 (3.03)	6.37 (1.63)	20 (21.2)	25.48 (17.78)**	1.59 (0.54)*
	T24	10.86 (5.54)*	3.59 (3.84)	1.65 (2.22)	1.30 (1.50)	3 (2.99)	5 (0.11)	20 (20.9)	20.73 (18.76)***	1.33 (0.69)***
not full ASAS axSpA	T0	21.68 (21.19)	4.24 (3.28)	2.76 (3.76)	2.47 (2.68)	3 (3.97)	9.83 (8.54)	20.69 (21.56)	53.48 (24.97)	2.66 (0.88)
	T6	19.73 (17.01)	6.35 (10.33)	2.85 (3.91)	2.66 (2.74)	3 (3.16)	9.56 (8.28)	21.34 (20.17)	45.66 (27.37)	2.51 (1.19)
	T12	16.06 (13.06)	4.22 (3.67)	2.94 (3.50)	1.74 (1.71)	3 (3.12)	6.71 (5.46)	20.18 (19.78)	31.26 (19.67)*	2.02 (0.99)
	T24	14.25 (10.21)**	3.63 (2.03)	1.50 (2.10)	1.11 (0.54)	3 (2.89)	5.31 (4.99)	20.12 (15.65)	24.80 (19.67)**	1.34 (0.61)*
Total patients with IBP, n=75	T0	17.99 (15.85)	3.98 (3.42)	2.11 (2.50)	3.01 (2.80)	3 (4.11)	12.58 (11.69)	21.53 (60.56)	46.01 (25.07)	2.54 (0.77)
	T6	16.09 (13.05)	4.29 (6.33)	2.75 (3.62)	2.84 (2.93)	3 (3.75)	10.43 (8.95)	21.79 (22.13)	36.90 (26.51)	2.19 (1.01)
	T12	14.48 (10.80)*	4.10 (4.62)	2.84 (3.27)	1.94 (1.91)	3 (3.10)	9.34 (5.84)	20.52 (19.87)	28.37 (21.05)**	1.79 (0.82)**
	T24	11.94 (7.30)**	3.46 (2.75)	1.90 (2.18)	1.88 (1.56)	3 (3.84)	7.37 (6.12)	20.81 (15.31)	21.34 (18.07)***	1.34 (0.61)***

Data are expressed as mean ± SD. The test Kruskal Wallis repeated measures test and Dunn's multiple comparison test were used * p<0.0001 vs t0, **p<0.001 vs T0, ***p<0.01 vs t0.
ESR, erytrocyte sedimentation rate; CRP, C-reactive protein; hs-CRP, high sensitive C-reactive protein; MMP3 matrix-metallo-proteinase 3; IL, Interleukine; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS, Ankylosing Spondylitis Disease Activity Score.





Figures 7a-7b. P calculated according to the test Kruskal -Wallis.
ASDAS, Ankylosing Spondylitis Disease Activity Score. BASDAI: Bath Ankylosing Spondylitis Disease Activity Index.

f) Regression analysis to identify the predictors of disease activity and radiological progression evaluated by mSASSS, score SIJ mNY, SPARCC-SIJ and SPARCC-spine scores at T0.

At T0 a regression analysis was performed to identify any predictors of radiological activity on MRI (measured by continuous variables such as SPARCC-SIJ and SPARCC-spine) and of radiological progression (measured by continuous variables such as mSASSS and SIJ mNY score and dichotomous variables such as presence/absence of structural lesions on X rays of SIJ and spine). The following independent variables of activity and radiological progression were considered in the analysis: female sex, age of LBP onset, duration of LBP, presence of HLA-B27, increased inflammation indices, BASDAI> 4, use of NSAIDs. As it can be seen in the results reported in **Tables from XVII to XXI**, the multivariate analysis allowed to identify as possible independent predictors of radiological progression measured in the presence of structural lesions to the column: an early onset of IBP onset, a lower use of NSAIDs, a BASDAI>4. For the radiological progression measured by the presence of structural SIJ lesions, the only independent predictor was a high SPARCC SIJ score. An higher mSASSS score was independently predicted by a lower age of onset of IBP at multivariate analysis. Predictive factors of increased radiological activity were, respectively, for a higher SPARCC-spine score, a higher use of NSAIDs and for a higher SPARCC-SIJ score, the presence of HLA-B27 and increased serological inflammatory markers (ESR and CRP).

Table XVII. Predictors factors of mSASSS score at T0

Independent Variables	Univariate analysis		Multivariable analysis	
	coeff (IC)	p	coeff (IC)	p
Female sex	-0.80 (-2.55, 0.93)	0.359	-	-
LBP onset age	**0.21 (0.11, 0.30)**	**0.000**	**0.19 (0.09, 0.29)**	**0.000**
Duration of LBP	0.02 (-0.12, 0.16)	0.770	-	-
HLA-B27+	-0.72 (-2.5, 1.08)	0.428	-	-
Increased ESR/CRP	0.97 (-0.80, 2.75)	0.278	-	-
BASDAI>=4	-0.48 (-2.22, 1.26)	0.583	-	-
Use of NSAIDs	2.63 (-0.22, 5.48)	0.070	1.22 (-1.46, 3.90)	0.367

Table XVIII. Predictors factors of SPARCC-spine score at T0.

Independent Variables	Univariate analysis		Multivariable analysis	
	coeff (IC)	p	coeff (IC)	p
Female sex	-0.14 (-0.56, 0.28)	0.511	-	-
LBP onset age	-0.01 (-0.04 , 0.00)	0.152	-0.02 (-0.05, 0.00)	0.124
Duration of LBP	0.02 (-0.01, 0.05)	0.154	0.02 (-0.01, 0.05)	0.192
HLA-B27+	0.35 (-0.06, 0.78)	0.099	0.12 (-0.33, 0.58)	0.582
Increased ESR/CRP	0.11 (-0.31, 0.54)	0.583	-	-
BASDAI>=4	-0.39(-0.81, 0.01)	0.058	-0.33 (-.73, 0 .07)	0.104
Use of NSAIDs	**0.60 (-0.26, 1.48)**	**0.168**	**0.88 (0.01, 1.75)**	**0.046**

Table XIX. Predictors factors of SPARCC-SIJ score at T0

Independent Variables	Univariate analysis		Multivariable analysis	
	coeff (IC)	p	coeff (IC)	p
Female sex	-3.59 (-9.54, 2.36)	0.233	-	-
LBP onset age	-0.36 (-0.72, -0.004)	0.047	-0.25 (-0.60, 0.08)	0.140
Duration of LBP	0.00 (-0.48, 0.49)	0.980	-	-
HLA-B27+	**9.41 (3.64, 15.18)**	**0.002**	**8.87 (3.08, 14.65)**	**0.003**
Increased ESR/CRP	**4.46 (-1.59, 10.51)**	**0.146**	**5.74 (0.15, 11.34)**	**0.044**
BASDAI>=4	-3.94(-9.85, 1.96)	0.187	-3.14 (-8.55, 2.26)	0.250
Use of NSAIDs	3.87 (-6.09, 13.84)	0.441	-	-

Table XX. Predictors factors of spine structural lesions (presence=1; absence=0) at T0

Independent Variables	Univariate analysis		Multivariable analysis	
	RRR (IC)	p	coeff (IC)	p
Female sex	0.93 (0.69, 1.26)	0.661	-	-
LBP onset age	**0.90 (0.84, 0.97)**	**0.006**	**0.89 (0.82, 0.97)**	**0.010**
Duration of LBP	0.97 (0.90, 1.05)	0.495	-	-
HLA-B27+	1.22 (0.48, 3.08)	0.672	-	-
Increased ESR/CRP	0.46 (0.16, 1.31)	0.147	0.57 (0.16, 1.93)	0.369
BASDAI>=4	**2.15 (0.79, 5.85)**	**0.131**	**4.05 (1.15, 14.24)**	**0.029**
Use of NSAIDs	**0.07 (0.008, 0.65)**	**0.019**	**0.09 (0.008, 0.99)**	**0.049**

Table XXI. Predictors factors of pelvis structural lesions (presence=1; absence=0) at T0

Independent Variables	Univariate analysis		Multivariable analysis	
	RRR (IC)	p	coeff (IC)	p
Female sex	0.45 (0.17, 1.18)	0.107	0.65 (0.21, 2.01)	0.462
LBP onset age	0.98 (0.92, 1.03)	0.527	-	-
Duration of LBP	1.00 (0.92, 1.08)	0.951	-	-
HLA-B27+	1.45 (0.58, 3.60)	0.423	-	-
Increased ESR/CRP	1.27 (0.48, 3.35)	0.629	-	-
BASDAI>=4	0.43 (0.16, 1.14)	0.091	0.60 (0.19, 1.84)	0.375
Use of NSAIDs	1.77 (0.32, 9.84)	0.511	-	-
SPARCC SIJ	**1.07 (1.026, 1.13)**	**0.003**	**1.07 (1.01, 1.12)**	**0.007**

g) Evaluation of the radiographic progression of SIJ and of the spine from T0 to T24.

The radiographic progression of SIJ defined according to the criteria formulated by the DESIR study [**208**] was evaluated. In our population there was no significant radiographic progression of SIJ from T0 to T24. The radiographic progression of the spine was also evaluated according to the method provided by Poddubnyy tested in the GESPIC cohort [**209**]. In our study there was no a significant radiological progression of the spine.

DISCUSSION

Diagnosis and follow up of axSpA is still difficult to define, especially in early forms and in those associated to comorbidities or other manifestations belonging to SpA itself (psoriasis, inflammatory bowel disease or reactive arthritis) or to fibromyalgia. The use of SIJ and spine imaging in the diagnosis, classification and monitoring of axSpA is of considerable help. The standard radiography of the pelvis is currently used to determine structural changes and bone alterations, expression of a well-established and advanced pathological process, such as the sacroiliitis in AS. For the diagnosis of AS, radiographic sacroiliitis has played a role of considerable importance for a long time and has therefore been included among the classification criteria of New York (1984) [**9**]. In the last two decades, with the advent and development of new imaging methods, the interest to diagnose AS and axSpA forms atearlier stages, before structural damage has occurred, has grown, in order to establish an as earlier and more effective treatment as possible [**29**,**31,32,210**]. The MRI, due to the peculiar property of visualizing inflammatory lesions affecting the spine and SIJ, has been widely used in clinical practice and is increasingly requested and used in the process of diagnosing the patient with a history of chronic inflammatory low back pain, with suspicion of axSpA [**35,36,38**]. Thanks to the ability to guarantee a multiplanar vision, this method offers the great advantage of both identifying early inflammatory lesions (bone marrow edema) and simultaneously studying all the joint structures, including the bone, the synovial membrane, the tendons and ligaments, cartilage and periarticular soft tissues, thus allowing visualization of structural lesions [**42**,**99**,**194**]. A recent systematic review of the literature, conducted with the purpose of formulating the imaging criteria for the classification of axSpA [**35**], has highlighted the usefulness of the SIJ MRI in the diagnostic procedure of the SpA, trying to define the aspects on MRI that can be considered positive. Based on the definition of the ASAS / OMERACT criteria, a SIJ MRI is considered positive if at least one bone inflammatory lesion (BME),

highly suggestive for SpA, is present on at least two consecutive slices or if at least two bone inflammatory lesions are visible on a single slice [**37**,**81**,**191**]. The presence of synovitis, enthesitis or capsulitis alone, unaccompanied by BME, is not sufficient to consider a SIJ MRI as positive. Recently, some studies [**36,38**] have also considered inflammatory and structural lesions that can be observed at the spinal MRI. The most frequent and specific lesion which might be found in SpA patients is the BMO of the anterior vertebral corners, an expression of anterior osteitis [**198**]. Another type of lesion found is the replacement of vertebral corners with fatty tissue (fatty lesions), although it seems to be less specific for SpA and of later appearance[**199-201**]. The presence of BMO in the posterior vertebral corners (posterior osteitis) was found to be highly specific for this form of disease, but the possibility of using it as a diagnostic criterion is limited by its poor sensitivity. In accordance with the data obtained from previous studies, including ASAS/OMERACT MRI study group, in our work the prevalence and the type of lesions both of inflammatory and structural nature were analyzed in subjects with CBP and with diagnosis or suspicion of early axSpA. In our study population, a higher prevalence of BME lesions to respect than those of structural lesions, has been highlighted at MRI, demonstrating the peculiar capacity of this method in the visualization of inflammatory lesions. A significant prevalence of BME lesions in MRI was observed for both SIJ and for the spinal district, with predominant localization in the vertebral anterior corner. This observation, in accordance with the data already reported in previous studies, highlights the importance of spine involvement, from the initial phases of the inflammatory process in axSpA [**31, 200, 211, 212**]. This data appears to be interesting especially in the nr-axSpA forms without signs of sacroiliitis on MRI, and the inclusion of positivity for spine BME lesions at MRI among the classification criteria for axSpA would allow to include subjects with probable SpA. In some studies [**201, 213-216**], it has been shown that the determination of at least two inflammatory lesions (BME) in the anterior vertebral site (anterior spondylitis) in subjects younger than 45 years increases the likelihood for the diagnosis of axSpA. However, it has been highlighted that other

non-rheumatic diseases involving the rachis may present similar patterns similar at MRI, e.g Scheuermann disease, septic spondylodiscitis, erosive osteochondrosis and other degenerative discs of the intervertebral disc [**217-220**]. The determination of multiple (at least three) structural lesions, such as fatty lesions, also increases the probability of having an axSpA [**221**], although in other studies it has been observed that the prevalence of these lesions tends to increase with increasing age and is also significant in healthy subjects or with other degenerative or non-rheumatic vertebral diseases [**217-220**]. Therefore, the inclusion of positive MRI of the spine among classification criteria is currently under discussion, as a reduction in specificity could be observed in favor of increased sensitivity. In our work, the spine MRI also showed a high prevalence of inflammatory signs attributable to enthesitis (47 patients), especially in the thoracic district (58%) [**222**]: this aspect of imaging suggests early involvement of this site in the initial phases of axSpA, since in our series 50 patients had a confirmed diagnosis of axSpA. From data reported in the literature so far, it is not clear whether the localization of MRI lesions can be correlated also to the site of axial pain [**35,36**]. In our work we showed a significant association between dorsal and gluteal pain and the location of inflammatory lesions (BME) in the thoracic spinal region and in the buttocks [**222**]. This data could suggest that the characteristics and the site of axial pain (thoracic and buttock) can be used as a specific predictive index for the positivity of inflammatory lesions on MRI. This association between the pain site and the localization of alterations on MRI is less striking for structural lesions, which was significant only for buttock pain and structural lesions of SIJ. The radiological progression of SIJ and the spine was also evaluated in terms of SIJ mNY and mSASSS scores from T0 to T24, according respectively to the definitions provided by Dougadas [**208**] and Poddubnyy [**209**]: we did not observe any significant variation. The absence of radiographic progression of the rachis and sacroiliac was probably due to the reduced sample size, the presence of a cohort that did not completely satisfy the criteria for axSpA and the limited duration of the observation period. The variations in time from T0 to T24 of the radiological activity level measured in terms of

ΔSPARCC-SIJ and ΔSPARCC-spine were also taken into consideration: there was no significant reduction in the score either in total patients or in individual cohorts, although there was a reduction trend in the 2 cohorts that met the ASAS criteria for axSpA. These results can be justified by two possible reasons: 1) the early onset of CBP or these axSpA forms; 2) the excessively short follow-up time (24 months) to observe significant changes in radiological terms. The tendency to SPARCC score reduction in the 2 cohorts that met the axial ASAS criteria is attributable to the pharmacological treatment undertaken after baseline evaluation and the diagnosis of axSpA. However, due to non-homogeneity in therapy and reduced sample size (n = 54 patients) to T24 imaging, it was not possible to conduct a comparison analysis on the ΔSPARCC-SI and ΔSPARCC-spine degree between patients divided by type of treatment. Several studies have systematically evaluated the concomitant use of the spine and pelvis MRI in patients with suspected axSpA and healthy subjects controls. Some of these [**90,192**] have shown that a simultaneous evaluation of the spine and SIJ on MRI can determine an increase in the diagnostic capacity. However, other authors who have not confirmed this observation [**41**] state that the combined use of spinal and pelvis MRI only moderately increases the level of diagnostic probability in patients with suspected axSpA, because of the inclusion of false positives. In fact, other rheumatic diseases involving the spine such as Scheuermann's disease, spondylodiscitis, erosive osteochondrosis and other degenerative diseases of the intervertebral disc, may show similar patterns on MRI [**36**]. Even the appearance of multiple structural lesions (at least three), just as fatty lesions, increases the probability of axSpA [**221**], although, according to other studies, the prevalence of these forms of lesions tends to increase with aging and can be significant even in healthy individuals or affected with other spinal degenerative diseases. Patients with non specific LBP and healthy subjects may have some signs suggestive of SpA such as fatty lesions on spinal MRIs [**217-221**]. According to Weber et al. [**41**] using a MRI of the SIJ alone is less sensitive but more specific than the combined use of the spinal and pelvis MRI, while inclusion of a spinal MRI leads to increased sensitivity and reduced specificity. The cost and

time necessary to carry out MRIs cannot, in any case, be ignored. In our study population the prevalence of patients with spinal MRI lesions in absence of SIJ involvement was considered: eighteen patients (24%) with inflammatory lesions on spinal MRIs showed no abnormalities on SIJ MRIs, while 11 (14.7%) patients with sacroiliitis on MRI did not present lesions on spinal MRIs. Our data seem to indicate that the use of spinal MRIs together with SIJ MRIs can add further, relevant information during both the diagnostic process and the therapeutic follow-up. We also evaluated the presence of predictors of radiological progression and of radiological activity on MRI in our population: the early age onset of IBP, a long duration of IBP, increased inflammation indices, an higher use of NSAIDs, male sex and the presence of HLA B27 were identified as predictors, in accordance with the data already reported in the literature [**50,51**].

The current study investigated if there were differences in the clinical indices of disease activity commonly used in clinical practice in relation to the presence or absence of signs of sacroiliitis on plain radiographs and on MRIs. Although a significant difference was found in the three cohorts with regard to the prevalence of sacroiliitis on MRIs and X-rays and on the SPARCC SIJ score, we did not find any differences in clinical and disease activity indices. Higher indices were not found in the patients with active sacroiliitis on MRI with respect to those without inflammatory changes in the SIJ or with initial signs of radiographic sacroiliitis. This result may depend on both the early stages of axSpA and the small sample size. In fact, several studies [**88-90,92,93,223**] reported higher values of clinical, functional and disease activity indices in AS and r-axSpA, in patients with long disease duration with respect to subjects with nr-axSpA. Our study results also uncovered at T0 a significant correlation between inter- and intra- clinical and disease activity indices and between ESR and hs-CRP, as reported in literature [**88,89**]. The correlation between serological MMP3 levels and mSASSS, indicative of metalloproteinases' role in the bone formation process, was an interesting finding and it is in accordance with the data described by Maksymowych et al. [**137**] who reported that independently of other indices, serum MMP3 levels could predicted

two-year radiographic progression in 97 axSpA patients. Our data also showed that MMP3 correlates with other inflammatory, functional and disease activity indices (such as CRP, BASFI and BASDAI) and it is increased in the serum of patients with active axSpA, in agreement with previous studies [**132,135-137**]. It has been shown that MMP3 correlates with disease activity indices even in PsA [**140**]. As reported by Maksymowych et al., anti-TNF treatment induces a significant decrease in serum MMP3 levels as well as in ESR and BASDAI [**137**]. Van Kuijk et al. [**141**] who studied 24 SpA patients who were randomized to receive 40 mg of adalimumab once every 14 days or placebo found a reduction in serum MMP3 levels after only 4 weeks after the onset with anti -TNFα therapy, while no change was noted in the serum levels of the placebo group. The molecule was elevated in active forms of SpA or in cases presenting important clinical involvement, supporting the hypothesis that MMP-3 could represent a reliable marker of disease activity. The correlation between mSASS values and serum biomarkers should be evaluated by prospective studies.

Recently, several studies have focused on the research and evaluation of interleukins (IL) involved in the pathogenesis of SpA [**137**]. IL-6 and IL-1 are the most widely studied proinflammatory cytokines [**88,101,107,108**], produced by a variety of immune cells and implicated in the production of a series of acute phase proteins such as amyloid serum A and the CRP. These two mediators are involved in the initiation and maintenance of the inflammatory process by stimulating the migration and proliferation of neutrophils at the site of inflammation and by regulating the activation and differentiation of T lymphocytes. Some recent studies have shown associations of IL-1 and of IL-6 with other interleukins with role of biomarker of inflammation [**101,107,108**]. Among these, IL-17 and IL-23 are key cytokines in the Th17 pathway [**115-128**]. A series of studies have reported that elevated levels of IL-17 and IL-23 are found in plasma and serum of patients with AS, which are associated with a higher degree of disease activity [**69,109,112,113**]. On the contrary, other studies have not confirmed this observation [**114**]. In our study we assessed whether there were significant differences in IL-17, IL-22 and

IL-23 levels at T0 in the three cohorts of patients (**axSpA Imaging Arm +, axSpA Clinical Arm +, not full ASAS axSpA**) and no difference was detected. The absence of any significant differences in serum concentration of the studied biomarkers in the patients as having radiographic disease and non radiographic disease with or without MRI pelvis positive findings is primarily due to the very early stage of the disease (inflammatory low back pain≥3 months and <2 years) and secondary due to the very small size samples for single groups. It has also to be underlined that the inclusion criteria for this study were primarily the presence of inflammatory LBP and clinic/immunogenetic and imaging SpA features, in accordance with ASAS criteria, while the study population was divided in three cohorts only afterwards, following the characteristics evidenced on imaging (presence or absence of SIJ involvement in MRI and X-Rays and of spine involvement in MRI). Another possible reason for the absence of elevated serum levels of inflammatory markers such as CRP and ESR may be the fact that inflammation is restricted to specific tissue compartments and does not extend to the systemic circulation and/or lymphoid organs in axSpA. Accordingly, previous studies reported that the immupathology of affected tissues and inflammatory alterations were not always reflected in the peripheral blood compartement [**224,225**]. These findings are similar to those reported in other study [**150**]: the authors did not find differences of some biomarkers levels in patients with early axSpA versus "control"back pain patients. We did not find a significant correlation of IL-17 and IL-23 values with other serological, clinical and disease activity indices. Therefore, the dosage of these biomarkers appears to be just indicative in the early stages of the disease, differently from IL-22 levels which correlated with some clinical indices, such as BASFI, BASG1, HAQ, VAS pain [94]. In some studies [**139,226,227**], conducted on patients with other forms of SpA, such as PsA, elevated levels of IL-22 expression have been reported and the correlation of this molecule with clinical and disease activity indices suggest its inflammatory role in the peripheral synovitis and in the diffuse skin psoriasis. However in our study, no significant correlation was found between skin involvement and serum levels of IL-

22, because patients with psoriasis had a very narrow skin involvement or only onicopathy with PASI <1. Higher IL-22 levels even in the axial form of SpA might suggest that it plays a role in active sacroiliitis. However, in our study population, we did not find increased IL-22 levels in patients with active sacroiliitis compared to patients without inflammatory changes of SIJ on pelvis MRI or in patients with radiographic sacroiliitis [**94**]. It would be interesting to evaluate if there is a correlation between the IL-22 levels and the presence/absence of active sacroiliitis on a larger sample size.

During the follow-up from T0 to T24, in all patients, a significant reduction offunctional and disease activity indices was observed MASES ($p=0.008$), BASG1 ($p=0.02$), BASG2 ($p<0.0001$), HAQ ($p=0.0002$), VASpain ($p=0.01$), VAS pain night ($p=0.04$), VAS disease activity ($p=0.05$), BASFI ($p=0.02$), ESR ($p=0.04$), BASDAI ($p<0.0001$), ASDAS ($p<0.0001$). On the other hand, BASMI and CRP did not decrease significantly. There were no differences in the serological markers (ILs, MMP3 and hsCRP) values during the 2 years follow up period in the three groups. Considering, patients subdivided among the 3 cohorts, we observed a downward trend for all functional indices (HAQ, BASFI, BASG1, BASG2, VAS pain, VAS night pain and VAS disease activity) and disease activity indices (BASDAI, ASDAS), which in some cases was statistically significant, particularly in two cohorts (axSpA Imaging Arm +, axSpA Clinical Arm +), but no more markedly significant decrease of these indices levels was observed in one cohort than the others. The improvement of the clinical, functional and disease activity indices is probably due to the pharmacological therapy undertaken after the diagnosis of axSpA, at the sametime it was observed a reduction of prevalence of inflammatory lesions (BME) on spinal and pelvis MRI, whose prevalence had decreased during the follow-up period. An accurate analysis of the above-reported indices in relation to the type of therapy was not possible, due to the reduced sample size and the diversification of the treatment in the three cohorts.

CONCLUSIONS

It has become increasingly urgent to detect axSpA in its earliest stages in order to initiate treatment as early as possible. MRI can detect inflammatory lesions and signs of active disease process even before structural bone damage has occurred. Pelvis MRI has recently been included in the ASAS classification criteria whose positivity is defined on the basis of the exclusive presence of inflammatory signs of SIJ. It remains a matter of debate whether the inclusion of inflammatory and structural lesions of spinal MRIs in the ASAS classification criteria could help to identify patients with suspected axSpA. A high prevalence of inflammatory lesions on MRIs of the SJI and of the spine (anterior osteitis and enthesitis) were found in our patients. As inflammatory lesions on spinal MRIs can occur in the absence of SIJ involvement, the use of spinal MRIs together with SIJ MRIs may add further, relevant information to the diagnostic process, especially with regard to nr-axSpA patients without signs of sacroiliitis on MRI. A standard radiograph of the pelvis continues to be a crucial step in the diagnostic investigation in patients with suspected axSpA, especially in those with a longer history of symptoms. Studies on the involvement of the thoracic spine, which has until now never been considered by methods scoring spinal structural damage, are warranted. A significant involvement of the thoracic region was noted in the patients studied.

In the current study in early axSpA patients with different types of axial involvement (presence or absence of radiographic sacroiliitis and of active sacroiliitis on pelvic MRI), significant differences in clinical severity, disease activity indices, and in biomarker levels were not found in the three patient cohorts. ILs, MMP3 and hs-CRP values were not significantly higher in any of the cohorts and were not correlated to radiographic SIJ involvement. A significant correlation between IL-22 and some disease activity indices and between MMP3 and hs-CRP were instead noted. The importance of early diagnosis and treatment of axSpA has been highlighted by many studies. Although the utility of some genetic and serological markers in diagnosing AS and axSpA, in monitoring disease activity,

and in predicting patients most at risk for poor outcome has been investigated by numerous researchers, ESR and CRP have proven to be inadequate parameters for monitoring SpA activity [**150**]. Our data suggest that the disease processes, driving axial spondyloarthritis are not reflected by alterations in the peripheral blood compartment. Limits of this study are due to the small sample size and to the early disease stage (low back pain ≥3 months, ≤2 years, onset < 45 years). Further studies are warranted to assess in a more exhaustive manner the validity and reproducibility of the disease activity biomarkers considered here in identifying and monitoring early stages of axSpA.

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ICONOGRAPHY

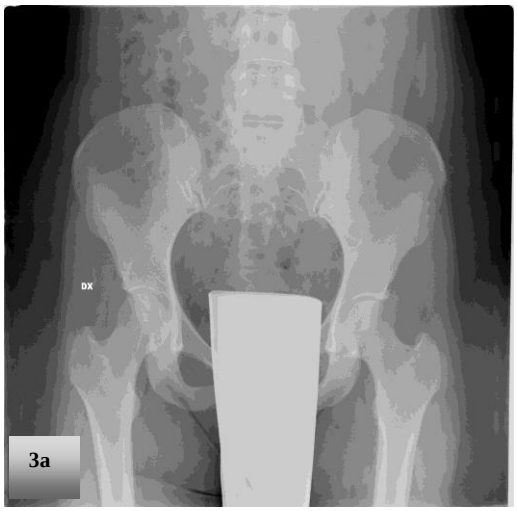


Image 3a. Sacroiliitis grade I

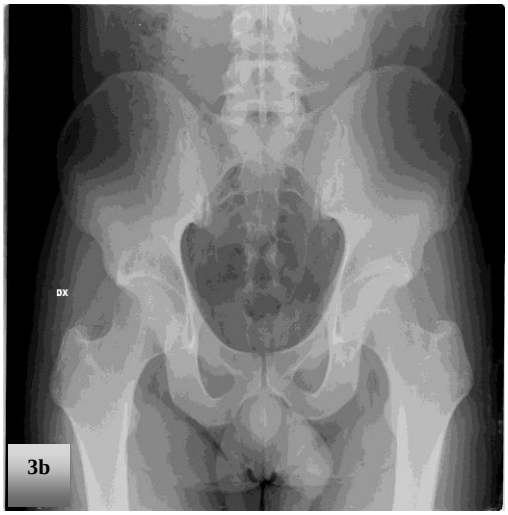


Image 3b. Sacroiliitis grade II

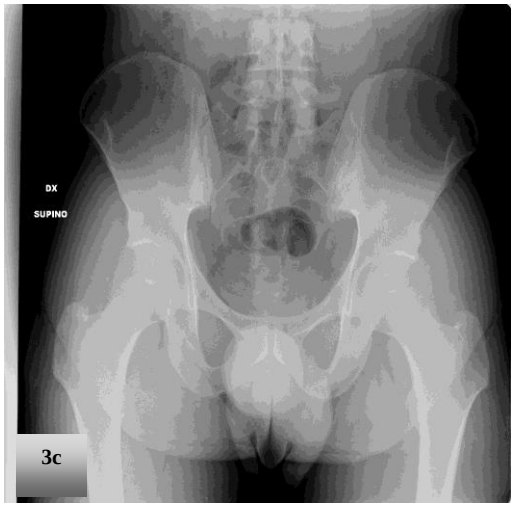


Image 3c. Sacroiliitis grade III

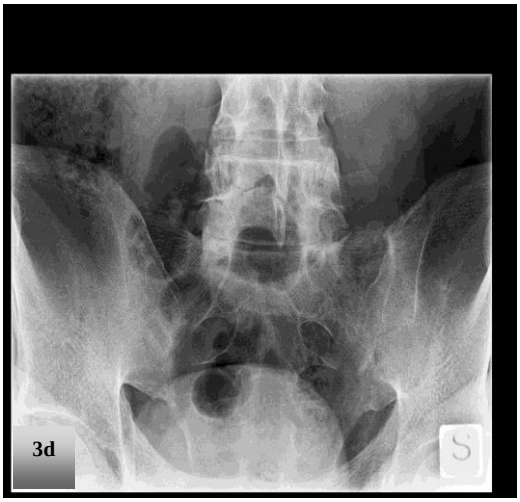


Image 3d. Sacroiliitis grade IV

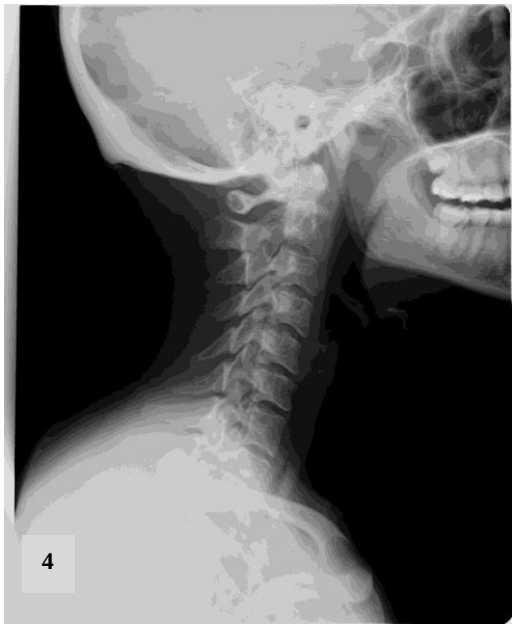


Image 4. Straightening of the cervical column

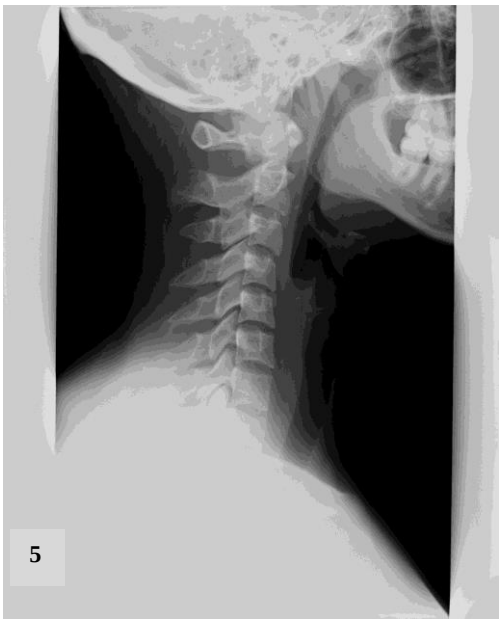


Image 5. Squaring of vertebral body of C7

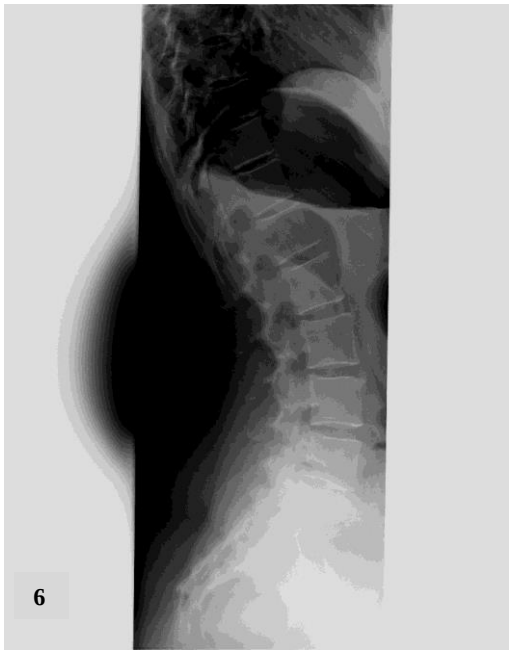


Image 6. Erosion of anterior corner of vertebral body of L4

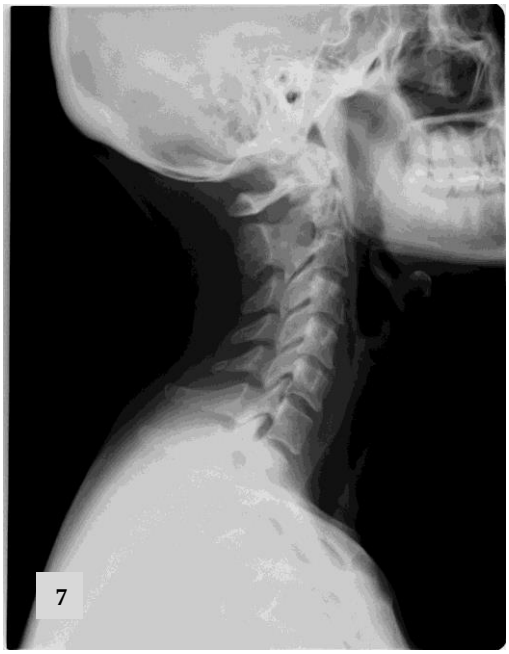


Image 7. Fusion of vertebral bodies of C2-C3

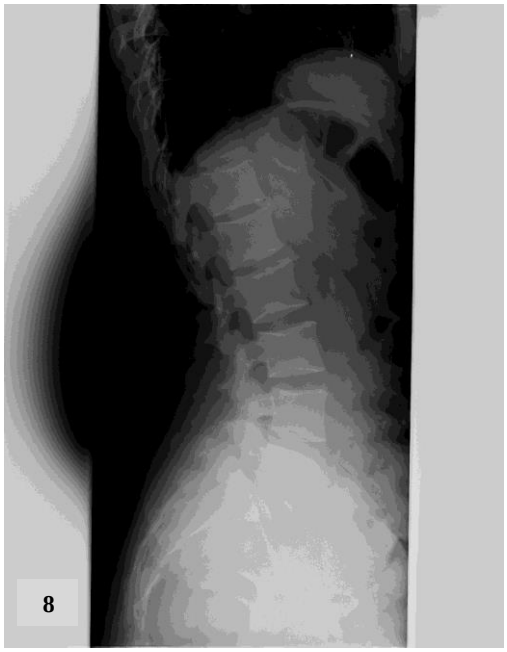


Image 8. Syndesmophytes of thoracic district of T12-L1

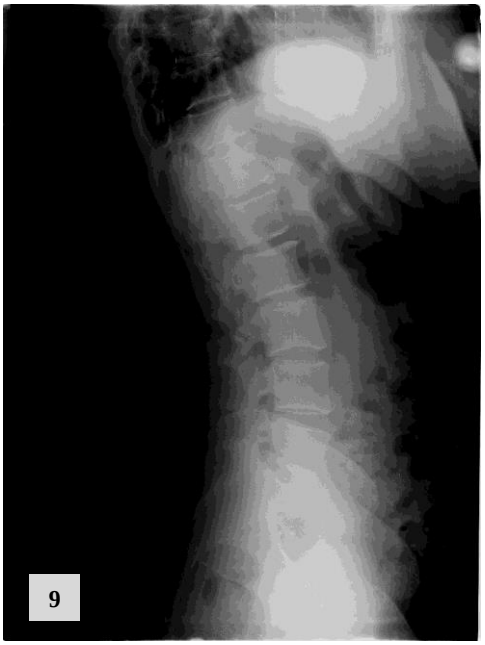


Image 9. Parasyndesmophytes of thoracic district of T11-T12-L1-L2 e squaring of vertebral bodies of L2-L3-L4

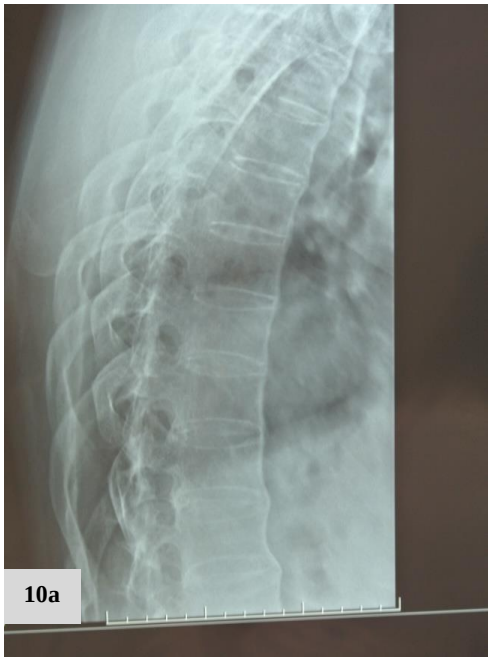


Image 10a. Calcification of the anterior longitudinal ligament (sign of the "bamboo cane")

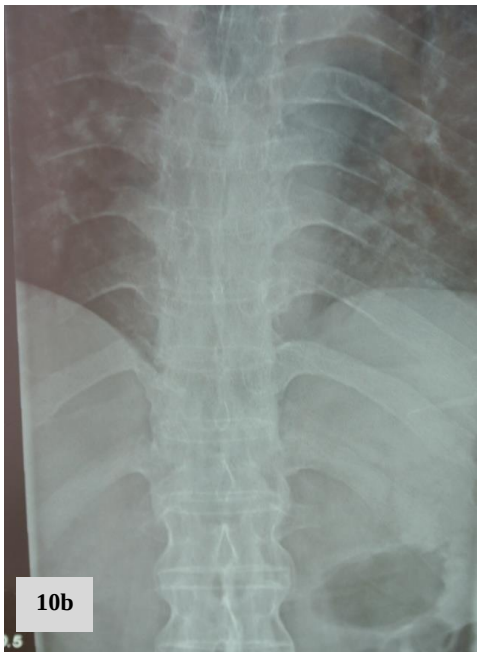


Image 10b. Calcification of the anterior longitudinal ligament, of the inter-apophyseal joints and inter-spinous ligaments (sign of the "cog railway")

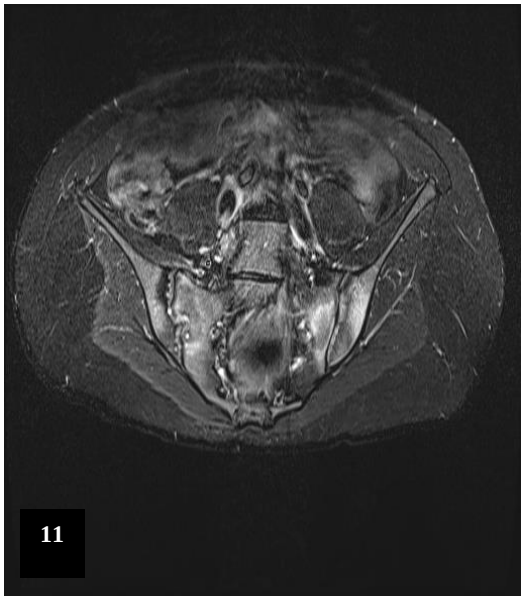


Image 11. Bone oedema of both SIJ, with main inflammatory involvement of left SIJ and with signs of sclerosis on right SIJ (STIR sequence)

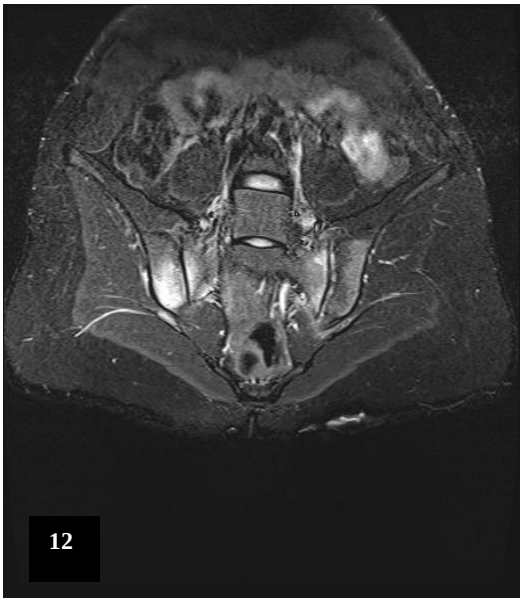


Image 12. SIJ bilateral bone oedema, with greater phlogistic involvement of the right SIJ, in STIR sequence

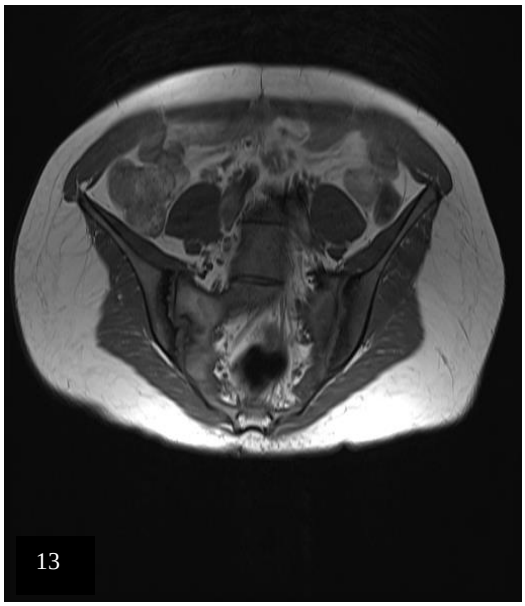


Image 13. Bilateral sclerosis and erosions of SIJ, with main involvement of the right SIJ, in sequence T1

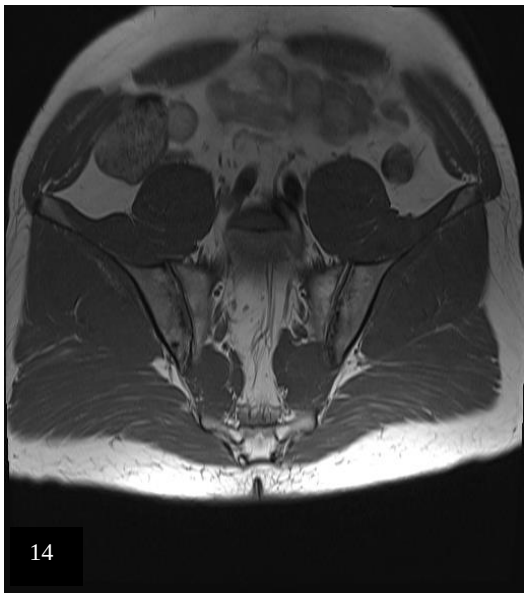


Image 14. Initial sclerosis and some areas of adipose metaplasia of both SIJ in sequence T1

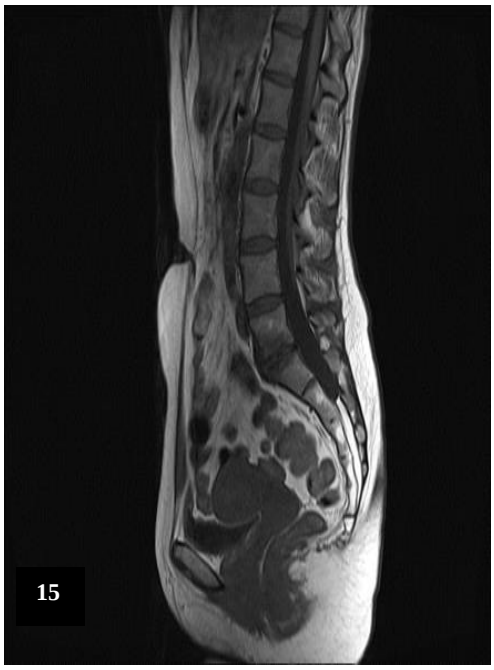


Image 15. Spondylodiscitis at the level of L5-S1 in sequence T1

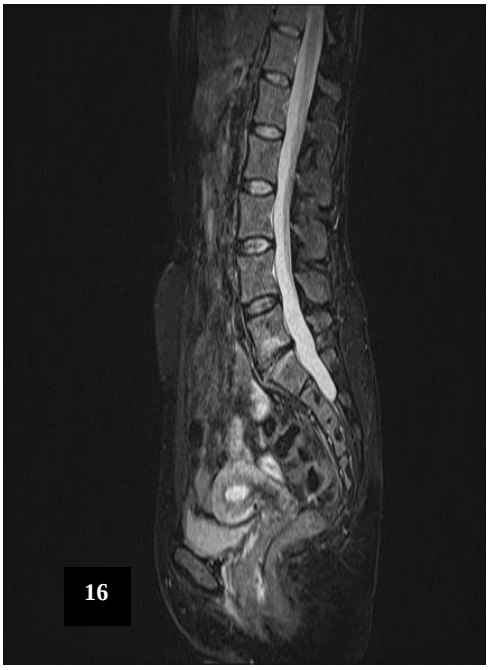


Image 16. Spondylodiscitis at the level of L5-S1 in sequence STIR

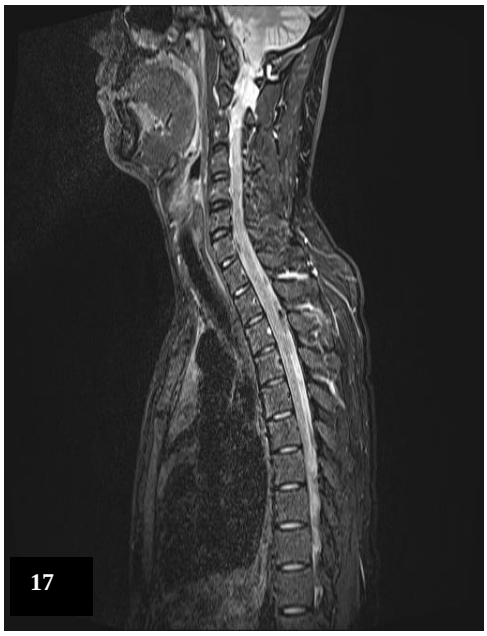


Image 17. Osteitis of anterior-superior corner of C6, of anterior-lower corner of T6 and anterior-superior corner of T7. Signs of enthesitis of D1-D5 district in STIR sequence

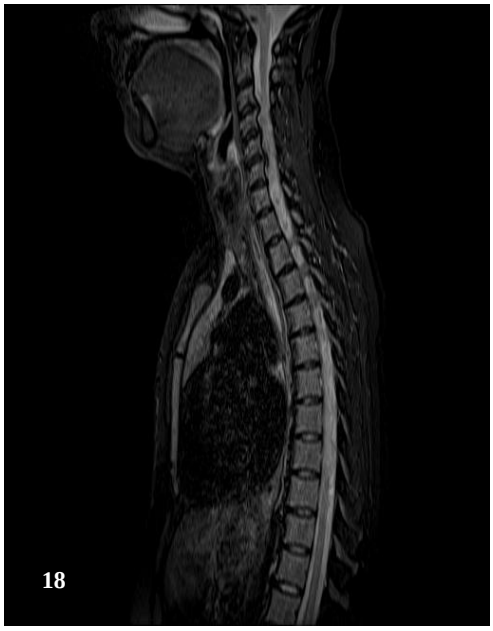


Image 18. Signs of enthesitis of D5-D11 district in STIR sequence

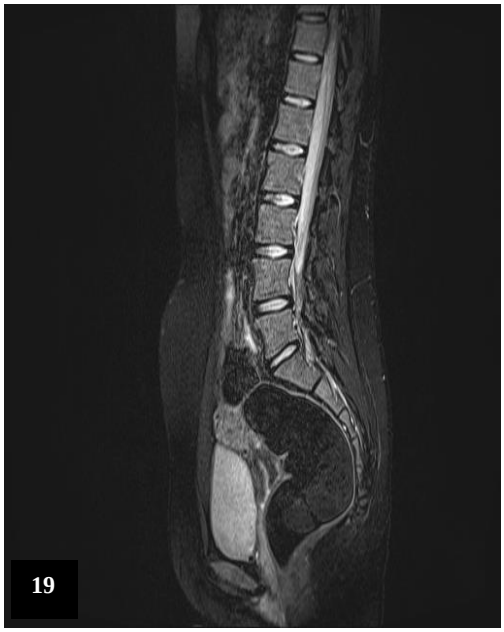


Image 19. Osteitis of anterior-superior corner of L3-L4-L5 in sequence STIR

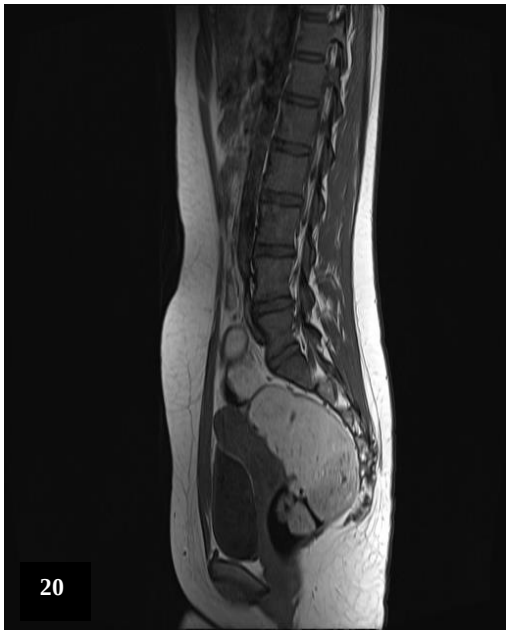
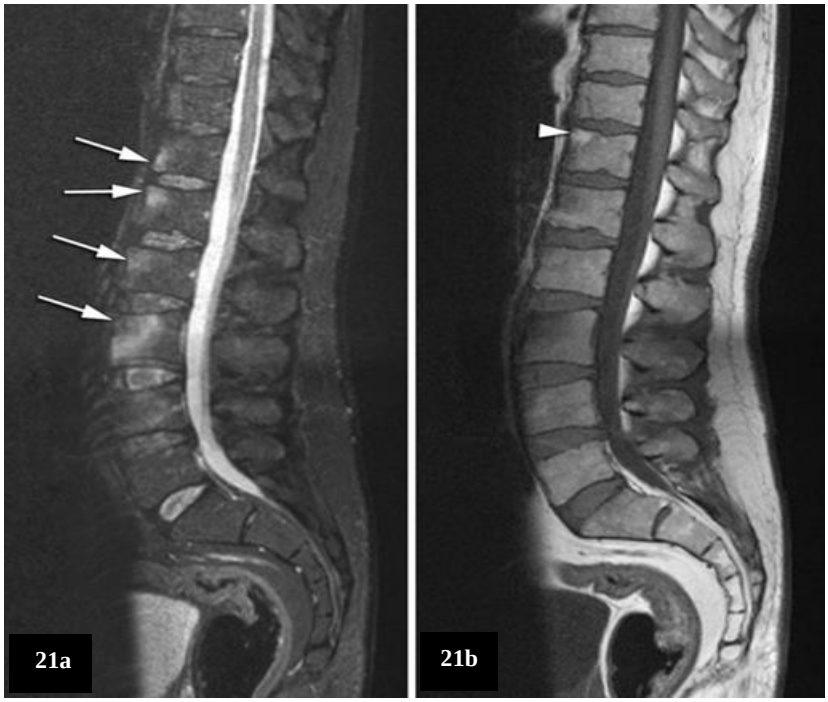


Image 20. Osteitis of anterior-superior corner of L3-L4-L5 in sequence T1



Images 21a e 21b. Anterior spondylitis. a) hyperintense signal from anterior osteitis from T12 to L3 in the STIR sequence (white arrows). b) corresponding hypointense signal in the same locations in sequence T1. Hyperintense signal at the antero-superior corner of T12 (white arrowhead) from adipose metaplasia