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# Juvenile spondyloarthritis: From basic science to clinical translation

**Edited by**
Miroslav Harjaček, Ruben Burgos-Vargas and Rik Joos

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# Juvenile spondyloarthritis: From basic science to clinical translation

**Topic editors**

Miroslav Harjaček — United Arab Emirates University, United Arab Emirates

Ruben Burgos-Vargas — General Hospital of Mexico, Mexico

Rik Joos — Ziekenhuisnetwerk Antwerpen Dept Rheumatology, Belgium

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# Table of contents

04 Editorial: Juvenile Spondyloarthritis: From Basic Science to Clinical Translation
Miroslav Harjacek, Rik Joos and Ruben Burgos Vargas

07 The Increased Levels of Fecal Calprotectin in Children With Active Enthesitis Related Arthritis and MRI Signs of Sacroiliitis: The Results of a Single Center Cross-Sectional Exploratory Study in Juvenile Idiopathic Arthritis Patients
Lovro Lamot, Marijana Miler, Rudolf Vukojević, Mandica Vidović, Mirta Lamot, Ivana Trutin, Nora Nikolac Gabaj and Miroslav Harjaček

15 Tapering of Biological Agents in Juvenile ERA Patients in Daily Clinical Practice
Chun-Hua Liao, Bor-Luen Chiang and Yao-Hsu Yang

24 The Development of Extra-Articular Manifestations in Children With Enthesitis-Related Arthritis: Natural Course or Different Disease Entity?
Ilaria Pagnini, Mariangela Scavone, Ilaria Maccora, Maria Vincenza Mastrolia, Edoardo Marrani, Federico Bertini, Lovro Lamot and Gabriele Simonini

33 Juvenile Spondyloarthritis: What More Do We Know About HLA-B27, Enthesitis, and New Bone Formation?
Shi Huan Tay, Joo Guan Yeo, Jing Yao Leong, Salvatore Albani and Thaschawee Arkachaisri

46 Outcomes in Juvenile-Onset Spondyloarthritis
Judith A. Smith and Ruben Burgos-Vargas

56 Similarities and Differences Between Juvenile and Adult Spondyloarthropathies
Corinne Fisher, Coziana Ciurtin, Maria Leandro, Debajit Sen and Lucy R. Wedderburn

72 Immunopathophysiology of Juvenile Spondyloarthritis (jSpA): The "Out of the Box" View on Epigenetics, Neuroendocrine Pathways and Role of the Macrophage Migration Inhibitory Factor (MIF)
Miroslav Harjacek

90 Inflammatory Foot Involvement in Spondyloarthritis: From Tarsitis to Ankylosing Tarsitis
José Pablo Romero-López, Dirk Elewaut, César Pacheco-Tena and Rubén Burgos-Vargas

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# Editorial: Juvenile Spondyloarthritis: From Basic Science to Clinical Translation

*Miroslav Harjacek*[1]*, *Rik Joos*[2,3] and *Ruben Burgos Vargas*[4]

[1] Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates, [2] Department of Rheumatology, Ziekenhuisnetwerk Antwerpen, Antwerp, Belgium, [3] Pediatric Rheumatology, University Hospital, Gent, Belgium, [4] General Hospital of Mexico, Mexico, Mexico

**Keywords: juvenile spondyloarthritis (JSpA), classification, translational science, pathophysiology, therapy, biomarkers, outcome**

**Editorial on the Research Topic**

**Juvenile Spondyloarthritis: From Basic Science to Clinical Translation**

## INTRODUCTION

Juvenile spondyloarthritis (JSpA) concept emerged relatively recently. Although through many years, attempts have been made to define this group of diseases, no uniform consensus could be reached. In a recent advanced course endorsed by Pediatric Rheumatology European Association PReS, in 2019. Authorities from all over the World joined in Zagreb, Croatia to exchange points of view and most recent knowledge. This Special Issue is the final result of this continuous effort of both rheumatologists and pediatric rheumatologists to grasp on JSpA. Without the close collaboration between these two specialties the long ongoing discussions and even misunderstandings would remain. Through a series of articles authors underscore the recent progress in our knowledge of JSpA classification, pathophysiology, diagnosis, similarities, and differences between adult and juvenile forms and treatment. It also pinpoints to unmet needs in translational research (e.g., biomarkers) to improve suboptimal outcomes in JSpA.

Juvenile spondyloarthritis is an umbrella term for a group of inflammatory diseases that involve the spine (sacroiliitis and spondylitis), joints (asymmetric peripheral arthritis), and tendons (enthesitis). Juvenile spondyloarthritis is now considered as a distinct subtype of juvenile arthritis (JIA). It is characterized by male predominance and later onset in childhood. Juvenile ankylosing spondylitis (jAS), reactive arthritis, juvenile psoriatic arthritis (JPsA), arthritis associated with inflammatory bowel disease (IBD), and undifferentiated JSpA (u-JSpA) or enthesitis related arthritis (ErA) are all fitting into the JSpA concept. While spondyloarthritis (SpA) in general, is one of the most widespread chronic rheumatic diseases, JSpA is also seen globally. Enthesitis related arthritis is the most common at about 10–40%, while JPsA represents ∼2 to 10% of all JIA patients and jAS, as the most severe form, is seen in about 1–7% of JIA children worldwide (1). The diagnosis of JSpA can often be difficult because the symptoms are sometimes episodic and unpredictable and the initial presentation can be subtle. Main symptoms include a peripheral asymmetrical arthritis, frequently hip involvement, and enthesitis as a hallmark of the disease. Spine disease is rare, and it may take several years for axial disease to develop in children. The HLA-B27 positivity is well-known as a risk factor for axial disease, but is not diagnostic for JSpA. Many children diagnosed with JSpA will eventually fulfill criteria for adult SpA suggesting common clinical patterns and pathophysiology. Similarly, to adults, when compared to healthy controls, recent studies have shown clear alterations in gut microbiome in patients with JSpA. It appears

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***Edited and reviewed by:***
*João Eurico Fonseca, University of Lisbon, Portugal*

***Correspondence:**
*Miroslav Harjacek*
*mharjacek@uaeu.ac.ae*

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convincingly that innate immune system, and in particular, interleukin 17A (IL-17/23 axis) are playing a pivotal role in JSpA pathophysiology. No pathognomonic laboratory biomarker(s) for the disease(s) diagnosis exists, and the radiographic findings are often negative or illusive in the children with growing skeletons. Treatment of peripheral arthritis usually includes non-steroidal anti-inflammatory drugs, and joint steroid injections. Juvenile patients are virtually unresponsive to MTX treatment, whereas TNF inhibitors are the main biologic therapy for treatment of axial disease and conventional therapy resistant peripheral disease. Although other biologics such as IL-17 blockers are beneficial in adult SpA, none are currently approved for the use in children. Yet, studies in adults, and recent JUNIPERA study in children with ErA and JPsA are promising (2).

With fewer than half of children achieving remission off medication 5 years after diagnosis and hence, rapid identification and early treatment is paramount (3, 4). Following revised (Edmonton 2011) ILAR criteria patients are most often classified as ErA, JPsA, or unclassified JIA (5). Presently pediatric rheumatologists do not diagnose JPsA in all children whose disease signs and symptoms fulfill adult classification criteria for Psoriatic Arthritis (CASPAR), the unification of CASPAR and pediatric PsA classification criteria is needed (6).

## OVERVIEW OF THE ISSUE

In the article by Joos author tries to illustrate the way of thinking about JSpA in recent decades. Starting from the criteria formerly used in adult rheumatology, gradually the concept of JSpA is becoming clearer. A clear description of the characteristics by which a patient can be considered as suffering from a JSpA is essential to achieve reproducible research.

The similarities and differences between adult and juvenile form of SpA are outlined in the article by Fisher et al. As they emphasize, adult SpA and JSpA share many common signs and symptoms, implying that they are a spectrum of the same disease. Yet, the authors noticed main differences in classification criteria, clinical picture, disease activity, and pathophysiology. In particular, section on the effects of adolescence, sheds additional light on the role of sex hormones and mechanical stress on development of JSpA.

In his "Out of box view" author (Harjacek) offers his understanding of epigenetics, various stressors exposure, neuroendocrine pathways, and macrophage migration inhibitory factor (MIF) in the immunopathophysiology of jSpA. In affected patients subclinical gut inflammation, initiated by intestinal dysbiosis, is essential to the prospective development of inflammation in the synovial–entheseal complex. Although the crucial role of IL17/23 axis, TNF-α, and IL-7 in the pathophysiology of SpA, including JSpA, is well-known, the role of MIF is generally ignored, but recently validated (7, 8). The strong correlation of osteitis with low-grade IBD, established in children with ErA by elevated concentration of fecal calprotectin (fCAL) (Lamot et al.), emerges as the central event in the early stages of SpA.

Furthermore, in the review by Huan Tay et al. progresses in understanding the role of HLA-B27, development of enthesitis and new bone formation in JSpA pathophysiology and therapy, were critically discussed. As authors emphasize, enthesitis as the pathognomonic sign of SpA, caused mainly by exaggerated response to biomechanical stress, emerges paradoxically as osteoproliferation at inflamed periarticular sites in a setting of increased systemic bone resorption. It is still unclear if inflammation and osteoproliferation are directly linked or separated events.

Children with JSpA often present with extra-articular manifestations as is shown in the retrospective, observational, monocentric study of 53 ErA patients followed at the Mayer Childrens Hospital of Florence, Italy (Pagnini et al.). The authors hypothesized the existence of two distinctive disease phenotypes in children with ERA, whether extra-articular manifestations are present or absent. In particular, tarsitis was associated with ErA diagnosis and the absence of extra-articular manifestations. The development of extra-articular manifestations over period of time, might evolve to different disease, such as Crohn disease or SAPHO syndrome. Authors concluded that older age of disease onset, HLA-B27 positivity, development of the hip arthritis within the first 6 months and tarsitis, are all risk factors associated with worse prognosis of ErA patients.

Along the same lines, Romero-López et al. discuss the clinical and translational features of inflammatory foot involvement in SpA. The authors highlight the predominance of tarsal affection in JSpA patients among several cohorts. The most severe form of this disease has been named Ankylosing Tarsitis (AT). This review provides a proposal for classifying the clinical features in three stages (prodromal phase, disease continuum, and bone ankylosis) that differ in clinical and radiographic findings. Few reports are approaching the specific pathogenesis of tarsal inflammation and ankylosis. Many experimental models of SpA can start with midfoot inflammation and progress to axial disease, somehow resembling the clinics of young patients with SpA. Foot enthesitis and ossification are usually neglected, although there is an increasing evidence of its relevance as an initial clinical manifestation that can either alert about future progression, or act as a more accessible source of tissues or cells for translational studies.

The search for reliable and prognostic biomarkers in JSpA has been extensive but results so far have been disappointing. In the single center study by Lamot et al., the role of fCAL, a proposed biomarker of gut inflammation, was evaluated in 71 JIA patients. In patients with ERA, moderate correlation was detected between fCAL concentration and the disease activity measured by JSpADA. Moreover, fCAL concentration was able to discriminate between ErA patients with inactive or active disease. Contrary to some previous studies, the fCAL levels were not linked to the use of NSAID, nor with the use of other therapy for JIA. Intriguingly, ErA patients with signs of SIJ inflammation detected by contrast MRI, had a significantly higher fCAL concentration than those without the signs of SIJ involvement, suggesting that gut inflammation might be a integral part of a systemic inflammation existing in patients with ErA. Authors concluded that even in asymptomatic ErA patients

with increased levels of fCAL, performing a contrast MRI of SIJ, might be helpful.

The real-life data, opposed to the data collected from the clinical trials are generally more useful for the daily clinical practice. Important questions about tapering/withdrawing classical DMARDs (cDMARDs) and biological therapy, in 75 ErA patients, were addressed in the retrospective study by Liao et al. Authors reached several important conclusions. Firstly, patients with a longer time interval between disease onset and initiation of cDMARDs had a higher risk of flare-ups during tapering of biologics. Secondly, they also disclosed that patients who had a shorter time to achieve clinical inactive disease once biological agents were started, seemed to have a lower chance of experiencing flare-up during biologics tapering. Thirdly, they confirmed that ANA positivity was present more frequently in patients under biologics, as previously reported. Due to insurance requirements Etanercept was the most commonly used first-line biologics in their cohort, yet showing less efficacy in those patients with higher risk of IBD development (9).

The focus of the final review in the series was the clinical burden of disease, prognostic indicators and outcomes in JSpA (Smith and Burgos-Vargas). Reviewing a number of articles regarding the clinical picture, and the data that allows the differentiation from other forms in the classification of JIA. The authors retake issues regarding the concept and nomenclature of SpA. The way this condition has been called throughout the years depends on who was the physician seeing the case. The indicial presentation to the pediatrician is surely peripheral arthritis and enthesitis and some years later, axial symptoms. Specialists will search for sacroiliitis and spondylitis and their differentiation from other forms of JIA. In reviewing the series of cases or the few cohort studies, investigators dedicated to adult onset Spa neglect the juvenile onset categories. In contrast, the pediatric rheumatologist tends to concentrate on the search of axial arthritis even in children and adolescents who have no evidence of sacroiliitis. Studies showing a transition in patients with JSpA toward adult forms confirm common clinical and genetic characteristics. The chapter approaches the relatively scarce information on the consequence of a disease, relatively rare, yet devastating. Therefor early recognition either by the presence of characteristic peripheral involvement or inflammatory back pain and sacroiliitis is needed.

## SUMMARY

Through this Research Topic efforts have been made to precisely classify and describe the pathophysiology and clinical features of JSpA. In order to accomplish unmet clinical needs, early diagnosis and treatment of children with JSpA is essential. The search for more sensitive and specific biomarkers is ongoing. Customized therapy for each and every patient should improve outcome as an ultimate goal, and diminish unfavorable consequences of the disease burden for those children.

## AUTHOR CONTRIBUTIONS

MH wrote initial version. RJ and RV wrote revisions and then MH submitted the final version. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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# The Increased Levels of Fecal Calprotectin in Children With Active Enthesitis Related Arthritis and MRI Signs of Sacroiliitis: The Results of a Single Center Cross-Sectional Exploratory Study in Juvenile Idiopathic Arthritis Patients

*Lovro Lamot[1,2*†], Marijana Miler[3], Rudolf Vukojević[4], Mandica Vidović[1], Mirta Lamot[5], Ivana Trutin[6], Nora Nikolac Gabaj[3] and Miroslav Harjaček[1,2†]*

[1] Division of Clinical Immunology and Rheumatology, Department of Pediatrics, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia, [2] Department of Pediatrics, University of Zagreb School of Medicine, Zagreb, Croatia, [3] Universirty Department of Chemistry, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia, [4] Department of Diagnostic and Interventional Radiology, Sestre Milosrdnice University Hospital Center, University of Zagreb, Zagreb, Croatia, [5] Division of Neonatology, Department of Gynecology and Obstetrics, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia, [6] Division of Nephrology and Cardiology, Department of Pediatrics, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia

Enthesitis related arthritis (ERA) is a specific subtype of juvenile idiopathic arthritis (JIA), often regarded as an undifferentiated form of juvenile spondyloarthritis (jSpA). While gut is increasingly recognized as origin and/or target of inflammation in adult onset spondyloarthritis (SpA), the incidence of gut involvement in ERA patients is largely unknown. The aim of this study was to measure the concentration of fecal calprotectin (fCAL), a surrogate marker of gut inflammation, in patients with different subtypes of JIA, as well as to correlate the results with various demographic, clinical, laboratory, imaging, and treatment characteristics. The cross-sectional exploratory study involving 71 patients with ERA, other forms of JIA and children complaining musculoskeletal symptoms was therefore conducted. Along with fCAL assessment, a detailed clinical and laboratory examination was performed, including the calculation of a composite disease activity scores. Moreover, MRI of the sacroiliac joints was performed in all ERA and other patients complaining of low back pain. The median concentration of fCAL was highest in ERA patients (33.2 mg/kg, $p = 0.02$), with a significant difference between those with inactive and active disease (20.0 vs. 57.4, $p = 0.01$), as well as those with and without MRI signs of sacroiliitis (22.6 vs. 54.3, $p = 0.04$). The fCAL did not differ depending on the NSAID use (23 vs. 20, $p = 0.18$), although weak correlation was observed with the treatment duration ($r = 0.25$, $p = 0.03$). In conclusion, our findings indicate that a parallel inflammation in musculoskeletal system and gut can occur not just in adults with SpA, but in children with ERA as well.

**Keywords: juvenile idiopathic arthritis, enthesitis related arthritis, juvenile spondyloarthritis, fecal calprotectin, magnetic resonance imaging, sacroiliitis, juvenile spondyloarthritis disease activity, juvenile arthritis disease activity score**

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***Edited by:***
*Garifallia Sakellariou, University of Pavia, Italy*

***Reviewed by:***
*Teresa Giani, University of Florence, Italy*
*Silvia Magni-Manzoni, Pediatric Hospital Bambino Gesù, Italy*

***Correspondence:**
*Lovro Lamot*
*lovro.lamot@gmail.com*

***Present address:**
*Lovro Lamot, Department of Pediatrics, University of Zagreb School of Medicine, University Hospital Centre Zagreb, Zagreb, Croatia*
*Miroslav Harjaček, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates*

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## INTRODUCTION

During the past decades, several studies have shown that a substantial number of children with juvenile idiopathic arthritis (JIA) have some kind of gastrointestinal (GI) symptoms, while up to 85% of JIA patients with significant GI symptoms have histological evidence of mild non-specific inflammation (1–5). Although it has been reported that JIA patient might experience abdominal pain related to NSAID use, other causes should be suspected as well, most importantly gut inflammation (6–9).

Among different subtypes of JIA, gut inflammation is most commonly associated with enthesitis related arthritis (ERA), often regarded as undifferentiated form of juvenile spondyloarthritis (jSpA) (10–12). Despite some differences between spondyloarthritis (SpA) in children and adults, mostly in tendency to involve axial joints which is more remarkable in adults, there are emerging views that spondyloarthritis (SpA) surpasses this arbitrary age-based divide (13). Nevertheless, it is still unclear if clinically silent macroscopic and microscopic gut inflammation which occurs in about 60% of adult patient with ankylosing spondylitis (AS) is present in children with ERA as well (14). This is largely due to the challenges imposed by the use of endoscopy, the gold standard for detailed assessment of the inflammation in the gut, which are considerably important in children (15). Since this procedure is invasive, has to be performed under general anesthesia, and has a potential for rare procedural accidents, such as bleeding and perforation, many children, and their parents experience discomfort, anxiety, and dissatisfaction (16, 17). Besides, symptoms of gut inflammation, such as abdominal pain and diarrhea, are rather vague, particularly in children, and overlap with symptoms of functional gastrointestinal disorders, which makes distinguishing those two rather challenging (18). Therefore, non-invasive tests such as blood and fecal biomarkers are increasingly used in clinical practice to help select patients who might benefit from endoscopies and other more detailed investigations.

In recent years, fecal calprotectin (fCAL), a member of the S100 calcium-binding protein family expressed in phagocytes, monocytes, macrophages and granulocytes, has emerged as a valuable screening tool for the gut inflammation, both in adults and children (19). Moreover, it has been shown that fCAL correlates with endoscopic and histologic inflammatory activity (20). Although many studies have shown increased fCAL in adult SpA patients, there are only few studies of fCAL concentration in JIA and/or jSpA patients (21–25). Furthermore, to best of our knowledge, no study investigated the possible association of fCAL concentration in JIA patients with disease activity indices, presence of sacroiliitis, treatment modalities, and various demographic data, which are all characteristics associated with microscopic gut inflammation in adult SpA patients (26–31).

The aim of this study was therefore to assess the fCAL concentration in patients with various subtypes of JIA and children complaining musculoskeletal symptoms, as well as to assess the correlation with various demographic, clinical, laboratory, imaging, and treatment characteristics.

## METHODS

### Study Design and Population

This was a cross-sectional exploratory study in a cohort of patients followed during the year 2019 at the Division of Clinical Immunology and Rheumatology at the Department of Pedaitrics in Sestre Milosrdnice University Hospital Center, Zagreb, Croatia. All of the oligo- and polyarticular JIA and ERA patients fulfilled the ILAR criteria, while in children complaining musculoskeletal symptoms after careful clinical examination, elimination of inflammatory cause of pain, and exclusion of other diseases, only the diagnosis of flat foot was established.

All of the ERA and oligo- and polyarticular JIA patients complained of an abdominal pain for more than seven days, but none had more severe symptoms that interfered with activity, haematochezia, persistent diarrhea, poor growth, prior abnormal studies of the gastrointestinal tract and/or other defined organic cause (e.g., urinary tract infection) (32). Along with fCAL assessment, a detailed clinical and laboratory examination was performed for each patient, including the calculation of a composite juvenile arthritis disease activity score with 27-joint reduced count (JADAS-27) for those diagnosed with oligo- and polyarticular JIA and ERA, as well as juvenile spondyloarthritis disease activity (jSpADA) for those diagnosed with ERA (33, 34). Moreover, as a part of a standard diagnostic procedure, in all ERA patients, as well as in other patients who complained of an inflammatory low back pain, MRI of the sacroiliac joints (SIJ) along with MRI of thoracic and lumbar spine was performed and analyzed by experienced musculoskeletal radiologist according to consensus definitions of components of the Juvenile Arthritis Magnetic Resonance Image Sacroiliac Joint Scoring System (JAMRIS-SIJ) (35, 36). In all ERA and oligo- and polyarticular JIA patients antinuclear antibodies (ANA) and rheumatoid factor (RF) were determined (data not shown). Moreover, in all ERA patients, as well as in some oligo- and polyarticular JIA patients, the presence of HLA-B27 antigen was determined. The disease in ERA and JIA patients was regarded as inactive if jSpADA was $\leq$0.5 and JADAS-27 $\leq$1, respectively, according to the previously reported cut-offs (33, 37). Detailed patients characteristics are shown in **Tables 1A–D**.

### Ethics Statement

The study was conducted per the Helsinki Declaration with the approval from Ethics Committee of the Sestre Milosrdnice University Hospital Center (1-2019-EP). The data were anonymous, and informed consent was obtained from parents/legal guardians as well as from children older than 12 years who participated in the study. All experiments were performed in accordance with relevant guidelines and regulations.

### Fecal Calprotectin (fCAL)

Fecal calprotectin was measured by PETIA (particle enhanced turbidimetric immunoassay) on automatic biochemistry analyzer Architect c8000 (Abbott Laboratories, Illinois, USA) using Bühlmann fCAL® turbo assay (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland). Fecal samples

**TABLE 1 |** Demographic data **(A)**, disease characteristics **(B)**, laboratory **(C)** and MRI **(D)** findings, and treatment modalities **(E)** of study participants.

| | | ERA | JIA | NIC |
|---|---|---|---|---|
| **(A) Demographics** | | | | |
| ***N*** (% female) | | 26 (46%) | 33 (58%) | 12 (67%) |
| **Age** (median yrs, IQR) | | 12 (7.7–14.5) | 11 (7–14) | 13 (6.1–14) |
| **(B) Disease characteristics** | | | | |
| **Disease duration** (median mo, IQR) | | 21 (6–54) | 18 (6–66) | / |
| **Active joints** (median N, IQR) | | 0 (0–2) | 0 (0–3.5) | / |
| **Active enthesis** (median N, IQR) | | 1 (0–2) | 0 | / |
| **Pain** (median VAS, IQR)[a] | | 3 (0–5) | 1 (0–3) | / |
| **PGA well-being** (median VAS, IQR)[b] | | 2.5 (0.2–4) | 1.5 (0–2.5) | / |
| **PGA disease activity** (median VAS, IQR)[c] | | 1.5 (0.37–3) | 1 (0–2) | / |
| **Morning stiffens**[d] (*N*) | | 6 | 7 | / |
| **Clinical sacroiliitis**[e] (*N*) | | 5 | 0 | / |
| **Abnormal back mobility**[f] (*N*) | | 4 | 0 | / |
| **Uveitis**[g] (*N*) | | 1 | 1 | / |
| **Positive family history**[h] (*N*) | | 13 | 14 | / |
| **(C) Disease activity** | | | | |
| **JADAS-27** (median, IQR) | | / | 2 (0–4) | / |
| **JSpADA** (median, IQR) | | 1 (0–3) | / | / |
| **(D) Laboratory findings** | | | | |
| **HLA-B27*** (N of positive/N analyzed) | | 12/26 | 1/17 | / |
| **CRP** (median mg/L, IQR) | | 1.0 (0.3–2.9) | 1.3 (0.3–2.6) | 0.5 (0.3–1.9) |
| **ESR** (median mm/3,6 ks, IQR) | | 5 (4–11) | 8 (5–11.5) | 4.5 (3.2–12.5) |
| **(E) MRI findings** | | | | |
| **MR of SIJ** (*N*) | | 26 | 10 | 0 |
| **Inflammation components**[i] | **Bone marrow edema** (*N*) | 14 | 0 | 0 |
| | **Joint space inflamation** (*N*) | 9 | 0 | 0 |
| | **Capsulitis** (*N*) | 0 | 0 | 0 |
| | **Enthesitis** (*N*) | 2 | 0 | 0 |
| **Structural components**[i] | **Sclerosis** (*N*) | 4 | 0 | 0 |
| | **Erosions** (*N*) | 1 | 0 | 0 |
| | **Fatty lesion** (*N*) | 0 | 0 | 0 |
| | **Ankylosis** (*N*) | 0 | 0 | 0 |
| **(F) Treatment modalities** | | | | |
| **NSAID** | ***N*** | 14 | 25 | 4 |
| | **Duration** (median mo, IQR) | 1 (1–15) | 4 (1–21) | 1 (1–2) |
| **cDMARD** | ***N*** | 2 | 5 | 0 |
| | **Duration** (median mo, IQR) | 9 (3–15) | 15 (6–46.5) | / |
| **bDMARD** | ***N*** | 1 | 4 | 0 |
| | **Duration** (median mo, IQR) | 3 | 11 (2.5–17.2) | / |

*ErA, enthesitis related arthritis; JIA, oligo- and polyarticular juvenile idiopathic arthritis; NIC, non-inflammatory controls; NSAID, non-steroidal anti-inflammatory drug; cDMARD, conventional disease modifying antirheumatic drug; bDMARD, biological disease modifying antirheumatic drug.*

[a] *patient reported pain over the past week, recorded on a visual analog scale (0, 10).*

[b] *patient/parent's global assessment of a child's overall well-being in range from 0 (best) to 10 (worst).*

[c] *physician global assessment of disease activity in range from 0 (best) to 10 (worst).*

[d] *morning stiffness for >15 min.*

[e] *defined as the presence of 2 or more of the following: tenderness on examination, positive Patrick's or FABER test and inflammatory back pain.*

[f] *abnormal back mobility defined as modified Schober's <20 cm.*

[g] *presence of any uveitis (including acute/symptomatic and chronic/asymptomatic disease).*

[h] *ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a 1 relative.*

[i] *as defined by Consensus definitions of components of the Juvenile Arthritis Magnetic Resonance Image Sacroiliac Joint Scoring System (JAMRIS-SIJ) (36).*

[*] *the presence of HLA-B27 was not determined in all of the patients.*

were extracted with extraction buffer using the CALEX® Cap extraction device. The extracts were incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies. Calprotectin from the sample mediated the immunoparticle agglutination. Sample turbidity caused by calprotectin-immunoparticle complex formation was proportional to calprotectin concentration. The lower and upper limits of the detection were 20–8,000 mg/kg, respectively. According to the manufacturer's instructions, the fCAL levels below 50 mg/kg were considered normal, those between 50 and 200 mg/kg as slightly elevated, and those above 200 mg/kg as elevated, both in adults and children between 4 and 17 years (38).

## Statistical Analysis

Data were analyzed using GraphPad Prism 8 and $p < 0.05$ was considered statistically significant. Normality of distribution was tested with Saphiro-Wilk test and data are presented as interquartile ranges (IQR) and medians. Statistical comparisons between two groups were performed using the Student's *t*-test (normal distribution) or the Mann-Whitney *U* test, while the comparisons between three or more groups were performed by Kruskall-Wallis test, following a *post-hoc* test using Tukey's method. Correlations between the parameters were calculated using the Spearman rank correlation. For the calprotectin test, the results below the detection limits were equalized to 20 mg/kg.

# RESULTS

In total, 71 patients were enrolled in the study. Among them, 26 patients were diagnosed with ERA and 33 patients were diagnosed with oligo- and polyarticular forms of JIA, while the rest were 12 children complaining musculoskeletal symptoms, regarded as non-inflammatory controls (NIC).

## Fecal Calprotectin in Patients With Various Forms of JIA

Overall, the median concentration of fCAL was highest in ERA group [33.2 (20–84.8), $p = 0.02$] (**Figure 1A**). A *post-hoc* test revealed that ERA patients had significantly higher fCAL than other JIA patients (adjusted $p = 0.04$). Moreover, 30.8% of ERA patients had values above 50 mg/kg, which was regarded as abnormal by test manufacturer (38). This percentage was twice lower in other JIA and NIC patients, 15.1 and 12.1%, respectively.

No significant correlation was observed between fCAL concentration and age at the time of sampling, duration of the disease, number of active joints and/or enthesis, physician global assessment, CRP or ESR concentrations, nor disease activity in JIA patients measured by JADAS-27 (data not shown). There was no difference between the fCAL values in JIA patients with inactive (JADAS-27 $\leq$1) or active (JADAS-27 $\geq$1) disease (20.0 vs. 20.0 mg/kg, $p = 0.934$).

## Fecal Calprotectin in Patients With ERA

In patients with ERA, moderate correlation was observed between fCAL concentration and the disease activity measured by jSpADA ($r = 0.46$, $p = 0.02$). Besides, there was a significant difference in fCAL concentration between ERA patients with inactive (jSpADA $\leq$0.5) or active (jSpADA $\geq$0.5) disease (20.0 vs. 57.4 mg/kg, $p = 0.01$). Moreover, ERA patients with one or more sign of SIJ inflammation detected by MRI (**Table 1E**) had a significantly higher fCAL concentration than those without the signs of inflammation (22.6 vs. 54.3 mg/kg, $p = 0.04$). Among all patients with ERA, the median levels of fCAL were highest in those with active disease (jSpADA $\geq$0.5) and MRI sign(s) of sacroiliitis [77.7 (26–226.1) mg/kg, $p = 0.04$] (**Figure 1B**), with three patients having a fCAL concentration above 200 mg/kg, and three more in 50–200 mg/kg range.

## Fecal Calprotectin and Various Treatment Modalities

Of 71 patients, 43 (60.5%) were treated with non-steroidal anti-inflammatory drugs (NSAID) at the time of sampling for the average duration of 1 (1–17) months (**Table 1F**). Seven patients were receiving conventional disease modifying antirheumatic drugs (cDMARD) for a median duration of 15 (5–21) months. Biological disease modifying antirheumatic drugs (bDMARD) were used in four patients with oligo- and polyarticular JIA for a median duration of 11 [2.5–17.2] months and in one patient with ERA for 3 months. All of them were receiving TNF-alfa inhibitor adalimumab. Finally, three patients were treated with glucocorticoids (GC) at the time of sampling for the median time of 12 (2–27) months. Of those, two had oligo- and polyarticular JIA [median duration of treatment 19.5 (12–27)] and one had ERA (duration of treatment 2 months).

In all patients, the fCAL concentration did not significantly differ among those receiving and not receiving NSAID [the median value was 23 (20–49.6) mg/kg in patients receiving NSAID vs. 20 (20–33.6) mg/kg in patients not receiving NSAID, $p = 0.18$], although weak correlation was found with the duration of use ($r = 0.25$, $p = 0.03$). No correlation was observed between fCAL levels and other treatment modalities and duration. The median value of fCAL concentration in patients receiving DMARD was 32.6 (20–44.4) mg/kg, higher than in patients not receiving these medications in which the median value was 20 mg/kg (20–46.7, $p = 0.27$). Moreover, the median value of fCAL in patients receiving bDMARD was 26.3 mg/kg (20–51.6), while in those who were not receiving bDMARD, the median value was 20 mg/kg (20–46.7, $p = 0.95$). Comparing the patients receiving and not receiving GC, the fCAL median value was the same, 20 mg/kg (20–39.4 vs. 20–46.7, respectively, $p = 0.66$). Finally, patients receiving medications (NSAID, DMARD, and GC) and patients not receiving any medication had the same median fCAL values of 20 mg/kg (20–46.3 vs. 20–46.1, respectively, $p = 0.64$).

# DISCUSSION

The association of epithelial barrier and joint inflammation has become a focus of attention in both basic and clinical research, with a task to understand the immunopathogenic link and the diagnostic utility (39). Interestingly, it has been proposed recently that a key event in the early stages of ankylosing spondylitis appears to be the association with subclinical Crohn's-like colitis (12). Although there is no consistent confirmation

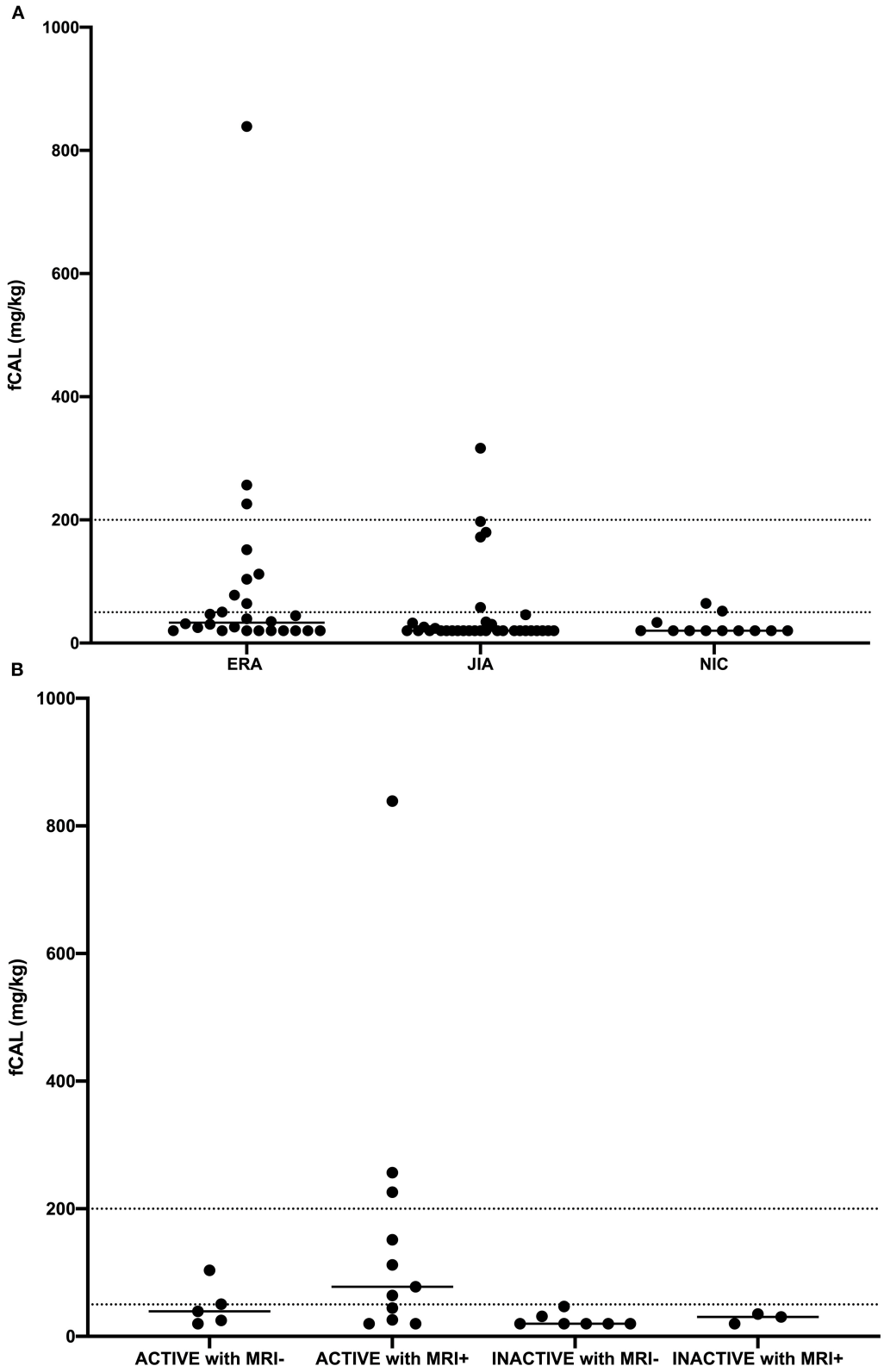


**FIGURE 1 |** Fecal calprotectin (fCAL) concentration in **(A)** enthesitis related arthritis (ERA), oligo- and polyarticular juvenile idiopathic arthritis (JIA), and non-inflammatory control (NIC) patients, and **(B)** enthesitis related arthritis (ERA) patients with active and inactive disease, with (MRI+) or without (MRI-) inflammatory changes detected by MRI. Each dot represents fCAL concentration in a single patient, while horizontal lines represent median values.

for the genetical, immunological and environmental ties between gut and joints, there are several clinical indications suggesting a close link between gut inflammation and SpA in adults, while the results of our study might help to further establish this link in children (12, 14, 40).

In our study, we have shown that among patients with various subtypes of JIA, the fCAL concentrations were highest in those with ERA subtype, which is alongside similar studies in adult SpA patients and two studies in children (21–25). Intriguingly, almost a third of patients with this particular type of JIA had fCAL concentrations above the range regarded as normal by the test manufacturer (38). The novel finding of our study was that fCAL concentration was significantly higher in ERA patients with MRI sign(s) characteristic for the SIJ inflammation, which adds to the growing number of evidences for a clinical association of gut inflammation and axial spondyloarthritis in adult and pediatric patients (40–43). Moreover, to best of our knowledge, our study was the first to correlate the fCAL concentration with somewhat novel disease activity scores, such as JADAS-27 and jSpADA, with the results showing significantly higher fCAL concentration in ERA patients with active disease (33, 34). Ultimately, the highest level of fCAL was observed in group of ERA patients with active disease and MRI sign(s) of sacroiliitis, suggesting that gastrointestinal inflammation might be a part of a wider inflammatory process present in patients with ERA. Therefore, the results of our study could encourage the wider diagnostic approach to ERA patients, which involves measuring of fCAL especially in patients with active disease and/or MRI signs of sacroiliitis, even without the signs of GI involvement, and vice versa, performing an MRI of SIJ in ERA patients with increased levels of fCAL and active disease, even without the presence of lower back pain. Moreover, since recurrent abdominal pain is very common in children, especially those with JIA and/or taking NSAID, it is useful to inquire about the potential inflammatory cause by performing a simple, economic and non-invasive test such as fCAL (1, 32, 44). Finally, our results could further inform the translational research on the ties between gut and joints. For this cause, fCAL should be observed merely as a surrogate marker correlating well with the inflammatory activity in the gut (20). Therefore, the significantly different concentrations of fCAL between children with oligo- and polyarticular JIA, ERA, and NIC, implies the different level of inflammatory activity and not necessarily the presence of the clinical symptoms characteristic for the inflammatory bowel disease. Nevertheless, as suggested by previous studies, this "subclinical inflammation" could lead to a certain degree of dysbiosis and further development of inflammatory rheumatic diseases (45–48).

As opposite to some previous studies, the fCAL levels in our study were not associated with the use of NSAID, nor with the use of other treatment modalities for JIA (25). It has been shown that NSAID use in adults may result in the intestinal inflammation and an increase in fCAL levels, while the drug discontinuation results in the decline of the FC levels, suggesting healing of the gut mucosa (49). Since we performed a cross sectional exploratory study with the focus on real-life data, with all of our JIA and ERA patients experiencing only light abdominal pain, none of the treatment modalities was omitted before the sampling for fCAL measurement. Regardless, the fCAL levels were highest in ERA group in which less proportion of patients (14/26) was treated with NSAID than in oligo- and polyarticular JIA group (25/33).

There are several important limitations to our study. Although the best available evidence in the literature support the use of fCAL in inflammatory bowel disease (IBD) diagnosis and monitoring, as well as in distinguishing between IBD and irritable bowel syndrome, the gold standard for the detection of (sub)clinical gut inflammation remains the endoscopy and biopsy, which was in our study performed only in one patient (data not shown) (50, 51). Moreover, we didn't report on repeated values of the fCAL measurement. Nevertheless, this might be of lesser importance, since the primary aim of our study was to assess the presence of the gut inflammation in patients with various forms of JIA via the use of surrogate non-invasive biomarker in a prospective cohort of consecutive patients followed at a single pediatric rheumatology center, regardless of their clinical characteristics, disease status and/or concomitant therapy. Additionally, although sensitive for mucosal inflammation, fCAL is non-specific and environmental exposures such as low fiber intake, lack of physical exercise and increasing age, and/or use of certain drugs such as proton pump inhibitors, can cause its elevation (52–54). Nevertheless, since children in our cohort were of similar age (**Table 1A**) and from same geographic area, it is reasonable to assume they also had a comparable nutritional habits and physical activity levels, while none of them was taking drugs such as proton pump inhibitors. Finally, the number of the study participants was not high, thereby limiting power of the analysis.

In conclusion, the results of our study show that ERA patients have significantly higher fCAL levels than those with other form of JIA or children complaining musculoskeletal symptoms. Moreover, the concentration was highest in ERA patients with active disease and the MRI sign(s) of the inflammatory process in SIJ, which emphasizes that a parallel inflammation in musculoskeletal system and gut can occur not just in adults with SpA, but also in children with undifferentiated SpA. Although this observation still needs to be confirmed in a multicentric studies involving larger number of patients, it could already contribute to planning of diagnostic procedures and treatment modalities in the clinical care for ERA patients, but also further encourage the translational research of the link between gut and joints.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Sestre Milosrdnice University Hospital Center. Informed consent was obtained from

the participant's legal guardian/next of kin, as well as from participants older than 12 years.

## AUTHOR CONTRIBUTIONS

LL: study design, charts review, data interpretation, statistical analysis, and manuscript preparation. MM: data interpretation, statistical analysis, and final review of the manuscript. RV and MV: data interpretation and final review of the manuscript. ML and IT: charts review and manuscript preparation. NG: laboratory analysis, statistical analysis, and final review of the manuscript. MH: final review of the manuscript. All authors contributed to the article and approved the submitted version.

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# Tapering of Biological Agents in Juvenile ERA Patients in Daily Clinical Practice

*Chun-Hua Liao*[1,2], *Bor-Luen Chiang*[2,3] *and Yao-Hsu Yang*[2,4]*

[1] Department of Pediatrics, National Taiwan University BioMedical Park Hospital, Hsin-Chu, Taiwan, [2] Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, [3] Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan, [4] Department of Pediatrics, National Taiwan University Hospital, Hsin-Chu Branch, Hsin-Chu, Taiwan

**Objectives:** We aim to evaluate the proportion and characteristics of enthesitis-related arthritis (ERA) patients in whom medications can be withdrawn in daily practice and to analyze the factors associated with flare-ups during medication tapering of these patients.

**Methods:** We retrospectively reviewed records of patients under 16 years old diagnosed with ERA from April 2001 to March 2020 in one tertiary medical center in Taiwan. Patients were categorized by different medication uses: conventional disease modifying anti-rheumatic drugs (cDMARDs) only and cDMARDs plus biologics. Demographics, laboratory data, presence of uveitis, and medication withdrawal rate were analyzed. Subgroup analysis was performed in the patients with cDMARDs plus biologics to identify factors associated with flare-ups during medication tapering of these patients. Statistical analysis was performed using R (v3.6.0).

**Results:** There were 75 juvenile ERA patients with a median onset age of 10.28 years old. Nineteen (25.3%) patients used cDMARDs for disease control; 56 (74.7%) patients depended on cDMARDs plus biologics. Poly-articular involvement was noted in 29 (38.7%) patients, and it occurred more frequently in the cDMARDs plus biologics subgroup (cDMARDs only, 5.3%; cDMARDs plus biologics, 53.6%; $P = 0.0001$). ANA positivity was observed in 18 (24.0%) patients, and it occurred more frequently in the cDMARDs plus biologics subgroup (cDMARDs, 0%; cDMARDs plus biologics, 32.1%; $P = 0.0038$). The overall medication withdrawal rate was 34.7%, and it occurred more frequently in patients with cDMARDs only (cDMARDs only, 84.2%; cDMARDs plus biologics, 17.9%; $P < 0.001$). In the subgroup analysis of patients with cDMARDs plus biologics, patients on biologics tapering with flare-up had a significantly longer time interval between disease onset and initiation of cDMARDs (biologics tapering without flare-up: 0.27 (0.11–0.73) years; biologics tapering with flare-up: 1.14 (0.39–2.02) years; ever withdrawing biologics: 0.26 (0.18–0.42) years, $P = 0.0104$).

**Conclusion:** Juvenile ERA patients with polyarticular involvement had a higher risk of developing cDMARDs refractory and progressing to biologics use. Patients with a long time interval between disease onset and initiation of cDMARDs were prone to experience flare-up during tapering of biologics.

**Keywords: enthesitis-related arthritis, biologics, tapering, withdrawal, flare-up**

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*Miroslav Harjaček,*
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*Marco Fornaro,*
*University of Bari Aldo Moro, Italy*

***Correspondence:**
*Yao-Hsu Yang*
*yan0126@ms15.hinet.net*

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## INTRODUCTION

Juvenile spondyloarthritis (SpA) is a distinct entity of chronic pediatric arthritis with characteristics of male predominance, strong association with human leucocyte antigen (HLA)-B27, and involvement of the entheses and axial bones (1). Currently, there are seven subtypes of juvenile idiopathic arthritis (JIA), which are classified by the International League of Association for Rheumatology (ILAR) criteria (2). However, juvenile SpA was not one of the seven subtypes, and most juvenile SpA was categorized as enthesitis-related arthritis (ERA) according to the ILAR criteria (1).

Among the seven subtypes of JIA classified by ILAR, ERA is the most common in a large part of eastern and southern Asia, accounting for up to 30% of JIA cases (3, 4). In contrast, oligoarthritis is the most frequent subtype in North American JIA cohorts, while ERA only accounts for 10% of all JIA cases (5). Compared with other subtypes of JIA, children with ERA are prone to have higher disease activity and pain severity (6). However, possibly because of the relatively low prevalence of ERA in Western countries, limited literature has focused on the outcome and treatment response as well as the medication withdrawal rate in ERA patients (7–9).

Timely diagnosis and treatment of JIA with conventional disease-modifying anti-rheumatic drugs (cDMARDs) as well as biologics has dramatically changed the prognosis in the past two decades. Biologics, such as tumor necrosis factor inhibitors (TNFis), interleukin-6 antagonists, and T cell activation inhibitors, can be used in patients with active JIA refractory to cDMARDs. A large proportion of JIA patients have gained inactive disease status or even remission on medication. However, with the economic burden and other potential costs for patients, families, and society, as well as safety concerns regarding the long-term use of cDMARDs and/or biologics (10), important questions have arisen on how and when physicians can taper and/or withdraw medications. Another serious issue of post-withdrawal recurrence should also be emphasized. Studies on JIA treatment tapering and/or withdrawal varied in many aspects, such as enrolled population, medication studied and tapering protocol and outcome assessed (11). We aim to evaluate the proportion and characteristics of ERA patients in whom medications can be tapered in daily practice and to analyze the factors associated with flare-ups during medication tapering.

## MATERIALS AND METHODS

We retrospectively reviewed the medical records of patients under 16 years old with a diagnosis of ERA from April 2001 to March 2020 at one tertiary medical center in Taiwan. The diagnosis of ERA was based on validated criteria defined by the ILAR (2). Patients were divided into two subgroups according to treatment: cDMARDs only and cDMARDs plus biologics. We retrieved demographic variables, laboratory parameters of inflammation, antinuclear antibody (ANA) positivity (titer $>$ or $=$ 1:80), HLA-B27 positivity, number of active joints at initiation of medication, presence of uveitis, presence of enthesitis, presence of axial involvement, type of cDMARDs administration, type of biologics use, time interval between disease onset and the start of cDMARDs, time interval between disease onset and the initiation of biologic therapy, time to achieve clinical inactive disease once biologic agent was started, time interval between clinical inactive disease and the initiation of biologics tapering, and medication withdrawal rate as well as post-withdrawal recurrence rate.

### Definition of Clinical Inactive Disease, Clinical Remission, Flare-Up, and Recurrence

We used Wallace criteria (12) to define clinical inactive disease, which included (1) no joints with active arthritis, (2) absence of systemic manifestations (fever, rash, serositis, splenomegaly or generalized lymphadenopathy resulting from JIA), (3) no active uveitis, (4) normal ESR or CRP (if both are tested, both must be within normal limits), and (5) physician's global assessment of disease activity indicating no disease activity.

Flare-up was defined as loss of at least two items of the Wallace criteria as well as when the attending physician intensified treatment because of elevated disease activity.

Clinical remission on medication was defined as clinical inactive disease for a minimum of 6 continuous months. Recurrence was defined as disease flare-up after clinical remission and discontinuation of cDMARDs and biologics for at least 2 months.

### Biologics Tapering Strategy

In our institution, most physicians reached a consensus to carefully extend the administration interval of biologics in patients with inactive disease, though there was no prespecified protocol. Tapering of cDMARDs was initiated before biologics. During tapering, concomitant administration of non-steroidal anti-inflammatory drugs (NSAIDs) was allowed. The decision of when to start tapering or the schedule of tapering biologics was left to the treating physician. The minimal follow-up period was 6 months after medication withdrawal.

### Statistical Analysis

Laboratory data are presented as the median (interquartile range, IQR). Continuous data were compared using the Kruskal-Wallis test. We compared categorical variables and proportions by using the chi-square test. Survival analysis was calculated by the Kaplan–Meier method. A threshold of $P < 0.05$ was used for statistical significance. Statistical analyses were conducted with R software (version 3.6.0).

## RESULTS

### Patient Characteristics

There were 75 patients enrolled in this retrospective study. The demographic data and clinical characteristics of all patients and the two subgroups are summarized in **Table 1**. Among all patients, 19 (25.3%) patients took cDMARDs only, and 56 (74.7%) of them took cDMARDs plus biologics for disease control. There were 62 (82.7%) boys among all patients. The median onset age was 10.28 (IQR: 8.24–12.05) years old. The

**TABLE 1 |** Characteristics of ERA patients with cDMARDs only and cDMARDs plus biologics.

| | Total (*N* = 75) | cDMARDs only (*N* = 19) | cDMARDs plus biologics (*N* = 56) | *P*-value |
|---|---|---|---|---|
| Onset age, | 10.28 | 10.60 | 10.27 | 0.9127 |
| years old | (8.24–12.05) | (8.28–12.20) | (8.24–12.04) | |
| Male sex | 62/75 (82.7%) | 14/19 (73.7%) | 48/56 (85.7%) | 0.2944 |
| Poly-articular | 29/75 (38.7%) | 1/19 (5.3%) | 30/56 (53.6%) | **0.0001** |
| Oligo-articular | 46/75 (61.3%) | 18/19 (94.7%) | 26/56 (46.4%) | |
| ANA positivity | 18/75 (24.0%) | 0/19 (0%) | 18/56 (32.1%a) | **0.0038** |
| HLA B27 positivity | 75/75 (100.0%) | 19/19 (100%) | 56/56 (100%) | 1.0 |
| Uveitis | 9/75 (12.0%) | 1/19 (5.3%) | 8/56 (14.3%) | 0.4337 |
| Enthesitis | 20/75 (26.7%) | 5/19 (26.3%) | 15/56 (26.8%) | 1.0 |
| Axial involvement | 28/75 (37.3%) | 7/19 (36.8%) | 21/56 (37.5%) | 1.0 |
| Time to cDMARDs, years | 0.40 | 0.33 | 0.48 | 0.10006 |
| | (0.20–1.24) | (0.11–0.53) | (0.23–1.39) | |
| MTX | 54/75 (72.0%) | 8/19 (42.1%) | 46/56 (82.1%) | **0.0021** |
| SAL | 31/75 (41.3%) | 15/19 (78.9%) | 16/56 (28.6%) | **0.0003** |
| AZA | 21/75 (28.0%) | 7/19 (36.8%) | 14/56 (25.0%) | 0.3796 |
| HCQ | 9/75 (12.0%) | 5/19 (26.3%) | 4/56 (7.1%) | **0.0406** |
| PEN | 3/75 (4.0%) | 1/19 (5.3%) | 2/56 (3.6%) | 1.0 |
| CsA | 3/75 (4.0%) | 0/19 (0%) | 3/56 (5.4%) | 0.5667 |
| Follow-up period, years | 6.20 | 3.01 | 6.87 | **0.00025** |
| | (2.91–9.56) | (1.04–5.30) | (4.59–11.35) | |

*Data shown are median (IQR) or number (%) of patients as appropriate.*
*ERA, enthesitis-related arthritis; cDMARDs, conventional disease-modifying antirheumatic drugs; ANA, antinuclear antibody; HLA, human leucocyte antigen; MTX, methotrexate; SAL, sulfasalazine; AZA, azathioprine; HCQ, hydroxychloroquine; PEN, mesalazine; CsA, cyclosporine.*
*Values in the bold indicate p values less than 0.05.*

**TABLE 2 |** Medication withdrawal rate and post-withdrawal recurrence rate in ERA patients with cDMARDs only and cDMARDs plus biologics.

| | Total *N* = 75 | cDMARDs only *N* = 19 | cDMARDs plus biologics *N* = 56 | *P*-value |
|---|---|---|---|---|
| Medication withdrawal rate | 26/75 (34.7%) | 16/19 (84.2%) | 10/56 (17.9%) | **<0.00001** |
| Recurrence[a] rate | 10/26 (38.5%) | 5/16 (31.3%) | 5/10 (50.0%) | 0.425 |
| Recurrence within 1 year | 5/10 (50.0%) | 2/5 (40.0%) | 3/5 (60.0%) | 1.0 |
| Recurrence after 1 year | 5/10 (50.0%) | 3/5 (60.0%) | 2/5 (40.0%) | 1.0 |

*Data shown are number (%) of patients as appropriate.*
*ERA, enthesitis-related arthritis; cDMARDs, conventional disease-modifying antirheumatic drugs.*
*[a]Recurrence was defined as disease flare-up after clinical remission and discontinuation of cDMARDs and biologic agents for at least 2 months.*
*Values in the bold indicate p values less than 0.05.*

percentage of male patients and disease onset age showed no significant difference between the two subgroups.

Overall, polyarticular involvement was noted in 29 (38.7%) patients, and it occurred more frequently in the cDMARDs plus biologics subgroup (cDMARDs only, 5.3%; cDMARDs plus biologics, 53.6%; $P = 0.0001$). ANA positivity was observed in 18 (24.0%) patients, and it occurred more frequently in the cDMARDs plus biologics subgroup (cDMARDs only, 0%; cDMARDs plus biologic agents, 32.1%; $P = 0.0038$). Nine (12.0%) patients had associated uveitis, and the incidence of uveitis showed no significant difference between the two subgroups. ($P = 0.4337$) Twenty (26.7%) patients had enthesitis, while 28 (37.3%) patients presented with axial involvement. The incidence of enthesitis and axial involvement showed no significant difference between the two subgroups.

The median time interval between disease onset and the start of cDMARDs was 0.40 (IQR: 0.20–1.24) years, and there was no significant difference between these two subgroups. The two most commonly used cDMARDs were methotrexate and sulfasalazine, which were prescribed to 72.0% and 41.3% of the patients, respectively. Methotrexate (82.1%) was the most commonly prescribed cDMARD in the cDMARDs plus biologics subgroup, while sulfasalazine (78.9%) was the most frequently used cDMARD in the cDMARDs only subgroup.

## Medication Withdrawal Rate and Post-withdraw Recurrence Rate

The overall medication withdrawal rate was 34.7%, and it occurred more frequently in patients with cDMARDs only (cDMARDs only, 84.2%; cDMARDs plus biologics, 17.9%; $P < 0.001$). Post-withdrawal recurrence occurred in 10 (38.5%) patients, and half of them occurred within 1 year after discontinuation of all medication. The post-withdrawal recurrence rate showed no significant difference between these two subgroups (cDMARDs only, 31.3%; cDMARDs plus biologics, 50.0%; $P = 1.0$) (see **Table 2**).

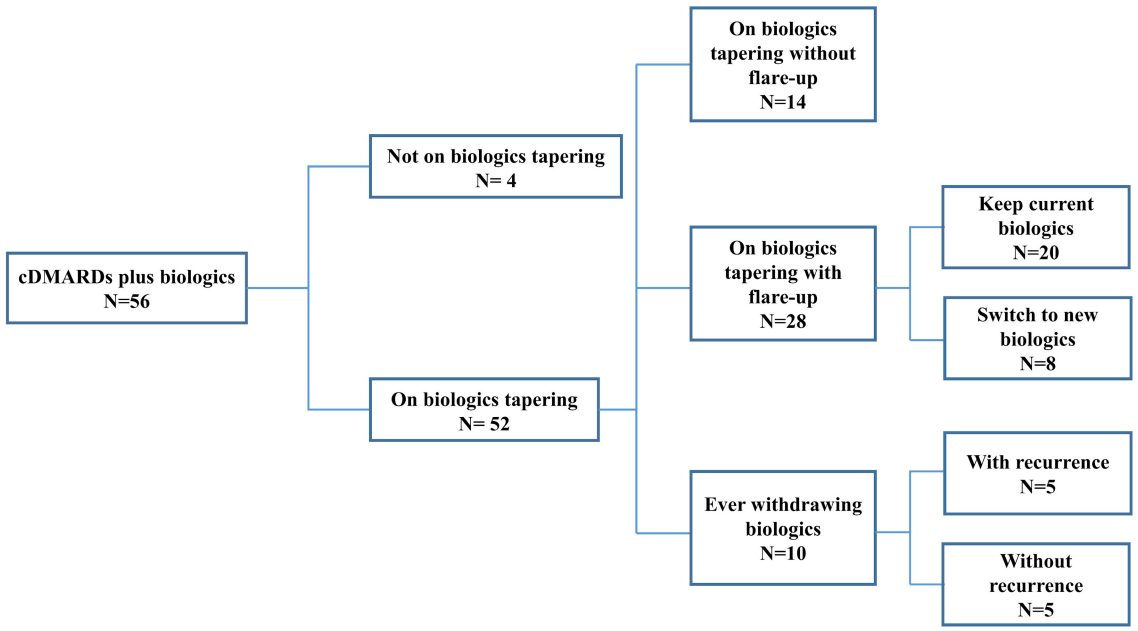


**FIGURE 1 |** ERA patients stratified by biologics tapering status. ERA, enthesitis-related arthritis; cDMARDs, conventional disease-modifying antirheumatic drugs.

## Factors Associated With Flare-Up During Biologics Tapering

To further investigate clinical predictors of successful tapering and then discontinuation of biological agents, we categorized the patients with cDMARDs plus biologics into four subgroups based on whether they experienced flare-up during tapering of biologics: not on biologics tapering, on biologics tapering with flare-up, on biologics tapering without flare-up, and ever withdrawing biologics. There were 4 patients who had not been on biologics tapering, 14 on biologics tapering without flare-up, 28 on biologics tapering with flare-up, and 10 ever withdrawing biologics. Half of the ever withdrawing biologics subgroup experienced recurrence during the follow-up period (see **Figure 1**). Among all patients with cDMARDs plus biologics, 39 (69.6%) patients used etanercept as first-line biologic, 16 (28.6%) patients used adalimumab as first-line biologic, and only one patient (1.8%) used abatacept as first-line biologic. In the biologics tapering with flare-up subgroup, eight patients switched to a second-line biological agent for better disease control. The detailed biologic switches of the eight patients is illustrated in **Figure 2**. Seven (87.5%) of them who had biologic switches were male. Seven (87.5%) patients received etanercept as first-line biologic, and one (12.5%) patient took abatacept as first-line biologic. Abatacept was used as first-line treatment instead of TNFi in this patient due to a history of severe skin eruption after TNFi injection. (He previously received a single etanercept injection, which precipitated the skin eruption.) The most commonly used biologic as a second-line treatment during flare-ups was adalimumab (87.5%). One patients used abatacept as second-line treatment after etanercept failed and due to an etanercept-related adverse effect (pulmonary tuberculosis). More than one biologic switch occurred in three patients, and two of them used tocilizumab as a third-line treatment. Flare-up with presentation of active arthritis was noted in seven patients, and uveitis was noted in one patient. Among 56 patients who received biologics, only one patient was found to have pulmonary tuberculosis after 2.5 years of etanercept (see **Figure 2**).

Among the three subgroups (on biologics tapering without flare-up, on biologics tapering with flare-up, ever withdrawing biologics), the disease onset age, percentage of male patients, poly-articular involvement, ANA positivity, presence of uveitis, presence of enthesitis, presence of axial involvement, laboratory parameters of inflammation, and type of administered cDMARDs and biologics showed no significant difference. Patients on biologics tapering with flare-up had a significantly longer time interval between disease onset and initiation of cDMARDs (on biologics tapering without flare-up: 0.27 (0.11–0.73) years; on biologics tapering with flare-up:1.14 (0.39–2.02) years; ever withdrawing biologics: 0.26 (0.18–0.42) years, $P = 0.0104$). Patients on biologics tapering with flare-up also seemed to take a longer time to achieve clinical inactive disease once the biological agent was started though with only trend significance (on biologics tapering without flare-up:0.35 (0.33–0.44) years; on biologics tapering with flare-up:0.38 (0.21–0.52) years; ever withdrawing biologics: 0.27 (0.16–0.30) years, $P = 0.0948$). The median time interval to biologics tapering after achieving clinical inactive disease was 0.57 (0.30–0.84) years, and it showed no significant difference among the three subgroups (see **Table 3**).

When they achieved clinical inactive disease, 25 (48.1%) patients were taking cDMARDs, 12 (23.1%) patients were taking NSAIDs, and NSAIDs were mostly used for pain relief only, not

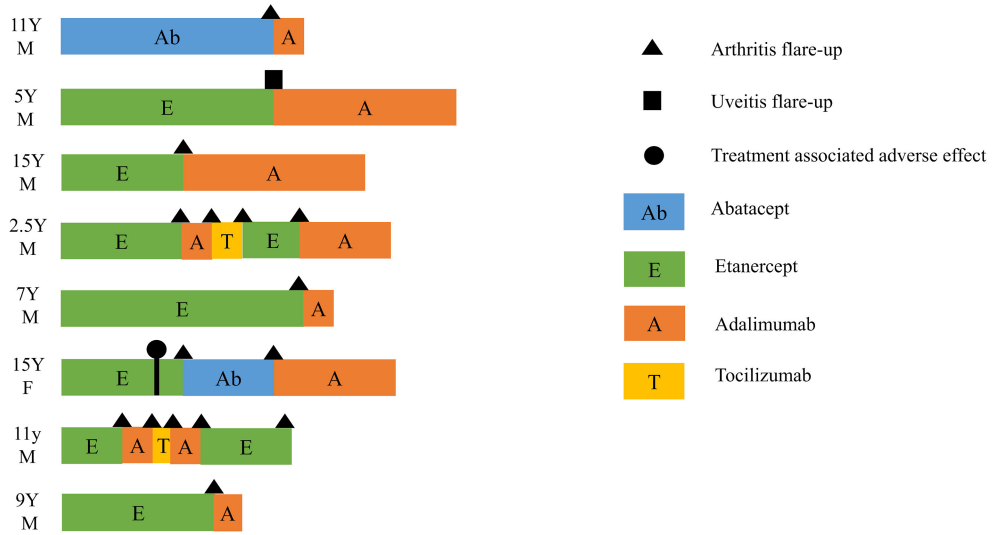


**FIGURE 2 |** Switch between biologics in eight patients who had flare-up during biologics tapering. Triangle, flare-up with presentation of arthritis; square, flare-up with presentation of uveitis; circle, treatment-associated adverse effect (pulmonary tuberculosis infection); Ab, abatacept; E, etanercept; A, adalimumab; T, tocilizumab; Y, years old; M, male; F, female.

on a daily basis. Two (3.8%) patients were under corticosteroids, and the corticosteroid dose was 5 mg of prednisolone per day. When biologics tapering was initiated, 10 (19.2%) patients were taking cDMARDs, 7 (13.5%) patients were taking NSAIDs, and 1 (1.9%) patient was taking corticosteroids (see **Table 3**).

Kaplan–Meier survival analysis demonstrated that the flare-free survival rate was significantly higher in the biologics tapering without flare-up group than in the biologics tapering with flare-up group and in the ever withdrawing biologics group ($P < 0.0001$). The median time to flare-up was 1.69 years in the biologics tapering with flare-up group versus 5.19 years in the ever withdrawing biologics group (see **Figure 3**).

## DISCUSSION

With more JIA patients achieving constant remission under cDMARDs and/or biologics, tapering or even withdrawing medications has been taken into account by the patients as well as the attending physicians. However, in a multicenter prospective observational study conducted by Otten et al. a sustained remission status could not be achieved in 22 pediatric patients with ERA, and none of them discontinued TNFi successfully. The study may not fully reflect the real-world data of biologics use in ERA patients not only because of limited case numbers but also because of the relatively high drop-out rate (up to two-thirds of them were lost to follow-up after 2.25 years) (7). Herein, we evaluated the proportion and characteristics of 75 ERA patients in whom medications can be tapered in daily practice and analyzed the factors associated with flare-ups during medication tapering.

In the present study, the overall medication withdrawal rate was 34.7% within the median follow-up period of 6.2 years. The withdrawal rate was significantly higher in patients with cDMARDs only (84.2%), and this may be correlated with variable disease severity (active joint number at initiation of medications) between cDMARDs only and cDMARDs plus biologic subgroups. The incidence of polyarticular involvement was 10-fold higher in patients receiving cDMARDs plus biologics than in patients receiving cDMARDs only.

The majority of our ERA patients had oligo-articular involvement (61.3%); however, the percentage was significantly lower compared to previous cohort studies (74–90%) (13–15). This discrepancy might relate to different ethnicities or study design. In previous literature, oligoarthritis or polyarthritis was defined by the active joint count at disease onset or diagnosis; however, we defined oligo-articular or poly-articular involvement by the active joint count at initiation of cDMARDs. Referral bias could also contribute to the difference in patient characteristics. Our institution, as a tertiary referral center, often cares for patients with higher disease severity, who often have polyarthritis, high inflammatory biomarkers, or are refractory to conventional treatment. Therefore, patients with poly-articular involvement may be overrepresented in our cohort study.

We also found that patients with a longer time interval between disease onset and initiation of cDMARDs had a higher risk of flare-ups during tapering of biologics. This finding demonstrated that earlier initiation of cDMARDs increased the likelihood of successful treatment withdrawal, possibly owing to the prevention of chronic and irreversible joint damage. Our report also disclosed that patients who had a shorter time to achieve clinical inactive disease once biological agents were started seemed to have a lower chance of experiencing flare-up during biologics tapering, though only with trend significance, which may be related to the limited number of cases.

**TABLE 3 |** Demographics and clinical manifestations in ERA patients stratified by biologics tapering.

| | On biologics tapering without flare-up (*N* = 14) | On biologics tapering with flare-up (*N* = 28) | Ever withdrawing biologics (*N* = 10) | *P*-value |
|---|---|---|---|---|
| Onset age, | 10.41 | 10.19 | 10.27 | 0.9147 |
| years old | (8.43–11.91) | (6.85–12.25) | (9.03–10.78) | |
| Male | 12/14 (85.7%) | 24/28 (85.7%) | 10/10 (100.0%) | 0.6114 |
| Polyarticular | 7/14 (50.0%) | 14/28 (50.0%) | 6/10 (60.0%) | 0.8686 |
| oligoarticular | 7/14 (50.0%) | 14/28 (50.0%) | 4/10 (40.0%) | |
| ANA positivity | 4/14 (28.6%) | 9/28 (32.1%) | 3/10 (30.0%) | 1.0 |
| Uveitis | 3/14 (21.4%) | 4/28 (14.3%) | 1/10 (10.0%) | 0.7697 |
| Enthesitis | 4/14 (28.6%) | 9/28 (32.1%) | 2/10 (20.0%) | 0.9188 |
| Axial involvement | 2/14 (14.3%) | 11/28 (39.3%) | 6/10 (60.0%) | 0.0743 |
| CRP | 0.98 | 2.05 | 1.91 | 0.5828 |
| mg/dl | (0.33–3.30) | (1.14–2.93) | (0.69–3.16) | |
| ESR | 31.50 | 37.00 | 29.00 | 0.4141 |
| mm/hr | (17.75–44.25) | (27.00–62.00) | (14.00–53.00) | |
| **Initial biologics** | | | | |
| Etanercept | 8/14 (57.1%) | 21/28 (75.0%) | 8/10 (80.0%) | |
| Adalimumab | 6/14 (42.9%) | 6/28 (21.4%) | 2/10 (20.0%) | |
| Abatacept | 0/14 (0%) | 1/28 (3.6%) | 0/10 (0%) | 0.4975 |
| Time to cDMARDs, | 0.27 | 1.14 | 0.26 | **0.0104** |
| years | (0.11–0.73) | (0.39–2.02) | (0.18–0.42) | |
| MTX | 11/14 (78.6%) | 23/28 (82.1%) | 9/10 (90.0%) | 0.8906 |
| SAL | 3/14 (21.4%) | 9/28 (32.1%) | 2/10 (20.0%) | 0.7637 |
| AZA | 3/14 (21.4%) | 8/28 (28.6%) | 2/10 (20.0%) | 0.8346 |
| HCQ | 2/14 (14.3%) | 2/28 (7.1%) | 0/10 (0%) | 0.6351 |
| PEN | 1/14 (7.1%) | 0/28 (0%) | 1/10 (10%) | 0.2081 |
| CsA | 1/14 (7.1%) | 1/28 (3.6%) | 1/10 (10%) | 0.7605 |
| Time to biologics, | 0.88 | 1.39 | 0.92 | 0.5136 |
| years | (0.58–2.01) | (0.54–4.72) | (0.40–2.30) | |
| Time to achieve clinical inactive disease | 0.35 | 0.38 | 0.27 | 0.0948 |
| once biological agent was started, years | (0.33–0.44) | (0.21–0.52) | (0.16–0.30) | |
| **Concomitant treatment while achieving clinical inactive disease** | | | | |
| cDMARDs | 8/14 (57.1%) | 12/28 (42.9%) | 5/10 (50.0%) | 0.6597 |
| NSAIDs | 5/14 (35.7%) | 7/28 (25.0%) | 0/10 (0%) | 0.1062 |
| Corticosteroid | 0/14 (0%) | 2/28 (7.1%) | 0/10 (0%) | 0.7043 |
| Time to biologic tapering after clinical | 0.55 | 0.73 | 0.49 | 0.7418 |
| inactive disease was achieved, years | (0.24–1.26) | (0.34–1.28) | (0.37–0.57) | |
| **Concomitant treatment while initiating biologics tapering** | | | | |
| cDMARDs | 2/14 (14.3%) | 6/28 (21.4%) | 2/10 (20.0%) | 0.8982 |
| NSAIDs | 1/14 (7.1%) | 5/28 (17.9%) | 1/10 (10.0%) | 0.8607 |
| Corticosteroid | 0/14 (0%) | 1/28 (3.6%) | 0/10 (0%) | 1.0 |
| Follow-up period, | 5.34 | 9.71 | 6.85 | **0.0225** |
| years | (3.05–7.43) | (6.32–13.35) | (6.21–7.90) | |

*Data shown are median (IQR) or number (%) of patients as appropriate.*

*ERA, enthesitis-related arthritis; cDMARDs, conventional disease-modifying antirheumatic drugs; ANA, antinuclear antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MTX, methotrexate; SAL, sulfasalazine; AZA, azathioprine; HCQ, hydroxychloroquine; PEN, mesalazine; CsA, cyclosporine; NSAIDs, non-steroidal anti-inflammatory drugs.*

*Values in the bold indicate p values less than 0.05.*

*The value with underline indicates p values between 0.05 and 0.1.(trend).*

When patients experienced flare-ups during biologics tapering, the treating physicians would pause the tapering attempt, escalate treatment to previous step, or even shift to alternative biologics in order to keep disease activity at inactive status. Further attempts at tapering might be considered with great prudence either by the treating physicians or patients themselves (16). Thus, patients with flare-ups during biologics tapering might receive a long period of follow-up with multiple stops and starts. We only analyzed the first tapering attempt for each patient, but the follow-up period was extended since the patients were still in need of active treatment. By contrast, some patients who completed tapering achieved total withdrawal of biologics, and were lost to follow-up, since these patients had no more active medical needs. This explains the shorter period of clinical follow-up for the ever withdrawing biologics group.

ANA positivity was noted more frequently in patients under biologics, and it corresponded to previous studies disclosing that TNFi treatment was associated with the development of ANA (17). It remains controversial whether ANA can be a biomarker predicting poor response to biologic treatment. Some studies reported that ANA and anti-ds DNA were associated with poor outcome to biological agents in patients with rheumatoid arthritis (18, 19). However, ANA was not associated with flare-ups in ERA patients receiving biologics in our study, and this finding

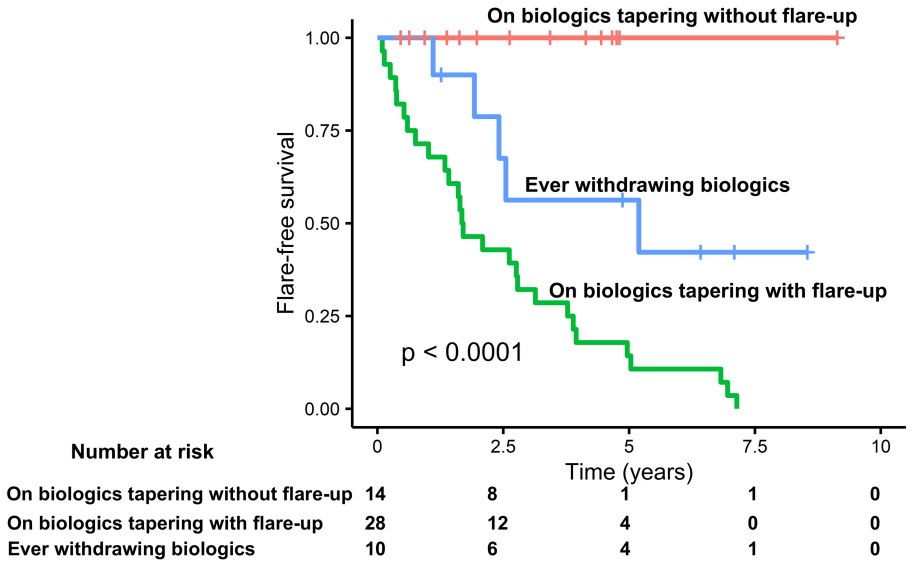


**FIGURE 3 |** Kaplan–Meier plot of time to enthesitis related arthritis (ERA) disease flare-up. Flare-up was defined as loss of at least two items of the Wallace criteria as well as when the attending physician intensified treatment because of elevated disease activity. Time to flare was defined as the time from the date initiating biologics tapering to the date of the first flare visit. Patients without flare-up were censored at the date of the latest visit.

was compatible with the study conducted by Simonini et al. in patients with JIA (20).

The most frequently used cDMARD in patients receiving cDMARDs only is sulfasalazine. This result was compatible with the therapeutic recommendation advised by the American College of Rheumatology in 2011 (21). Although methotrexate is known to be less effective in patients with ERA (21), JIA patients in Taiwan have to take methotrexate first for at least 3 months before applying biologics regardless of the subtypes to which these patients belong. If methotrexate usage of 3 months proved to be ineffective or intolerable to patients, patients could apply for biologics covered by national health insurance instead of at their own expense. This explained why patients under biologics took methotrexate more frequently than others. Twenty-one (28%) and nine (12%) patients took azathioprine and hydroxychloroquine, respectively. Though less commonly used in ERA patients, azathioprine has been reported as a beneficial alternative for SpA (22, 23) and JIA associated uveitis (24). Hydroxychloroquine, though less commonly used in patients with SpA, showed greater efficacy while in combination with methotrexate and sulfasalazine compared with sulfasalazine alone (25).

TNFis were the most commonly used first-line biologics in our cohort study, comprising 98.2% subjects (etanercept: 69.6%, adalimumab: 28.6%), and this result was compatible with the therapeutic recommendation advised by the American College of Rheumatology in 2019 for JIA children with active enthesitis or sacroiliitis (26). Switching between biologics occurred in 8 patients (six to adalimumab after failing etanercept, one to adalimumab after failing abatacept, and one to abatacept after failing etanercept). Three (37.5%) patients discontinued second-line biologics due to ineffectiveness and switched to third-line biologics. The therapeutic options of biologics were limited, because only four kinds of biologics were reimbursed by the national health insurance for JIA patients in Taiwan (etanercept, adalimumab, abatacept, and tocilizumab). Treatment of JIA patients whose disease failed to respond to TNFi or who could not tolerate TNFi was challenging. When the first TNFi failed to show efficacy, physicians might choose another TNFi, abatacept, or tocilizumab as alternatives. Although there was no strong evidence about the effectiveness of biologics switching, it is still attempted since only limited treatment options were available.

Scant literature focused on the efficacy of abatacept and tocilizumab in ERA children; however, both biologics failed to demonstrated major clinical improvement in adult patients with spondyloarthritis (27). Recent research interest has concentrated on the IL-23/IL-17 axis. Blockade of IL-23 or IL-17 worked well in adult patients with ankylosing spondylitis (28, 29). Trials of secukinumab (an anti-IL-17A monoclonal antibody) have been launched in children with ERA (NCT03031782, NCT03769168).

Etanercept was the most commonly used first-line biologics in our cohort, up to 69.6%. One patient was infected with pulmonary tuberculosis after 2.5 years of etanercept. TNFi has already been proven to increase the risk of severe infection, especially tuberculosis (30). Therefore, latent tuberculosis infection screening before TNFi is warranted, especially in countries with a high tuberculosis burden (31).

There were several limitations in our cohort study. With its retrospective nature, a risk of missing data or incorrect documentation may exist. Second, it is difficult to derive definitive conclusions from this single-center experience with limited case numbers. However, few previous investigations exist on juvenile ERA patients with long follow-up periods, and the current pilot study provides new insights in this subgroup.

Further research with multiple centers or nationwide databases is warranted. Finally, there was no strict medication tapering protocol in this study. Attending physicians mostly tapered cDMARDs first and then extended intervals between doses of biologics. The speed of tapering was based on physicians' judgment. Although this tapering strategy was more practical in daily care, a rigorous study design with a fixed tapering protocol (whether dose reduction or dose interval extension) is still needed to identify factors associated with successful biologic tapering.

Thus far, there are few studies based on daily routine care that focus on the medication withdrawal rate in children with ERA. We found that approximately half of the biologics users experienced flare-ups during tapering, and half of those who halted medication successfully had post-withdrawal recurrence. A longer time interval between disease onset and initiation of cDMARDs was associated with flare-up during medication tapering. Therefore, early intervention with cDMARDs may decrease the incidence of flare-ups during tapering and further increase the medication withdrawal rate in ERA patients.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of National Taiwan University Hospital (202010068RIND). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

C-HL led the overall study, contributed to the data collection and interpretation, and wrote the manuscript. B-LC contributed to the data interpretation and manuscript editing. Y-HY contributed to the study design and manuscript editing. All authors read, contributed to the research design, and approved the final manuscript.

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# The Development of Extra-Articular Manifestations in Children With Enthesitis-Related Arthritis: Natural Course or Different Disease Entity?

*Ilaria Pagnini[1]*, Mariangela Scavone[1], Ilaria Maccora[1], Maria Vincenza Mastrolia[1], Edoardo Marrani[1], Federico Bertini[2], Lovro Lamot[3] and Gabriele Simonini[1]*

[1] Rheumatology Unit, Meyer Children Hospital of Florence, University of Florence, Florence, Italy, [2] Radiology Unit, Meyer Children Hospital of Florence, Florence, Italy, [3] Department of Pediatrics, School of Medicine, University of Zagreb, University Hospital Centre Zagreb, Zagreb, Croatia

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***Correspondence:**
*Ilaria Pagnini*
*ilaria.pagnini@meyer.it*

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**Introduction:** Enthesitis-related Arthritis (ERA) is a specific category of juvenile idiopathic arthritis (JIA) characterized by axial and/or peripheral arthritis, and enthesitis, although other different extra-articular manifestations may encompass its clinical spectrum.

**Materials and Methods:** In order to examine if ERA-JIA with extra-articular involvement may represent a different entity from ERA without extra-articular involvement, we performed a retrospective, observational, monocentric study, in a cohort of ERA patients followed between 2001 and September 2020 at the Pediatric Rheumatology Unit of Meyer Children Hospital of Florence. We analyzed the demographic, clinical, laboratory and imaging data at the disease onset, as well as after 3, 6, and 12 months follow up.

**Results:** We have enrolled 53 patients, 33 males. At the time of diagnosis, average age was 10.9 years, 53 patients had active arthritis and 25 active enthesitis. The middle foot involvement was present in 20 patients. Twenty-five children achieved clinical remission on medication. Extra-articular manifestations were observed in 14 patients, of whom 3 had inflammatory bowel disease, 5 uveitis, one uveitis associated with Crohn disease, 4 SAPHO syndrome, one celiac disease. The cohort was stratified according to the presence/absence of extra-articular manifestations. It was observed that middle foot involvement was more frequent in patients with no extra-articular manifestations (18/39 vs. 2/14; $\chi^2 = 4.45$, $p = 0.05$). Additionally, patients presenting extra-articular manifestation needed more frequently (12/14 vs. 21/39, $\chi^2 = 4.45$, $p = 0.05$), and preciously (months: $3.7 \pm 5.4$ vs. $16.7 \pm 26.5$, $p = 0.02$), treatment with biologic agents. Finally, these patients achieved belatedly (months: $31.6 \pm 32.3$ vs. $22.9 \pm 18.3$, $p = 0.01$) and less frequently (3/14 vs. 22/39; $\chi^2 = 5.50$, $p = 0.03$) the clinical remission on medication. Eventually, extra-articular involvement inversely correlated with the middle-foot arthritis ($\rho_S$ −0.29, $p = 0.03$), the chance to achieve remission on medication ($\rho_S$ −0.31 e $p = 0.02$), as well as the chance to keep overall remission, with and without medication ($\rho_S$ −0.28, $p = 0.04$).

**Conclusion:** In our cohort, children diagnosed with ERA-JIA at the onset of disease and then developed extra-articular manifestations show the absence of middle foot involvement and worse prognosis with an early need for the use of biologic agents, and overall low chance to achieve remission.

**Keywords: enthesitis-related arthritis, extra-articular, midfoot, biologic, sacroiliitis, children, JIA**

## INTRODUCTION

Enthesitis-related Arthritis (ERA) is one of the more common types of Juvenile Idiopathic Arthritis (JIA) (1), frequently affecting males after 6-year-old. In Europe and North America, ERA comprises almost 10% of JIA cases (2, 3) as opposite to certain countries in Asia where it represents the most common JIA subtype (35–40% of JIA patients) (4–7). The main findings of this particular JIA subtype are axial and/or peripheral arthritis, inflammatory back pain, enthesitis and a specific association with the HLA-B27 typing.

While the criteria of International League of Associations for Rheumatology (ILAR) are most commonly used by pediatric rheumatologists to classify this entity in children, ERA patients are often regarded as having an undifferentiated form of juvenile spondyloarthritis (jSpA) (1, 8). However, as opposite to ERA, jSpA encompasses differentiated forms such as juvenile ankylosing spondylitis (jAS), psoriatic arthritis (PsA), reactive arthritis (ReA) and arthritis associated with inflammatory bowel disease (IBD), as well (8). Moreover, different extra-articular manifestations, such as uveitis inflammatory bowel disease, celiac disease, Synovitis Acne Pustulosis Hyperostosis Osteitis (SAPHO) syndrome or less common cardiac and/or pulmonary involvement may encompass jSPA spectrum. Conversely to other JIA subtypes, uveitis in ERA patients is typically characterized by an acute, symptomatic onset with red, painful, and photophobic eye, usually unilateral and frequently recurrent (9, 10). Inflammatory bowel disease, including Crohn disease, ulcerative colitis and undifferentiated colitis, occurs in 5–10% of patients affected by ERA, more frequent in males, at the onset or during disease course (11). In addition, celiac disease occurs in 1–8% of patients with ERA (12), while SAPHO syndrome is an even less frequent complication characterized by axial involvement along with enthesitis and peripheral arthritis and typical cutaneous findings (13, 14).

Although uncommon, aortic insufficiency as well as myocarditis, endocarditis and pericarditis, often with spontaneous resolution, can occasionally occur in ERA patients (15–17). Pulmonary involvement, characterized by diminished chest expansion with decreased vital capacity, are very rare (18, 19), whilst central nervous system (CNS) diseases are seldom reported in ERA (19).

In order to define the clinical phenotype of ERA-JIA with extra-articular features, we enrolled patients affected by ERA and stratified them by the presence of extra-articular manifestations. Moreover, to address if ERA-JIA with extra-articular involvement may represent a different entity from ERA without extra-articular involvement, we compared the clinical features, laboratory data, treatment modalities, disease activity and outcome of a pediatric cohort of ERA-JIA patients.

## MATERIALS AND METHODS

We performed a retrospective, observational, monocentric study, in a cohort of patients affected by ERA and followed between 2001 and September 2020 at the Pediatric Rheumatology Unit of Meyer Children's University Hospital in Florence.

All patients fulfilled the International League of Associations for Rheumatology (ILAR) criteria (1, 20) for the diagnosis of ERA-JIA. We analyzed the demographic, clinical, laboratory and imaging data at the disease onset, and thereafter at 3, 6, and 12 months follow up. All data were enrolled in a customized database, including:

- **Demographic variables:** (1) gender; (2) age at the onset of clinical manifestation; (3) age at the diagnosis, entered as the age the child met the (ILAR) criteria for ERA; (4) history of HLA-B27-related disease in first-degree relatives, including ankylosing spondylitis, ERA, inflammatory bowel disease, reactive arthritis (Reiter's syndrome), and acute anterior uveitis. Psoriasis was excluded since is a mandatory exclusion criterion for ERA diagnosis if present in patient and/or in a first-degree relatives.
- **Clinical variables:** (1) disease duration; (2) interval between disease onset and diagnosis; (3) assessment of the number and type of affected joints. Active arthritis was defined as a joint with swelling not caused by bone enlargement, or limitation of motion in combination with pain or tenderness; (4) assessment of the number and type of affected enthesis. Enthesitis was defined as discretely localized tenderness at the point of insertion of ligaments, tendon, joint capsules, or fascia to bone (21), and assessed according to the Maastricht Ankylosing Spondylitis Enthesitis Score (MASES), but including in additional plantar fascia and calcaneus enthesis (22); (5) middle foot involvement, mono or bilateral; (6) inflammatory back pain, defined according to the Assessment of Spondylarthritis International Society (ASAS), expert criteria (23), as lumbosacral spinal pain persisting at least 3 months in patients with: age $<$ 40 years, insidious onset, improvement with exercise, not improved with rest, pain at night (at least 4 of 5 requirements need to be present) (21, 23); (7) tenderness of sacroiliac (SI) joints, compression of pelvis, distraction of the SI joints by Patrick's test (21, 23); (8) limited anterior spinal flexion, assessed by the modified Schoeber test (23); (9) limited lateral spinal flexion, according to the ASAS expert criteria (23); (10) extra-articular features, at the onset or

during disease course (range was referred in months): uveitis, diagnosed by slit lamp examination; inflammatory bowel disease diagnosed by endoscopy and; celiac disease, diagnosed by laboratory test (antigliadin antibody, ant-transglutaminasis antibody detected by Enzyme-linked immunosorbent assay [ELISA] and anti-endomision antibody detected by Immunofluorescence [IFI]) and by biopsy of duodenum tract; SAPHO syndrome was diagnosed by the presence of the key clinical features; (11) Disease activity measures, according to the American College of Rheumatology pediatric criteria (24); (12) Disease remission indices, according to the preliminary criteria for clinical remission in JIA (25), including no active entheses.

- **Laboratory variables:** Hemoglobin value (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), presence of the HLA-B27 allele, and anti-nuclear antibody (ANA) positivity.
- **Imaging SI assessment:** Magnetic resonance imaging (MRI) study of the SI joints: dynamic contrast-enhancement MRI before and after administration of contrast medium was performed as described (26, 27). In all patients MRI images were obtained with a 1.5 Tesla unit (Philips Intera: Philips, Eindhovenn, The Netherlands) using a pelvic array body coil with reg following sequences: semicoronal Short tau inversion recovery (STIR) sequences, semiaxial Turbo spin echo (TSE) T1-weighted sequences, semicoronal Spectral Presaturation with Inversion Recovery (SPIR) T1-weighted sequences, semiaxial TSE T2-weighted sequences, semicoronal dynamicc T1fat-saturated (FS), and semiaxial T1 SPIR after administration of intravenous gadolinium (0.1 mmol/kg). Assessment of the MRI examinations included a grading of 0–3 (0 normal, 1 minimal, 2 moderate, 3 severe) of the following findings: erosion, sclerosis (low signal intensity on T1and/or T1 FS), bone marrow edema (high signal intensity in STIR), contrast enhancement in the bone and in the joint space, and joint space narrowing and/or Widening. All assessment and grading were performed at four anatomical sites for each SI joint: the sacral and iliac sites of the cartilaginous and ligamentous portions go the joint. In addition, gadolinium contrast enhancement was performed and acute/active sacroiliitis on MRI was defined if bone marrow edema on STIR or bone marrow osteitis on T1 post-gadolinium was detected and located in subchondral or periarticular bone marrow (28). Moreover, monolateral and bilateral sacroiliitis was graded 0–4 corresponding to the New York criteria, according to Aarhus criteria accepted by Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) Rheumatoid Arthritis Magnetic Resonance Imaging Scoring System (RAMRIS) (29);
- **Therapeutic variables,** as therapy administered at onset and throughout the disease course in terms of Disease-modifying anti-rheumatic drugs (DMARDs) and/or biologic treatment.

## Statistical Analysis

All results were expressed as mean and standard deviation (SD), or median and range. Mann-Whitney *U* test, Kruskal-Wallis test, Wilcoxon signed-rank test for paired samples, chi-square test ($\chi^2$) and Fisher exact test, when appropriate, were used to compare data. Pearson and Spearman correlation tests were used to determinate correlation coefficients for different variables (sex, age at diagnosis, number of active joints at diagnosis, number of active enthesis at diagnosis, middle foot involvement, inflammation of SI joints, increased ESR, increased CRP, ANA positivity, HLA-B27 positivity, DMARDs treatment and timing of therapy, biologic treatment and timing of therapy, remission time). Multiple stepwise regression was performed to determine variables that could correlate independently with the development of extra-articular involvement and a confirmed diagnosis of ERA at last available follow-up. The predictors used in the final model were those showing a significant correlation in the univariate analysis. Non-parametric tests were used, where necessary, due to the small size of our groups and to the skewness of data. A p level $< 0.05$ was considered statistically significant. All analyses were performed on SPSS for MAC, version 26.0 (SPSS Inc., Chicago, IL, USA).

# RESULTS

Fifty-three children fulfilled the criteria for ERA at the time of diagnosis and were then enrolled into the study: 33 males and 20 females with a median age at diagnosis of 10.9 years (range 3–16 years). Except for eight patients, all children were Caucasian. This represents 10% of the total cohort of patients with JIA followed in our center in the same period.

As regards family history, the presence of an autoimmune disease (Ankylosing Spondylitis, Rheumatoid Arthritis, Hashimoto thyroiditis, type 1 Diabetes Mellitus, celiac disease, vitiligo and inflammatory bowel disease) was described in 23 subjects (43.4%) of whom five reported multiple autoimmune disease.

At diagnosis, active arthritis was observed in all 53 children, whilst enthesitis, SI involvement and the middle foot in 25 (47.2%), 23 (43.3%), and 20 (37.7%) patients, respectively. **Table 1** details the clinical features, laboratory parameters and therapeutic approaches in our cohort of ERA-JIA patients.

At last available follow-up (median time from disease onset 42 months, range: 4–193), 25 patients (47.2%) reached clinical remission on medication after a median time of 14 months (range 6–62). Clinical remission on medication lasted for a median time of 67 months (range 9–142). Fifteen patients (28.3%) reached clinical remission without medication for a median time of 16 months (range 6–35).

Over the disease course, 14 patients (26.4%) developed extra-articular manifestations, which were not present at diagnosis but complained during the clinical course. In particular three patients had inflammatory bowel disease, one child had acute anterior uveitis associated to IBD, five patients had uveitis, four patients had SAPHO syndrome, and one patient had celiac disease. The mean time between ERA-JIA diagnosis and the extra-articular manifestation onset was 18.8 months (range 9–60). In three subjects, extra-articular symptoms (uveitis) were concomitant at the time of ERA onset and diagnosis. Thus, once the patients developed a confirmed diagnosis of IBD,

**TABLE 1 |** Clinical features, laboratory parameters and therapeutic approaches of our cohort of diagnosed as ERA-JIA at the onset of disease.

| Clinical features | N of pts | Non-extra-articular involvement | Extra-articular involvement |
|---|---|---|---|
| Arthritis at diagnosis | 53 | 39 | 14 |
| - Symmetrical involvement | 18 | 12 | 6 |
| - Asimmetrical involvement | 35 | 27 | 8 |
| - Oligoarticular | 33 | 22 | 11 |
| - Polyarticular | 20 | 17 | 3 |
| Enthesitis at diagnosis | 24 | 20 | 4 |
| - Monolateral involvement | 13 | 12 | 1 |
| - Bilateral involvement | 11 | 8 | 3 |
| **Number of enthesitis at diagnosis** | | | |
| - One | 13 | 12 | 1 |
| - Two | 8 | 6 | 2 |
| - Three | 2 | 1 | 1 |
| - Four | 1 | 1 | - |
| SI involvement | 23 | 16 | 7 |
| - Monolateral | 18 | 12 | 6 |
| - Bilateral | 5 | 4 | 1 |
| Middle foot involvement | 20 | 18 | 2 |
| - Monolateral | 20 | 18 | 2 |
| - Bilateral | 7 | 7 | - |
| **Laboratory values** | | | |
| Increased ESR | 27 | 19 | 8 |
| [mean value ± SD (mm/h)] | 37.1 ± 29.6 | 36.1 ± 30.9 | 40.1 ± 26.2 |
| Increased CRP | 27 | 20 | 7 |
| [mean value ± SD (mg/dl)] | 2.26 ± 2.9 | 2.17 ± 3.1 | 2.51 ± 2.9 |
| Anemia | 12 | 9 | 3 |
| [Hb mean value ± SD (g/dl)] | 10.0 ± 1.4 | 9.9 ± 1.3 | 10.3 ± 1.2 |
| ANA positivity | 16 | 11 | 5 |
| HLA B27 positivity | 21 | 18 | 3 |
| **Therapeutic approach** | | | |
| DMARDs | 48 | 37 | 11 |
| - Methotrexate | 28 | 37 | 10 |
| - Sulfasalazine | 20 | 19 | 1 |
| Biologic agents | 33 | 21 | 12 |
| - Adalimumab | 23 | 14 | 9 |
| - Etanercept | 8 | 5 | 3 |
| - Golimumab | 1 | 1 | - |
| - Abatacept | 1 | 1 | - |

*SI, sacroiliac; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; Hb, hemoglobin; ANA, antinuclear antibodies; DMARDs, disease modifying anti-rheumatic drugs.*

documented by typical histopathology then that affected patients have been classified as IBD-associated arthritis. The same for children at disease onset classified as ERA and later on as SAPHO syndrome due to the development of additional clinical findings. Therefore, eight (15%) children did not fulfill anymore the ERA diagnosis.

Persistent arthritis was the indication for the biologic treatment, along with the development of uveitis, IBD and SAPHO syndrome in the cohort who exhibited over the disease course extra-articular involvement. Among these 14 children, two children, one with coeliac disease and one with uveitis, did not receive biologic treatment.

Moreover, of the 23 patients affected by sacroiliitis in ERA, 13 patients were in treatment with DMARDs (12 with sulphasalazine and 1 with Methotrexate), while 10 patients were in treatment with anti-TNF alpha inhibitors. Of these 10 patients, 7 developed extra-articular manifestation. In particular: two patients developed acute anterior uveitis, one patient developed gastrointestinal involvement, one patient developed celiac disease and three patients developed SAPHO syndrome. Eventually, among 23 children with sacroiliitis, 4 out of 23 (17%) did not fulfill anymore the ERA diagnosis.

Patients' stratification according to the presence/absence of extra-articular manifestations revealed that the middle foot involvement was more frequent in patients without extra-articular manifestations (18/39 vs. 2/14; $\chi^2 = 4.45$, $p = 0.05$). Additionally, patients presenting extra-articular manifestations needed more frequently (12/14 vs. 21/39, $\chi^2 = 4.45$, $p = 0.05$), and preciously (months: 3.7 ± 5.4 vs. 16.7 ± 26.5, $p = 0.02$), treatment with biologic agents (**Figure 1**). Moreover, this group of patients achieved less frequently (3/14 vs. 22/39; $\chi^2 = 5.50$, $p = 0.03$) and belatedly (months: 31.6 ± 32.3 vs. 22.9 ± 18.3, $p = 0.01$) the clinical remission on medication (**Figure 2**). Overall, considering all the children on clinical remission at last available follow-up, including patients receiving and stopped treatment, this group of patients had less chance to maintain remission (4/14 vs. 26/39: $\chi^2 = 6.08$, $p = 0.01$) (**Table 2**).

Eventually, extra-articular involvement inversely correlated with the middle-foot arthritis ($\rho_s$ −0.29, $p = 0.03$), the achievement of remission on medication ($\rho_s$ −0.31 e $p = 0.02$), as well the chance to keep remission, with and without medication ($\rho_s$ −0.28, $p = 0.04$)

No further correlations considering the number of active joints, the number of enthesitis, the presence of SI, the elevation of inflammatory markers, ANA and HLA-B27 positivity, type and duration of treatment with DMARDs were detected. In multiple regression analysis, where the development of extra-articular involvement at last available follow-up was set as dependent variable, the middle foot involvement and the clinical remission on medication remained as negative predictors of extra-articular involvement (multiple R = 0.41, multiple adjusted R2 = 0.14, F: 5.19, $p < 0.009$).

Considering that eight children, four with IBDs and four with SAPHO syndrome, did not fulfill anymore the criteria for ERA-JIA over the disease course according ILAR classification, we additionally performed a sub-analysis limited to the 45 ERA-JIA children who fulfilled the diagnosis of ERA according ILAR criteria even over the diseases course.

The same statistical significance findings have been detected regarding the middle foot involvement: it was more frequent in patients without extra-articular manifestations (18/39 vs. 0/6; $\chi^2 = 4.61$, $p = 0.03$), and extra-articular involvement in ERA-JIA inversely correlated with the middle-foot arthritis ($\rho_s$ −0.32, $p = 0.03$). Conversely, the others statistical significance results have not been kept.

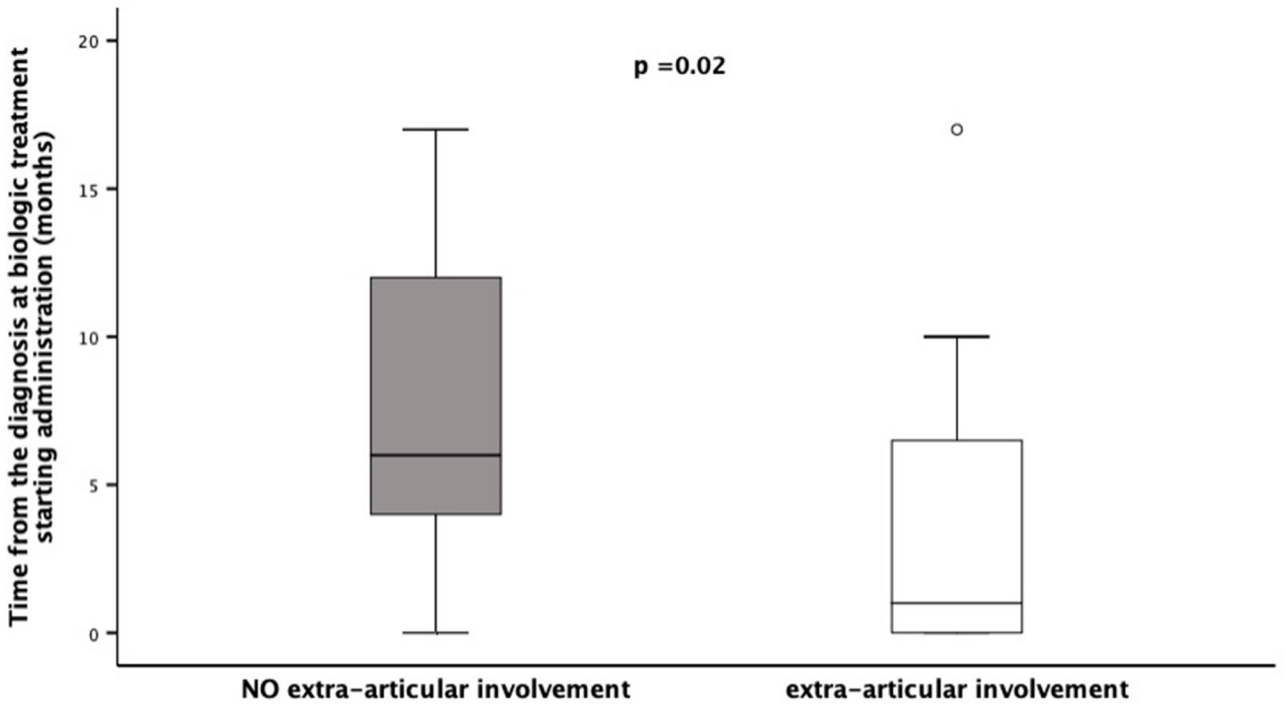


**FIGURE 1 |** Time of Biologic Treatment starting in a cohort of 53 children diagnosed as ERA-JIA at the onset of disease. Figure shows the time from the diagnosis at biologic treatment starting administration (months) in 12 children with extra-articular involvement, diagnosed ERA-JIA at the onset (white box) and in 21 ERA-JIA children without extra-articular manifestations (gray box). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots are values higher than the 90th percentile. ($p = 0.002$). ERA-JIA, enthesitis-related arthritis juvenile idiopathic arthritis.

## DISCUSSION

The central pathogenic event in various forms of arthritis in adults and children alike is certainly chronic inflammation of the synovial tissue and joint destruction (30, 31). In the specific types of the arthritis, such as spondyloarthritis in adults and ERA subtype of JIA in children, other structures, such as enthesis and/or axial joints, can be affected by inflammation in similar fashion (11, 32). Finally, the inflammatory process can spread beyond the musculoskeletal structures throughout the body causing the various extra-articular manifestations (33). However, for reasons not entirely clear, this scenario occurs in less than quarter of patients with SpA (34), while, to the best of our knowledge, our study was the first to report the incidence of several extra-articular manifestations in a single cohort of patients with ERA-JIA.

The results of our study showed that 26% of the enrolled subjects had and/or developed over the disease course extra-articular manifestations: three IBD, five uveitis, one uveitis associated with Crohn disease, four SAPHO syndrome and one celiac disease.

Therefore, eight children, representing the 15% of this monocentric cohort, and fulfilling the criteria for ERA at the onset of disease, developed additional clinical findings and were then reclassified accordingly: IBD-associated chronic arthritis, not included in ILAR criteria, and SAPHO syndrome.

In our monocentric cohort, it appears that the current ILAR criteria failed to properly classify the 15% of patients. The complexity of childhood rheumatic diseases makes them difficult to classify in coherent set criteria, as patients may present simultaneously a various range of manifestations shared by different disorders or develop over time additional findings. Mostly in childhood, the phenotype of each patient may evolve over time and extend beyond defined schemes, creating overlapping entities challenging to systemize while relying on the present knowledge (35). Although the ILAR criteria for ERA-JIA may fit jSpA peculiarities better than other classifications utilized mainly for adults, in these set of criteria, jSpA is covered by different subtypes of JIA, namely psoriatic arthritis, ERA and undifferentiated arthritis (1). However, certain phenotypes are excluded (e.g., reactive arthritis, arthritis associated with IBD) and the axial involvement is not specifically recognize, since the ERA subtype does not discriminate between axial and peripheral disease. Indeed, how to categorize JSpA still remains a debated and unsolved issue (8, 36, 37).

Any attempt to classify SAPHO has been equally difficult, considering that the articular sings may be the first presentation of the latter cutaneous findings. While several authors consider this disorder as a group of autoinflammatory bone disorders others highlighted the strong link with SpA, especially in later life stage (38).

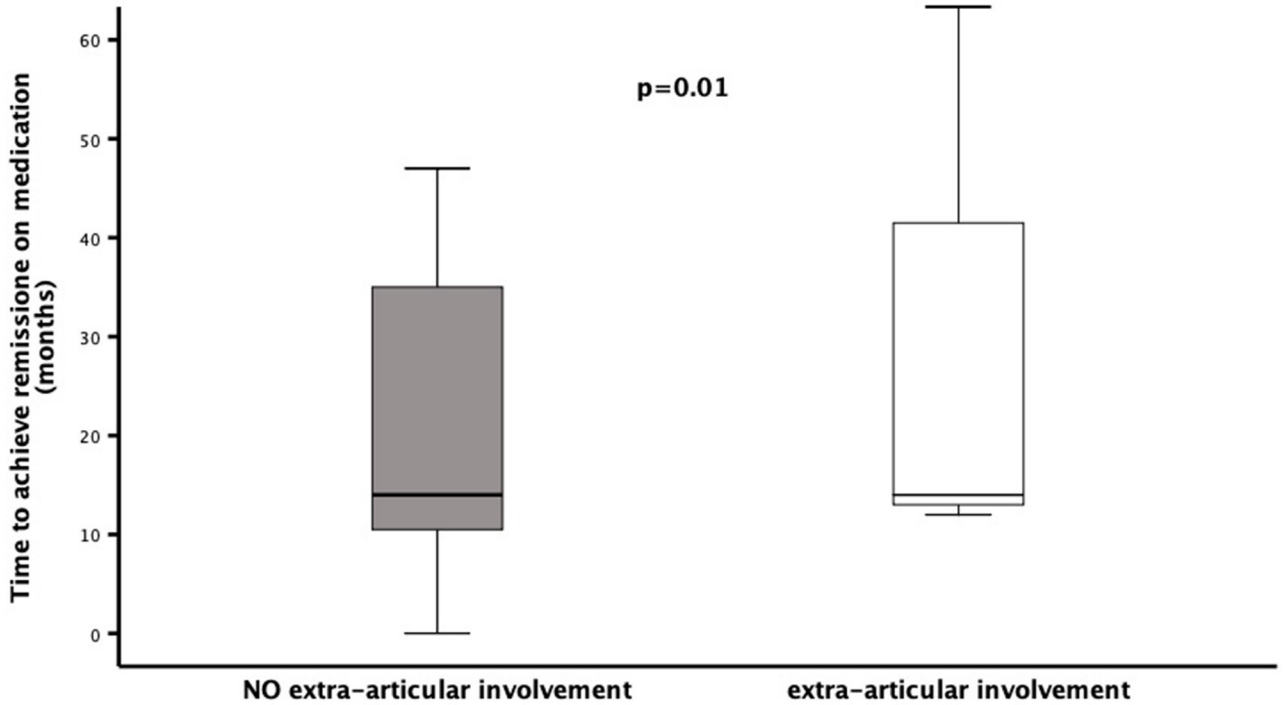


**FIGURE 2 |** Time to reach clinical remission on medication in a cohort of 53 children diagnosed as ERA-JIA at the onset of disease. Figure shows the time to reach clinical remission on medication (months) in 3 ERA-JIA children with extra-articular involvement (white box) and in 22 ERA-JIA children without extra-articular manifestations (gray box). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots are values higher than the 90th percentile. ($p = 0.002$). ERA-JIA, enthesitis-related arthritis juvenile idiopathic arthritis.

**TABLE 2 |** Comparison statistics stratifying into extra-articular- vs. non-extra-articular group.

| | Non-extra-articular involvement | Extra-articular involvement | $\chi^2$ *P*-values |
|---|---|---|---|
| Middle foot involvement (*n*/*N*, %) | 18/39 (46%) | 2/14 (14%) | 4.45, 0.05 |
| Number of children receiving biologic treatment (*n*/*N*, %) | 21/39 (54%) | 12/14 (85%) | 4.45, 0.05 |
| Number of children on clinical remission on medication (*n*/*N*, %) | 22/39 (56%) | 3/14 (22%) | 5.50, 0.03 |
| Number of children on clinical remission at last available follow-up (*n*/*N*, %) | 26/39 (67%) | 4/14 (29%) | 6.08, 0.01 |

Since some pathognomonic manifestation often occur late in disease course, the best chance to properly identify a disease may be a close clinical monitoring over time and reassessing the diagnosis when new sings/symptoms appear.

Interestingly, there were several important differences among patients with and without extra-articular manifestations in our cohort. Stratifying our cohort according to the presence/absence of extra-articular manifestations, we identified that the midfoot involvement in our cohort of ERA-JIA patients seems to correlate with no extra-articular manifestations, which was not observed in previous studies.

However, in accordance with recent literature, the midfoot involvement seems to be one of the key characteristics of ERA-JIA and may significantly affect the ERA prognosis. Phatak et al. (39), in an elegant prospective study, reported that the midfoot disease produced important functional limitation. During the course of the last years, numerous studies addressed the midfoot involvement as characteristic feature of ERA-JIA (40–42). The inflammation of midfoot in spondyloarthritis can engage pathological processes such as tarsal swelling, synovial inflammation, bone overgrowth, enchondral ossification, enthesophytosis, bone bridging, and finally ankylosis of the tarsal bones, leading to the distinctive form of the severe involvement of the feet, named ankylosing tarsitis (43). Interestingly, the similar changes are noticed in ankylosing spondylitis (AS), which is considered the differentiated form of spondyloarthritis, as opposite to the undifferentiated forms such as ERA-JIA. Therefore, the increased frequency of midfoot involvement in ERA-JIA patients, that do not develop extra-articular manifestations, could indicate the distinctive type of inflammation which spreads mainly

throughout the musculoskeletal structures, avoiding the other body systems.

When our analysis was limited to the 45 children who still meet the ILARA criteria over the disease course, only the middle foot involvement remained statistically significant related to the ERA-JIA group with no extra-articular involvement. In our opinion, this result may strengths the hypothesis that middle foot involvement strictly belongs to the ERA phenotype, since other clinical data, even those included into the ILAR criteria were not able enough to clearly differentiate a group of ERA children, who at the onset met the ILAR criteria, and were then diagnosed with another different disease.

Another important finding in our cohort was that ERA-JIA children that later developed extra-articular manifestations needed more frequently and preciously treatment with biologic agents. Moreover, these patients achieved belatedly and less frequently the clinical remission, on medication as well as without medication. Therefore, development of a different disease, such as IBD, as well as a systemic syndrome such as SAPHO, clearly modifies the prognosis and outcome in these children initially diagnosed as ERA-JIA according to ILAR criteria. Clinical experience from adult patients with AS reports a common association with extra-articular manifestations, although often subclinical (44). The presence of these comorbid conditions negatively affects the patients' quality of life and overall outcome (45). Emerging knowledge suggests that extra-articular manifestations in AS may be the expression of a unique inflammatory process involving the whole body (46). Therefore, the adoption of a therapeutical strategy that takes into account patients' symptoms in their entirety without focusing on a single area should be considered. In this perspective, an early recourse to a TNF-inhibitor (adalimumab and infliximab to be preferred above etanercept) for the management of AS with extra-articular manifestations and comorbid conditions may represent the most appropriate therapeutic approach (45–47).

According to several studies, ERA is associated with worse function, poorer quality of life, and increased pain intensity (48–50). In particular, HLA-B27 positivity, tarsitis, hip arthritis within the first 6 months, and older age of disease onset are associated with worse function, quality of life and pain (51–53). However, some of these studies reported some unexpected potential associations. Specifically, it has been reported that normal ESR correlates with a lower likelihood of attaining inactive disease (54). Interestingly, the authors suggested that TNF-alpha inhibitors may be more effective in patients whose active disease is accompanied by robust active systemic inflammation mirrored by raised inflammatory markers. Conversely, disease manifestations, such as joint pain and enthesalgia, that may not be strongly associated with systemic inflammation, tend to respond less well to TNF-alpha inhibitors. In this complicated scenario, non-randomized studies showed that adding methotrexate to TNF-alpha inhibitors seems to be more effective than TNF-alpha inhibitors alone, which could be explained by different mechanisms of these two DMARDs (55, 56). Nevertheless, whatever the reason, these findings are in favor of the concept of the widespread disease in some patients with ERA-JIA.

We fully acknowledge the limitation of our results imposed by the small sample size which limits the strength of the conclusions. However, the present cohort represents the 10% of all JIA children followed at our unit over the course of 20 years, which is the percentage reported in other cohorts as well (2, 3).

Additionally, another advocated shortcoming might be that enthesitis was not confirmed by imaging studies, thus biasing the ERA-JIA diagnosis at the onset of the disease. However, none of the enrolled patients has been classified at diagnosis as ERA-JIA only by the clinical presence of enthesis, without a concomitant peripheral arthritis. Currently, according ILAR criteria, confirmation of Enthesitis by imaging is not a required criterion.

In conclusion, based on clinical observations from our cohort of patients, we hypothesize that there could be two distinctive disease phenotypes in JIA children with ERA, depending on the presence or absence of the extra-articular manifestations. Specifically, midfoot involvement was associated with ERA-JIA diagnosis and the absence of extra-articular manifestations. Conversely, development of extra-articular manifestations over the time increases the chance of a different disease, such as Crohn disease and SAPHO syndrome, thus associated with worse prognosis, an early need for the use of biologic agents, longer time to achieve remission with and/or without medication. According to this mono-centric cohort, middle foot involvement seems to be a specific feature of JIA-ERA, since children with tarsitis, classified as ERA at the onset, still fulfilled the same criteria over the course of the disease.

While these findings might already have an important implication in the diagnostic and therapeutic approach to JIA patients with ERA, results from a prospective study involving larger multicenter cohort are mandatory for their confirmation.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

IP designed the study and drafted the preliminary paper. MS and EM collected the patients' data. IM, MM, and LL participated in drafting the paper. FB helped in imaging assessment. GS performed statistical analysis, design the study, and finalized the paper. All authors contributed in writing the papers and approved the final draft of it.

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REVIEW
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# Juvenile Spondyloarthritis: What More Do We Know About HLA-B27, Enthesitis, and New Bone Formation?

*Shi Huan Tay*[1,2], *Joo Guan Yeo*[1,2,3], *Jing Yao Leong*[1,2], *Salvatore Albani*[1,2,3] *and Thaschawee Arkachaisri*[2,3]*

[1] SingHealth Duke-National University of Singapore Academic Medical Centre, Translational Immunology Institute, Singapore, Singapore, [2] Duke-National University of Singapore Medical School, Singapore, Singapore, [3] Rheumatology and Immunology Service, Department of Pediatric Subspecialties, KK Women's and Children's Hospital, Singapore, Singapore

Juvenile spondyloarthritis (JSpA) refers to a diverse spectrum of immune-mediated inflammatory arthritides whose onset occurs in late childhood and adolescence. Like its adult counterpart, JSpA is typified by a strong association with human leukocyte antigen-B27 (HLA-B27) and potential axial involvement, while lacking rheumatoid factor (RF) and distinguishing autoantibodies. A characteristic manifestation of JSpA is enthesitis (inflammation of insertion sites of tendons, ligaments, joint capsules or fascia to bone), which is commonly accompanied by bone resorption and new bone formation at affected sites. In this Review, advances in the role of HLA-B27, enthesitis and its associated osteoproliferation in JSpA pathophysiology and treatment options will be discussed. A deeper appreciation of how these elements contribute to the JSpA disease mechanism will better inform diagnosis, prognosis and therapy, which in turn translates to an improved quality of life for patients.

**Keywords: juvenile arthritis, spondyloarthritis, enthesitis related arthritis, HLA-B27, osteogenesis, enthesitis**

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***Correspondence:**
*Thaschawee Arkachaisri*
*thaschawee.arkachaisri@singhealth.com.sg*
*orcid.org/0000-0002-1387-1169*

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## INTRODUCTION

Juvenile spondyloarthritis (JSpA) is a heterogeneous group of inflammatory arthritides whose onset occurs before the age of 16. A diverse spectrum of disorders, JSpA is characterized by varying degrees of peripheral and axial arthritis, enthesitis, and a strong association with human leukocyte antigen-B27 (HLA-B27). Thus, JSpA not only includes children meeting the criteria for juvenile idiopathic arthritis (JIA) categories of enthesitis related arthritis (ERA), juvenile psoriatic arthritis (JPsA), but also juvenile ankylosing spondylitis (JAS), reactive arthritis and inflammatory bowel disease (IBD)-associated arthritis. The underlying etiology of JSpA remains unknown, though robust familial aggregation and increased risk in HLA-B27-positive individuals allude to significant genetic susceptibility interacting with a plethora of environmental triggers.

JSpA is a common rheumatic disease reported in children worldwide, of which ERA is the most prevalent at about 10 to 40% whereas JPsA represents ∼2 to 10% of all JIA patients (1–7). JAS, on the other hand, accounts for 1 to 7% of children in national pediatric rheumatic disease registries in Europe and North America (8–11). While the disease course of JSpA is highly variable, patients (especially ERA relative to other JIA subtypes) tend to show a worse prognosis with greater pain, poorer physical function, and persistent disease activity (5, 12). Much of the morbidity may be attributed to unrelenting inflammation and excessive new bone formation at inflamed sites that eventually lead to loss of joint mobility. Even though biologics like anti-tumor necrosis factor

(TNF) drugs have attained commendable success in reducing inflammation, only around 20% of patients achieve remission off medication within 4 to 5 years and radiographic progression persists in a significant number of treated individuals (13, 14). Thus, disentangling the causative relationship between inflammation (in particular at the entheses) and new bone formation as well as delineating the role of HLA-B27 in both processes are two critical gaps in knowledge. Addressing these questions will aid in identifying reliable prognostic biomarkers and therapeutic targets to limit inflammation and structural disease progression.

To better appreciate pathological new bone formation in JSpA, it is prudent to first understand how skeletal development and turnover take place under physiological conditions (15). There are two main types of osteogenesis: intramembranous and endochondral. Both commence with a mesenchymal tissue precursor, but they differ in how bone is formed and how they contribute to skeletal development. Intramembranous ossification sees direct differentiation of mesenchyme into bone, and is responsible for the development of flat bones (e.g., skull, mandible, and lateral clavicles). In contrast, endochondral ossification relies on chondrogenic differentiation whereby mesenchymal progenitors first condense into an "anlagen" before further differentiating into hypertrophic chondrocytes (16). Angiogenesis subsequently occurs and cartilage is progressively replaced by bone matrix synthesized by osteoblasts. Endochondral ossification steers the formation of the rest of the skeleton (i.e., axial and appendicular). In response to biological and biomechanical stimuli, bone homeostasis is maintained throughout life primarily via careful balancing of local bone resorption by osteoclasts and new bone formation by osteoblasts (17). While endochondral bone formation is crucial for skeletal growth and development, it can also be reactivated in pathological conditions such as fracture repair and local inflammation leading to syndesmophyte and enthesophyte formation (18, 19).

The structural hallmark of JSpA, comparable to its adult counterpart, is an altered bone remodeling process that paradoxically manifests as excessive new bone formation at inflamed peri-articular sites whilst in a background of increased systemic bone resorption (20, 21). How inflammation is associated with new bone formation remains an enigma, especially since inflammation classically promotes RANKL-mediated osteoclastogenesis and osteoclast activation to stimulate bone loss (22). Thus, several fundamental questions have dominated over the past decades: (1) if inflammation and new bone formation in JSpA are closely linked or uncoupled, (2) why and how inflammation co-localizes with new bone formation at entheseal sites, and (3) the contribution of HLA-B27 to these processes. Thus, this Review seeks to critically evaluate recent literature in both JSpA and its closely related adult SpA to resolve the aforementioned issues and appraise their translatability to improving diagnosis, prognosis, and therapy for JSpA patients.

## DECIPHERING THE LINK BETWEEN INFLAMMATION AND NEW BONE FORMATION IN JSPA

The relationship between inflammation and new bone formation in JSpA remains disputed. Two schools of thought, based on key findings from studies on adult SpA, dominate the field. One espouses a direct and sequential link between inflammation and new bone formation, while the other advocates an uncoupling of both processes. Even though inflammation can manifest separately or concurrently as enthesitis and synovitis, this Review will focus on evidence regarding enthesitis as it is pathognomonic of SpA.

## LINK BETWEEN INFLAMMATION AND NEW BONE FORMATION: EXISTING IDEAS AND EVIDENCE

The first view proposes that active inflammation may temporarily act as a brake on bone repair or remodeling. Inflammation induces inhibitors of new bone formation and simultaneously promote erosive cartilage and bone destruction. As the initial inflammation resolves or fluctuates, osteoproliferation follows (23). However, new bone formation in JSpA is hypothesized to be an excessive form of physiologic bone repair, thereby resulting in the formation of syndesmophytes and enthesophytes, as well as ankylosis.

This concept is supported by early magnetic resonance imaging (MRI) studies as entheseal sites with prior inflammation tend to have an increased frequency of bony appositions (23, 24). Building upon these topographical associations, contemporary studies focused on the effect of TNF on SpA pathogenesis. Evidence from animal models informed how TNF-induced inflammation may regulate new bone formation in the entheses through some candidates, notably the Wingless-related integration site (Wnt) pathway. TNF, acting through the Wnt pathway inhibitor Dickkopf-related protein 1 (DKK-1), may be important in repressing new bone formation driven by Wnt signaling as demonstrated in human TNF-overexpressing murine models (25, 26). Thus, there may be a window of opportunity to halt new bone formation with early and sustained anti-TNF treatment, which should prevent significant bone destruction and preclude the excessive bone repair response. Also, late anti-TNF treatment may even accelerate new bone formation. However, findings from TNF inhibitor (TNFi) studies have cast aspersions on this hypothesis. In the DBA/1 mouse model of ankylosing enthesitis, TNF blockade with the soluble TNF receptor etanercept did not inhibit the formation of new cartilage and bone at the enthesis (27). In TNFi placebo-controlled trials, ankylosis neither accelerated nor slowed down in the TNFi-treated cohorts despite significantly reduced clinical signs and symptoms (28–30). All in all, it is difficult to conclude from these studies as apart from their conflicting results, the studies

have critically overlooked other inflammatory mediators and also inconsistently used animal models.

The second perspective suggests that inflammation and new bone formation are simultaneously induced by common triggers (e.g., biomechanical stimuli, infection) but are uncoupled. Yet, interactions between signaling pathways involved are likely to be present (31). This hypothesis hinges upon the concept of entheseal stress, which entails mechanical load and microdamage at the entheses, in promoting inflammation and activating stromal progenitor cells for tissue remodeling and new bone formation. Therefore, ankylosis might not necessarily be a repair process initiated by bone damage, but more of a response to direct entheseal damage that may be partially dependent on chronic or recurrent inflammation.

Histological evidence supports this concept—syndesmophyte formation preferentially localized to the posterolateral vertebral rim where there is significant mechanical stress, and inflammation-associated bone loss and enthesophyte formation occurred at anatomically distinct regions of the Achilles tendon enthesis of SpA patients (32, 33). Furthermore, collagen-antibody-induced arthritis (CAIA) mice that were subjected to tail suspension had limited enthesophyte formation (34). Patients with radiographically advanced ankylosis might still suffer from persistent inflammation that is responsive to TNFi, and there are disorders (e.g., diffuse idiopathic skeletal hyperostosis i.e., DISH) that appear to rely on non-inflammatory-driven pathways of new bone formation (18, 35). Genetic and environmental factors could modulate the chronicity and intensity of inflammation as well as the extent of new bone formation, since ankylosis and its rate of progression are highly variable traits that do not correlate well with the degree of inflammation in SpA patients (18).

## NEW DATA ON THE LINK BETWEEN INFLAMMATION AND NEW BONE FORMATION

Advances in the field of osteoimmunology have shed light on the role of inflammation and its associated cytokines in the bone pathology of JSpA. Additionally, there is also a greater recognition of how local mechanical stress may promote disease.

### Interleukin-17/23

The interleukin (IL)-17/23 axis has been increasingly implicated in disease pathogenesis since early observations of increased serum levels of IL-17 and IL-23 alongside a T helper 17 (Th17) predominance in the circulation of SpA patients relative to healthy controls (36). IL-23, a cytokine primarily secreted by macrophages and dendritic cells, induces the production of IL-17 by T cells. IL-17 is pro-inflammatory and can synergize with TNF to orchestrate synovitis and enthesitis (37). However, their effects on bone physiology are unclear. In particular, IL-17 exerts a Janus-faced influence on bone formation as seen from various *in vitro* studies (38–45). IL-17 can stimulate osteoclastogenesis and block osteogenesis, but it may also promote bone-forming phenotypes in osteoblasts and their mesenchymal precursors under certain situations. Findings from animal and human SpA studies seem to favor IL-17A as a driver for bone formation, given that IL-17A enhances bone formation in C57BL/6 mice by stimulating the proliferation and osteoblastic differentiation of mesenchymal progenitor cells and it appears to do so via the JAK2/STAT3 pathway at least in adult AS patients (46, 47).

Recent studies have described entheseal resident immune cell subsets that can respond to IL-23 and produce IL-17A, which hint at pathological roles on exposure to appropriate triggers. In the CAIA mouse model of arthritis, IL-23 promoted highly specific entheseal inflammation reminiscent of SpA by acting on a distinct $CD3^+CD4^-CD8^-$ IL-$23R^+ROR\gamma t^+$ entheseal resident T cell subset (48). Upon IL-23 stimulation, these T cells produced IL-17 and IL-22 of which the latter is likely to be important in bone remodeling. Osteoproliferative changes were reduced *in vivo* with anti-IL-22 administration and could be reproduced with systemic IL-22 overexpression. In healthy human donors, subsets of IL-17A-producing group 3 innate lymphoid cells (ILC3) and γδ T cells reside in the spinal entheses (49). Similarly, in newly diagnosed SpA patients, innate-like T cells possessing a Th17-skewed phenotype ($ROR\gamma t^+$T-$bet^{lo}$PLZF$^-$ invariant NKT and γδ-hi T cell subsets) were enriched within inflamed sites, albeit in the joints rather than the entheses (50).

The importance of the IL-17/23 axis is further highlighted via studies investigating the effects of blocking IL-17 and IL-23. IL-17A knockout mice models displayed impaired bone regeneration and fracture repair at the femur when compared to wild-type mice (46). IL-17A inhibition concurrently reduced synovial inflammation (peripheral more than axial) and bone formation in animal models and peripheral SpA patients (51, 52). Surprisingly, in AS clinical trials, IL-17A inhibition (secukinumab, ixekizumab) was more effective than IL-23 blockade (ustekinumab, risankizumab) on spinal disease progression (42, 53, 54).

In summary, currently available evidence pinpoints the IL-17/23 axis as an integral component in SpA pathogenesis. The effects of the IL-17/23 axis may vary at different anatomical locations (i.e., peripheral vs. axial) owing to differences in biomechanical stress, which culminate in divergent molecular mechanisms of inflammation and bone remodeling. The preferential alleviation of spinal inflammation and ankylosis with IL-17A blockade in AS patients convincingly suggests that IL-17, not IL-23, is the major cytokine directing disease pathogenesis at least in axial SpA and that it is likely to be generated in an IL-23-independent manner. Indeed, there is evidence of an IL-23-independent pro-inflammatory loop incorporating Th17 autocrine IL-17 secretion induced by local prostaglandin E2 (PGE2) production, albeit in an *in vitro* rheumatoid arthritis (RA) system (55). Nonetheless, IL-23 overexpression in an HLA-B27-negative mouse model was still sufficient to trigger peripheral ankylosing enthesitis and appeared to bypass the requirement for mechanical overload, which signified that IL-23-dependent mechanisms may still be relevant in JSpA (48). While approximately a third of JSpA patients develop axial symptoms within several years of disease onset, peripheral disease is strongly associated with disease onset before 16 years of age (56). Thus, IL-23 could be critical especially in JSpA disease initiation and further research should focus

on resolving this quandary of IL-23 dependence to inform therapeutic strategies.

The entheseal non-Th17 sources of IL-17A may be useful as prognostic and therapeutic targets, but their reliance on IL-23 induction and downstream functional roles have yet to be fully clarified. Additionally, these immune cell subsets are also rare and limited in tissue distribution, so this calls into question their contribution to disease initiation and progression. For instance, ILC3s were found not to be a major source of IL-17A in the joints of adult peripheral SpA patients (47, 57). On top of considering IL-17 production by those cells, it is also worthwhile to explore alternative sources since it is possible that IL-17A may be secreted from distant sites (e.g., in the gut) to influence synovial cells that may, in turn, be abnormally sensitive to the cytokine.

Regarding IL-23, its cellular origins in JSpA also require further delineation. Going against the traditional view of entheses being largely devoid of myeloid cells, a recent study identified in healthy human entheses and adjacent bone a resident $CD14^+$ population that produces most of the inducible IL-23 (58). Peripheral monocytes isolated from patients with enthesitis also displayed increased IL-23 secretion following stimulation (58). It would thus be beneficial to make out how resident vs. tissue-infiltrating myeloid cells modulate IL-23 generation in inflamed entheses, albeit the pronounced difficulty in classifying myeloid subsets in tissues. Epithelial cells are also capable of secreting IL-23, which may portend gut, skin, and even lung involvement in the disease mechanism (59). It also remains to be seen how the remarkable polymorphism of the IL-23 receptor (IL-23R) functionally impacts JSpA pathogenesis, since various IL-23R single nucleotide polymorphisms (SNPs) have been strongly associated with AS, psoriasis, and IBD (58, 60, 61).

## TNF Superfamily

The role of TNF as a key driver of entheseal inflammation and its influence on bone pathology are definite. However, the contradictory effects of TNFi on radiographic progression and the inability of experimental models to accurately recapitulate SpA features substantially restrict how much we know about the cellular and molecular mechanisms by which TNF supports JSpA pathogenesis.

The TNFi conundrum may be partly explained by the duration for which patients were followed up after receiving anti-TNF therapy. Despite earlier placebo-controlled trials reporting that anti-TNF treatment was more effective in hampering inflammation than spinal radiographic progression in AS patients, they only had a 2 year follow-up period as it is unethical to expose patients to ineffective treatment for a longer duration. Recent studies have circumvented this hurdle by comparing TNFi-treated patients to biologics-naïve controls, and they indicated that prolonged TNFi therapy (especially for more than 4 years) may potentially slow down the progressive structural change in AS (62, 63). As such, this suggests that sustained TNFi treatment may confer beneficial effects on limiting inflammation and hence ankylosis.

Nevertheless, these results do not demonstrate total abolition of structural changes, so the extent of coupling between TNF and new bone formation in SpA remains equivocal. Given that inflammation is mediated by a plethora of factors (including the IL-17/23 axis) and not solely by TNF, more work is needed to delve into the relative contributions and interactions among such factors. Current anti-TNF literature has also focused more on axial rather than peripheral SpA, so this raises doubts concerning their impact on the peripheral disease-dominant JSpA. Conventional radiography, whilst favored to measure radiographic progression as the primary outcome for structural disease in human studies, has limited sensitivity to change (62, 64). A potential remedy may be to rely on alternative modalities such as high-resolution quantitative computerized tomography (HR-qCT) and whole-body MRI. These techniques should complement plain radiography by concurrently assessing enthesitis as well as its associated bone marrow oedema, bone erosion, and enthesophyte formation in peripheral SpA and JSpA (65, 66). Yet, age-related anatomical variations (e.g., physiologic subchondral oedema vs. pathological bone marrow oedema in children) may confound interpretations of inflammatory and structural lesions in JSpA, particularly in the absence of pediatric-specific definitions (56). Even so, the increased resolution of the entheseal organ and adjacent bony ultrastructure will prove invaluable in supplementing mechanistic studies to address why inflammation and new bone formation co-localize in JSpA.

The TNF-overexpressing experimental models, though commonly employed, have largely failed to phenocopy SpA. The human TNF transgenic (hTNFtg) and the TNFΔARE mice lack typical SpA features like spondylitis and enthesitis but instead develop destructive polysynovitis reminiscent of RA (25, 26, 34). Moreover, the latter has minimal endochondral bone formation and ankylosis at affected sites despite focal degradation of bone and cartilage (34). Despite these limitations, hTNFtg mice have been used to demonstrate the reversal of a destructive bone phenotype into a remodeling one characterized by new bone formation in synovial joints after blocking DKK-1, while TNFΔARE mice exhibited the suppression of peripheral enthesitis with reduction of mechanical strain. As such, these conclusions only partially reveal the causative relationship between inflammation (at least TNF-driven) and pathological new bone formation in JSpA.

A series of recent studies using another preclinical mouse model of TNF overexpression have shed light on how the structure of TNF may contribute to SpA pathogenesis (52, 67). The TgA86 mice systemically overexpress a mutant murine TNF gene that is defective at the ADAM17 cleavage site, thereby causing a specific increase of transmembrane-bound TNF (tmTNF) but not soluble TNF (sTNF) (68). Both tmTNF and sTNF are biologically active, but they may have varying affinities for the TNF receptors I and II (TNFRI and TNFRII) (69). The TgA86 mice appear more SpA-like: they not only develop significant peripheral arthritis but also enthesitis, spondylitis, osteitis, and endochondral bone formation leading to eventual axial and peripheral ankylosis. The same studies also demonstrated relative overexpression of tmTNF over sTNF in the synovial environment of SpA vs. RA patients, thereby hinting at probable mechanistic relevance. Indeed, it was found that stromal tmTNF overexpression could drive inflammation, especially at peripheral sites (52). Moreover, tmTNF-driven

inflammation and bone erosion seemed to precede new bone formation, which involved both endochondral and membranous ossification (67). However, this model lacks extra-articular manifestations of the SpA disease spectrum, which suggests that additional triggers may be required to induce IBD, uveitis, and/or psoriasis in the background of increased susceptibility owing to the tmTNF-sTNF imbalance. It also proposes that the inability of TNFi to induce full inflammatory remission and arrest radiographic progression in many patients is due to the subpar blockade of tmTNF in a poorly-vascularized environment like the enthesis. Although illuminating, it is not entirely clear how a single cytokine can foment distinct pathologies. To address this question, we will need to determine how TNFRI and TNFRII are differentially activated, how tmTNF is differentially expressed across cells, as well as how TNF and other pro-inflammatory molecules (e.g., IL-17) interact in the entheseal microenvironment.

## Other Modulators of Inflammation

The intensity of inflammation may help to direct the switch between bone catabolism and anabolism in SpA. Constitutive low TNF levels, but not short-term or high-intensity TNF stimulation, induced persistent expression of Wnt proteins and downstream bone formation through NF-κB and JNK/activator protein 1 (c-Jun) signaling pathways *in vitro* (42). When either the canonical Wnt/β-catenin or non-canonical Wnt/protein kinase Cδ (PKCδ) pathway is inhibited, new bone formation is significantly suppressed *in vitro* and in mouse models of SpA. Thus, the findings add nuance to the TNF brake hypothesis: inflammation does not have to be completely resolved before osteoproliferation can commence, and prolonged subclinical inflammation may be detrimental to bone physiology at entheseal sites. This may explain why structural disease, fueled by sustained low-intensity inflammation, continues to progress in many patients receiving anti-TNF therapy. As such, there is merit in exploring the feasibility of an early, aggressive and constitutive anti-inflammatory treatment for better outcomes in JSpA. Concurrent efforts should also further look into the molecular workings of this putative relationship since we do not fully understand how different Wnt family members and other pro-inflammatory cytokines contribute to this process, as well as how other osteogenic pathways (e.g., bone morphogenetic proteins; i.e., BMPs) come into play. There may also be interindividual differences in the threshold for inflammation-mediated structural progression, which could help to explain why some patients are refractory to TNFi treatment.

The role of the calcium-sensing receptor (CaSR) has recently received close scrutiny, as $CaSR^{+}$ osteoblasts were reported to have accumulated in entheseal sites of AS patients and animal models (70). Systemic administration of a CaSR antagonist NPS-2143 attenuated osteogenic differentiation *in vitro* and pathological new bone formation *in vivo*. Moving upstream, inflammation directly induced CaSR upregulation in osteoblasts through the NF-κB and JAK/Stat3 signaling pathways. Collectively, it appears that inflammation may directly promote osteogenic differentiation of precursor cells, on top of inducing the secretion of osteogenic growth factors, in the pathological bone-forming microenvironment. Yet, the study did not examine if non-osteoblast CaSR expression contributes to ankylosis and how flux in extracellular $Ca^{2+}$ levels at inflamed entheses may alter CaSR activity to influence bone remodeling.

## Biomechanical Stress

As the entheses are essential for transducing mechanical forces to bone and providing stability, they are sites of concentrated stress. Their distinct microanatomy consisting of fibrocartilaginous tissue is well-adapted for the high mechanical demands, as it grants both stiffness and elasticity (32, 71). While it is possible to induce enthesitis with repeated mechanical overloading like during sports, such pathology is usually limited to one entheses and resolves spontaneously. It has been proposed that excessive mechanical stress causes extracellular matrix damage and a rapid loss of mechanotransduction in tenocytes. Subsequently, a positive feedback loop of cell death, immune cell recruitment and production of pro-inflammatory molecules gives rise to sterile inflammation. When the stressor is removed, inflammation resolves and repair is collaboratively driven by immune and stromal cells (72).

However, SpA patients often suffer from long-term inflammation at multiple entheses, even though they may not report of significant trauma or mechanical strain at affected sites. Thus, SpA patients may possess a lower threshold for developing enthesitis reminiscent of an overexuberant response to stress (73). Moreover, the normal healing process at the entheses might be compromised by perturbed local and systemic immunity, which leads to persistent inflammation and ectopic bone formation. Contributing factors are likely to include genetics (e.g., IL-23R polymorphisms, HLA-B27) and environmental triggers (e.g., microbial infection and accompanying immune dysregulation), but the exact molecular mechanisms have yet to be determined (72). A study using TNFΔARE mice showed that hind limb unloading suppressed Achilles tendon enthesitis supposedly in a mitogen-activated protein kinase (MAPK) signaling-dependent but T cell-independent fashion, but it remains to be seen if the same mechanism can be demonstrated in other preclinical models and human patients (34). The impact of age-related differences in skeletal anatomy and kinematics on biomechanical stress has also been underexplored in JSpA. For example, could walking with adult-like velocity despite immature lower limbs (74) and aberrant tendon stiffening amidst increasing body mass during childhood (75) apply increased forces on the entheses of susceptible children to favor disease development?

Recognizing the significance of microtrauma in promoting site-specific inflammation, Benjamin and McGonagle proposed the concepts of "synovio-entheseal complexes" (SECs) and "functional entheses" to rationalize how stress concentrations may lead to simultaneous enthesitis, adjacent osteitis and synovitis in SpA (18). The SEC hypothesis captures the functional interdependence between entheses and synovial membrane in entheseal organs. As biomechanical stress at the entheses is dissipated, the secretion of pro-inflammatory factors from focal bony attachment sites may trigger secondary osteitis and synovitis. A functional enthesis refers to a region proximal to the

bony attachment sites where tendons or ligaments wrap around bone pulleys. This region is similar to true fibrocartilaginous entheses in anatomy, biomechanics and pathology, and are well-documented sites of pathology in SpA. Even though the theories may explain the diffuse nature of enthesitis and provide a unifying biomechanical basis for SpA pathology, they are exceedingly difficult to prove in animal models and human patients as the contribution of autoimmunity cannot be easily debarred.

## CONTRIBUTION OF HLA-B27 TO INFLAMMATION IND NEW BONE FORMATION

Possession of HLA-B27 is strongly associated with the development of JSpA, though adult-onset disease has a higher prevalence of HLA-B27 positivity (56). However, the exact pathogenic role of HLA-B27 is unknown despite intense investigation. Additionally, no more than 5% of HLA-B27$^+$ individuals develop SpA, which suggests the involvement of other genetic and environmental influences.

## HLA-B27 IN INFLAMMATION: DRIVER OR MODULATOR?

There are several theories explaining how HLA-B27 can stimulate and sustain inflammation in SpA, and the most favored ones will be critiqued in greater detail alongside data that has recently emerged. In a nutshell, HLA-B27 may present arthritogenic peptides to cytotoxic T lymphocytes (CTLs), but B27 can also assume abnormal forms at the cell surface.

### Presentation of Arthritogenic Peptides

Like other major histocompatibility complex (MHC) class I molecules, HLA-B27's natural function is to present endogenous peptides to T cells, especially CTLs. In this process, cytosolic proteins are first degraded into peptide fragments and loaded onto HLA-B27. Next, the peptide-MHC complexes are transported to the cell surface where they interact with their cognate T cell receptors (TCRs) to elicit antigen-dependent, cell-mediated immune responses (76, 77). Thus, it was hypothesized that HLA-B27 could present arthritogenic peptide(s) to autoreactive CTLs, which may subsequently induce inflammation at target tissues in SpA (78). This was thought to be an undesirable consequence of prior infections, whereby microbial peptides elicit a CD8$^+$ T cell response cross-reactive with combinations of HLA-B27 and self-peptides.

Several lines of evidence from human studies support this theory. Firstly, patients with reactive arthritis exhibited HLA-B27-restricted CD8$^+$ T cell responses specific for causative bacteria, which may be possible triggers for SpA (79, 80). This finding is buttressed by the oligoclonal T cell expansion in the inflamed joints of SpA patients, which implies an ongoing antigenic-driven process (81, 82). Moreover, HLA-B27 subtypes possess unique peptide specificities owing to variation at the peptide-binding groove, so this provides structural evidence for the existence of distinct autoantigens in SpA (83).

Current studies have employed high-dimensional technologies to interrogate the specificity of the B27-restricted T cell responses, and to define putative autoantigens responsible for these responses. Using next-generation sequencing-aided high-throughput T cell receptor (TCR) repertoire analysis (Rep-seq), a recent study demonstrated a positive association between disease activity and the oligoclonal expansion of not just CD8$^+$ but also CD45RA$^+$ effector memory CD4$^+$ ($T_{EMRA}$; CD45RA$^+$CD45RO$^-$CD62L$^-$) T cells in the inflamed joints of HLA-B27$^+$ AS patients (84). It also identified common complementarity determining region 3 (CDR3) motifs unique to the pathological CD4$^+$ and CD8$^+$ T cells. Therefore, both T cell compartments appear critical for SpA pathogenesis, and they may be stimulated by shared arthritogenic autoantigens. Another study, via data-independent acquisition (DIA) and multiple reaction monitoring (MRM) mass spectrometry, identified 26 candidate autoantigens that are presented in less abundance by the protective HLA-B*27:06 and HLA-B*27:09 relative to six AS-associated variants (85).

However, evidence from animal studies is at odds with this hypothesis. The HLA-B27$^+$ transgenic arthritic/colitis rat model persistently developed SpA-like features even after the depletion of CD8$^+$ T cells with either anti-CD8α monoclonal antibodies or CD8α knockout (86, 87). Many murine models are also capable of phenocopying SpA to varying degrees in the absence of human HLA-B27, notably through the overexpression of human TNF (25, 26, 34, 52, 67). IL-23 overexpression alone in an HLA-B27$^-$ background was also sufficient to drive murine SpA-like disease by activating IL-17-producing entheseal resident non-αβ T cells (48). These animal data complicate the significance of the arthritogenic peptide model in initiating disease. Even so, it was shown in HLA-B27/hβ2m–transgenic rats that B27-restricted T cell responses may augment entheseal inflammation preferentially through the IL-17/23 axis as IL-17A inhibition significantly diminished inflammation and new bone formation (88).

All in all, it is difficult to conclusively demonstrate pathogenic T cell responses to arthritogenic peptides from existing literature. New approaches to distinguish the specific T cell subsets at fault and the autoantigens that stimulate them will likely assist in this search, especially in light of the latest advancements in our understanding of antigen presentation. For instance, the proteasome-generated spliced peptide pool unexpectedly contributes to a significant portion of the MHC-I immunopeptidome (i.e., epitopes presented by MHC-I molecules), which suggests that there is a far greater antigenic peptide diversity with overlapping sequences derived from either human or pathogen proteomes (89). Moreover, the strong association of AS with endoplasmic reticulum aminopeptidase 1 (ERAP1), whose expression is restricted to HLA-B27$^+$ individuals (90), argues for aberrant MHC-I antigen processing in the disease mechanism since ERAP1's only known function is to trim peptides prior to MHC-I presentation (91). Thus, future prediction tools must account for these complexities

to optimize the set of candidate autoantigens to be tested for immunogenicity.

### Cell Surface Free Heavy Chain Forms

HLA-B27 normally exists as a heterodimer consisting of a polymorphic α-chain (encoded by the MHC gene) that is non-covalently linked to a non-polymorphic $\beta_2$-microglobulin chain. Peculiarly, HLA-B27 can also assemble as cell surface free heavy chain (FHC) forms, such as the $\beta_2$m-free, Cys67-mediated disulfide-bonded homodimers ($B27_2$), on immune cells of SpA patients (92, 93). FHC molecules can elicit TCR-independent immune responses by binding to innate immune receptors on natural killer (NK), $CD3^+$ T and myeloid cells (93). These receptors include the killer immunoglobulin receptors KIR3DL1, KIR3DL2, and LILIRB2 in humans (93) and the rodent paired immunoglobulin receptors (PIR) (80). In particular, the KIR3DL2/B27 interaction is of exquisite affinity and exerts pro-inflammatory effects by enhancing NK cell survival and $CD4^+$ T cell proliferation in HLA-$B27^+$ SpA patients (94). It is also associated with a Th17 phenotype in SpA: the circulation and inflamed joints of HLA-$B27^+$ SpA and ERA patients were enriched with $KIR3DL2^+$ $CD4^+$ T cells (95), of which a subset produced IL-17 upon stimulation with $B27_2$-expressing antigen presenting cells (96).

Nevertheless, more direct evidence on the function of these FHC forms in JSpA pathogenesis is needed. Future work has to explore the conditions favoring FHC and $B27_2$ formation, the relationship between homodimer expression and JSpA disease severity, as well as the extent of protection afforded by inhibiting the KIR/B27 interactions.

### Other Theories

HLA-B27 can misfold in the endoplasmic reticulum (ER), which may lead to ER stress and the inflammatory unfolded protein response (UPR) (97). A possible consequence of the UPR is thought to be increased IL-23 production, which correlated with the degree of HLA-B27 misfolding in stimulated HLA-B27 transgenic rat bone marrow–derived macrophages (98, 99) and dendritic cells derived from healthy human volunteers (100). Yet, the failure of excess β2m in relieving ER arthritis in HLA-B27 transgenic rats (91) and the lack of direct evidence in human SpA (101) warrant caution.

HLA-B27 may also promote microbial dysbiosis to result in entheseal inflammation. There is evidence of local gut inflammation in more than half of AS patients and shared genetic associations between AS and IBD (102). The cecal microbiome is also altered in HLA-B27 transgenic rats (30). However, the jury is still out on how HLA-B27 alters the gut microbiome and promotes inflammation at distant sites.

## HLA-B27 IN NEW BONE FORMATION: A PREDISPOSING FACTOR?

HLA-B27 positivity has been associated with worse radiographic damage, more typical marginal syndesmophytes, and more frequent syndesmophyte symmetry in SpA patients (103). How does HLA-B27 specifically direct osteoproliferation in JSpA? The first line of evidence comes from the observation that HLA-$B27^+$ individuals appear predisposed to exaggerated bone formation regardless of SpA disease status (104). This inflammation-agnostic osteoproliferative state may be attributed to increased Wnt signaling evident by lower serum concentrations of the Wnt pathway inhibitors DKK-1 and sclerostin (SOST), as well as elevated circulating levels of the ossification-enhancing Indian hedgehog (IHH). Yet, the molecular mechanisms by which these bone regulators exert and coordinate their effects in disease are not fully known.

A recent study suggests another possibility: HLA-B27 directly activates the tissue non-specific alkaline phosphatase (TNAP) pathway in mesenchymal stem cells (MSCs) to enhance bone mineralization and ectopic bone formation at inflamed entheses (105). RNA interference (RNAi)-mediated HLA-B knockdown reduced the expression of TNAP and its upstream transcription factor retinoic acid receptor-β (RARB), as well as the amount of mineralization, in MSCs derived from HLA-$B27^+$ AS patients. Reciprocally, HLA-B27 overexpression by lentiviral transformation of control MSCs reversed the phenotype. Furthermore, TNAP blockade inhibited new bony appositions induced by AS MSCs that were implanted into surgically decorticated spinal sites of NOD-SCID mice. Collectively, HLA-B27 may encourage ectopic new bone formation in a TNAP-dependent manner. The study also suggests HLA-B27 misfolding as a potential trigger for this mechanism since HLA-B27 FHC forms substantially accumulated and the inositol-requiring 1 (IRE1)/spliced X-box–binding protein 1 (sXBP1) pathway was upregulated in AS MSCs. As such, the findings put forth a compelling paradigm directly implicating HLA-B27 in both inflammation and new bone formation. However, the relative importance of this mechanism in JSpA and how the entheseal stroma interacts with other players (e.g., immune cells, cytokines, and mechanical stress) to induce the HLA-B27-mediated TNAP pathway remain unclear.

Contrary to the previously discussed literature, a study argues against a direct role of HLA-B27 in mediating new bone formation in axial SpA (106). HLA-B27 overexpression in various *in vitro* mouse and human differentiation systems mimicking endochondral ossification did not result in differences in bone formation when compared to the HLA-B7 overexpression controls. While the experimental set-up was deliberately reductionist to exclude the influence of possibly intervening variables (e.g., pro-inflammatory cytokines, immune cells), its oversimplicity may render the study's conclusions premature.

In summary, there is sufficient evidence to surmise an ancillary role for HLA-B27 in pathological new bone formation, but how HLA-B27 specifically influences or signposts osteoproliferation deserves continued investigation. Future work should also aim to reconcile the pro-inflammatory and osteogenic capacities of HLA-B27.

## INTEGRATING NEW TO OLD DATA: TRANSLATING FINDINGS TO CLINICAL PRACTICE FOR JSPA

Taking a leaf from adult SpA, entheseal-specific bone turnover in JSpA is likely to depend on concomitant inflammatory and

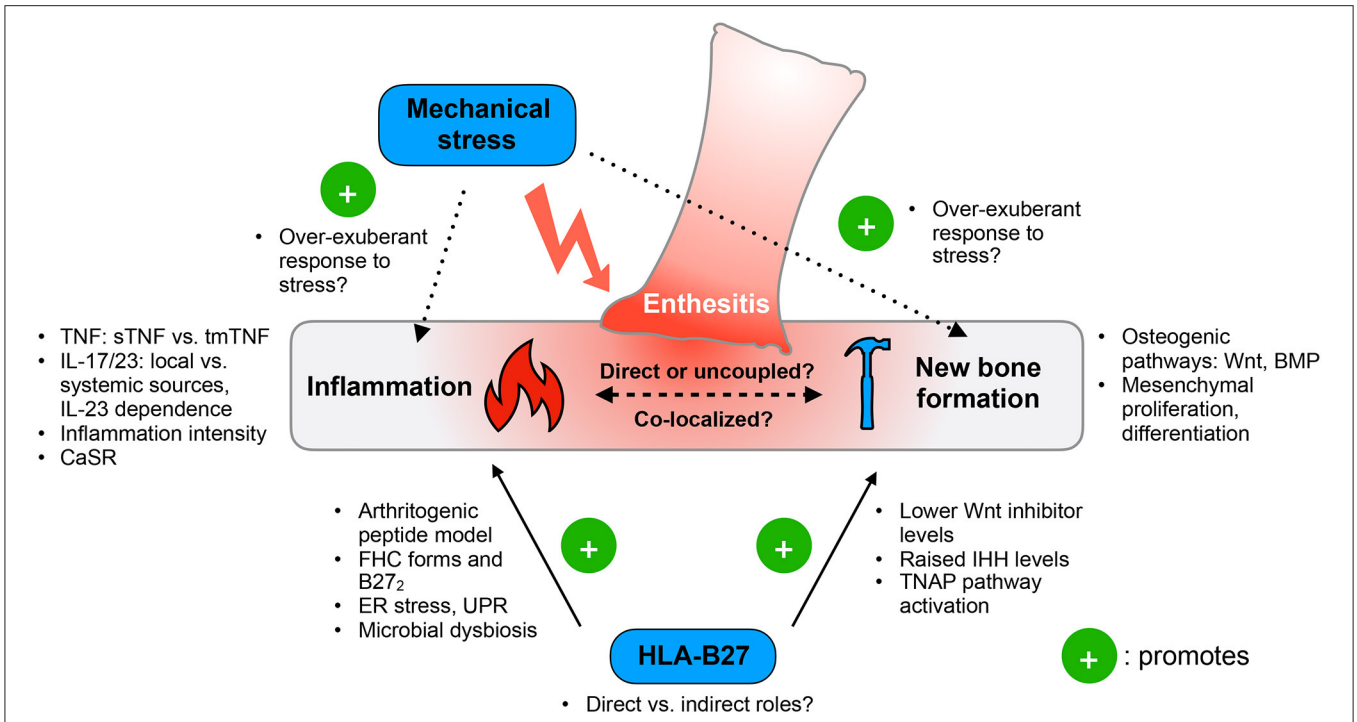


**FIGURE 1 |** Functional model of ankylosing enthesitis in JSpA. Enthesitis is primarily driven by the tumor necrosis factor (TNF) and IL-17/23 pathways. Inflammation appears to influence osteoproliferation by acting on the Wnt and BMP pathways, as well as controlling mesenchymal proliferation and differentiation. The intensity of inflammation may determine the switch between bone loss and formation. However, it is still unclear if inflammation and pathologic new bone formation are directly linked or uncoupled. Mechanical stress has emerged as an important trigger of disease, yet the exact molecular mechanisms are unknown. HLA-B27 may contribute to both inflammation and pathologic new bone formation, but how crucial this genetic risk factor is to JSpA pathogenesis remains to be clarified. sTNF, soluble TNF; tmTNF, transmembrane TNF; CaSR, calcium sensing receptor; FHC, free heavy chain; B272, HLA-B27 homodimers; ER, endoplasmic reticulum; UPR, unfolded protein responses; Wnt, Wingless-related integrated site; IHH, Indian hedgehog; TNAP, tissue non-specific aminopeptidase; BMP, bone morphogenetic protein.

mechanical effects on bone physiology. Inflammation, by acting both directly and indirectly, drives bone loss and influences osteoproliferation. Genetic and environmental risk factors (e.g., HLA-B27, local biomechanical stress) may amplify the effects of inflammation at entheseal sites to promote new bone formation (**Figure 1**).

In JSpA, the treatment of enthesitis aims to resolve inflammation and prevent downstream inflammation-induced tissue responses. Non-steroidal anti-inflammatory drugs (NSAIDs) are the initial pharmacological management and can adequately control early disease as seen in adult AS patients (107), though no trials of NSAIDs have been reported in JSpA. NSAIDs may also hamper new bone formation since they inhibit PGE2, which possesses potent osteoinductive properties (108–110). When enthesitis becomes chronic and increases in severity, therapy is escalated to include disease-modifying anti-rheumatic drugs (DMARDs) and biological agents (111). The conventional DMARDs sulfasalazine and methotrexate have been shown to be effective for managing peripheral enthesitis in ERA and JAS patients (112, 113), but the same benefits may not extend to axial disease. As such, TNFi treatment is indicated for peripheral disease refractory to DMARDs or when axial disease is present since several trials in ERA have guaranteed its efficacy and safety (114).

Taking into account how inflammation pervades disease induction and progression, early and sustained anti-inflammatory treatment should form the bedrock of JSpA management. A sizeable number of patients have been unable to sustain disease remission despite receiving prolonged TNFi therapy (115–118). However, this refractoriness may be partly ascribed to (1) interindividual differences in the intensity threshold for structural progression, as well as (2) the contributions of other pro-inflammatory cytokines and local pathways. Thus, future treatment strategies demand greater personalization and combinatorial use of multiple therapeutic options, both pharmacological and non-pharmacological.

Promising therapeutic targets to complement TNF blockade include the IL-17/23 axis, especially in light of its purported role in both inflammation and new bone formation. Moreover, clinical trials of IL-17 and IL-23 inhibitors have been successful in reducing disease activity and structural progression in adult SpA. The therapeutic benefits of IL-17/23 inhibition are currently under evaluation in children, but it is expected that a similarly striking responsiveness will be replicated in JSpA. Whether or not synergy exists between anti-TNF and anti-IL-17/23 drugs on curbing JSpA is also a tantalizing prospect to follow up on. In addition, the recent discoveries of IL-17-producing entheseal resident innate-like T cells and ILC3s, as well as stromal tmTNF

expression, draw attention to local mediators of inflammation in perpetuating disease. Coupled with the poor vascularity of the enthesis, it may be advantageous to assess how local anti-TNF and anti-IL17/23 treatment can dovetail systemic drug administration. An extension of the IL-17/23 axis is the Janus kinase (JAK)/STAT pathway, which is thought to activate the IL-17/23 axis so its blockade could augment the effects of IL-17/23 inhibition (119). An early trial using the JAK1/3 inhibitor tofacitinib has been moderately successful in reducing disease activity and radiographic disease in AS (120), but evidence is still sorely lacking in the JSpA realm.

The heightened awareness of local players in entheseal inflammation and new bone formation has also emphasized the advantages of relieving biomechanical stressors and targeting stromal remodeling pathways in JSpA. As JSpA patients may be predisposed to a pathologically exaggerated inflammatory response to mechanical stress, physical therapy could help by maintaining a safe loading environment for the entheses. Short-term interventions should aim to immediately reduce mechanical forces imparted to affected sites, such as through the use of custom-made foot and ankle orthotics (121, 122). On the other hand, long-term strategies ought to enhance the entheseal loading environment. Emulating the restorative treatment approaches of osteoarthritis (123), physical therapy in JSpA should concomitantly strengthen muscles of the limbs, trunk and back, as well as engage in neuromuscular training. Doing so will not only slow down the loss of range of motion (ROM) and correct poor functional positioning (114), but also minimize a major environmental risk for enthesitis to complement the action of anti-inflammatory drugs. Studies have also implicated various bone remodeling pathways (e.g., Wnt, BMP) in JSpA new bone formation, but pharmacological modulation of these processes (especially with systemic administration) may upset normal skeletal development (124, 125). As such, future studies are needed to assess feasibility and fine-tune therapeutic doses for maximal effectiveness.

HLA-B27 continues to be an important genetic risk factor for JSpA, but the molecular mechanisms behind this disease association remain at large. Nevertheless, progress has been made in clarifying the arthritogenic peptide model using high-dimensional technologies, and in identifying candidate pathways through which HLA-B27 exerts its influence on bone formation.

## CONCLUSION

JSpA is a largely inherited disease that is almost certainly influenced by ubiquitous environmental triggers. The recurrence of enthesitis, despite substantial heterogeneity in clinical presentation, emphasizes a common pathological process in a unique anatomical and immune microenvironment. This process is likely to depend on multiple steps consisting of initiation and augmentation of inflammation followed by local tissue responses leading to new bone formation. Current data provide strong support for the central roles of TNF, IL-17/23 axis, and HLA-B27 in JSpA pathogenesis, albeit incomplete evidence. Nevertheless, questions on temporality and gene-environment interaction in the relationship between inflammation and new bone formation remain, especially when the field is beset by inconsistencies in clinical trial design and deficiencies in preclinical disease models. Moving forward, one would have to integrate information from wide-ranging studies and clarify disease nomenclature. Doing so would be critical not just for translating findings in adult SpA to JSpA, but also for facilitating the otherwise tricky transition between pediatric and adult care.

## AUTHOR CONTRIBUTIONS

ST, JY, and TA contributed to the conceptualization and writing of the article. JL and SA helped in the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Outcomes in Juvenile-Onset Spondyloarthritis

*Judith A. Smith*[1]* *and Ruben Burgos-Vargas*[2]

[1] Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States,
[2] Departamento de Reumatologia, Hospital General de Mexico, Mexico City, Mexico

Some studies have suggested children with juvenile onset spondyloarthritis (JoSpA) have a relatively poor outcome compared to other juvenile idiopathic arthritis (JIA) categories, in regards to functional status and failure to attain remission. Thus, in the interest of earlier recognition and risk stratification, awareness of the unique characteristics of this group is critical. Herein, we review the clinical burden of disease, prognostic indicators and outcomes in JoSpA. Of note, although children exhibit less axial disease at onset compared to adults with spondyloarthritis (SpA), 34–62% have magnetic resonance imaging (MRI) evidence for active inflammation in the absence of reported back pain. Furthermore, some studies have reported that more than half of children with "enthesitis related arthritis" (ERA) develop axial disease within 5 years of diagnosis. Axial disease, and more specifically sacroiliitis, portends continued active disease. The advent of TNF inhibitors has promised to be a "game changer," given their relatively high efficacy for enthesitis and axial disease. However, the real world experience in various cohorts since the introduction of more widespread TNF inhibitor usage, in which greater than a third still have persistently active disease, suggests there is still work to be done in developing new therapies and improving the outlook for JoSpA.

**Keywords: juvenile spondyloarthritis, enthesitis-related arthritis (ERA), disease manifestations and outcomes, prognosis, TNF inhibitor, sacroiliitis**

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***Correspondence:**
*Judith A. Smith*
*jsmith27@wisc.edu*

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## INTRODUCTION

As a whole, the group of children with JoSpA/ERA have worse reported outcomes than other categories of juvenile idiopathic arthritis (JIA) in regards to remission rates, pain, and quality of life (1, 2). Some of the challenges for this group derive from treatment-refractory complications and insidious, sometimes asymptomatic axial disease progression in JoSpA/ERA. The long lag between symptom onset and diagnosis remains problematic as well (3, 4). Greater awareness of the unique clinical attributes in JoSpA/ERA could aid providers in stratifying their patients toward more aggressive therapy. It is also important to identify unmet clinical needs regarding outcomes. Although the therapeutic options have changed over time with the advent of biologics, the real-world impact on outcome is not clear. Thus, the goals of this review are to highlight the clinical characteristics of this group that contribute to the burden of disease, prognostic indicators, and the remaining gaps in outcomes.

In adults, axial SpA encompasses a spectrum of symptoms including pain and stiffness affecting the spinal and sacroiliac joints and axial entheses, and more rarely peripheral arthritis and enthesitis. Familial aggregation (genetics) and significant association with HLA-B27 antigen play

an important role in the pathogenesis of the disease (5–7). Besides musculoskeletal involvement, adults with SpA have a variable percentage of anterior uveitis, psoriasis, and gut disease (8). Children and adolescents, by definition those with disease onset ≤ to 18 years of age, present with clinical disease that clearly overlaps with this adult spectrum, although with some differences, likely reflecting the developing immune system, mechanical differences and potentially the microbiome (9, 10). However, because of some of these key clinical differences, the adult classification criteria, particularly those for axial SpA, may not perform particularly well in capturing children. For instance, inflammatory back pain (by definition, pain for more than 3 months), which serves as an entry point for adult disease, is much less common in children early in their disease course (11–13). Related to the lower frequency of sacroiliitis at disease onset in juvenile Spondyloarthritis (JoSpA), one group has reported a sensitivity of only 25% for ASAS axial SpA criteria (14). The ASAS peripheral SpA critera may perform better in children, capturing >90% of subjects (15–17). Unfortunately, there is currently no official JoSpA classification equivalent. The current SpA monikers and classification criteria applied to children is a muddle, including a pot-pourri of terms such as "seronegative enthesopathy and arthropathy" (SEA syndrome), "enthesitis related arthropathy" (ERA), and worse yet, "undifferentiated arthritis" (18). The problems surrounding nomenclature are described in detail in chapter 1 of this issue and so will not be addressed further here. In this chapter, we will generally use the inclusive acronym JoSpA/ERA (juvenile onset spondyloarthritis/ERA), unless specific International League Against Rheumatism (ILAR) classification categories (e.g., ERA, PsA, undifferentiated arthritis) are being described Petty et al. (19).

## AXIAL AND PERIPHERAL ARTHRITIS AND ENTHESITIS IN JOSPA/ERA

Compared with other types of JIA, children with JoSpA/ERA have a higher male representation and older age of onset (typically 10–11 years) (basic clinical characteristics in **Table 1**) (18, 20, 25, 29, 30). HLA-B27 positivity in various JoSpA/ERA cohorts and case series is variable, potentially reflecting ethnic differences. For instance HLA-B27 is present in ∼6–8% in Europeans, but rare in Africans and in Japanese (<1%) (31). In general, the prevalence of ankylosing spondylitis (AS) and other related SpA conditions strongly associates with HLA-B27 antigen in different populations around the world (32). Although in some populations, for instance in Africans, SpA may associate more with other HLA molecules (e.g., HLA-B14:03) (33). In the multi-national studies cited here, HLA-B27 prevalence ranges from 35 to 97% [see **Table 1** and (26)]. Despite this ethnic variability, HLA-B27 still accounts for the greatest known influence on genetic susceptibility to AS and SpA (6, 34, 35), and is overrepresented in children with SpA compared to the general population (30, 36, 37). Moreover, distribution of HLA-B27 disease-associated subtypes in JoAS (juvenile onset ankylosing spondylitis) mirrors the prevalence in subjects with adult onset AS (36).

In comparison with adult onset AS, children with JoAS tend to present with more enthesitis and peripheral arthritis and less lumbar pain and stiffness, a pattern also characteristic of the greater spectrum of JoSpA/ERA (4, 11, 38). Presence of enthesitis varies by study location and definition of SpA, ranging from 37% in ERA to >100% in SEA (**Table 1**, with specific anatomic distribution described in **Table 2**) (18, 23). The most frequent areas of involvement are the calcaneal insertion of the Achilles tendon, patellar tendons and insertions of the plantar fascia. Clinical assessment of enthesitis can be challenging; however, ultrasound has been an extremely useful, though operator-dependent adjunct, as has MRI (41, 42). Interestingly enthesitis is not exclusive to ERA. One of the largest studies to date on the topic of enthesitis in different JIA categories comes from Rumsey et al. (43). In a Canadian JIA inception cohort, enthesitis was defined by entheseal tenderness in more than one body site on more than one occasion during 60 months follow up. Enthesitis affected 16% of this large JIA cohort (1,406 patients), and ERA, PsA and undifferentiated arthritis accounted for 64, 2, and 18%, respectively, of those with enthesitis. In this cohort, children with enthesitis tended to be older at disease onset (10.7 vs. 7.5), male (57 vs. 31%), have polyarthritis (57 vs. 41%), and sacroiliitis (30 vs. 4%). Within ERA, 141/202 (70%) had enthesitis. In this JIA cohort, the most common anatomical locations were the plantar fascia (39%), Achilles (31%), and tibial tuberosity (30%). The course of enthesitis tended to follow active joint count (43).

Regarding arthritis phenotype, children with JoSpA/ERA typically present with asymmetric oligoarticular arthritis affecting the large weight-bearing joints (knees), ankles, mid-foot, and root joints (hips and shoulders) (17, 18, 26, 39, 44). **Table 2** presents the anatomic distribution described in several JoSpA/ERA cohorts and case series. In a long-term study from Norway, 73% had oligoarticular onset, and in US and Taiwanese cohorts, 78 and 97% had oligo articular onset, respectively (2, 26, 27). A few studies have reported >50% prevalence of polyarticular involvement in JoSpA/ERA, and patients can accumulate 5 or more joints over time (17, 21, 39, 43). The most commonly affected joints include the knees (46–100%) and ankles/subtalar joints (27–80%). Hip arthritis is also common (19–83%, **Table 2**) and can be relatively aggressive and severe (45). Indeed, 2 studies comparing juvenile and adult onset AS described increased rates of hip arthroplasty in the JoAS group (17.7 vs. 8.7% in the Genseler study and 17 vs. 4% in the Calin study), although no difference was reported in a Canadian study (7% for both, O'Shea et al.) (38, 45, 46).

Foot arthritis, particularly mid-foot arthritis or tarsitis, is highly characteristic of this population, though the prevalence varies depending upon the study (**Table 2**). Indeed, in one study comparing JoAS and other Juvenile Rheumatoid Arthritis (JRA), 85.7% of children with JoAS experienced tarsitis, vs. 10.7% in JRA within 1 year of presentation (39). Anatomic involvement of the feet was particularly well described in a study from India (40). Phatak et al. described a case series of 55 children diagnosed with ERA for <60 months. This population was 96% male and 80% HLA-B27 positive, with sex most likely skewed by referral

**TABLE 1 |** JoSpA/ERA clinical features.

| Years (y) | Country | Cohort/ sample size | Y f/u | SpA #(%) of total cohort | Age onset ±SD or (25%, 75%) or (range) | % Male | % HLA-B27 | % Spine involved[e] | % periph. Arthritis | % Enthesitis | % Eye | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <1982 | Canada | 39 | 1.9 | 39 (100) SEA[b] | 9.8 (2, 16) | 90 | 72 | 44 exam<br>28 X-ray SI<br>15 X-ray TL spine | 74 | 100 | 15 | (18) |
| 1980–1985 | Norway | 175 | 15.3 | 55 (33) ERA | 11.1 ± 2.8 | 65 | 85 | 35 X-ray<br>47 IBP<br>75<br>decreased mobility | – | – | – | (2) |
| 1997–2000 | Nordic | 410 | 8 | 46 (11.2) ERA<br>14 (3.4) PsA<br>63 (15.4) UA | 10.5 (8.6, 12.3)<br>5.9 (3.2, 7.2)<br>8.1 (3.5, 11.9) | 65.2<br>50<br>28.6 | 72<br>21<br>21 | – | – | – | – | (20, 21) |
| <2001 | England | 246 | 28 | 32 (13.1) ERA<br>15 (5.3) PsA | 10.0 ± 3.3<br>9.9 ± 3.3 | – | – | – | – | – | 28<br>23 | (22) |
| 2002–2003 | Germany | 118 | 4 | 118 (100) SpA (mNY or ESSG)[b] | – | 73 | 66 | 32 IBP | 96 | 44 | 6.8 | (17) |
| 1994–2006 | India | 235 | >1.5 | 84 (36) ERA<br>3 (1) PsA<br>11 (5) UA | 13 (7, 16)<br>12 (5, 12)<br>11 (2–15.5) | 91<br>33<br>55 | 89[d]<br>–<br>40 | 19 SI, 37 spine<br>0<br>18 SI, 20 spine | – | 37[f]<br>0<br>27 | 8.3<br>0<br>0 | (23) |
| 2000–2006 | Italy | 59 | 3 | 59 (100) ERA | 9.3 (6.5–13.3) | 68 | 66 | 36 IBP or decreased mobility (in 1y) | – | – | – | (24) |
| 2006–2009 | Brazil | 253 | | 253 (100) JSpA (ESSG) | | 86 | 80 | 60 IBP | 60 lower limb, 20 upper limb | 58 | 25 | (4) |
| 1995–2010 | Taiwan | 195 | >1.5 | 73 (37) ERA<br>3 (1.5) PsA<br>11 (5) UA | 10.8 (8.9, 12.3)<br>9.4 (6.2, 10.8)<br>10.2 (8.7, 15.1) | 85<br>67<br>20 | 82<br>33<br>0 | 48 SI or lumbar<br>33<br>0 | – | – | 9.6<br>0<br>0 | (25) |
| 1989–2012 | USA | 234 | –[a] | 234 (100) ERA | – | 72 | 59 | 26 clinical SI<br>56 MRI | 92 | 75 | 5.6 | (26) |
| 2008–2015 | France | 114 | 2.5 | ERA/JSpA[c] | 9.6 (6.9, 12.3) | 59 | 43 | 63 (47 with SI, 24 thoracic and 44 lumbar) | 87 | 86 | – | (16) |
| 1993–2018 | Taiwan | 181 | 7.7 | 72 (40) ERA | 11.0 ± 3.2 | 86 | 97 | 16 (clinical or X-ray SI) | – | – | 10 | (27) |

*Cohorts/series are listed by years of patient recruitment and nationality in left columns. Unless otherwise indicated, features are cumulative at follow up rather than baseline. ILAR, International League against Rheumatism; ERA, enthesitis related arthritis; PsA, psoriatic arthritis; UA, undifferentiated arthritis. Follow up years refers to mean or median, depending upon the study. For cohorts not describing a specific manifestation (no data) or with insufficient data (based on <1%), the missing data are designated with a dash (–). IBP, inflammatory back pain; SI. sacroiliitis.*

[a] *Cross-sectional study.*

[b] *Other JoSpA. SEA, seronegative enthesopathy and arthropathy; ESSG, European Spondyloarthropathy Study Group; Mny, modified New York criteria for ankylosing spondylitis (28).*

[c] *Physican diagnosed JSpA. At last followup, 92% met clinical criteria for ASAS peripheral SpA and 75% for either ERA or PsA.*

[d] *Percentages are from 62 patients with ERA and 10 with PsA were tested for HLA-B27.*

[e] *Description of axial involvement was heterogeneous between sources and included symptoms, clinical assessment, radiology (X-ray), and MRI.*

[f] *Relatively low proportions of enthesitis in "ERA" are not explained.*

**TABLE 2 |** Anatomic distribution of peripheral arthritis and enthesitis.

| **Years (y) recruited** | **<1982** | **1980–1985** | **2002–2003** | **2000–2006** | **1995–2010** | **1989–2012** | **2008–2015** | **2015–2016** |
|---|---|---|---|---|---|---|---|---|
| **Country** | **Canada** | **Mexico** | **Germany** | **Italy** | **Taiwan** | **USA** | **France** | **India** |
| Cohort size | 39 | 110 | 118 | 59 | 195 | 234 | 114 | 55 |
| Y f/u | 1.9 | 12.2 | 4 | 3 | >1.5 | –[b] | 2.5 | <5 |
| SpA # (%) | All SEA | 35(32) JoAS | All SpA mNY or ESSG | All ERA | 73(37) ERA | All ERA | All SpA | All ERA |
| Peripheral arthritis | | | | | | | | |
| % Knee | 83 | 100 | 77 | 65 | 52 | 46 | 58 | – |
| % Ankle | – | 80 | 40 | 48 | 38 | 36 | 38 | 27 |
| % Hip | – | 83 | 38 | – | 43 | 19 | 46 | – |
| % Mid-foot | – | 89 | 9.3 | 58 | – | – | 9 | 36 by ultrasound 54 by MRI |
| % Fingers | – | 23 | 25 | – | 18[a] | – | 12 | – |
| % Toes or MTP | – | 86 | 27 | – | 16[a] | – | 17 | 4 toes 16 MTP |
| % Wrist | – | 14 | – | – | 16 | 20 | 25 | – |
| % Dactylitis | – | – | 13 | – | – | – | 13 | 7.3 (toes) |
| Enthesitis | | | – | | – | | | |
| % Achilles | 51 | 34 | | 28 | | 33 | 74[c] | 44 |
| % Plantar front insertion | – | – | | – | | – | 39 | 20[d] See note |
| % Plantar calcaneal insertion | 67 | 54 | | 38 | | – | See note[c] | See note[d] |
| % Knee | 49 | 23 | | – | | 44 | 46 | – |
| % Pelvis | 5 | 9 | | – | | 30 | 22[c] | – |
| % Greater trochanter | – | 14 | | – | | – | See Note[c] | – |
| **References** | (18) | (39) | (17) | (24) | (25) | (26) | (16) | (40) |

*Cohorts are listed across the top by years of recruitment and nationality. ERA, enthesitis related arthritis; ESSG, European Spondyloarthropathy Study Group; mNY, modified New York criteria for ankylosing spondylitis (28). ASAS, Assessment of Spondyloarthritis International Society; F/u, median or mean follow up, depending upon the study; MTP, metatarsal-phalangeal joints. No data or insufficient data (<1%) designated as –. Knee includes tibial tuberosity, infrapatellar, and suprapatellar sites. Pelvis includes ischial tuberosity, iliac crest, and interosseous ligaments of sacroiliac joint.*

[a] *Specified as "small joints" of fingers or feet.*

[b] *Cross sectional inception cohort without specified follow up.*

[c] *74% had Achilles or plantar calcaneal insertion enthesitis. "Pelvis" was lumped with greater trochanteric enthesitis in this study.*

[d] *Not specified if frontal or calcaneal plantar fasciitis.*

bias. This sex bias may over-represent the incidence of this complication in JSpA but anatomic distribution should still be generally informative. Foot pain occurred at presentation in 56% and another 27% developed foot pain during <5y follow up. Foot/ankle arthritis was apparent by exam in 65%, with talo-Achilles enthesitis in 47% and plantar fasciitis in 20%. Ultrasound was abnormal in 56% and MRI in 54%. Most of the MRIs that were positive in clinically asymptomatic subjects only had bone marrow edema, which may or may not have been pathologic. By exam, the most commonly involved arthritic joints were the midfoot (44%) followed by tibiotalar (27%), subtalar (15%), and MTP (16%). By ultrasound and MRI, the most commonly affected joints were talonavicular and tibiotalar, followed by calcaneocuboid and subtalar. Ankle arthritis prevalence was 27%. In those with mid-foot disease, half had mid-foot enthesitis and 2/3 tenosynovitis. Foot involvement correlated with significant functional impact, based on their juvenile arthritis foot index (JAFI). None of the children received biologics. There was no correlation with sacroiliitis or HLA-B27. Similar early prevalence was described in a Spanish cohort, where 35% had tarsitis at presentation (47). In this series, the children with tarsitis were much less likely to present with axial pain (8 vs. 54%) or develop axial involvement, and were often initially misdiagnosed with infection. These results contrast with those obtained by Burgos-Vargas et al., in which tarsitis (in conjunction with enthesitis) was highly predictive of a diagnosis of AS at 10 years (39).

"Inflammatory Back Pain" seldom occurs in children. In addition, some of the metrics used to follow adults are not helpful in children. For instance, in one study, chest expansion was the same for children with JoAS and SEA and healthy children (48). Moreover, there is poor correlation between symptoms, exam findings and disease indicated by MRI, making clinical assessment challenging (49). However, it is important to maintain a high index of suspicion during initial assessment and to remain vigilant for the development of axial disease. A significant portion of children with JoSpA/ERA have axial involvement at presentation or develop axial disease within the first 5 years, with some variability by study. For example, in the predominantly male and HLA-B27+ ERA case series from India, 55% had inflammatory back pain (IBP) and 38% clinical sacroiliitis during the first 5 years of disease (mean duration of disease 1.9 years) (40). Of these, 33% had radiographic sacroiliitis. In one of

the largest multinational MRI-imaged inception cohort of 540 children with clinically suspected JoSpA, 20% had sacroiliitis. Interestingly 42% had incidental findings unrelated to the sacroiliac joints that potentially contributed to axial symptoms, including enthesitis, hip arthritis, and degenerative disease (50). Even in cohorts where sacroiliitis incidence was initially <30%, more than half of the subjects developed axial disease within 5 years (16). In a 1989 report, in an SEA cohort of 20 children, 47% fulfilled modified NY criteria for ankylosing spondylitis (evidence by X-ray) within 3 years, 75% in 5 years and >90% in long-term follow up (51). In another study of children with JoAS, only 14% had lumber or sacroiliac pain 1 year after disease onset, although 100% reported sacroiliac/lumbar symptoms by 10 years (39).

Recent MRI-imaging studies of children with JoSpA/ERA have revealed an alarming percentage with asymptomatic axial disease. In a SpA cohort reported by Weiss at al. 20% had MRI-detected active sacroiliac disease at presentation. A high proportion (88%) of these cases already exhibited erosions, but only 38% of those with positive MRIs reported any back symptoms (49). In another cohort of 143 JoSpA patients, 53 (37%) of the patients had imaging or clinical suggestions of axial involvement. Eighteen had abnormal sacroiliac X-rays and 20 had sacroiliitis by MRI (32 total with abnormal imaging), but a third of these (11) had no back symptoms (52). Given the prevalence of axial inflammation at baseline, propensity for developing axial disease, and occurrence of axial disease in the absence of reported back pain in some children, there should be a low threshold for evaluating children with JoSpA/ERA by MRI. In adults with AS, early disease is a time-limited opportunity to gain the greatest response from biologic medication such as TNF inhibitors (53, 54). Unfortunately lag between symptom onset and diagnosis is even longer in children than in adults (8–9 vs. 5 years) (3, 55).

## EXTRA-ARTICULAR MANIFESTATIONS

Depending upon the study and length of follow up, uveitis has been reported in 5–28% of JoSpA/ERA subjects [(22, 26), **Table 1**]. A comprehensive cross-sectional/retrospective study came out recently describing uveitis among 118 children with JoSpA, including ERA (62% of the SpA cases), PsA (18% of the cases), undifferentiated arthritis (14%), and IBD-associated arthritis (6%). Uveitis was reported in 24 subjects (11%), with the highest proportion in ERA (13% of those patients) and 7% in the other ILAR SpA categories. Seventy nine percent of the uveitis was symptomatic. HLA-B27 prevalence, at 45%, was similar between groups and did not correlate with likelihood of uveitis, nor with symptomatic uveitis (56).

Skin and nail involvement characterizes the PsA ILAR subgroup. Clinical manifestations for this subgroup have been reviewed extensively elsewhere and will not be described in detail here (57–59). Briefly, children with nail involvement may exhibit nail pitting and onycholysis. Children do not always manifest the pathognomonic discrete erythematous scaly plaques. Psoriasis may be subtle and confused with eczema in children. Places that may exhibit scaling are along the hairline, behind the ears, around the umbilicus and intergluteal cleft. Dactylitis, or diffusely swollen "sausage" digits are also common in children with PsA. Prevalence in JSpA overall is ∼10% (**Table 2**) (16, 17, 40).

In adults, between 6 and 14% of patients with AS develop frank IBD (60). However, colonoscopies from asymptomatic or mildly symptomatic patients have revealed a shocking prevalence of subclinical inflammation in ∼60% of subjects (61). These findings and other studies have suggested the potential involvement of a gut-joint axis in disease pathogenesis (62, 63). In children, gut involvement in JoSpA/ERA is less clear, not the least because of the difficulties in classification. However, one study found 67% of children with ERA had elevated calprotectin compared to 18% in other types of JIA, supporting the concept of a disease continuum between childhood and adult onset SpA (15, 64).

Heart disease is certainly less common in children with AS vs. adults, though a 1995 study in a 36 patients with JoAS revealed 2 patients with mild mitral regurgitation and 3 with aortic regurgitation (65). None had functional impact or conduction abnormalities. In the initial description of SEA, 2 of 39 subjects (5%) had aortic insufficiency (18). By way of comparison, 5–10% of adults with AS have a conduction disorder or aortic insufficiency (66). Although functional cardiac complications may be relatively infrequent in JoSpA/ERA, these studies suggests the heart might be an organ worth monitoring in children. At the very least, more data on this topic would be helpful.

## PROGNOSTIC INDICATORS AND OUTCOMES

Various studies, particularly those from before the biologic era, paint a relatively dismal prognosis for children with SpA (**Table 3**), particularly when considering disability, pain, and remission rates. In a long-term Norwegian cohort, at 15 years patients had a lower level of physical function indicated by HAQ scores (0.38 vs. 0.16) and poorer physical health (SF-36, 46.4, vs. 52.4) and pain (2.88 vs. 2.09 on 1-6 scale) vs. polyarticular and oligoarticular JIA (2). In the 30-year follow up of that same cohort comparing ILAR subgroups, only 37% of ERA patients were in remission off medications, and the only group that fared worse than ERA was RF+ poly JIA (67). Similarly, in a large cross-sectional study using CARRA registry data, Weiss et al. reported that children with ERA had worse pain, function (CHAQ) and health status than other forms of JIA (1). In the Canadian ReACCh-Out JIA cohort, a diagnosis of ERA carried an OR of 0.67 and undifferentiated arthritis an OR of 0.49 for attaining inactive disease (72).

Multiple studies point to baseline enthesitis as a poor prognostic indicator, despite its responsiveness to current therapeutic approaches (1, 43, 71, 73). One possible explanation for this association is that enthesitis often portends sacroiliitis (16). Indeed, several studies have directly implicated sacroiliitis as a poor prognostic indicator. In a Taiwanese JIA cohort followed over 8 years, any sign of sacroiliitis (clinical or radiologic) predicted active disease, as none of these subjects attained

**TABLE 3 |** Remission vs active disease in Juvenile Spondyloarthritis cohorts.

| Years recruited | Country | Y f/u | SpA #(%) of total cohort | % Active | % Remission (total) | % Remission (on med) | % Remission (off med) | % Continuous active | % Remit for 1y then flare | % TNFi | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1980–1985 | Norway | 15.3 | 55(33) ERA | 56 | 44 | – | – | – | – | 0 | (2) |
| 1980–1985 | Norway | 30 | 27(15) ERA<br>21(12) PsA<br>11(6) UA | –<br>–<br>– | –<br>–<br>– | –<br>–<br>– | 37<br>48<br>64 | –<br>–<br>– | –<br>–<br>– | 0<br>–<br>– | (67) |
| 1970–1998 | Italy | 10 | 67 (10) SpA (ILAR/ESSG) | 64 | 36 | – | – | 52 | 15 | – | (68) |
| 1997–2000 | Nordic | 7 | 49(11) ERA<br>14(3.2) PsA<br>66(15) UA | 61<br>54<br>53 | 39<br>46<br>47 | 8<br>23<br>6 | 31<br>23<br>41 | 31<br>27<br>40 | – | 17.5 for all JIA | (20) |
| 2002–2003 | Germany | 4 | 118 SpA (mNY or ESSG) | 54[a] | – | 43[a] | 23[a] | – | 14 | 6 | (17) |
| 1995–2010 | Taiwan | >1.5 | 73(37) ERA<br>3(1.5) PsA<br>5(2.6)UA | 56<br>0<br>20 | 44<br>100<br>80 | 11<br>33<br>0 | 33<br>67<br>80 | 48<br>0<br>0 | 8<br>0<br>20 | 12.8 for all JIA | (25) |
| 2005–2010 | Canada | 5 | 157(14) ERA<br>64(6) PsA<br>110(10) UA | 53<br>53<br>54 | 47<br>47<br>46 | – | – | – | – | <20 | (69) |
| 2005–2010 | Canada (2 of above centers) | 5.6 | 52(21) ERA<br>10(4) PsA<br>13(5) US | 35<br>20<br>15 | 65<br>80<br>69 | 13<br>30<br>15 | 52<br>50<br>54 | – | – | 22 for all JIA | (70) |
| 2013–2014 | Germany | 1 | 74(11) ERA<br>28(4) PsA<br>50(7) UA | 72<br>73<br>58 | 28[b]<br>29<br>42 | – | – | – | – | 25<br>32<br>36 | (71) |
| 2008–2015 | France | 2.6 | 114 ERA or ASAS | 45 | 55 | 35 | 20 | – | – | 42 | (16) |
| 1993–2018 | Taiwan | 7.7 | 73(40) ERA<br>1(0.5) PsA<br>1(0.5) UA | 66 | 34 | 7 | 27 | – | – | 78 | (27) |

*Cohorts are listed by years of patient recruitment and cohort nationality (left columns).*

*Cohort acronyms: GESPIC, German Spondyloarthritis Inception Cohort; ReACCh-Out, Research in Arthritis in Canadian Children Emphasizing Outcomes); ICON, Inception Cohort of Newly Diagnosed Children with JIA; JCA, juvenile chronic arthritis; JRA, juvenile rheumatoid arthritis; JIA, juvenile idiopathic arthritis; ERA, enthesitis related arthritis; PsA, psoriatic arthritis; UA, undifferentiated arthritis; ESSG, European Spondyloarthropathy Study Group; mNY, modified New York criteria for ankylosing spondylitis; ILAR, International League against Rheumatism; ERA, enthesitis related arthritis; PsA, psoriatic arthritis; UA, undifferentiated arthritis; ASAS, Assessment of Spondyloarthritis International Society. Follow up years refers to mean or median, depending upon the study. Cohort specific details are in footnotes. TNFi: TNF inhibitor usage by end of study.*

[a] *54% in active disease after 4y. 43% in remission on meds and 23% in remission off meds at 4y or within past 6 months.*

[b] *Status of active or inactive disease during months 9–12. In ERA, 55% eventually attained disease remission, but at a mean of ∼16 months.*

inactive disease (27). In the Berntson cohort, any signs of sacroiliitis (OR 4.1), enthesitis (OR 2.4) or hip arthritis (OR 2.1) increased the risk for persistent disease. For sacroiliitis alone, 84% exhibited active disease (21). Herregods et al. noted the association between enthesitis and sacroiliitis in an imaging study, where sacroiliitis was present in 74% of children with pelvic enthesitis (74). In an Italian MRI study in ERA, early predictors of sacroiliac disease were numbers of active joints and active entheses at onset (24). Earlier studies had also reported an association between high joint count and ultimate development of sacroiliitis; in a study comparing initial patterns of arthritis (pauci vs. poly) between children with JoAS and those with SEA who did not develop axial disease, polyarticular disease at 1 year was highly associated with the development of radiographic sacroiliitis/AS (75). In another study examining joint accumulation over time in a group of children ultimately diagnosed with JoAS, at 6 months after disease onset, only 28.6% of patients had polyarticular disease, but by 1 year, that number increased to 80% (39). In a Norwegian ERA cohort, high active joint count at 6 months predicted physical limitation at 23 years (2). Besides high joint count and enthesitis, other risk factors for the development of sacroiliitis include family history of SpA, persistently high ESR and hip arthritis (2, 16, 21, 24, 76).

Several studies have examined the influence of HLA-B27 positivity, sex, and their interaction on the presence of sacroiliitis and more directly on prognosis. In a study from Berntson et al. focusing on HLA-B27 across JIA categories in a Nordic cohort, HLA-B27 positivity associated with clinical signs of sacroiliitis (including inflammatory back pain, sacroiliac and buttock pain), enthesitis, and tenosynovitis in boys, but not girls (21). In the whole JIA cohort, boys were more often HLA-B27+ (26%) vs. 18% of girls, and boys with ERA had a trend toward clinical signs of sacroiliitis more often than girls (21). In a Norwegian ERA cohort followed over 15 years, male sex also associated with the development of decreased spinal mobility (abnormal Schober test) in 67% of 36 boys vs. 37% of 19 girls) (2). However, in a French JoSpA/ERA cohort followed over 5 years, axial disease and sacroiliitis prevalence was equally distributed between sexes (16). In several studies from Taiwan, Germany and Norway, HLA-B27 positivity associated with failure to attain remission (17, 21, 25, 27). The relative odds ratios were around 2 (1.7–2.2), correlating with persistence of active disease after 8 years of 73.2 vs. 59.4% in the whole JIA cohort (21, 25). Interestingly, in the GESPIC and Norwegain cohorts, female sex carried a worse prognosis (2, 17). Thus, there have been some cohort or analysis-specific differences regarding sex.

## REMISSION RATES AND BIOLOGIC DMARDS

One possible reason for the relatively poor outcomes noted in various studies, is that the most prominent aspects of JoSpA/ERA (enthesitis and sacroiliitis) do not respond well to conventional synthetic disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate or sulfasalazine (reviewed in another chapter in this issue) (77, 78). The data supporting these contentions, mostly obtained from adult studies, has led to revisions in current ACR treatment guidelines supporting earlier use of biologic DMARDs (e.g., TNF inhibitors) following NSAID failure in children with sacroiliitis or enthesitis (79). The IL-17 inhibiting monoclonal antibodies are too new to assess in children, however TNF inhibitors have become much more widespread in use in the 2000s. Trial data has been promising (reviewed in another chapter), but cohorts indicate "real world" application, and how outcomes may be shifting (or not). For simplicity, we will focus on active disease vs. remission over time (**Table 3**). Over time, there has been a steady increase in biologic DMARD usage, yet a corresponding increase in remission vs. persistent active disease is not yet clear.

A few studies suggest the outlook for ERA may be improving (**Table 3**), particularly in the short term. For instance, in an open-label study of JSpA treated with TNF inhibitors, 81% (13/16) achieved clinical remission within 6 months. However, 6/16 (38%) subsequently flared a median of 2 years after attaining remission (80). In a French study, 69% of subjects treated with TNF inhibitors experienced inactive disease at 1y, with boys exhibiting a greater response (OR 6.94) (16). Experience in a Canadian cohort (ReACCh-Out) also suggested good short-term gains in SpA; the probability of attaining inactive disease some time during 5y follow up was extremely high, at >92% for all SpA categories. Probability of coming off meds during the 5y was 71% for ERA, 74% for PsA and 59% for undifferentiated arthritis. However, overall remission rates at the end of the study were still <50% (69). A subset of this same cohort examined a few years later achieved remissions >60% (20–35% with active disease), marking an improvement in outcome, though this may be specific to the two centers examined (70). In a German etanercept cohort, only 52% of ERA patients attained inactive disease, with 22% in remission on medication (81). Comparable outcomes were reported in a 2020 comprehensive study on unmet needs in JIA. Brunner et al. described 2 large cohorts, one from Cincinnati Children's (CHMC) and another from the multi-site Childhood Arthritis and Rheumatology Research Alliance (CARRA) registry, including 279/1351 with ERA/PsA and 50/164 with undifferentiated arthritis (82). In the two cohorts, 79 and 72% of children with ERA/PsA were treated with biologics. Even among those treated with two biologics, 31% (CHMC) and 55% (CARRA) still had active disease. While these outcomes are much better than those reported by Minden et al. in 2000 [17% remission at 5y (83)], there is still obviously room for improvement.

Children with JoSpA/ERA treated with TNF inhibitors may still experience a worse outcome compared to other types of JIA. In the German ICON cohort, even though 30% of ERA patients were treated with biologic DMARDs, this group took longer than other categories of JIA to attain a state of inactive disease (9m) and spent only 27% of time in inactive disease within the first year (compared to 40% in the whole JIA cohort). PsA patients spent 25% in inactive disease. Only 55% of ERA patients reached inactive disease at a mean of 15.9m, leaving 45% with active disease more than a year from enrollment (71). In a study from Taiwan, only 33% achieved inactive disease, despite high levels of treatment with TNF inhibitors (78%), an

outcome that was still significantly worse than for other JIA groups (27). Even within a JIA cohort started on TNF blockers, patients with ERA were much less likely than those with poly JIA (RF-) to attain inactive disease ever (43 vs. 76%) or be in inactive disease at 1y (24 vs. 57%) (73). Part of the issue may reflect TNFi refractory disease manifestations typical of JoSpA/ERA. For instance, the aggressive hip arthritis may be resistant to TNFi therapy (80). Similarly, TNFi may have limited capacity to suppress progression of sacroiliitis. In the 2014 open label study of etanercept and infliximab, 42% of children met modified NY AS criteria prior to treatment, and 92% fulfilled criteria 7 years later (80).

In summary, children with JoSpA/ERA have significant disease burdens and relatively poor outcomes compared to other types of JIA (1). Although most patients initially present with peripheral arthritis and enthesitis, a very high proportion go on to develop axial disease, within the first 5 years of diagnosis (11, 51). An alarming number of these children (one third to over one half!) develop "silent" axial disease and have MRI evidence for both acute disease and chronic destructive changes, even in the absence of reported back pain (49, 52). Moving forwards, it will be critical to determine if there are other clinical features that correlate more reliably with axial disease. Another possible solution is to treat children diagnosed with JoSpA more aggressively early on, incorporating TNF inhibition for their peripheral arthritis and enthesitis prior to the development of axial disease. Greater TNF inhibitor use may be improving the outcome in this difficult to treat JIA category, particularly in the short term, though more data would be helpful for supporting this idea. The development of sacroiliitis portends a relatively poor prognosis and increased refractoriness to treatment (27). Limited data also suggests axial disease may progress despite TNF inhibitor treatment (80). Thus, the window of opportunity may be limited. At this time, persistently active disease in 30 to >50% of children indicates that there is still much to accomplish toward improving the outlook for JoSpA/ERA.

## AUTHOR CONTRIBUTIONS

JS and RB-V conceived the review. JS wrote the first draft of the manuscript. RB-V contributed to revision of the manuscript prior to submission.

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REVIEW
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# Similarities and Differences Between Juvenile and Adult Spondyloarthropathies

*Corinne Fisher*[1,2,3]*, *Coziana Ciurtin*[1,2,4], *Maria Leandro*[1,2,4], *Debajit Sen*[1,2,3†] and *Lucy R. Wedderburn*[1,5,6†]

[1] Centre for Adolescent Rheumatology Versus Arthritis at University College London, University College London Hospital and Great Ormond Street Hospital, London, United Kingdom, [2] Department of Adolescent Rheumatology, University College London Hospitals NHS Foundation Trust, London, United Kingdom, [3] National Institute for Health Research University College London Hospitals Biomedical Research Centre, London, United Kingdom, [4] Division of Medicine, Department of Rheumatology (Bloomsbury), University College London, London, United Kingdom, [5] National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children, London, United Kingdom, [6] Infection, Immunity & Inflammation Teaching and Research Department University College London Great Ormond Street Institute of Child Health, London, United Kingdom

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**Edited by:**
*Miroslav Harjaček, United Arab Emirates University, United Arab Emirates*

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***Correspondence:**
*Corinne Fisher*
*corinne.fisher@ucl.ac.uk*

[†] *These authors share senior authorship*

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Spondyloarthritis (SpA) encompasses a broad spectrum of conditions occurring from childhood to middle age. Key features of SpA include axial and peripheral arthritis, enthesitis, extra-articular manifestations, and a strong association with HLA-B27. These features are common across the ages but there are important differences between juvenile and adult onset disease. Juvenile SpA predominantly affects the peripheral joints and the incidence of axial arthritis increases with age. Enthesitis is important in early disease. This review article highlights the similarities and differences between juvenile and adult SpA including classification, pathogenesis, clinical features, imaging, therapeutic strategies, and disease outcomes. In addition, the impact of the biological transition from childhood to adulthood is explored including the importance of musculoskeletal and immunological maturation. We discuss how the changes associated with adolescence may be important in explaining age-related differences in the clinical phenotype between juvenile and adult SpA and their implications for the treatment of juvenile SpA.

**Keywords: juvenile spondyloarthropathy, enthesitis related arthritis, adolescence, enthesitis, adult spondyloarthropathy**

## INTRODUCTION

The spondyloarthropathies (SpAs) are a heterogeneous group of inflammatory arthropathies affecting both the peripheral and axial joints. In adults the most common form of SpA is axial SpA (axSpA) which includes ankylosing spondylitis (AS) and non-radiographic axSpA (nr-axSpA). Other forms of SpA include psoriatic arthritis, enteropathic arthritis, reactive arthritis, and undifferentiated SpA (1). Apart from axial and peripheral arthritis, common clinical features across all these subtypes can include enthesitis, dactylitis, and extra-articular manifestations such as acute anterior uveitis, inflammatory bowel disease (IBD), and psoriasis. There is a strong association between these conditions and the major histocompatibility complex (MHC) class 1 antigen human leucocyte antigen (HLA)-B27 although the mechanism for this association remains unclear (2). The prevalence of SpA varies widely by geographical area with the highest rates reported in North America and Europe which may correspond to the prevalence of HLA-B27 (3).

The onset of SpA occurs across a broad age range from childhood to adolescence and adulthood with a peak incidence in late adolescence and early adulthood (4). Although the common features remain the same, the clinical phenotype is different across the ages with peripheral arthritis and enthesitis predominant in juvenile SpA (JSpA) and axial manifestations more typical of adult onset disease. JSpA is classified differently from adult SpA as enthesitis related arthritis (ERA) which encompasses both axial and peripheral arthritis, and juvenile onset psoriatic arthritis, both of which are classified within the umbrella term juvenile idiopathic arthritis (JIA) (5).

The reasons for differences in clinical phenotype between adult onset disease and JSpA are not well-understood but may be due to the changes associated with musculoskeletal maturation and development of the immune system during childhood and adolescence. This review will discuss the similarities and differences between JSpA and adult onset SpA and highlight areas where better alignment between paediatric and adult disease would enhance research and clinical care.

## CLASSIFICATION CRITERIA

Adult SpA is classified by the Assessment of SpA International Society (ASAS) criteria which have been developed separating axial and peripheral SpA but encompassing the broad spectrum of disease including psoriatic arthritis, enteropathic arthritis, and reactive arthritis (**Table 1**) (6). There is a focus on the range of typical clinical features associated with SpA, including the association with HLA B27, enthesitis, and extra-articular manifestations. In addition, the inclusion of magnetic resonance imaging (MRI) assessment of the sacroiliac joints (SIJs) enables much earlier diagnosis of axial disease (including nr-axSpA) and therefore earlier treatment. This is in contrast to the older but still widely used modified New York criteria, which define AS by the grading of sacroiliitis using plain radiographs (7) and therefore capture more longstanding disease (8).

In contrast to adult SpA, JSpA is classified as part of the International League of Associations for Rheumatology (ILAR) classification criteria for JIA. JIA encompasses the broad spectrum of idiopathic inflammatory arthritis occurring before the age of 16 years, divided into seven mutually exclusive subtypes. Six subtypes are defined according to the pattern of joint involvement and extra-articular features during the first 6 months of disease. Those patients who do not meet the classification criteria for one of these six groups, or who meet the criteria for more than one subtype are classified in the seventh group as "undifferentiated JIA" (5). In contrast to adult SpA, patients with JSpA are not confined to one group but are divided between three subtypes of JIA: ERA, juvenile onset psoriatic arthritis and undifferentiated JIA (**Table 1**). The term ERA was developed in recognition of the distinct clinical presentation of childhood disease and replaced others including seronegative enthesopathy and arthropathy (SEA) (9), juvenile AS and JSpA.

There are well-recognised problems with the ILAR criteria (10–12). Compared to the ASAS criteria, the ILAR criteria lack the differentiation between axial and peripheral disease which is important for disease course and outcome. There is no inclusion of MRI assessment or inflammatory markers. The criterion of male sex in the ERA classification criteria increases the chance of diagnosis in males and leads to more females being classified as other JIA subtypes such as oligoarticular JIA. This may not be appropriate given recent evidence from studies in adults that the male predominance in SpA has significantly reduced in recent years (13). Patients with psoriatic arthritis and with a family history of psoriasis in a first degree relative are separated into the psoriatic arthritis category; despite these patients often having an overlapping disease phenotype with ERA (12). In addition, patients with enteropathic arthropathy and reactive arthritis are excluded from the psoriatic arthritis subtype (although are included in the ERA subtype) (5) even though they would all be considered as having SpA according to the adult ASAS criteria. A study of adult patients diagnosed with ERA in childhood found that 95% fulfilled the classification criteria for adult SpA (14).

In practice, the ILAR criteria result in a high proportion of patients with JSpA being classified in the undifferentiated JIA group and this, and the other reasons above, has led to a resurgence of the use of the term JSpA. The difficulties with the classification criteria and lack of alignment between paediatric and adult classification criteria has hampered the development of collaborative research studies in this field resulting in relatively few studies examining the clinical patterns, outcomes, and pathogenesis of JSpA.

## PATHOGENESIS

Research investigating the pathogenesis of JSpA is sparse compared to the extensive study of the pathogenesis of adult SpA. It is assumed that the pathogenesis is the same but studies including or comparing patients with adult SpA and JSpA are rare. Patient numbers in studies of JSpA are often small or are combined with other JIA subtypes and therefore it is difficult to draw conclusions from the current published data.

HLA-B27 is strongly associated with both adult SpA and JSpA and many subtypes exist. The most common allele is HLA-B27:05 which is associated with an increased risk of SpA across all races, ethnicities and ages (15). The HLA-B27:04 allele, which confers an increased risk in Eastern Asians was found to be the most prevalent subtype in a population of south Indian patients with ERA (16). Despite the strong association with SpA and in particular AS, HLA-B27 is only estimated to account for around 20% of the total heritability of AS (2, 17, 18) with other genetic risk alleles accounting for a further 8% (19). Many genetic variations related to pro-inflammatory pathways known to be associated with SpA have been found in patients with AS (20). After HLA-B27, genetic variations in endoplasmic reticulum aminopeptidase (ERAP) 1 are the most commonly recognised in adults (21) and have also been found in patients with ERA (22). Variations in the IL23 receptor (IL23R) gene are strongly associated with AS and other related conditions such as psoriasis and IBD (23) and have also been linked with

**TABLE 1 |** Classification criteria for the diagnosis of adult SpA and JSpA.

**ASAS CRITERIA**

| **Axial SpA** | | | **Peripheral SpA** |
|---|---|---|---|
| For patients with ≥3 months back pain (with/without peripheral manifestations), aged <45 years | | | For patients with peripheral manifestations only |
| Sacroiliitis on imaging plus ≥1 SpA feature | OR | HLA-B27 plus ≥2 other SpA features | Arthritis or enthesitis or dactylitis plus |
| SpA features:<br>• Inflammatory back pain<br>• Arthritis<br>• Enthesitis (heel)<br>• Uveitis<br>• Dactylitis<br>• Psoriasis<br>• Crohn's disease/Ulcerative Colitis<br>• Good response to NSAIDs<br>• Family history of SpA<br>• HLA-B27<br>• Elevated CRP | | | ≥1 SpA feature:<br>• Uveitis<br>• Psoriasis<br>• Crohn's disease/ Ulcerative Colitis<br>• Preceding infection<br>• HLA-B27<br>• Sacroiliitis on imaging<br>OR<br>≥2 other SpA features:<br>• Arthritis<br>• Enthesitis<br>• Dactylitis<br>• Inflammatory back pain ever<br>• Family history of SpA |

**ILAR JIA CRITERIA**

| **ERA** | **Juvenile onset psoriatic arthritis** |
|---|---|
| Arthritis AND enthesitis<br>OR<br>Arthritis OR Enthesitis plus ≥2 of:<br>• Current or past sacroiliac joint tenderness and/or inflammatory lumbosacral pain<br>• HLA-B27<br>• Onset of arthritis in a male > 6 years old<br>• Acute anterior uveitis<br>• History of AS, ERA, sacroiliitis with inflammatory bowel disease, reactive arthritis or acute anterior uveitis in a first degree relative | Arthritis AND psoriasis<br>OR<br>Arthritis plus ≥2 of:<br>• Dactylitis<br>• Nail pits or onycholysis<br>• Family history of psoriasis in a first degree relative |
| Exclusions:<br>• Psoriasis or a history of psoriasis in the patient or a first degree relative<br>• Presence of immunoglobulin M rheumatoid factor on at least two occasions 3 months apart<br>• Presence of systemic JIA in the patient | Exclusions:<br>• Arthritis in an HLA-B27 positive male beginning after their 6th birthday<br>• AS, ERA, sacroiliitis with inflammatory bowel disease, reactive arthritis or acute anterior uveitis, or a history of one of these in a first degree relative<br>• Presence of immunoglobulin M rheumatoid factor on at least two occasions 3 months apart<br>• Presence of systemic JIA in the patient |

*ASAS, Assessment of SpA International Society; ILAR, International League of Associations for Rheumatology; JIA, juvenile idiopathic arthritis.*

juvenile onset psoriatic arthritis but not ERA (22). Differentially expressed genes in the mitogen-activated protein kinase (MAPK) pathway have been identified in both adult SpA and JSpA (24–26). Thus, similarities exist in the genetic associations between adult onset SpA and JSpA, but study of other genetic variants in the paediatric population is limited by low study participant numbers compared to the large numbers studied in adult SpA.

The interleukin (IL)23/IL17 pathway has been strongly implicated in the pathogenesis of SpA. IL23 plays a crucial role in enthesitis, the primary pathological feature of SpA (27). Increased production of IL23 is noted from macrophages of adult patients with AS compared to healthy volunteers (28) and IL23 is found in higher concentrations in the inflammatory lesions from facet joints of patients with AS compared to those with osteoarthritis (29). IL23 is less well-studied in ERA. One study demonstrated higher levels of IL23-producing intermediate monocytes in patients with ERA (30) but serum levels were no different between patients and healthy controls (31). Studies of serum IL23 levels in adult SpA have produced mixed

results with some reporting higher concentrations and others no difference between patients and healthy controls (32–35). Recent studies suggest that IL23 may be critical in the early phases of disease pathogenesis (36) and therefore further investigation is important to understand its role in the pathogenesis of JSpA.

IL23 is crucial for the expansion and survival of Th17 cells (37) which are increased in the peripheral blood and synovial fluid of adult patients with SpA (38, 39). IL17 has also been found at higher concentrations in the serum and synovial fluid of adult patients with active SpA (40, 41). However, Th17 levels were not found in higher numbers in a small study of patients with ERA compared to controls (42) and levels of serum IL17 were no different between patients and healthy controls in another study by the same group (31). However, a subset of Th17 cells expressing the killer immunoglobulin receptor (KIR)3DL2, which interacts with aberrant forms of HLA-B27 and promotes Th17 cell survival, is increased in patients with ERA as well as adults with SpA compared to healthy controls (43). Levels of IL27, a regulatory cytokine in the IL23/17 pathway, are reduced in patients with ERA and this negatively correlates with Th17 cell concentration, suggesting dysregulation of the IL23/IL17 pathway is important in ERA (31). IL27 has been implicated in adult SpA through genetic studies (20).

Other pro-inflammatory cytokines such as tumour necrosis factor (TNF), IL6, IL1, and granulocyte-macrophage colony-stimulating factor (GMCSF) have also been implicated in the pathogenesis of SpA (44). The use of TNF blockade is highly effective in treating both JSpA and adult SpA therefore implying the importance of TNF in the pathogenesis of SpA across the ages. One study of synovial fluid TNF levels did not find any differences in levels between patients with ERA and another subtype of JIA (polyarticular JIA) or rheumatoid arthritis (45) but this is perhaps not surprising given the efficacy of TNF blockade across the inflammatory arthritides. In the same study, levels of IL6 were found to be higher in patients with ERA compared to those with polyarticular JIA. Increased serum levels of IL6 are found in patients with AS (46, 47) and IL6 is found in the inflamed SIJs of patients with AS (48). However, clinical trials of IL6 blockade in adults with SpA have failed to show clinical benefit (49) and therefore the significance of this is unclear. GMCSF has been implicated in the pathogenesis of inflammatory arthritis and in particular in the pathogenicity of Th17 cells (50) perhaps through a synergistic relationship with IL23 (51, 52). Higher numbers of GMCSF-producing Th17 cells are found in the peripheral blood of adult patients with SpA compared to patients with rheumatoid arthritis and healthy volunteers (53). GMCSF has also been implicated in oligoarticular and polyarticular course JIA but has not been studied specifically in JSpA (54).

Higher levels of interferon gamma (IFNγ) are found in synovial fluid from patients with ERA compared to those with polyarticular JIA (45). This is in contrast to adult SpA where lower levels of IFNγ are found in the synovium compared to those with rheumatoid arthritis and studies suggest dysregulation of IFNγ genes (55–57). Further study in both adult SpA and JSpA is important to understand the role of IFNγ in the pathogenesis of these conditions.

Chemokines have been studied in adult SpA as potential biomarkers for active disease and radiographic progression. Serum MMP3 levels have been shown to be higher in adult patients compared to healthy volunteers (58). In some studies, MMP3 levels reflect disease activity and may also predict structural damage (59). In contrast, for patients with ERA, MMP3 levels were found to be no different to healthy volunteers but did correlate with disease activity (60). MMP8 and 9 have been shown to be closely associated with disease activity in adult SpA in one study (61) but have not been studied in JSpA.

Another potential biomarker studied in both adult and JSpA is calprotectin or myeloid-related protein (MRP) 8/14. Serum levels are elevated in patients with SpA and may correlate with disease activity. High levels at baseline may predict radiographic progression and levels reduce on treatment with TNF inhibitors (62–65). Similarly in JSpA, plasma levels have been shown to be higher in patients compared to healthy volunteers; high levels correlate with active disease and reduce in those who respond to treatment (66). MRP8/14 is helpful in predicting response to treatment in patients with JIA (67, 68). In addition, faecal calprotectin is an established biomarker of disease activity in inflammatory bowel disease (69) and is elevated in adult patients with SpA (70). One small study of patients with ERA demonstrated higher levels compared to disease control patients with connective tissue diseases but further study is needed (71).

Evidence for the involvement of gut flora in the pathogenesis of SpA dates back many years with gram negative bacteria implicated in particular in both adult SpA (72–75) and JSpA (76). Microbes found in the gut shape host immune response from an early age (77) and several studies have shown differences in the gut microbiome between patients with SpA and healthy controls. A study by Costello et al. found differences in certain families of bacteria from terminal ileal biopsies from patients with AS (increases in Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Porphyromonadaceae and Bacteroidaceae and decreases Veillonellaceae and Prevotellaceae) compared to healthy volunteers (78). Another study demonstrated an increase in Ruminococcus gnavus in faecal DNA from adult patients with SpA compared to those with rheumatoid arthritis and healthy volunteers (79). A large Norwegian cohort study revealed increased Proteobacteria, Enterobacteriaceae, Bacilli, Streptococcus species, and Actinobacteria, but lower abundance of Bacteroides and Lachnospiraceae in patients with AS compared to healthy volunteers. A link with raised faecal calprotectin was observed in patients with a lower abundance of certain bacterial species with anti-inflammatory properties (80). The microbiome has also been studied in JSpA with one study of Indian patients showing similar findings to studies in adult SpA with increases in Bacteroidaceae and Enterobacteriaceae families and a reduction in the Prevotellaceae family in patients compared to healthy controls. A study of both adult patients with SpA and patients with ERA found that a strain of the anti-inflammatory bacterial family Faecalibacterium prausnitzii was reduced in both patients with ERA and SpA. However, a higher abundance of Bacteroides was noted in patients with ERA compared to SpA (81) and this has also been found in other JIA subtypes (82). The presence of HLA-B27 appears to

influence the gut microbiome (83). In addition, changes in the gut microbiome may result in upregulation of Toll Like receptors (TLRs) causing an inflammatory cascade and the production of pro-inflammatory cytokines (84). The expression of TLRs 2, 4, and 5 is upregulated in the synovium and peripheral blood mononuclear cells (PBMCs) of patients with SpA (85–87). Upregulation of TLRs 2 and 4 has also been found in patients with ERA (88).

Therefore, despite the similarities between the pathogenesis of adult SpA and JSpA, some differences exist. These may be explained by the lack of studies directly comparing adult SpA and JSpA or are perhaps due to lower patient numbers in studies of JSpA. However, a study comparing synovial biopsies in patients with JSpA, including both patients with ERA and juvenile onset psoriatic arthritis, to biopsies from patients with adult SpA found similarities but also marked differences. In particular, in JSpA there was a stronger lining layer hyperplasia and the number of infiltrating CD163+ macrophages were lower which meant JSpA failed to classify in the SpA group by class prediction analysis. Instead, there was partial overlap with other JIA subtypes suggesting that age may strongly influence the pathogenenic features of SpA (89). Factors such as changes in the immune system and gut microbiome with age, which have been demonstrated in detailed studies of healthy subjects and in smaller cohorts of patients (90, 91) may give rise to some of the differences described above and distinct clinical phenotype of JSpA compared to adult SpA. Some specific immune age-related changes have been demonstrated to occur at the time of puberty in both boys and girls such as IFN production in response to TLR stimulation (92). However, how these age-related immune function changes relate to the differences between JSpA compared to adult SpA, remains an area for future investigation.

## CLINICAL FEATURES

Multiple studies across different countries clearly demonstrate the predominance of peripheral arthritis and enthesitis in JSpA compared to adult SpA where axial arthritis is the most common clinical feature at onset and throughout disease course (93–99). Shoulder and hip joint involvement are also more common in JSpA (100) and the most frequently involved joints in JSpA include the knee (40–50%), ankle (25–40%), and hip (30–40%) (101, 102). As in adult SpA, enthesitis commonly affects the lower limb in JSpA and, in particular, the inferior pole of the patella, plantar fascial insertion into the calcaneum, Achilles tendon and the tibial tuberosity (103–105). In one study, enthesitis was shown to be present in 83% of patients with ERA on ultrasound but clinical examination yielded a much lower detection rate (106). Enthesitis is a key feature of early disease in SpA and the lack of sensitivity of clinical examination may contribute to diagnostic delay. This has been identified to be longer in patients with JSpA compared to adult SpA in several studies (96, 97, 107) although not consistently (95).

In general, comparisons between JSpA and adult SpA have not identified significant differences in the male to female ratio, HLA-B27 status, family history of HLA-B27-related disease and extra-articular manifestations such as psoriasis and inflammatory bowel disease. An increase in uveitis has been reported in JSpA compared to adult SpA in a small number of studies (97, 98) but this was not confirmed in a recent meta-analysis (108).

**TABLE 2 |** Comparison of adult and paediatric disease activity scores for SpA.

| AS disease activity score (ASDAS) | Juvenile SpA disease activity (JSpADA) index |
|---|---|
| - Back pain<br>- Peripheral pain/swelling<br>- Morning stiffness<br>- Patient global assessment<br>- CRP/ESR | - Active joint count<br>- Active enthesitis count<br>- Patient pain score<br>- CRP/ESR<br>- Morning stiffness<br>- Clinical sacroiliitis<br>- Uveitis<br>- Back mobility (modified Schober's test) |

*CRP, C reactive protein; ESR, erythrocyte sedimentation rate.*

## ASSESSMENT OF DISEASE ACTIVITY

Disease activity is measured differently in adult SpA and JSpA. The AS disease activity score (ASDAS) is now the most widely used assessment of disease activity in adults (109) and includes patient-reported measures of back pain, morning stiffness, peripheral joint pain and swelling, global assessment, and a measure of inflammation (either ESR or CRP) (**Table 2**). It has validated classifications for inactive, low, high, or very high disease activity (110) but has not been validated in JSpA. Other disease activity measures in use in adults include the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (111) and the Bath Ankylosing Spondylitis Functional Index (BASFI) (112). These have been validated in children and young people but focus on spinal disease and underestimate enthesitis, therefore may not accurately reflect disease activity (113, 114).

Until recently, disease activity in JSpA was measured using the same scores used for all JIA subtypes. The two scores in common use are the juvenile arthritis disease activity score (JADAS) which measures four domains including physician global assessment, patient global assessment, active joint count, and an inflammatory marker (either CRP or ESR) (115) and the core outcome variables which include a physician global assessment, patient/ parent global assessment, functional status, inflammatory marker (ESR), number of joints with active arthritis, and number of joints with restricted range of movement (116). However, neither of these measures assesses enthesitis or axial arthritis and therefore disease activity in patients with JSpA is likely to be underestimated. The need for a dedicated disease activity score for JSpA has been met with the development of the juvenile SpA disease activity (JSpADA) index. This assesses 8 domains including active joint count, enthesitis count, patient pain assessment, inflammation (either CRP or ESR), morning stiffness, clinical evaluation of sacroiliitis, uveitis, and back mobility measured by the modified Schober's test

(**Table 2**). Necessarily, for the paediatric population, it contains more objective measures and fewer patient reported measures compared to the ASDAS. It has undergone preliminary validation in a patient population with a mean age of 12 years and appears to be superior to other JIA disease activity measures in assessing disease activity in ERA (117). It has been subsequently validated in other populations with an older mean age (14.3 years) (113) and has started to gain wider use.

Further measures of disease severity in adult SpA include the Bath Ankylosing Spondylitis Metrology Index (BASMI) which measures spinal movement and the modified Stoke AS Severity Score (mSASSS) which evaluates disease severity on plain radiographs. However, neither of these measures is useful in JSpA as they focus predominantly on axial disease and are not sensitive for early SpA or peripheral arthritis (118, 119). Radiographic changes seen on the mSASSS are a reflection of late disease and plain radiographs of the SIJ in children and young people are often misleading with frequent false negative and false positive results (120).

Imaging, in particular MRI, is useful to assess disease activity and severity in axial disease, peripheral arthritis, and enthesitis in both adult SpA and JSpA. MRI features of axial SpA include both inflammatory lesions such as bone marrow oedema and osteitis and structural lesions such as erosions, sclerosis, and bony ankylosis. These features alone lack specificity and must be interpreted together with the clinical picture (121–123). Caution is needed in the interpretation of MRI of the SIJs in children and young people as significant variability is found (124). Features of sacroiliitis are comparable to those found in adults (125) although diagnostic criteria used in adults may have a lower sensitivity in children and young people (126). More significantly, MRI images of normal marrow and cartilage development can be mistaken for bilateral sacroiliitis. In particular, bilateral symmetrical metaphyseal high signal and cortical irregularities along the ilial margin of the SIJ which occur with normal maturation may appear similar to sacroiliitis leading to misdiagnosis of JSpA (127, 128). This was illustrated in a multicentre study in which significant variation in the interpretation of inflammatory and structural lesions on MRI of the SIJ in patients with JSpA was seen giving rise to frequent false positive results (129). Another single centre study showed low to moderate inter-reporter reliability when interpreting SIJ MRI in children and young people (130). Clinical examination, back pain and buttock pain all have a low sensitivity for identifying sacroiliitis in children and young people (131) and therefore novel MRI techniques which help to distinguish true sacroiliitis from the changes associated with normal maturation in children and young people are important (132).

Other imaging modalities such as ultrasound are helpful to assess enthesitis which may be difficult to detect clinically (121, 133). However, again, caution is needed in the interpretation of ultrasound findings in children and young people. There is significant variability in entheseal thickness with growth and development, making it difficult to define a "normal range." One study in healthy children revealed a correlation between entheseal thickness and age (134). However, another study found a correlation with weight. This study also noted that boys tended to have thicker entheses than girls but significant variability was found even between left and right sides in the same subject (135).

## TREATMENT

Multidisciplinary management is important in the treatment of both adult SpA and JSpA. There is good evidence for physiotherapy intervention in adult SpA but no studies in JSpA. However, the impact of diagnosis and symptoms (as well as delayed diagnosis) is often significant on psychosocial well-being as well as education and physical functioning in children and young people (136, 137).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the first line pharmacological therapy in adult SpA and improve symptom in both axial and peripheral arthritis (138). There is some evidence to suggest prolonged, continuous use of NSAIDs may slow radiographic progression in adult SpA (139, 140) but this was not confirmed in a prospective study (141). NSAIDs are also frequently used as first line therapy in JSpA (105, 142) but there is no evidence for continuous or prolonged treatment or of the effect on disease progression. Whilst rarely used in adult SpA, local, or systemic corticosteroids are sometimes used as a holding measure before treatment with either DMARDs or TNF inhibitors is effective in JSpA, although they may not be as effective as in other JIA subtypes.

Conventional disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate and sulfasalazine are only used if there is peripheral arthritis in adult SpA (143, 144) as they are ineffective in axial disease (145, 146). However, DMARDs are commonly used in JSpA because of the high prevalence of peripheral arthritis and enthesitis. Although methotrexate is the most commonly used DMARD for JIA (including ERA and psoriatic arthritis), there is no specific evidence for the use of methotrexate in JSpA as patients with ERA and psoriatic arthritis were excluded from a large trial of methotrexate in patients with JIA (147). Sulfasalazine was found to be effective in a randomised double blind placebo controlled trial in 33 patients with JSpA after 26 weeks treatment (148).

When the treatments above do not establish adequate disease control, TNF inhibitors are highly effective treatments for adult SpA and JSpA, especially for axial arthritis where conventional DMARDs are not effective. In adults, numerous randomised controlled trials (RCTs) have provided good evidence for the efficacy of etanercept (149), adalimumab (150), infliximab (151), golimumab (152), and certolizumab (153) with reported response rates of around 60%. Until recent years, the efficacy of TNF inhibitors in JSpA was established through open label studies, retrospective analyses and reports from registries. Treatment regimens for JSpA were often the same as those of other JIA subtypes without stratification between axial and peripheral disease. However, two RCTs of treatment in JSpA have been published demonstrating the efficacy of etanercept (154) and adalimumab (155). Adalimumab is effective for the treatment of axial disease in JSpA (156) and for treating extra-articular manifestations including uveitis (157), inflammatory bowel disease (158), and psoriasis (159). Of the other TNF inhibitors,

only Infliximab has been studied specifically in JSpA in one randomised trial (160). There does not appear to be any difference in the efficacy of the different TNF inhibitors in the treatment of adult SpA and JSpA but there is evidence that the monoclonal antibodies (adalimumab, infliximab, golimumab, certolizumab) are superior to etanercept in the treatment of extra-articular manifestations and therefore may be used in preference when these are present (161, 162).

In both adult SpA and JSpA, there is evidence that early treatment with TNF inhibitors may be more beneficial with the possibility of partial remission demonstrated in adults with axSpA (163, 164) and a greater improvement in disease activity and lower reported pain scores in patients with JSpA (165). Following the initiation of TNF inhibitors, sustained remission without treatment is rare in both adult SpA and JSpA, even in those treated early, with most patients relapsing within a year of treatment cessation (166–169). However, tapering treatment by increasing the interval between doses or reducing the dose may be possible (170–172).

Despite the clear benefit of treatment with TNF inhibition, the effect on radiographic progression has been debated in adults with axSpA. Several studies have demonstrated continued new bone formation despite treatment (173–175). However, more recent studies do suggest that TNF inhibitors slow radiographic progression, especially if used early in disease, suggesting a potential early window of opportunity for treatment (176–179). In JSpA, one small study demonstrated progression of sacroiliitis despite treatment with TNF inhibitors (180) and SIJ bony ankylosis occurs with rates correlating with the length of time taken to start treatment with TNF inhibition (181).

Given the importance of the IL23/IL17 pathway in the pathogenesis of SpA, biologics inhibiting this pathway are of particular interest for the treatment of patients with SpA. Ustekinumab, an inhibitor of IL23, was initially found to be effective in an open-label study of patients with active AS (182). However, no benefit was found in two subsequent RCTs, and the studies were terminated (183). Ustekinumab is an effective treatment for psoriatic arthritis (184) including axial arthritis in this group, and is also an effective treatment of enthesitis in patients with psoriatic arthritis (185). In JSpA, a case series of five patients treated with Ustekinumab demonstrated an improvement in disease activity in all but one patient (186) but no RCTs have been carried out. Other inhibitors of IL23, including guzelkumab, are effective treatments for psoriasis and psoriatic arthritis and demonstrate significant improvement in enthesitis (187). However, no benefit has been found in the treatment of axSpA and there have been no studies in JSpA (188). Given that enthesitis is an early feature of SpA and that there is a high prevalence in JSpA, investigating the effect of IL23 inhibition in patients with JSpA should be considered.

Treatments inhibiting IL17 have proved more effective than those inhibiting IL23 in adult SpA and for axSpA in particular, with response rates similar to those of TNF inhibitors (189). Secukinumab is now an established treatment for adults with SpA (including psoriatic arthritis) (190) and a clinical trial in JSpA has recently been completed (ClinicalTrials.gov: NCT03031782). Other agents blocking IL17 such as ixekizumab and brodalumab have proved effective for axSpA and psoriatic arthritis (191–193) and a clinical trial of ixekizumab is planned in JSpA (ClinicalTrials.gov: NCT04527380). It has been suggested that IL17 inhibitors may slow radiographic progression more effectively than TNF inhibitors (194) but this needs further investigation with "head to head" studies.

JAK inhibitors are another emerging treatment for SpA and Tofacitinib has shown similar efficacy to TNF inhibitors in adult SpA, including axSpA and psoriatic arthritis (195, 196). Clinical trials of JAK inhibitors are underway in patients with JIA, including patients with ERA and psoriatic arthritis subtypes (ClinicalTrials.gov: NCT02592434, ClinicalTrials.gov: NCT03773978). GMCSF inhibition is also under investigation in axSpA but not yet in JSpA (ClinicalTrials.gov: NCT03622658). Other biologic agents including IL6 and IL1 receptor inhibitors (197, 198), abatacept (199), and rituximab (200) have proved ineffective for the treatment of adult SpA and have not been studied in JSpA.

## OUTCOMES

Factors associated with a worse prognosis in adult patients with SpA have historically included the presence of hip arthritis and three or more of persistently raised ESR, unresponsiveness to NSAIDs, limitation of the lumbar spine movement, dactylitis, oligoarthritis, or onset younger than 16 years (201). AxSpa, the most studied form of SpA, is noted to be more common in men and is associated with higher levels of inflammation, more structural changes on MRI, higher levels of peripheral arthritis and enthesitis, and more uveitis compared to those with nr-axSpA (202). HLA-B27 status and severe inflammation on SIJ MRI in early disease are predictors of more severe radiographic changes after 8 years follow up (203). Obesity is associated with a worse outcome in axSpA (204). Differences in outcomes between males and females are reported (13).

There are relatively few outcome studies in JSpA and most compare JSpA to other JIA subtypes. These studies demonstrate that JSpA, in particular ERA, has low rates of remission and a worse prognosis compared to most other subtypes (205, 206). Despite treatment with TNF inhibitors and good initial response rates, this has not changed in recent years with ongoing disease activity seen in around 60% (142, 207). Higher pain scores and worse levels of physical function are also seen in patients with ERA compared to other JIA subtypes (208–210). Factors influencing prognosis in JSpA are similar to those in adult SpA. HLA-B27 in patients with JIA is associated with an increased chance of developing inflammatory lower back pain and, in males, enthesitis, and a lower chance of clinical remission after 8 years (211, 212). Other factors associated with a worse prognosis include male sex, ankle and hip arthritis and persistently raised inflammatory markers (206). Two studies comparing JSpA and adult SpA reported a greater requirement for hip arthroplasty in patients with JSpA (213, 214).

## THE EFFECT OF ADOLESCENCE: SEX HORMONES AND MECHANICAL STRESS

The peak age of onset of JSpA differs from most other JIA subtypes and corresponds to the onset of adolescence. Some HLA-alleles have been associated with early childhood arthritis. However, HLA-B27 appears to confer protection from the development early onset disease and has been associated with later onset arthritis in children which may explain the peak age of onset in early adolescence (215).

Increased oestrogen and progesterone production in females and increased testosterone production in males occur at the time of puberty. The effect of sex hormones on the immune system is well-documented and key changes in adolescence include higher levels of monocytes in males compared to females (216) and higher levels of T cells in females (217). The gut microbiome is also unique in adolescents and increased diversity of microbial species has been noted compared to adults. Sex hormones are also associated with differences in gut microbiota between males and females (218). Sexual dimorphism in adult SpA is well-recognised with higher levels of circulating Th17 cells in males with AS, in addition to higher serum levels of IL17, TNF, and IL18 (219, 220). Differences are also noted in clinical outcomes and response to treatment. In SpA, male sex is associated with more extensive bone marrow oedema of the SIJs (221) and a worse radiographic outcome (222–224). However, female sex is associated with higher disease activity and pain scores (222, 223, 225–227), less severe radiographic progression (222, 228–232) and a lower response rate to treatment with TNF inhibitors (233–235). These aspects have not been studied in JSpA but given the typical age of onset around the time of puberty, and the effect of sex hormones on the immune system and gut microbiome, it seems likely these would influence disease pathogenesis.

Adolescence is also a time of significant skeletal development and therefore increased mechanical strain on the musculoskeletal system. SpA tends to affect sites under the most mechanical strain such as the lower limb and the spine. Extra-articular manifestations of SpA are also found at sites of mechanical stress such as the aortic root and anterior uveal tract (236). In adult SpA, studies have shown more severe disease in those with manual jobs and with certain types of exercise (237, 238). One study of patients with JSpA showed nearly a quarter took part in intense physical training (95).

Enthesitis, the primary lesion in early SpA, occurs in susceptible individuals following repetitive microtrauma (239). Mechanical stress has been shown to exacerbate enthesitis in animal models and results in the release of pro-inflammatory cytokines at the enthesis (240). In heathy individuals, the resultant recruitment of innate immune cells leads to healing. However, in SpA, perpetuation of the inflammatory response occurs, perhaps due to IL23/IL17 pathway activation, HLA-B27 or gut dysbiosis, resulting in aberrant tissue healing and eventual new bone formation (241). Mechanical stress is heightened during periods of growth and development which may explain the prevalence of enthesitis and peripheral arthritis in children and young people with JSpA. Anatomical studies of the developing skeleton during puberty show that the most important factor in sacral development is the effect of mechanical force which occurs through body weight, load through the femur and strain on the pubic symphysis. Similarly, normal SIJ development is dependent torsion between the ilia and the sacrum (128, 242). Changes in SIJ orientation are seen with age and significant differences are found between adults and children and adolescents (243). In addition, distinct pelvic morphology develops in males and females after puberty with larger SIJ surface area in males thought to be related to higher biomechanical loading and larger ligamentous attachments (244). These changes in biomechanical loading and mechanical stress which occur during skeletal development may, in susceptible individuals, result in inflammation at the entheses and later at the SIJs.

## CONCLUSION

There are many similarities between adult SpA and JSpA suggesting that they are a spectrum of the same disease. Key differences in classification criteria, prevalence of clinical features, disease activity, and pathogenesis are evident which are influenced by age, pubertal development, changes in the immune response, and skeletal maturation. Further research is needed in to these factors which may influence treatment stratification for patients with JSpA. Better alignment between paediatric and adult classification and assessment criteria as well as studies encompassing the whole age spectrum of SpA will further our understanding and improve the treatment of this important disease.

## AUTHOR CONTRIBUTIONS

This article was drafted by CF and reviewed and revised by CF, CC, ML, DS, and LW. All authors agree to be accountable for the content of the work.

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REVIEW
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# Immunopathophysiology of Juvenile Spondyloarthritis (jSpA): The "Out of the Box" View on Epigenetics, Neuroendocrine Pathways and Role of the Macrophage Migration Inhibitory Factor (MIF)

*Miroslav Harjacek**

*Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates*

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**Edited by:**
*Fernando Manuel Pimentel-Santos, Universidade NOVA de Lisboa, Portugal*

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*Antonio Ferrante, South Australia Pathology, Australia*
*Herman Tam, University of Alberta, Canada*

***Correspondence:**
*Miroslav Harjacek*
*mharjacek@uaeu.ac.ae*

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Juvenile spondyloarthritis (jSpA) is a an umbrella term for heterogeneous group of related seronegative inflammatory disorders sharing common symptoms. Although it mainly affects children and adolescents, it often remains active during adulthood. Genetic and environmental factors are involved in its occurrence, although the exact underlying immunopathophysiology remains incompletely elucidated. Accumulated evidence suggests that, in affected patients, subclinical gut inflammation caused by intestinal dysbiosis, is pivotal to the future development of synovial–entheseal complex inflammation. While the predominant role of IL17/23 axis, TNF-$\alpha$, and IL-7 in the pathophysiology of SpA, including jSpA, is firmly established, the role of the cytokine macrophage migration inhibitory factor (MIF) is generally overlooked. The purpose of this review is to discuss and emphasize the role of epigenetics, neuroendocrine pathways and the hypothalamic-pituitary (HPA) axis, and to propose a novel hypothesis of the role of decreased NLRP3 gene expression and possibly MIF in the early phases of jSpA development. The decreased NLRP3 gene expression in the latter, due to hypomethylation of promotor site, is (one of) the cause for inflammasome malfunction leading to gut dysbiosis observed in patients with early jSpA. In addition, we highlight the role of MIF in the complex innate, adaptive cellular and main effector cytokine network, Finally, since treatment of advanced bone pathology in SpA remains an unmet clinical need, I suggest possible new drug targets with the aim to ultimately improve treatment efficacy and long-term outcome of jSpA patients.

**Keywords: juvenile spondyloarthritis, enthesitis-related arthritis, pathophysiology, epigenetics, neuroendocrine pathways, hypothalamic-pituitary axis, gut-joint axis, macrophage migration inhibitory factor**

## LITERATURE SEARCH

A systematic literature search was conducted in Ovid Medline (PubMed), Scopus, Science Citation Index expanded, and Google Scholar to find related articles. The key words were "juvenile spondyloarthirtis", "enthesitis-related arthritis (ERA)", "pathophysiology", "signal transduction", "epigenetics", "neuroendocrine pathways", "stress response", "HPA axis", "sex hormones", "gene expression", "proteomics", "Gut–joint axis", "dysbiosis" "NLRP3", "tissue hypoxia", "new bone formation (NBF)" in combination with "macrophage migration inhibitory factor (MIF)". I also manually screened the reference lists in relevant reviews and other non-primary data sources captured by the search strategy. Only publications in English were included.

## INTRODUCTION

Spondyloarthritis (SpA) is an umbrella term for a group of chronic inflammatory disorders that share common clinical and pathophysiological features. In children, enthesitis-related arthritis (ErA) is a subgroup of juvenile idiopathic arthritis (JIA) clinically characterized by enthesitis, chronic inflammatory arthritis, acute anterior uveitis, back pain, and low-grade gut inflammation. ErA also falls under the collective term of juvenile spondyloarthritis (jSpA) (1). Depending on the geographic region, ErA accounts for 15–30% JIA cases and is one of the commonest subtype of JIA seen in Asia (2). The jSpA commonly starts as "undifferentiated" disease (e.g., ERA) which differs between children and adults. For example, in juvenile-onset disease (jSpA), when compared to adults, hip arthritis is more frequently observed, there is a lower prevalence of human leukocyte antigen B27 positivity, axial involvement and acute anterior uveitis, but less peripheral arthritis and enthesitis (1). Although several classification criteria are used in children for uniformity of diagnoses, several other conditions share similar clinical features, thus resulting in either overlap or indistinct classifications. The juvenile spondyloarthritis (JSpA) is a perfect example of that, as the currently available criteria do not reflect their complexity and peculiarities (3).

The SpA family of diseases comprises undifferentiated jSpA (ERA), ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), and inflammatory bowel disease (IBD) associated arthritis. In general, SpAs are depicted by inflammation, bone erosions and new bone formation (NBF). Enthesis, representing the connective tissue junction where ligaments and tendons attach to the bone, is a primary target tissue for inflammation in SpA, with inflammation affecting both the enthesis soft tissue and the nearby anchoring peri-entheseal bone (PEB) (4). In fact, the anchoring PEB, or synovial–entheseal complex, is the main site of inflammation and osteitis in SpA (5).

As expected, the majority of the published genetic studies in SpA have been restricted to ankylosing spondylitis (AS), the classical form of SpA in adults. The genetic heritability of juvenile spondyloarthropathy remains incompletely understood, with HLA-B27 accounting for almost 25% of its identified heritability, with newly discovered gene mutations responsible for 2.1% of inherited cases (6). However, studies have shown that HLA-B27 positivity on its own is not sufficient to trigger disease as the concordance rate for HLA-B27 positivity in dizygotic twins was shown to be significantly lower than for monozygotic twins (24 vs. 63%, respectively), suggesting the important role of other relevant genes with an oligogenic model of familial transmission (7).

## GENE EXPRESSION AND PROTEOMIC STUDIES

Recent genome-wide association studies (GWAS) and single nucleotide polymorphisms (SNPs) analysis have further delineated the role of non-MHC genes in the development of adult AS, involving the interaction of endoplasmic reticulum aminopeptidase 1 (ERAP1) with HLA-B27 (8). The role of ERAP1 was also later confirmed for ErA and IL-23R for juvenile psoriatic arthritis (9). ERAP1 polymorphisms only affect the risk of development of SpA in HLA-B27-positive individuals, suggesting that they influence SpA pathogenesis by altering HLA-B27 function (10). Nevertheless, for better understanding of differences between genotype and phenotype as well as mechanisms of disease development, research methods such as quantification of gene expression are often necessary. So far, although a number of different gene expression studies in adult patients have been conducted, they included only a small number of patients with jSpA (11–13). In another cohort of patients diagnosed with ErA, using ILAR criteria, and with a known HLA genotype, none of the transcriptome studies was performed with RNA isolated from whole blood, nor was the calculation of the odds ratio (OR) for disease development performed, with absence of independent verification of data specificity and universality. Our group conducted a meticulous gene expression analysis in a very homogenous group of Croatian patients with enthesitis-related arthritis (ErA) diagnosed according to ILAR classification criteria. We documented increased expression of TLR4 and CXCR4 and decreased expression of NLRP3 and PTPN12 genes (13). In another ErA cohort from the USA, Barnes et al., found different genes or gene clusters, resulting in the under-expression of hemoglobin genes, with unknown significance so far (11). In a different study by Myles et al. involving Indian patients with ErA, gene expression in synovial fluid mononuclear cells (SFMCs) was compared to that in peripheral blood mononuclear cell (PMBCs). SFMCs were found to have a different gene expression profile from PBMCs, with overexpression of genes associated with various cell processes such as antigen presentation, scavenger function, chemotaxis and proteases, while genes involved in NK cell function, cell adhesion and inhibitors of apoptosis were under-expressed, suggesting a dysregulation of the innate immune system genes in that condition (12). The mechanism(s) responsible for those alterations, which differ among populations, remain largely unknown (see below).

Gene expression studies provide important information about the involvement of various signal pathways. They rely, however, on plasma which is frequently used as surrogate, instead of the synovial membrane proteome, actual site of pathology, from

early disease-stage, which would be most informative for precise determination of immunopathology of jSpA. However, synovial membrane is extremely difficult to acquire. Although control tissue from healthy children would have been useful, ethical considerations prevent it. On the other hand, as synovial fluid (SF) is in close proximity to tissues primarily altered during jSpA, analyzing it has significant potential to better understand the underlying immunopathogeneses. In the study of Rozenkranz et al. distinctively 24 proteins were identified as differentially abundant in SF between JIA subtypes, but jSpA patients were not included (drugi link). However, in the Taiwanese pilot study of two children with diagnosed enthesitis-related arthritis (ERA), the patients' plasma was studied before and after the administration of etanercept alone, using conventional two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry (MALDI-MS). They showed that etanercept therapy improved clinical ERA symptoms through the regulation of several cytokines (IL-2/IFN-γ), chemokines (MCP-1), and growth factors (GRO) that affect the expression of specific acute phase proteins such as haptoglobins, immunoglobulin A, and fibrinogen-γ chain (14). However, there are many challenges within the SF proteomics field including the requirement for standardized and stringent methods of sample collection and storage, the differences in sensitivity and specificity of various proteomic assays, the impossibility of including healthy controls, compounded by the lack of comprehensive biostatistical analysis of the data to exclude falsely detected biomarkers (15).

## EPIGENETIC STUDIES

The role of epigenetic mechanisms is essential in the regulation of gene expression, and consequently in the pathogenesis of various diseases, including rheumatic conditions (16, 17). The notion that these mechanisms could be influenced by external stimuli raises the possibility of a link between the environment and gene function, providing a potential clue for the potential contribution of these external stimuli to many diseases. Epigenetic mechanisms are traditionally defined as mitotically and/or meiotically heritable changes in gene expression that do not involve changes in DNA sequence. To contribute to the control of gene expression and repression, they are closely connected with other regulatory elements, such are transcription factors, and in some cases, with extracellular factors such as cytokines and growth factors (17). Although various studies have already confirmed the prevalence of epigenetic changes in both genetically complex and monogenic inflammatory rheumatic diseases, such are rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjorgen syndrome (SS), Cryopyrin-associated periodic syndrome (CAPS) and Familial Mediterranean Fever (FMF), to best of our knowledge, none has looked at patients with juvenile spodyloarthritis (16–19). In oligo-JIA, Chavez-Valencia et al. have found no substantial alterations in DNA methylation of $CD4^+$ T cells, but only modest alterations in genes of known or potential relevance to JIA (20). On the other hand, DNA methylation of the pro-inflammatory interleukin-32 (IL-32) gene was found to be reduced in JIA CD4+ T cells, suggesting an association between the reduction of IL-32 methylation and JIA (21). At present, there are at least three accepted mechanisms that can initiate and maintain epigenetic alterations: DNA methylation as pretranscriptional, histone modifications and non-coding RNA (ncRNA)-associated gene silencing like microRNAs (miRs) at the posttranscriptional level (22, 23). In our recent study in patients with ErA, we assessed the methylation levels of the *TLR4*, *CXCR4*, *NLRP3*, and *PTPN12* gene promoter, as well as the expression of several non-coding microRNAs (miR-150, miR-146a, miR-181a and miR-223) with reported interactions with the specific genes we were interested in. We collected PBMCs from 19 newly diagnosed patients with jSpA, according to ILAR classification criteria for enthesitis-related arthritis (ErA), and seven gender- and age-matched asymptomatic children. Out of four genes studied, we only found hypermethylated NLRP3 gene, while the expression analysis of selected microRNAs showed no significant difference (24, 25). DNA methylation studies in adults with AS have already identified over 1600 hypermethylated loci in the peripheral blood, most of which are located in HLA genes (26). In other studies, genes such as DNMT1 and BCL11B were found to be hypermethylated, but their expression did not correlate with the clinical manifestations of ankylosing spondylitis (27, 28). In a similar study of patients with AS, Coit et al. demonstrated an overexpression of hypermethylated genes like GTPase-related genes, as well as hypomethylated genes that included HCP5 gene encoding a lncRNA within the MHC region linked with a genetic risk for psoriasis and toxic epidermal necrolysis. Furthermore, the presence of an HLA-B*27 allele was associated with strong hypomethylation of HCP5, tubulin folding cofactor A (TBCA) and phospholipase D Family Member 6 (PLD6), of unknown relevance at this point (29). On the other hand, the miRNA expression profiles in the blood of patients with AS showed 19 differentially expressed miRNAs, with increased levels of miR-146a and miR-155 compared to controls, and with the disease index correlating only with miR-155 expression (30). Furthermore, IL-10 inhibits the pro-inflammatory microRNA miR-155 through STAT3 (31). This is relevant in the context of SpA where generally low IL-10 values are found in patients across the different phenotypes (*see below*).

## STRESSORS EXPOSURE AND NEUROENDOCRINE IMMUNO-MODULATION

When environmental strains exceed the human adaptive capacity or ability to cope, stress will occur. These environmental strains are collectively termed stressors, and appropriate responsiveness of the stress system to stressors translates into a sense of general wellbeing, adequate task performance and positive social interactions (e.g., homeostasis) (32, 33). By contrast, the effect of various stressors may hamper a child's growth and development, and may be responsible for various rheumatology, endocrine, metabolic, immune-mediated and psychiatric disorders (33). For example, in the extensive Swedish cohort of 2,453 adults with AS, increased risk for disease development was linked to

respiratory tract infections in childhood (34). As shown in both adult and pediatric patients with rheumatological conditions, the occurrence of stressful or traumatic life events frequently precede the onset of their illness or disease flares. It is well known that trauma or mechanical stress are frequent triggers or flare-inducers of JIA, and particularly for the induction of enthesitis (35). Moreover, stress can cause the brain to trigger the immune response, which can, in turn, induce changes in the central nervous system (CNS) suggesting bidirectional communication. However, in the course of chronic inflammation, an interruption of this communication might be possible.

Based on the observation of sex differences in AS, in studies performed over 50 years ago an etiological association with endocrine factors was suggested. A study of testicular function in 22 patients with AS demonstrated diminished testicular testosterone (T) reserve, elevated luteinizing hormone (LH) serum level, estradiol/testosterone ratio (E2: T) inversion and slightly increased estradiol (E2) serum level (36, 37). Interestingly, in the animal SKG mouse model *(SpA model*), estrogen was found to suppress TNF-$\alpha$ and arthritis development (38). Similarly, other animal model studies have shown that estrogen can suppress the differentiation of T helper (Th)17 cells from naive T cells (39).

Low serum levels of sex hormones, especially dehydroepiandrosterone sulfate (DHEAS) (i.e., **androgen drain**), may also contribute to bone loss in patients with AS, while patients with early or adult reactive arthritis have a high cortisol and DHEA serum levels that might change the course of disease (40, 41). After administration of a low-dose of adrenocorticotropic hormone (ACTH), the serum cortisol rise became significantly lower in patients with AS than in controls, suggesting an impaired hypothalamic-pituitary-adrenal (HPA) axis and reinforcing the possibility of involvement of the neuroendocrine system in the etiology of AS ("**the disproportion principle**") (42). More importantly, low cortisol and testosterone serum levels were found in subjects with active JIA, while the lowest androgen levels were found in those patients in whom disease extended into their adult life (43). In addition, Bravo et al. found elevated levels of serum prolactin (PRL), another pro-inflammatory hormone, in male patients with juvenile ankylosing spondylitis, with levels correlating with disease activity (44). The detailed description of the neuroendocrine pathways including HPA, arousal/sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), are beyond the scope of this paper but have been detailed in other reviews (45–51).

The role of the HPA axis extends to the glucocorticoid (GC) metabolism. Cortisol is converted to cortisone mainly by the kidney, *via* 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2, while the major organ for converting cortisone back to cortisol is the liver, *via* 11β-HSD1. Interestingly, in arthritis, conversion from cortisone to cortisol by 11β-HSD1 is increased (48). In addition, the circadian rhythm of the HPA axis may be defective in overcoming the signs and symptoms of the disease associated with inadequate cortisol secretion. This may augment negative feedback and explain the HPA dysfunction in inflammatory conditions (49). On the other hand, macrophage migration inhibitory factor (MIF) is secreted from identical corticotrophic pituitary cell type know to secrete ACTH, the hormone that stimulates the adrenal secretion of glucocorticoids. MIF was shown *in vivo* to neutralize the glucocorticoid-induced suppression of inflammatory cytokine secretion in activated macrophages (i.e. TNF-$\alpha$, IL-1-$\beta$, IL-6, IL-8) (**Figure 1**) (52). The circadian variation in plasma MIF closely parallels glucocorticoid levels. During stressful events or life-threatening infections coupled with high levels of glucocorticoids, the antagonistic effects of MIF on glucocorticoids probably represent the mechanism by which the host preserves a functioning immune response (52). Ralph et al. provided evidence that described the nuclear orphan receptor 1 (NURR1) as a target of MIF and GCs in RA, and a repressor of MAPK phosphatase 1(MKP1) expression. MKP1, the negative regulator of MAPK activation has been identified as a key gene that regulates MIF. Thus, NURR1, target of both GCs and MIF in mediating their opposing effects on MKP1, appears to be involved in the vital regulatory network that influences both innate and adaptive immune responses, as well as disease phenotypes (53). There is therefore a clear transition from a well-functioning HPA axis in the early phases of undifferentiated spondyloarthritis or reactive arthritis, to an inhibited HPA axis in late stages of chronic spondyloarthritis. This phenomenon is thought to be due to pro-inflammatory cytokine production such as MIF, IL-6, TNF-$\alpha$ and IFN-$\gamma$, which are likely to negatively influence steroidogenesis. Thus, in chronic spondyloarthritis, MIF might counter-regulate the suppressive effect of glucocorticoids on cytokine mRNA translation (**Figure 1**).

The combination of reduced parasympathetic with increased sympathetic tone, has been a consistent finding in chronic adult arthritis patients, suggesting an imbalanced autonomic nervous system (53–56). The sympathetic nervous system (SNS) has a bimodal effect in the chronic arthritis, by either increasing or decreasing serum levels of proinflammatory and anti-inflammatory cytokines. This depends on several factors, such as the time point of immune system activation, the cellular context, and the distinct adrenoceptors involved ($\alpha$ vs. $\beta$) (54). Although there is no published evidence in juvenile spondyloarthritis, the peripheral blood mononuclear cells (PBMC) of adult patients with JIA express mRNA-encoding α-adrenergic 1 receptors (α1-AR subtype), which are not found in healthy children. It seems, therefore, that the expression of α1-AR mRNA in PBMC during chronic inflammation might be associated with attenuated immune responses to stress (57). Functional α1-AR receptors seem to be upregulated on the leukocytes of patients with poliJIA, resulting in higher IL-6 levels upon stimulation of these receptors by a cold pressor test (58). Consequently, the α-ARs might become more relevant in a later stage of chronic inflammation, concurring with decreased numbers of β-ARs ("**β-to-α-adrenergic shift")** (59). The endogenous synergy of HPA axis (cortisol) and SNS is clearly demonstrated in patients with chronic synovitis, by the stiffness and/or the decrease of high cytokine levels in the morning (47).

On the other hand, the immunosuppressive effect of the parasympathetic nervous system is much more obvious. The cholinergic anti-inflammatory pathway (e.g.,

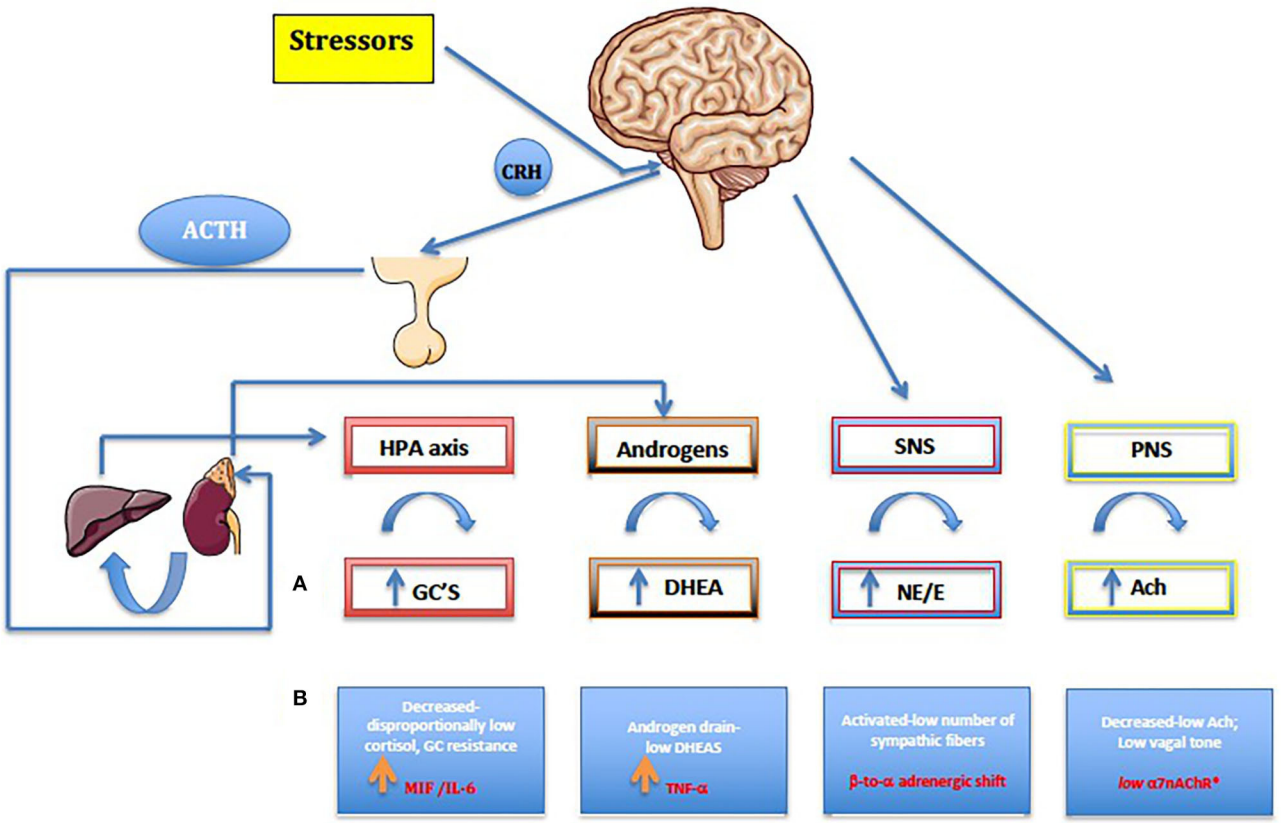


**FIGURE 1 | (A)** Normal, healthy neuroendocrine pathways seen also in reactive and early undifferentiated arthritis. **(B)** Chronic arthritis including jSpA. Along with the autonomic nervous system (SNS and PNS), the HPA axis is the main stress response mechanism, forming a carefully regulated signaling network. HPA axis dysregulation results in various downstream physiological consequences, like increasing risk for immune-mediated disease, mood disorders, metabolic disease, and cardiovascular disease. On the other hand, gonadal hormones play a master role in the formation, activation, and regulation of HPA axis. By affecting the response and sensitivity to cytokines, neurotransmitters, and hormones, gonadal steroids help to HPA axis to fine-tune the levels of stress hormones in the circulation. Furthermore, the behavioral responses to stress and obvious sex differences are particularly important considering their role in maintaining homeostasis. Pro-inflammatory cytokines namely MIF, TNF-$\alpha$ and IL-6 have a major role in shaping up final anti-stressor response during the chronic inflammation. Furthermore, centrally produced MIF by pituitary gland, was the first cytokine that can counter-regulate the inhibitory effects of glucocorticoids and, as a result, plays a critical role in the host control of inflammation and immunity in general. Imbalance in the autonomic nervous system (ANS) or dysautonomia has been detected in many established chronic autoimmune diseases, and in general, patients with chronic synovitis have increased activation of sympathetic fibers ($\beta$ to $\alpha$ adrenergic shift) and reduced PNS activity (*low a7nAChR*). For the details see text (32–65). HPA, hypothalamic-pituitary-adrenal axis; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; GC, Glucocorticoids; ACh, acetylcholine; NE, norepinephrine; E, epinephrine; DHEA, dehydroepiandrosterone.

"**anti-inflammatory reflex**") can suppress inflammation through release of acetylcholine (ACh) by the vagus nerve, involving the alpha7 nicotinic acetylcholine receptor (alpha7nAChR) expressed on CD68+ macrophages and other immune cells (60–62). As a result, a series of well-known proinflammatory molecules such as TNF-$\alpha$, IL-6, MIF, IFN-$\gamma$, high-mobility group box-1 (HMGB-1), free radicals, inducible nitric oxide (iNO), and others are inhibited (61). Interestingly, both the α7nAChR, ubiquitously expressed by $CD4^+$T lymphocytes, and the nAChR agonist nicotine, can inhibit the production of IL-17 in $CD4^+$ T cells in human peripheral blood (63). In various animal models, such as those for arthritis and colitis, nicotine has also been shown to have an anti-inflammatory effect by inhibiting the polarization to Th1/Th17 (64). This mechanism has been reinforced in a small pilot study of 37 adult patients with psoriatic arthritis or AS, where a transcutaneous vagal nerve stimulation (t-VNS) led to a significant reduction in ASAS scores (65).

## SUBCLINICAL GUT INFLAMMATION (GUT–JOINT AXIS)

The gut epithelial barrier is a first line of defense against harmful microorganisms. Disruption of the epithelial layer puts gut microbes in direct contact with the host's immune cells, thereby activating an aberrant inflammatory response. It has been shown that prenatal and early life bacterial gut colonization is thought to play a paramount role in shaping the immune system. This is translated into the gain of basic functions such as immunotolerance of commensal microorganisms. Early life exposures have been linked to the development of inflammatory

bowel disease (IBD) later in life. Infants born to mothers with IBD demonstrated enrichment in *Gammaproteobacteria* in their gut, often associated with intestinal inflammation, and also depletion in protective *Bifidobacteria*. Likewise, germ-free mice (GFM) inoculated with stools of third trimester IBD mother and of 90-day infants, showed a significant reduction in microbial diversity and fewer class-switched memory B cells and regulatory T cells in the colon (66). A study by Yatsuneko et al. suggests that gut microflora evolves toward a stable configuration by the age of 3 years (67). On the other hand, subclinical gut inflammation is a hallmark in all forms of juvenile spondyloarthritis and is associated with a high prevalence of inflammatory bowel disease (IBD) (66). The involvement of the gut–joint axis of inflammation in jSpA is strengthened by similarities in immunopathogenesis, and also by the clinical success of anti- TNF-$\alpha$ and IL-23 therapies in both IBD and in some forms of SpA (67). It is believed that inflammation in SpA originates in the gut and subsequently leads to joint inflammation. Both conditions share many genetic risk factors as well as changes in the composition of gut microbiota. Although conceptually attractive, some therapies targeting IL-17A are efficacious in the joint but not *vice versa,* and the targeting of adhesion molecules such as α4β7 in IBD can lead to onset or flares of SpA (67). Recent studies in ethnically different patient populations, and especially in patients with HLA-B27, have demonstrated dysbiosis in patients with SpA (68–76). Such dysbiosis is highly dependent on the host's genetic background and/or environment, implicating an "**ecological model of dysbiosis"**, with the effects of a multitude of microbes contributing all to the aberrant immunopathogenesis (77). At the functional level, different inflammation-associated microbes exhibit common metabolic pathways, including the synthesis of short-chain fatty acids (SCFA) such as butyrate, steroid biosynthesis as well as bacterial motility (78). The synthesis of butyrate, which has anti-inflammatory effects, promotes the development of regulatory T cells and is generally decreased in patients with SpA (78). The metabolomics data, in addition to less convincing 16S data, suggest differences in tryptophan metabolism in children with ErA, linked to the fecal microbiota, with a pro-inflammatory effect (79). Furthermore, as shown in early AS and HLA-B27 positive adults, dysbiosis and a leaky gut lead to adaptive immune activation associated with characteristic MRI phenotype of osteitis (80). Whether these changes are intrinsically inherent to the disease, or are a mere consequence of a more systemic inflammatory process that also involves the intestine it is not clear at this point. However, data from animal models and studies on relatives of patients with SpA, strongly suggest that these changes indeed precede the onset of the disease (76). In a some rheumatic disease it is possible that the use of specific probiotics, as an adjuvant therapy, correct the dysbiosis, resulting in the overall clinical efficacy. Our preliminary data has shown that VSL-3 medical probiotic, with a proven role in IBD, can improve clinical symptoms and decrease disease activity in jSpA patients (81). On contrary, different proof-of-concept studies from India, showed no clinical or immunological benefit in patients with JIA-ERA, with VSL-3 probiotic use compared to the regular use of NSAIDs (82).

## THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF)

While the dominant role of IL17/23 axis, TNF-$\alpha$, and IL-7 in pathophysiology of SpA, including jSpA, is well established, the role of cytokine MIF has generally been overlooked (83–89). The MIF is a critical upstream alarming-like mediator of innate immunity and inflammation. Under physiological conditions MIF circulates with serum concentrations between 2 and 6 ng/ml, with a circadian rhythm correlating with plasma cortisol (90). As mentioned earlier, it plays a pivotal role in the neuroendocrine axis mediated tissue-specific damage mechanisms, by counteracting the immunoregulatory effects of glucocorticoids (GCs) (52, 90). Unlike other cytokines, MIF is intrinsically expressed and stored in intracellular granules of various immune cells such as T- and B- lymphocytes, monocytes, macrophages, dendritic cells (DCs), mast cells, neutrophils, basophils, endothelial cells, tissue macrophages, and certain parenchymal cells (91). In response to liposaccharides (LPS) and stress, MIF is released from preformed cytoplasmic pools of mainly macrophages and dendritic cells. It up-regulates the expression of pattern recognition receptors, induces synthesis of downstream inflammatory cytokines, including IL-1β, IL-6, TNF-$\alpha$, IFN-$\gamma$, IL-17 and sustains the inflammatory responses by inducing recruitment of neutrophils, monocytes, macrophages and DCs and inhibiting their activation-induced apoptosis (92, 93). In order to regulate autophagy/mitophagy as well as glucose catabolism, MIF induces, in an autocrine or paracrine manner, enhancement of phagocytosis and an increase of the production of reactive oxygen species (ROS) and nitric oxide (NO) (94–96). In humans, MIF, a 114-amino-acid non-glycosylated peptide of 12.5 kDa, is encoded by a single gene located on chromosome 22q11.2m (92). Two distinct polymorphisms of MIF exist: rs755622 (– 173 G > C) and rs5844572 (– 794 CATT tandem repeat). They exist in linkage disequilibrium, and are associated, in different proportions, with various autoimmune diseases, such as SLE, systemic onset JIA, psoriasis and ulcerative colitis (97–100). Depending on the cellular context and disease state, MIF signaling is mediated by its receptors CXCR2, CXCR4 and/or CD74. The latter receptor alone mediates extracellular MIF binding, but MIF-induced MAPK signaling requires the co-expression of hyaluronan receptor CD44 leading to subsequent activation of proinflammatory transcription factor nuclear factor- κβ (NF-κβ) (101). The noncognate binding of MIF to CXCR2 and CXCR4 is the molecular basis for MIF-triggered recruitment of monocytes and T cells (102). In T cells and fibroblasts activation of JNK signaling by MIF involves the upstream kinases PI3K and SRC and is dependent on CXCR4 and CD74 (101). Besides, MIF inhibits p53-mediated apoptosis in macrophage with the induction of increased cytoplasmic phospholipase A2 (PLA2), arachidonic acid, COX2 and PGE2, which maintains the macrophage pro-inflammatory function (102). Increased gene expression of CD74 occurs in inflamed and noninflamed colonic mucosa of IBD patients, and it is also a possible T cell antigen in SpA, eliciting Th1 and Th17 responses (103, 104).

Intracellular MIF is involved in Toll-like receptor and inflammasome-mediated inflammatory responses. It upregulates Toll-like receptor 4 (TLR-4) expression, and consequently induces the release of proinflammatory cytokines such as TNF-$\alpha$ and interleukin IL-12, known to play an important role in pathogenesis of SpA (105). Loss of MIF has been shown to suppress the LPS-induced release of TNF-α by downregulating TLR4 expression (105). In response to the stimulation by LPS and Gram-negative bacteria (*canonical TLR4 activators*), the MIF-deficient macrophages have reduced production of TNF-α and IL-6, underlining a role for MIF in modulation of TLR4 downstream signaling pathways (106). We have already proposed that the Thr399Ile polymorphism of TLR4, found in variant carriers of Croatian patients with jSpA but undetectable in Indian patients, may be accountable for modified immune response to microbial infection (107, 108). MIF, via an interaction with JAB1/ CSN5, directly affects transcriptional activity of activator protein-1 (AP-1), a central regulator of several proinflammatory genes (109). This hints to a possibly interesting overlap between MIF and glucocorticoid mediated (GC) responses. An important mechanism of GC action is the ability to suppress AP-1- and NF-κβ-regulated genes, with steroid-resistant disease being often associated with increased AP-1 activity (110). Moreover, MIF is either directly involved in the assembly and activation of the NLRP3 inflammasome, or via intermediate filament protein vimentin, which is essential for NLRP3 activation (111). More importantly, this role is independent of its function as a cytokine, because recombinant and native MIF are unable to salvage NLRP3-dependent IL-1 release in $Mif^{-/-}$ macrophages (111). Depletion or inhibition of MIF in macrophages and DCs result in the inhibition of IL-1α, IL-1β and IL-18 in response to NLRP3-activating stimuli. It appears, therefore, that by regulating NLRP3 inflammasome activation and downstream IL-1β production, MIF has an upstream role in outlining the inflammatory characteristics of activated macrophages and DCs (111). Activation of caspase-1 is the main characteristic of inflammasome activation, with higher caspase-1 serum level in SpA, gout, inflammatory arthritis, and osteoarthritis than in other conditions (112).

Moreover, hypoxia initiated by microbiotome, plays a physiologic role in the normal intestine, and has also a disease-perpetuating role in the intestines of IBD patients (113). The oxygen used for butyrate metabolism is an important factor of intestinal homeostasis. Butyrate has a dual role: it is the primary fuel source for the colon, and also shapes the gut microbiotome (114). Hypoxia stabilizes hypoxia-inducible factor (HIF), a transcription factor that regulates many genes important for intestinal barrier function (115, 116). In addition, following a hypoxic stimulus, innate immune cells, including neutrophils, macrophages and dendritic cells, resist apoptosis, and in addition, intra-epithelial cells (IECs) are stimulated to produce TNF-$\alpha$ and other pro-inflammatory cytokines, causing increased barrier permeability (e.g., leaky gut) (117). This effect is furthermore perpetuated by oxygen consumption by the luminal bacteria, and also by inflammatory mediators and LPS, which also regulate HIF activity (117). The chronic HIF stimulation in the colon epithelial cells initiates a hyperinflammatory reaction and, at least in mice, HIF-1α enhances experimental colitis through a MIF-dependent inflammatory signaling cascade, reversed by MIF inhibition (118). This autoamplifying feedback loop could be interrupted by high doses of GCs via the GCR, or by the inhibition of HIF-1 an expression/stabilization under normoxia (119). MIF-JAB1 interaction also stabilizes HIF1α by preventing its hydroxylation, resulting in increased expression of pro-angiogenic factor such as VEGF (120, 121). These observations support the view that hypoxia is a key driving factor in chronic inflammation, and in case of jSpA on both gut and joint levels. Hypoxia, and in particular HIF-1α, are very potent inducers of MIF in the joints, as shown in cultured RA synovial fibroblasts stimulated by rhMIF (121). In macrophage cultures, hypoxia induces TLR-4 which is also important in the context of jSpA (122). Therefore, accumulating evidence supports hypoxia and HIFs in regulating a number of important pathophysiological characteristics of chronic arthritis, including synovial inflammation, angiogenesis, and cartilage destruction (123).

## SUGGESTED NOVEL HYPOTHESIS

Following the above discussion, I suggest a novel hypothesis in which decreased NLRP3 gene expression, due to epigenetic modifications of promotor site, is (one of) the cause for inflammasome malfunction leading to gut microbiota composition alterations observed in patients with early jSpA. This dysbiosis (*caused by NLRP3 dysfunction*) could potentially cause increased influx of TLR4 ligands and increased expression of the TLR4 gene (*possibly due to Thr399Ileu polymorphism of TLR4*), reduction of commensal bacteria with anti-inflammatory properties, namely *Faecalibacterium prausnitzii,* known to inhibit NF-κβ signaling, and finally leading to TNF-$\alpha$ abundancy, characteristic of jSpA (124).

The reduced expression of NLRP3 gene is a new and intriguing observation in jSpA/ErA. Studies on the role of NLRP3 inflammasome in IBD yielded controversial results. Earlier studies have reported that activated NLRP3 inflammasome stimulated production of IL-1β and IL-18 and contributed to intestinal inflammation (125). However, the concept of damaging inflammasome signaling in IBD is currently being reconsidered. This follows recent reports showing that IL-1β and IL-18 production can provide protection against colitis, and supported by recent GWAS studies showing that the polymorphisms which confer hypofunctional NLRP3 phenotypes are associated with the development of IBD (125). Note added in proof comes from the recent finding by Yao et al. who were using the gain of function NLRP3 R258W mice. They found that the hyperactive NLRP3 inflammasome, associated with local over-production of IL-1β, could maintain gut homeostasis resulting in strong resistance to experimental colitis. It appears that remodeled gut microbiota and increased induction of regulatory T cells were main mechanisms responsible for observed resistance (126). Therefore, it seems that a defective NLRP3 inflammasome signaling in the gut contributes to IBD, causing leaky gut and the induction of harmful immune responses against invading commensals (127).

I further speculate that hypermethylation of NLRP3 can be promoted by certain MIF genotypes, based on similar association of MIF rs755622C allele with hypermethylation of tumor suppressors $p14^{ARF}$ and $p16^{INK4a}$, both encoded by Cyclin Dependent Kinase Inhibitor 2A (CDKN2A). Hypermethylation of both $p14^{ARF}$ and $p16^{INK4a}$ was found in normal colonic mucosal tissues of patients with UC, as well in the precancerous lesions, suggesting that UC patients with this particular inflammatory genotype of MIF may be at a higher risk for developing colonic cancer (128). This rs755622C genotype association was also observed in patients with IBD and in Chinese patients with psoriasis, but not in Turkish patients with AS (97, 98, 129). Despite that, the authors have suggested that the time of onset and the duration of AS still might be affected by rs755622C allele (129). However, this hypothesis still needs to be proven in the laboratory, along with testing for *MIF*-rs755622C allele in more patients with early-onset jSpA. In summary, the downregulated NLRP3 gene in patients with early jSpA/ErA might reflect the occurrence of a subclinical inflammation of the gut mucosa *("low-grade IBD")*, leading to a leaky gut (**Figure 2**).

While the role of MIF in the early phases of SpA development is still speculative, its role in the late phases of disease is well established. Earlier reports have shown that inflammatory markers and serum MIF levels were significantly higher, and anti-inflammatory IL-10 levels were significantly lower, in patients with AS when compared to control patients. There is also a significant correlation between disease activity indices (BASFI) and MIF levels in these patients (130). It was therefore suggested that MIF may be involved in the pathogenesis of the chronic inflammation in AS. This was confirmed in a recent study where MIF was shown, not only to trigger inflammation, but also promote osteoblastic activity, suggesting its novel pathogenic role in new bone formation (NBF) in patients with SpA. It is important to mention that in SpA, NBF contributes to the disease burden independently of the pain and stiffness induced by chronic inflammation. In patients with AS increased levels of MIF have been demonstrated in the synovial fluid and ileum with a high number of MIF-producing macrophages and Paneth cells. Furthermore, increased MIF-induced TNF-$\alpha$ production was detected in monocytes and activated $\beta$-catenin in osteoblasts, both processes involved in promotion of the mineralization of osteoblasts leading to NBF causing spinal progression (131).

The level of expression of microRNA-451 was recently found to be lower in PMBCs of patients with AS, while MIF expression in PMBCs was significantly increased compared with those with pSpA and controls, indicating that MiR-451 suppresses

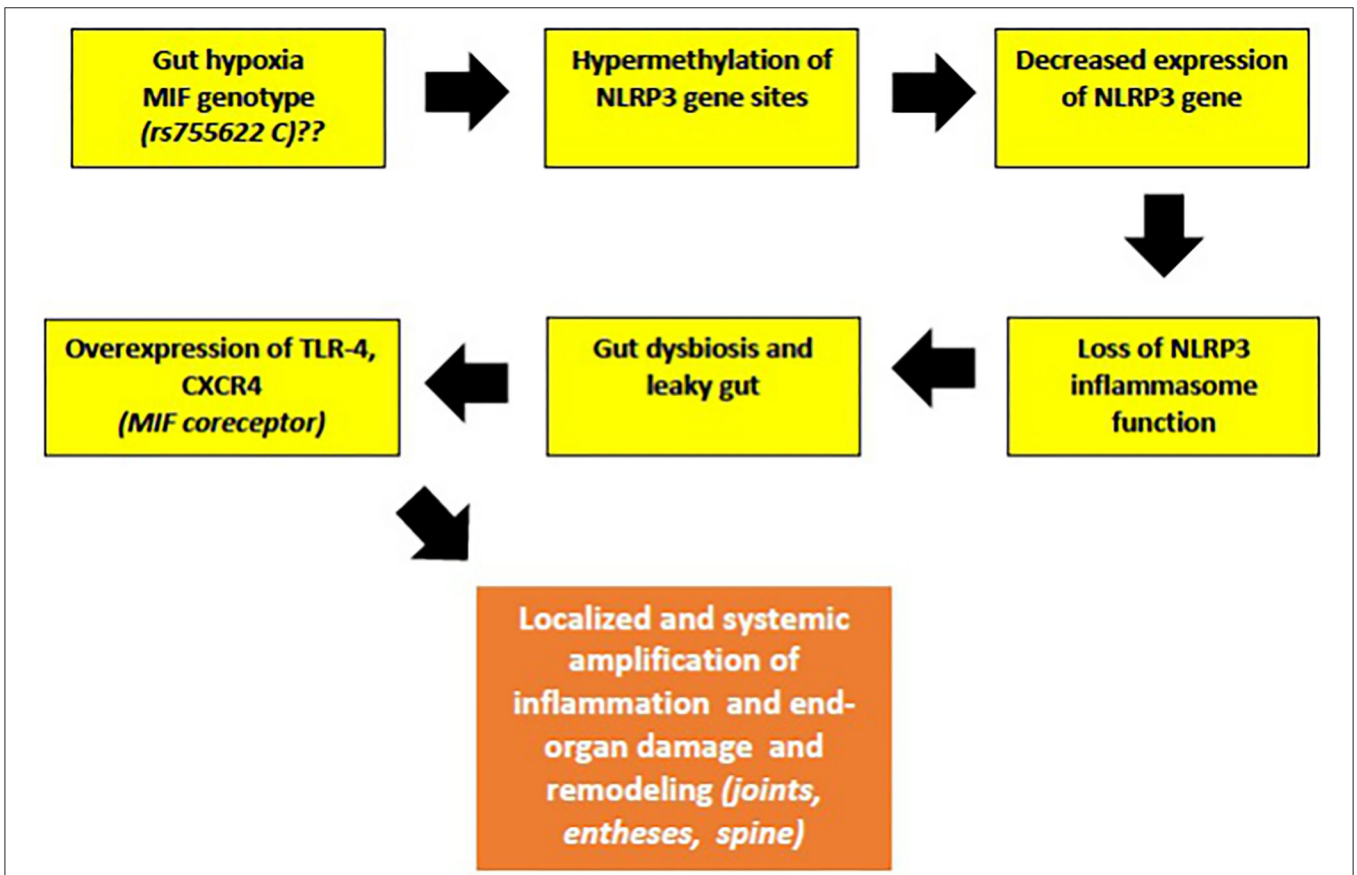


**FIGURE 2 |** The malfunction of NLRP3 inflammasome, a key tissue damage sensor, plays an important role in various autoinflammatory and autoimmune diseases. Proposed hypothesis of decreased NLRP3 gene expression and possible role of MIF in the early phases of jSpA development (see text for description).

inflammatory response in patients with AS by targeting MIF (132). Similarly to AS, the anti-inflammatory and anti-migratory effects of miR-451 that resulted in suppression of MIF, IL-6, TNF-$\alpha$ or RANTES expression, have been described *in vitro* in dendritic cells and synovial fibroblasts of RA patients and *in vivo* in mice with collagen-induced arthritis (133, 134).

In the joints themselves, MIF is also involved in synovial angiogenesis and neovascularization enhanced by loss of autophagy/ mitophagy (135). A dysregulation of these mechanisms is a critical mechanisms in the progression of inflammatory arthritis, including SpA (136). Transgenic mice overexpressing MIF exhibit high-turnover osteoporosis, while in different animal models MIF is able to enhance osteoclastogenesis through downmodulation of SDF-1 production in bone tissue and chemoattraction of circulating CXCR4+ osteoclast precursor cells (OCPs) (137–139). Furthermore, MIF (-/-) and CD74(-/-) mice also exhibit a practical absence of osteoclasts at the synovium-bone junction, as well as reduced osteoclast-related gene expression. This indicates that MIF and CD74 accelerate RANKL-induced osteoclastogenesis, suggesting that MIF contributes directly to inflammation and bone erosion in those animals (139). Nevertheless, the variety of bone pathology seen in SpA is unique in medicine and includes increased bone turnover, bone loss, osteitis, osteolysis and erosion, osteoproliferation as well as NBF, either at peripheral (enthesophytes) or axial (syndesmophytes) skeletal ligament, or tendon entheses and osteosclerosis (140). Notably, these effects can be present concurrently in the same patient.

The immunopathogenesis of SpA, with the complex interactions of cellular and main effector cytokine network mediated by MIF, are displayed in **Figure 3**.

## PUTTING IT ALL TOGETHER

Similar to adults, juvenile spondyloarthritis consists of chronic inflammation, articular bone erosions and pathologic new bone formation. Based on these differences with prototypical autoimmune diseases, such as rheumatoid arthritis or other connective tissue diseases, SpA may be better classified among autoinflammatory diseases (176). Children with clavicular cortical hyperostosis (CCH), a rare manifestation of jSpA, show complex patterns of gene expression related to several inflammatory pathways. These include STAT3 downregulation, B-cell activation, apoptosis, and MAP kinase with upregulated TRPM3/7 $Ca^{++}$ channels, and the most interestingly, genes closely linked to autoinflammatory diseases PTPN12 and MEFV. Interestingly, stimulation of TRPM3/7 $Ca^{++}$ channels can provide a second signal for NLRP3 inflammasome activation suggesting that CCH might be indeed an early autoinflammatory presentation of jSpA [manuscript in preparation, (177)].

A crucial event in the early stages of SpA appears to be the strong association of osteitis with low-grade IBD, confirmed in children with ErA by elevated concentration of fecal calprotectin (fCAL), a surrogate marker of gut inflammation (178, 179). Additionally, early studies with colonoscopy have shown that patients with SpA who had sub-clinical inflammation were more likely to have active arthritis on follow up, in particular in the hip, emphasizing therefore the prognostic value of this finding (180, 181). Nonetheless, the cell types that are principally involved in local inflammation in human SpA remain largely unclear (**Figure 3**). Circulation of immunological cells from the intestines (e.g., entero-synovial circulation) to the entheses, synovium and spine permits the enthesitis and synovitis to become chronic. The separation between the innate and adaptive immune system is largely artificial as neither works in isolation and cross-talks are well reported. The SpA also requires specific innate and adaptive immunological events targeting the synovium with several processes that run in parallel, such as dysregulated epigenetic control, tissue hypoxia (gut and joint) and neoangiogenesis, all leading to the final stage of tissue damage and remodeling characterized by chronic synovitis and enthesitis, syndesmophyte formation and ankylosis (**Figure 4**).

However, considering that the frequencies of HLA-B*27 alleles and ERAP1 polymorphisms ("*first hit*") are ethnic-specific, it is important to understand that jSpA pathogenesis could well be the result of various combinations of these mechanisms in different populations (182, 183). It is also important to underline sex differences. Examples include SLE and adult AS where different clinical phenotypes exist in males and females. Therefore, different sexes may require different biomarkers for proper diagnosis of the same disease (184). Nevertheless, two scenarios of disease development are possible: some patients who had reactive arthritis or early undifferentiated form like ErA, can reach remission ("*second hit*"), but the majority of the patients progresses to active chronic disease *("multiple hits"*). The neuroendocrine immune response of the HPA axis and sympathetic nervous system, intended to overcome a transient inflammatory episode, are uncoupled and can therefore lead to immune cell metabolic disease in the context of erroneous energy regulation (45, 66, 175). Furthermore, failed autophagy and apoptosis of immune cells, in addition to failure of negative immune regulation (e.g., immune suppression) due to decreased GC production (*high MIF production*), blocked AMP-activated protein kinase (AMPK) pathway, and decreased IL-10 production by TREGs, BREGs, regulatory DCs most likely due to IL-7/HIF-1 production, collectively result in the progression to chronic inflammation and subtype/endotype differentiation (185, 186) (**Figure 4**). Of note is that the activated AMPK, and mammalian target of rapamycin (mTOR), a downstream molecule of activated AMPK, represent key control points of a series of inter-connected inflammatory signaling pathways. These include NF-κβ and JAK/STAT, crucial drivers of maintaining energy balance, cytokine signaling, cell growth, and apoptosis (187). Interestingly, in the HLA-B27/h$\beta$2 transgenic rat model where *in vivo*, prophylactic treatment of rats with rapamycin (m-TOR inhibitor) significantly inhibited the development and severity of inflammation in peripheral joints and spine (*arthritis and spondylitis*), with histological evidence of reduced bone erosions and new bone formation, all hallmarks of SpA (188). This is relevant in view of the fact that mTOR pathway has been indeed activated in SpA synovitis, and because mTOR blockade by rapamycin or metformin in mouse model

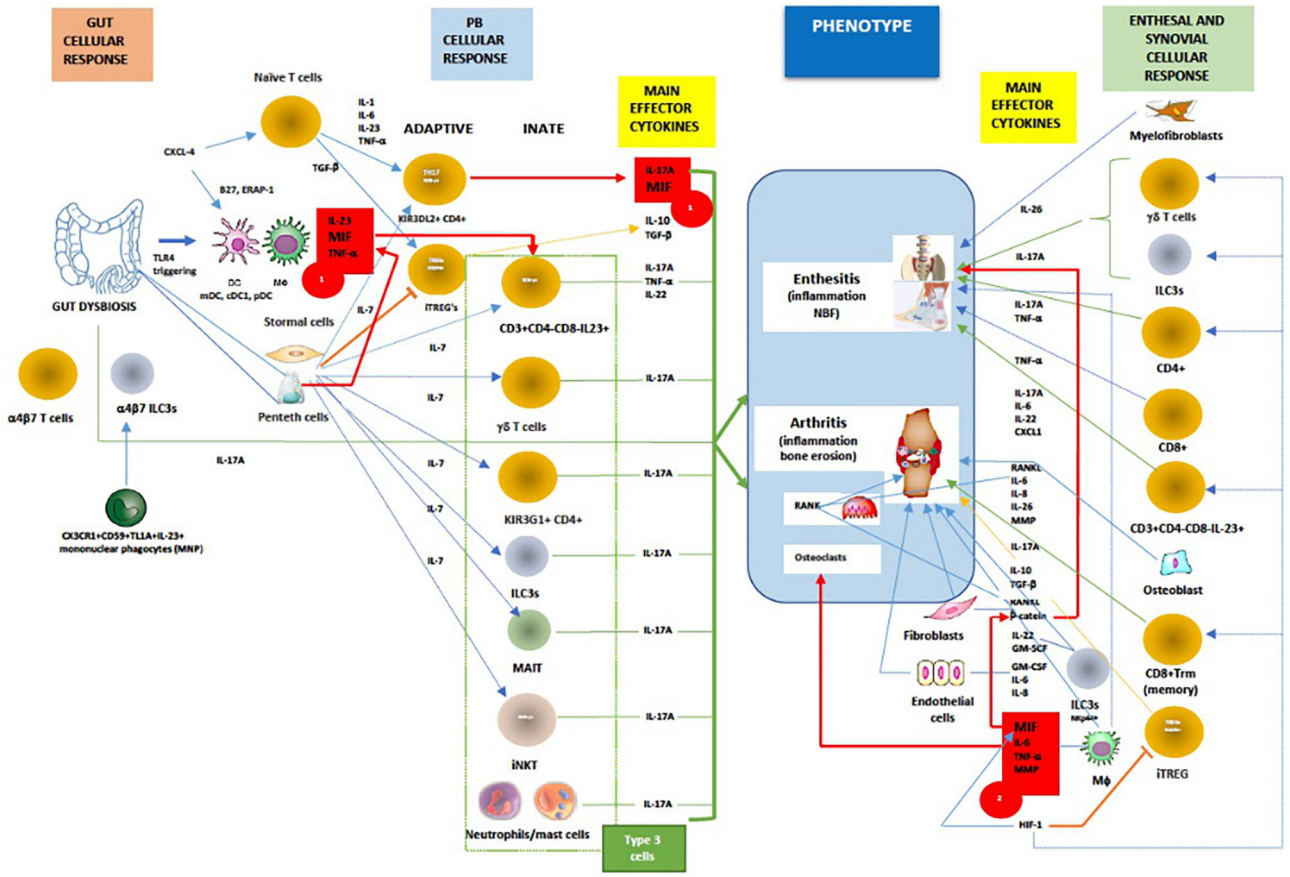


**FIGURE 3 |** In general, MIF is an proximal mediator of host defense that up-regulates the expression of pattern recognition receptors (eg TLR-4) and perpetuates inflammatory responses by inhibiting activation-induced apoptosis in monocytes and macrophages. As mentioned above in chronic spondyloarthritis the inhibitory effect of glucocorticoids on cytokine mRNA translation may be counter-regulated by MIF. Furthermore, dysbiosis could trigger TLR-4 ligands to induce ileal production of MIF, especially in CD163+ macrophages, DCs and Paneth cells, which consequently activates CD14+ monocytes to produce TNF-α/IL-23 and primes adaptive and innate (Type 3) immune cells to produce IL-17A (*Red box 1*). Chemokine CXCL4 (*MIF co-receptor*) promotes IL-17 production in human $CD4^+$ T cells by acting both directly on CD3/CD28-activated human (naive) $CD4^+$ T cells or indirectly via myeloid antigen presenting cells (mDCs). On the other hand, IL-7 is a cytokine that, better than IL-23, stimulates IL-17 production in both innate and adaptive immunity, and suppress the function of regulatory T cells (Treg). High levels of IL-7 have been confirmed both in the intestinal tissue and in the inflamed synovium of patients with AS. The tissue-resident γδ T cells, ILC3s, circulating and/or gut-derived α4β7 γδ T cells, $T_H17$ cells, or mucosal associated invariant T (MAIT) cells all are known to promote IL-17-driven joint/entheseal inflammation. MIF, along with TNF-α and other pro-inflammatory cytokines, also induces synovitis and enthesitis with bone erosions. Joint hypoxia induces hypoxia inducible factor (HIF) expression that augments inflammation-promoting Th17 cell development through recruitment to the IL-17 promoter. In parallel, HIF-1, by binding to Foxp3, restricts regulatory T cell (Treg) development. Through positive feedback loop HIF-1 induces MIF, which in turn causes HIF-1 expression via the MIF receptor (CD74). In contrast to early phases of SpA, axial inflammation is not dependent on IL-23, but rather on IL-17A and most likely MIF. MIF is inducing mineralization of primary osteoblasts in a dose-dependent manner, upregulates genes involved in osteogenesis and triggers stabilization of a known mediators of osteoblastic activity, namely β-catenin and wingless protein ligand (Wnt). (*Red box 2*). Beside MIF, upregulated PGE2, IL-1β, TNF-α, IL-17A, IL-22, IL-26 and IL-23, BMP-2, calcium-sensing receptor CaSR-PLCγ-**signaling** and downregulated sclerostin, ankylosis progressive homolog (ANKH) and Dikkopf-1 (DKK-1) protein are important pathways involved in bone remodeling and tissue repair. Finally, activation of both the canonical Wnt/β-catenin and noncanonical Wnt/PKCδ pathways is required for inflammation-induced new bone formation (NBF) in SpA. Taken all together, it still unknow what is/are the most important factors in NBF, even TREGS were recently proposed to paradoxically, via IL-10, promote NBF through suppressing TH17 production [adapted modified from (80, 84, 87, 119, 130–175)]. Red line/box- MIF pathway; green line -IL-17A production; orange line- iTREG suppression and signaling; dotted blue line-IL-7 signaling; blue line-cellular stimulation by other cytokines.

stops osteoclastogenesis. In humans with AS, that blockade also attenuates inflammation, inhibits production of IL-17A and TNF-α, bone remodeling and new periosteal bone formation (189–192). Also, rapamycin *in vitro,* may reduce inflammation in SpA by promoting autophagy of misfolded HLA-B27 (193).

In undetermined disease stage of jSpA, without well-defined and serological testing, genomic and/or imaging biomarkers become crucial because, despite biologic therapy, fewer than half of children achieve reach long-term and sustainable remission off medication 5 years after diagnosis (2, 194). Similarly, treatment of the various bone pathology in SpA remains an unmet clinical need. Although the beneficial effect of anti-TNF-α therapy might not only neutralize the effects of TNF-α, but also down-regulate Th17 and Th17-related cytokines associated with up-regulating

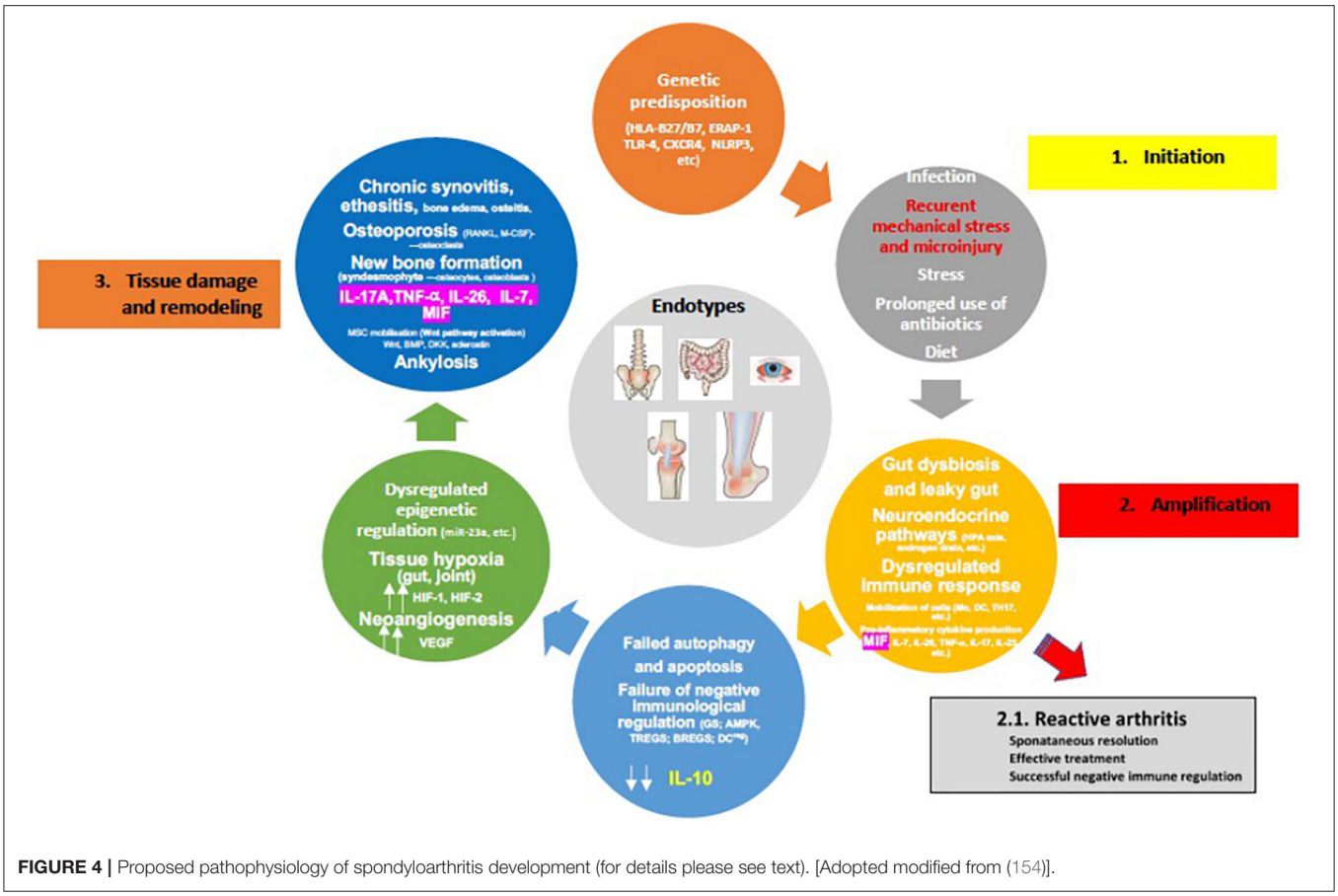


**FIGURE 4 |** Proposed pathophysiology of spondyloarthritis development (for details please see text). [Adopted modified from (154)].

the TREG/TGF-$\beta$ axis in responders, this can also passively cause new bone formation since TNF-$\alpha$ stimulates the expression of DKK-1. DKK-1 in turn suppresses signaling by Wnt, promoting consequently osteoblast and osteoclast formation as well as differentiation induced by BMP-2 (195).

The definition of disease subtypes on the basis of underlying pathophysiology and the concept of endotypes has emerged more recently. Phenotypes/endotypes are dynamic, clearly overlapping and may evolve into one another, thus making clear-cut definitions somehow difficult. Nevertheless, a phenotype-/endotype-based classification approach could direct toward the application of personalized/precision medicine in the SpA field. Discoveries from basic science research might, as mentioned above, define multiple complex molecular pathways involved in the pathogenesis of jSpA, which may provide biomarkers for the molecular endotyping of this complex disease. In addition, these molecular pathways might reveal potential therapeutic targets. An endotype might consist of several complex mechanisms that cannot be clearly separated into "*pure single molecular mechanism*" thus being a "complex" endotype (196). Therefore, new powerful biomarker like fCAL that is able to differentiate various JIA subtypes, would allow us to precisely define various potential endotypes of jSpA. Down that line it was recently demonstrated that in patients with AS, a small RNA molecule, miR-199a-5p was downregulated in T cells and associated with radiographic severity of disease when compared to controls (197). MiRNA-199a-5p expression levels also showed significant negative correlations with the Ankylosing Spondylitis Disease Activity Score (ASDAS) and modified Stoke Ankylosing Spon dylitis Spinal Score (mSASSS) of AS patients. It turns out that, in T cells of AS patients, miR-199a-5p has a novel role in regulating autophagy by modulating the mTOR signaling though direct inhibition of Rheb. Rheb is known to inhibit T cells autophagy and promotes pro-inflammatory cytokine production by activating mTOR signaling (197). These data suggest that miR-199a-5p participates in the regulation of AS pathogenesis by affecting T cell autophagy and mTOR inhibition (197). In addition, the level of expression of another miR-451 was lower in AS PBMCs than in both pSpA and control PBMCs, but MIF expression was significantly increased in AS PBMCs compared to AS patients and with greater radiographic damage. It turns out, that overexpression of miR-451 suppresses the MIF (132). These findings suggest miR-451/MIF may be a novel therapeutic target in the treatment of SpA. Besides that, epigenetics could potentially be used as preventive, diagnostic, and therapeutic biomarkers and should be included in any future jSpA classification and determination of endotypes.

Well established treatment for jSpA still includes NSAIDs, but only sulfasalazine, as one of the conventional DMARDs, was found to be effective in a randomized double blind placebo

controlled trial in 33 patients with jSpA after 26 weeks treatments (198). While adult with SpAs respond well to treatments that include TNF-$\alpha$ or IL-17-targeting biologics, they are mostly unresponsive to abatacept or MTX treatment (80, 199). Secukinumab clinical trial in children with jSpA has recently been completed (ClinicalTrials.gov: NCT03031782) but among other IL17 blocking agents, such as ixekizumab and brodalumab, that were proven to effective for adult axSpA and psoriatic arthritis, only clinical trial of ixekizumab is apparently planned in jSpA (ClinicalTrials.gov: NCT04527380) (200). Also, clinical trials of JAK inhibitors are underway in patients with JIA, including patients with ERA and psoriatic arthritis (ClinicalTrials.gov: NCT02592434, ClinicalTrials.gov: NCT03773978) (200). JAK inhibitors, in particular Tofacitinib, has shown similar efficacy to TNF inhibitors in adult SpA, including axSpA and psoriatic arthritis (201, 202).

Finally, to ultimately improve treatment efficacy and long-term outcome of patients with jSpA, consideration should be given for the use of new drugs such as iguratimod (IGU) that target simultaneously MIF, IL-17A and TNF-$\alpha$, or for those that only target IL-7, m-TOR, IL-26 and/or ERAP 1 (203, 204). Blockage of MIF by a monoclonal antibody provides *in vivo* antirheumatic effects, suggesting MIF as a suitable target for antirheumatic therapy (205). Furthermore, in recent experiments, treatment of RA patients with histone deacetylase inhibitors (HDACi) downregulate MIF, in particular with two distinct orally active molecules MS-275 and SAHA. They have shown *in vivo* anti-inflammatory activities in preclinical models of rheumatoid arthritis, and both MS-275 and SAHA strongly suppress MIF protein expression by interfering with the MIF transcriptional machinery in RA synovial fibroblasts (206). Givinostat, a pan-class I/II HDACi, is currently being investigated in JIA but no data about its potential use in jSpA is currently available (207). An additional benefit of anti-MIF therapy is that it could in addition be steroid-sparing in patients with chronic steroid dependence or refractory rheumatic disease requiring daily steroid therapy. In children and adults, combination of two biologic agents is not well documented. Safety of rituximab in combination with other biologic agents (*adalimumab, etanercept, infliximab*) in adults with RA was reported as an open-label study (208). Rigby et al. showed that no serious adverse events occurred within 24 h of any rituximab infusion, and that efficacy improved at week 48 compared with that at week 24 (208). However, none of the biologic combination therapy have ever been studied in children, but favorable adverse reaction profiles, with not significant increase in infection rates with mono biologic therapy, might stimulate future researchers to consider combination therapy in children as well. Finally, another interesting approach was performed using bispecific antibody where combining a well-established anti-TNF therapeutic domain [single-chain variable fragment (scFv) of adalimumab with a synovial tissue specific targeting domain (scFv-A7) (e.g., scFv-A7 antibody) was located on the human arthritic synovium *in vitro* and in a synovium xenograft in severe combined immune deficient (SCID) mouse model (209). This study provided the first description of a BsAb capable of direct drug delivery to synovium with potential applications to other existing biologics. In practical terms, due to the improved potency, the use of such BsAb molecules in the clinical care of chronic arthritis like jSpA may offer reduced duration of treatment and consequently reducing the associated healthcare costs.

Finally, future network analysis using multiomics approach to integrate emerging forms of data from multiple platforms, has the potential to further highlights overall imunopathogenesis of the jSpA and offer true biological classification of childhood arthritis as suggested recently (210, 211).

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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# Inflammatory Foot Involvement in Spondyloarthritis: From Tarsitis to Ankylosing Tarsitis

*José Pablo Romero-López*[1,2], *Dirk Elewaut*[3], *César Pacheco-Tena*[4] *and Rubén Burgos-Vargas*[5*]

[1] Laboratorio A4, Carrera de Médico Cirujano, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla de Baz, Mexico, [2] Laboratorio de Inmunología Clínica 1, Posgrado en Ciencias Quimicobiológicas, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional de México, Ciudad de México, Mexico, [3] Ghent University Hospital, Ghent University, Ghent, Belgium, [4] Facultad de Medicina, Universidad Autónoma de Chihuahua, Chihuahua, Mexico, [5] Department of Rheumatology, Hospital General de México, "Dr. Eduardo Liceaga", Ciudad de México, Mexico

Spondyloarthritis (SpA) is a group that includes a wide spectrum of clinically similar diseases manifested by oligoarticular arthritis and axial or peripheral ankylosis. Although axial SpA is predominant in Caucasians and adult-onset patients, juvenile-onset and Latin American patients are characterized by severe peripheral arthritis and particularly foot involvement. The peripheral involvement of SpA can vary from tarsal arthritis to the most severe form named ankylosing tarsitis (AT). Although the cause and etiopathogenesis of axSpA are often studied, the specific characteristics of pSpA are unknown. Several animal models of SpA develop initial tarsitis and foot ankylosis as the main signs, emphasizing the role of foot inflammation in the overall SpA spectrum. In this review, we attempt to highlight the clinical characteristics of foot involvement in SpA and update the knowledge regarding its pathogenesis, focusing on animal models and the role of mechanical forces in inflammation.

**Keywords: spondyloarthritis, ankylosing tarsitis, juvenile onset spondyloarthritis, foot arthritis, mechanical stress**

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***Correspondence:**
*Rubén Burgos-Vargas*
*r.burgos.vargas@gmail.com*

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## INTRODUCTION

Spondyloarthritis (SpA) is a group of chronic inflammatory diseases of the entheses and the synovial membrane of the joints, tendons, and bursae that affects the spine, the sacroiliac joint, and peripheral sites (1, 2). Currently, SpA is known as axial SpA (axSpA) (3, 4) or peripheral (pSpA) (5). Ankylosing spondylitis (AS) represents the most severe form of SpA in which episodes of disease activity merge with chronic irreversible manifestations such as bone proliferation and ankylosis. According to ASAS classification, the "SpA" name is kept, and ankylosing spondylitis (AS) corresponds to radiographic axSpA (r-axSpA). While axSpA predominates at onset and through disease's course in European and European descendants (6), the combination of axSpA with pSpA is the clinical pattern most frequently found in Latin America (7–16), India (17, 18), the Middle East (19), and Asia (20).

In the past, studies on SpA referred to peripheral involvement in children, adolescents, and young adults (21, 22). Usually, peripheral involvement was described as an asymmetrical affection of the lower limbs. Regarding adult-onset disease, particularly AS, the peripheral disease became recognized in the mid-1970s (23, 24) as part of AS and disorders such as reactive arthritis (ReA) (formerly Reiter's syndrome), psoriatic arthritis (PsA), Crohn's disease, ulcerative colitis, and undifferentiated SpA.

Amor et al. (25) first included peripheral arthritis of the lower limbs among the SpA criteria. Then, the European Spondyloarthropathy Study Group (ESSG) proposed a classification system with two entry arms axial and peripheral (26). This idea gained recognition in the ASAS classification as pSpA. In the meantime, mid-foot arthritis, enthesitis, or tarsitis appeared as important manifestations in adolescents or young adults with AS (27).

It was challenging to assess enthesopathy even though the concept of "the entheseal organ" turned fundamental in understanding the disease's pathophysiology (28–30). Synovitis and particularly enthesitis, have been the target for studying cellular infiltrates, pro-inflammatory cytokines, and bone proliferation. As discussed below, the mechanisms leading to such phenomena include mechanic forces that act upon mechano-receptors, HLA-B27, ERAP1, and IL-23. The approach to studying the disease's pathogenesis has been driven from two paths: throughout animal models and human surgical samples.

## FOOT INVOLVEMENT AND TARSITIS

Mid-foot arthritis, also known as, tarsitis, is a prominent feature in adolescents and young adult males with SpA (31, 32). Most adolescents and young adults have recurrent lower-limb arthritis and enthesitis combined with <20% axial symptoms. Five to 10 years later, 75% of such patients fulfill the AS criteria (33, 34). In contrast to juvenile-onset SpA (JoSpA), adults have back pain and 5 to 10 years later inflammatory back pain alongside sacroiliitis on magnetic resonance imaging (MRI) and radiographic studies (5, 35–39). Identifying the characteristic involvement of peripheral arthritis and enthesitis and its differentiation from other forms of juvenile idiopathic arthritis (JIA) as early as possible allows the use of biologic disease-modifying anti-rheumatic drugs (bDMARDs) years before the appearance of the spinal and sacroiliac joints symptoms (21). The same applies to eligibility criteria and outcome measures in clinical trials on the efficacy and safety of bDMARDs. Besides peripheral disease, some other variables are predictive of SpA in children and adolescents; specifically, a family history of SpA, HLA-B27 positivity, and clinically the history or presence of foot enthesopathy and arthropathy uveitis, inflammatory back pain, and sacroiliitis (40–45).

Tarsitis presents with mid-foot pain and swelling and often swollen ankles, inflammation of the plantar fascia, and Achilles tendon enthesitis (**Figure 1**). Radiographic studies show a spectrum of findings, such as diffuse osteopenia, joint space narrowing, and bone ankylosis. Erosions and enthesophytes are found in the extraarticular entheses, such as Achilles' tendon and plantar fascia bone attachments (**Figure 2**). MRI shows bone edema, synovial sheath and bursae swelling, and abundant synovial fluid in the joint space (**Figure 3**).

The most severe cases are those evolving into ankylosing tarsitis (AT), a condition characterized by a partial or complete fusion of the tarsal bones and by the formation of bone bridges resembling in certain aspects the long-term changes of the sacroiliac and particularly the spine of AS patients (**Figure 2**).

Besides our descriptions of Mexicans with JoSpA, there are sporadic descriptions of tarsal involvement in other geographic localization and ethnic groups. For example, unilateral ankylosis of the tarsal bones was described in a 19 year-old male with AS diagnosed at the age of 15 who had several mid-foot episodes of arthritis; unilaterality was attributed to radiotherapy (47). In another report, 15 of 40 patients with JoSpA that underwent therapeutic immobilization of the feet developed tarsometatarsal fusion (48).

Chinese and French large cohorts of patients with JoSpA have shown tarsitis in around 6 to 9% (49, 50). Data from India indicate that involvement of the mid-foot is common and severe (17). Likewise, tarsal bone ankylosis was seen in patients with oligoarticular juvenile rheumatoid arthritis and back pain from India (51) and Turkey (52, 53).

Based on the New York classification of sacroiliitis (5, 54), our group has developed an equivalent grading of radiographic

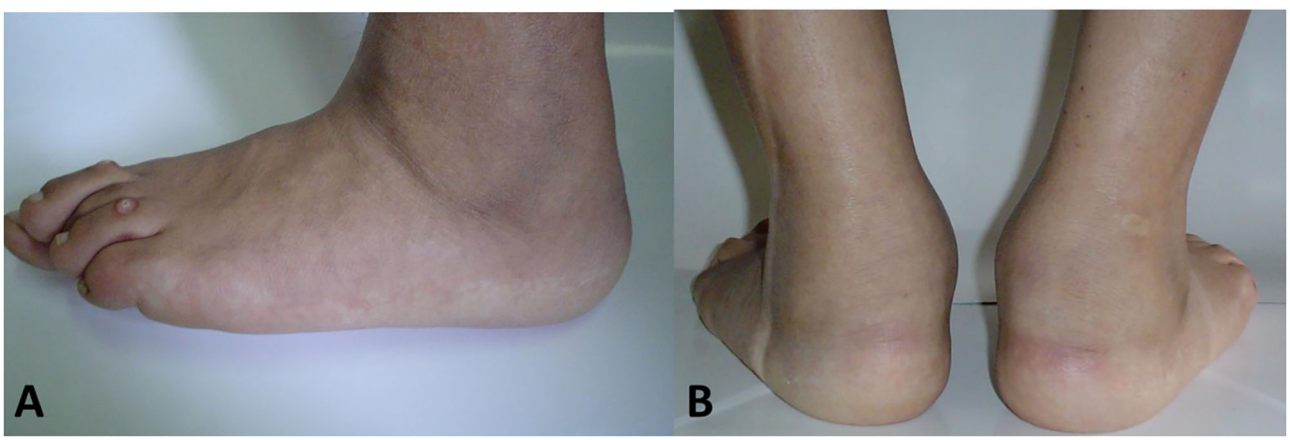


**FIGURE 1 |** Lateral **(A)** and coronal **(B)** T-2 weighted-fat suppressed MR imaging showing edema in various tarsal bones, joint spaces and soft tissues in a 16 year old boy with chronic ankylosing tarsitis [Modified from Burgos-Vargas (46)].

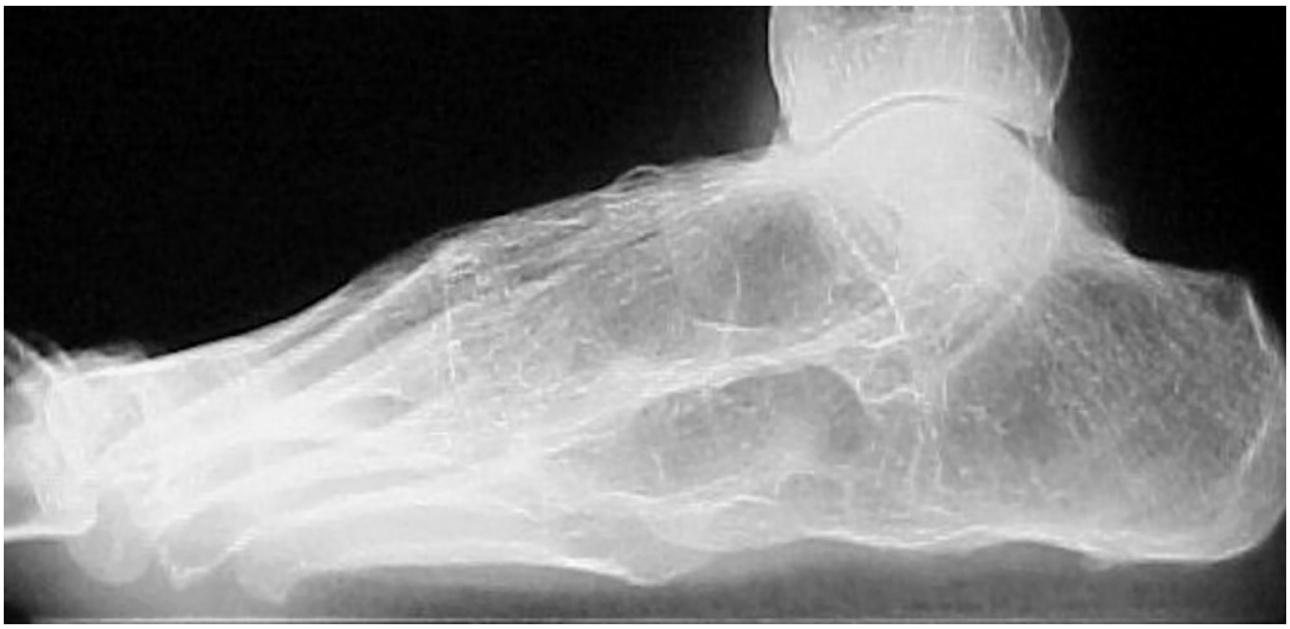

**FIGURE 2 |** Chronic changes in a patient with JoSpA. Lateral view showing complete tarsal ankylosis and plantar enthesophytosis. Courtesy of Dr. Rubén Burgos-Vargas.

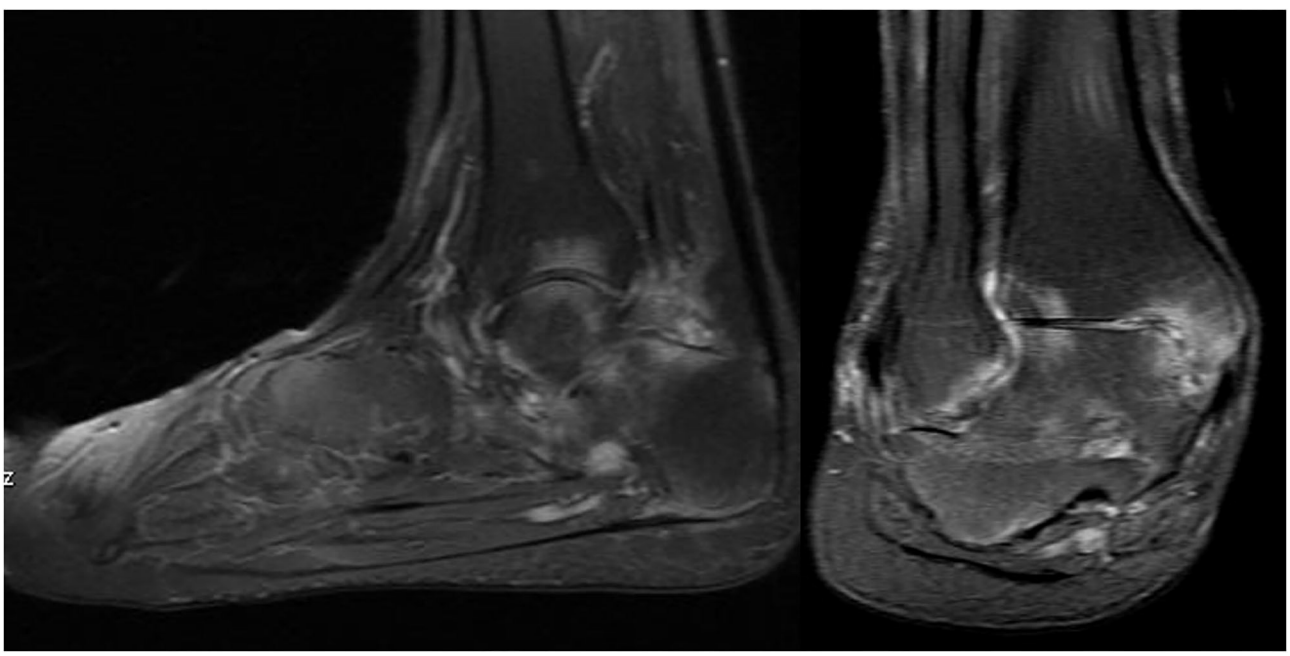

**FIGURE 3 |** T-2 weighted-fat suppressed MR imaging showing edema in various tarsal bones, joint spaces and soft tissues in a 16 year old boy with chronic ankylosing tarsitis [Modified from Burgos-Vargas (46)].

parameters of classification and interpretation of tarsitis (39). As a result, the Spondyloarthritis Tarsal Radiographic Index (SpA-TRI) has good sensitivity and specificity to evaluate structural but no inflammatory changes (39, 48).

Tarsal ankylosis (defined as tibiotarsal, intertarsal, or tarsometatarsal ankylosis) occurred in 18% of patients with juvenile rheumatoid arthritis (JRA) and 23% of adult-onset patients with rheumatoid arthritis. In another study, tarsal

ankylosis accounted for 25% of the seropositive and seronegative polyarticular JRA, 9 and 19% of the pauciarticular and systemic (51). Interestingly, most of these cases also had carpal ankylosis, and none had SpA. In addition, Tarsal and carpal ankylosis occurred in adult patients with JRA from India (51). Compared with patients without radiographic sacroiliitis, around 40% of such patients with radiographic tarsitis graded 3 or 4 with the SpA tarsal radiographic index (55).

## THE TARSITIS LINK WITH SpA

The characteristics of SpA in adults are inflammation and enthesitis, followed by bone proliferation and ankylosis involving the sacroiliac and spinal joints (56). While the cause and mechanisms involved in inflammation are partially known, those participating in bone proliferation remained unidentified. Research advances are associated with new treatment forms, which may induce remission of inflammation but not halter bone formation (57). Additionally, many studies focus on the axial skeleton and very little on peripheral sites. In contrast, striking findings occur in the mid and hindfoot in many animal models, highlighting a window of opportunity to study human samples from peripheral joints.

Unfortunately, pediatric and adult rheumatologists in our clinic and probably from other centers have neglected midfoot involvement despite its severity and consequences. Most people think of the ankle and metatarsophalangeal joints when children and even adults complain of midfoot/tarsus pain and swelling. The connection between the exacerbated inflammatory responses and the abnormal residual ossification remains a potential field to improve our therapeutic approach. Blocking the mechanisms subjacent to bone proliferation could improve the overall prognosis significantly in our patients.

Interest in peripheral arthritis as a critical manifestation of SpA developed in parallel with studies on psoriasis and psoriatic arthritis (PsA) and descriptions of enthesitis and dactylitis (24,

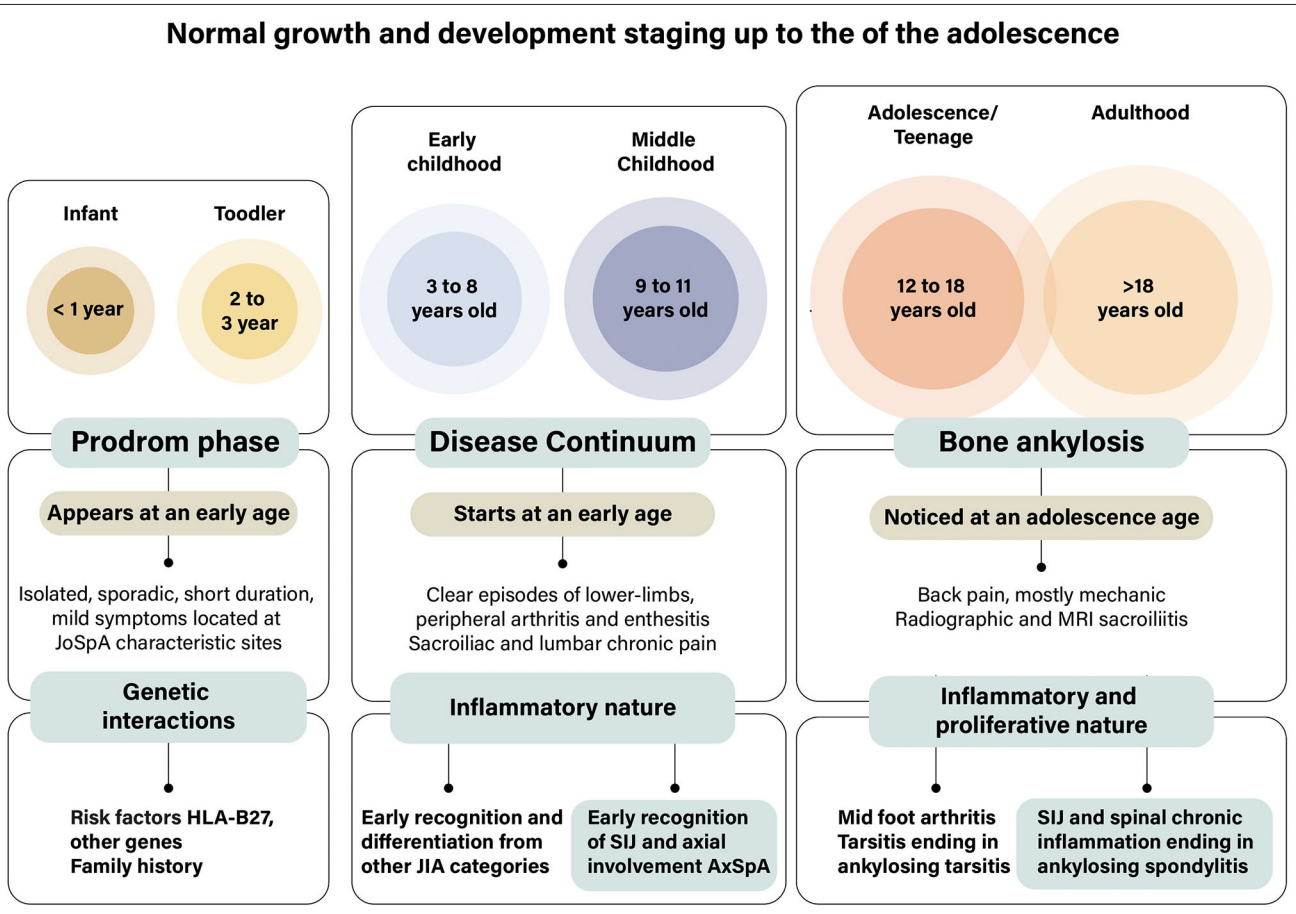


**FIGURE 4 |** Stages in the development of foot affection in juvenile-onset spondyloarthritis. We proposed three stages to approach the early symptoms of foot affections in patients with JoSpA. The duration of each of the stages is certainly unknown since information is scarce. Few symptoms, such as isolated, sporadic, short duration, episodes of joint pain often considered growing pains, characterize the prodrome stage. Around the age of 8 years starts the inflammatory stage of the disease, which is mostly characterized by arthritis, enthesitis, and axial involvement; this is the disease "continuum" stage, which merged with tarsal ankylosis in the adolescent years or sacroiliac and spinal ankylosis in early adulthood. This is a clinical picture needing differentiation from other diseases, particularly from subgroups of juvenile idiopathic arthritis. At the same time, the relation between children and adult diseases should be established via de sacroiliac and spinal demonstration in children. These events take place when there are several changes in growth and development in childhood and adolescence.

58, 59). Dactylitis often occurred in single toes as a companion to nail psoriasis. Recently, peripheral arthritis appeared again in clinical descriptions (48). An international study of 4,465 patients with SpA found that nearly 70% of the participants had at least one episode of peripheral arthritis (48, 60). Data splitting yielded 57% with arthritis, enthesitis in 44%, and dactylitis in 15%. The study confirmed the highest percentage of peripheral manifestations in around 80% of patients in Latin America, dactylitis in 37% of PsA, and enthesitis in 65% of JoSpA. Mid-foot involvement or tarsitis occurred in rank order in 13% of pSpA, 10% of PsA, 9% of reactive arthritis (ReA) and inflammatory bowel disease (IBD), and 5% of axSpA. The proportion of tarsitis in JoSpA was 19%. Per geographic region, tarsitis occurred in 24% of patients from Latin America. In children, inflammatory clinical events may progress throughout the years and end in bone ankylosis. We proposed three stages to approach the very early symptoms of the "Prodrome" stage, which evolve and progress in a rather slow and recurrent "continuum" of disease, ending up with bone ankylosis (**Figure 4**).

## INSIGHTS INTO THE PATHOGENESIS OF ANKYLOSING TARSITIS

There is very scarce information addressing the pathogenesis of peripheral and foot affection on SpA; nonetheless, genetic association studies have revealed that the HLA-B27 predisposition is shared with axSpA and that other genes like LMP2 (61), ERAP-1, ERAP-2 (62) and class II MHC are involved (63).

Although peripheral osteoproliferation seems to be the main problem in these patients, very few studies focus on the pathogenesis of tissue inflammation and proliferation (2). We have previously analyzed tendon, enthesis, and joint samples from the midfoot of Mexican patients with ankylosing tarsitis (AT) (64). Our results revealed a scarce leukocyte infiltration accompanied by an osteoid intrusion in the extracellular matrix (ECM), suggesting that, probably, intramembranous ossification of the enthesis and subchondral osteoproliferation could take place. We also found an important expression of bone lineage proteins like osteopontin (OPN) and osteocalcin (OCN) in mesenchymal tissues as well as parathyroid hormone-related protein (PTHrP) and basic sialoprotein on bone tissues. The role of osteocalcin in physiological and pathological bone formation remains an important question in SpA (65, 66); however, its expression on entheseal cells might involve its participation in inducing an osteoblastic phenotype (67).

## CONTRIBUTION OF ANIMAL MODELS

Remarkably, in different animal models of SpA, midfoot arthritis and ossification are the main clinical features that can happen either before or simultaneously that axial arthritis. Animal models of transgenic animals like the HLA-B27-transgenic rats develop spontaneous arthritis in the hind paws accompanied by spondylitis, uveitis, and gut inflammation, resembling human disease (68). Interestingly, a transgenic model of TNF overexpression in mice ($TNF^{\Delta ARE}$) is characterized by Crohn's-like ileitis, midfoot ossification and inflammation, sacroilitis, and spinal ossification (69, 70) that worsens with increased mechanical stress and can develop in the absence of mature T or B cells (71). A more recently described model involves the transmembrane expression of TNF; in this model, animals develop a disease characterized by axial and peripheral enthesitis with abundant leukocyte infiltration (72). These experimental approaches resemble human disease and point to the importance of peripheral arthritis and enthesitis in the onset of the disease with an interesting involvement of immune pathways and mechanical forces.

Another noteworthy model of SpA is induced after the transgenic edition of ZAP70 in SKG (Sakaguchi) mice, which develop SpA and Crohn's-like ileitis after the injection of microbial compounds like curdlan or zymosan (73, 74). In addition, the animals of other induced models like proteoglycan-induced arthritis (PGIA) (75–77), collagen-antibodies induced arthritis (CAIA) (70, 71, 78, 79), and DBA mice (80–85) also can show a certain degree of midfoot inflammation and, in chronic models, a severe ossification, accompanied by overexpression of inflammatory cytokines like IL-1B, IL-12B, IL-17A, and IL-6.

Experimental evidence from the IL-23 minicircle overexpression model points to an essential involvement of tendon and ligaments through altered stromal cell function, myeloid cell responsiveness, or gamma delta (γδ) T cell-dependent mechanisms (78). Furthermore, firm evidence for a link with mechanical stress has arisen from hind limb unloading vs. voluntary running experiments that decreased or increased mechanical stress. The studies firmly showed that both in the $TNF^{\Delta ARE}$ model (70) and CAIA (71), unloading prohibits arthritis onset, whereas the reverse was observed under voluntary running conditions. Intriguingly, while unloading prohibited the onset of arthritis in collagen-induced arthritis (CIA), it did not interfere with the development of anti-collagen antibodies, indicating that mechanostress regulates joint inflammation but uncouples it from induction of autoantibodies (71). The mechanostress effect is also apparent in the absence of adaptive immunity, suggesting that tissue-resident stroma may account for it. This is intriguing as several studies have pointed to a crucial role for entheseal and skin γδ T cells in the onset of IL-23-driven PsA both on skin and joints (86–88).

Several animal models point that canonical T cells appear not to be indispensable for mechanostress induced inflammation. In line with this, *in vitro* stretch of tendon and ligament-stromal cells induce an array of pro-inflammatory mediators, several of which are shared with skin keratinocytes. They include chemokines, cytokines, and several danger-associated molecular patterns (DAMPs). The induction of CCL2, for example, was shown to recruit classical monocytes. Mechanostress also led to a marked activation of complement, which attenuated mechanostress induced inflammation (89).

A summary of the characteristics of animal models that can present midfoot inflammation and ossification is showed in **Table 1**. Interestingly, many mechanisms can be involved in the

**TABLE 1 |** Foot and axial involvement in animal models of SpA.

| Experimental model | Pathogenic mechanisms | Foot involvement | Axial involvement | Role of mechanical stress | Inflammatory pathways | Ref. |
|---|---|---|---|---|---|---|
| Collagen-antibodies induced arthritis (CAIA) | Passive transfer of anti-type II collagen antibodies induces polyarthritis and synovitis | Enthesitis can be found on paws after 7 days of induction | Cartilage hypertrophy, bone damage, and kyphosis can be observed | Mechanical stress drives osteophyte formation and size | Inflammation in this model is dependent on IL-23 | (65, 66, 73, 74) |
| SKG mice | SKG (Sakaguchi) mice have a defect on the SH2 domain of ZAP70 and develop SpA-like disease after the injection of microbial components like Curdlan and Zymosan | Mice develop progressive ankle arthritis and swelling of the foot soft tissue. Foot arthritis and enthesitis precede axial affection | Sacroiliitis, tail, and lumbar arthritis appear after around 10 weeks of induction | Unknown | IL-23 mediates inflammation and bone proliferation. Also, extra-articular manifestations like ileitis and uveitis are involved | (68, 69) |
| Proteoglycan-induced spondyloarthritis | The injection of bovine proteoglycan and adjuvant induces T cell-induced synovitis with progressive ankylosis | A progressive polyarthritis with synovitis and cartilage destruction is evident in the initial 8 weeks after proteoglycan injection, followed by osteoproliferation and ankylosis of frontal and hind limbs | Spinal arthritis starts weeks after peripheral arthritis and ankylosis, followed by intervertebral ankylosis and bone proliferation | Unknown | T-cell induction of arthritis and TNF involvement | (70–72) |
| HLA-B27 transgenic rats | Transgenic expression of human HLA-B27 and β2-microglobulin in rats induces spontaneous arthritis, spondylitis, ankylosis, and gut inflammation | Tarsal affection is classically described as tenderness, swelling, and inflammation of one or two hind limbs | Axial bone proliferation and sacroiliitis are present | Unknown | Several mechanisms have been described, including HLA-B27 misfolding, homodimer formation, gut dysbiosis, and type 3 immunity pathways | (63) |
| $TNF^{\Delta ARE}$ Mice | A deletion in the regulators of TNF (AU-rich elements, or ARE) induces a prolonged overexpression of TNF, inducing gut inflammation, and a SpA-like phenotype | Arthritis initiates at enthesis in interphalangeal joints and Achilles' tendon | Radiographic spinal inflammation and sacroiliitis are evident after 4 months of development | Tail unloading experiments demonstrated that mechanical stress is an essential driver of arthritis | The model can be induced in the absence of mature T and B cells so that stromal cells might drive the mechanisms leading to arthritis and enthesitis | (64–66) |
| Transmembrane TNF | A defect in the cleavage site for ADAM17 drives overexpression of membrane-bound but not soluble TNF | Peripheral enthesitis and osteitis are accompanied by bone proliferation with entheseal and synovial leukocyte infiltration | Animals develop tail deformities and spondylitis with deformation with focal joint destruction. Also, inflammation of the spinal ligaments and bone marrow is present | Unknown | The inflammation is driven by TNF-receptor I and can be induced with the TNF overexpression of only stromal cells | (67) |
| Spontaneous arthritis in DBA Mice | Hormonal, aging, and behavioral factors are involved in the spontaneous development of arthritis, enthesitis, and ankylosis of 4 month old DBA/1 male mice | Initial signs are detected in interphalangeal, metatarsophalangeal, and ankle joints with further swelling of tarsal joints. Enthesitis, dactylitis, and psoriasiform nail changes are frequently observed | Scarce evidence of axial ankylosing or enthesitis | An aggressive behavior related to mice fight and microtrauma is involved; nevertheless, the specific role of the mechanical load has not been explored | There is evidence of a role of testosterone, BMP signaling, and inflammatory cytokines like IL-1, IL-12, IL-6, and IL-17. Arthritis and enthesitis can be induced in the absence of alpha-beta or gamma-delta T cells | (75–80) |

development of SpA, and although animal models have provided much of the current information, several studies on human samples reveal a complex immune network that modulates the response to risk factors and enhancing elements toward the onset of the disease (**Figure 5**).

## INFLAMMATORY PATHWAYS IN AXIAL AND PERIPHERAL SpA

Genetic studies have provided a significant step in the discovery of potential triggers of SpA pathogenesis. The most studied

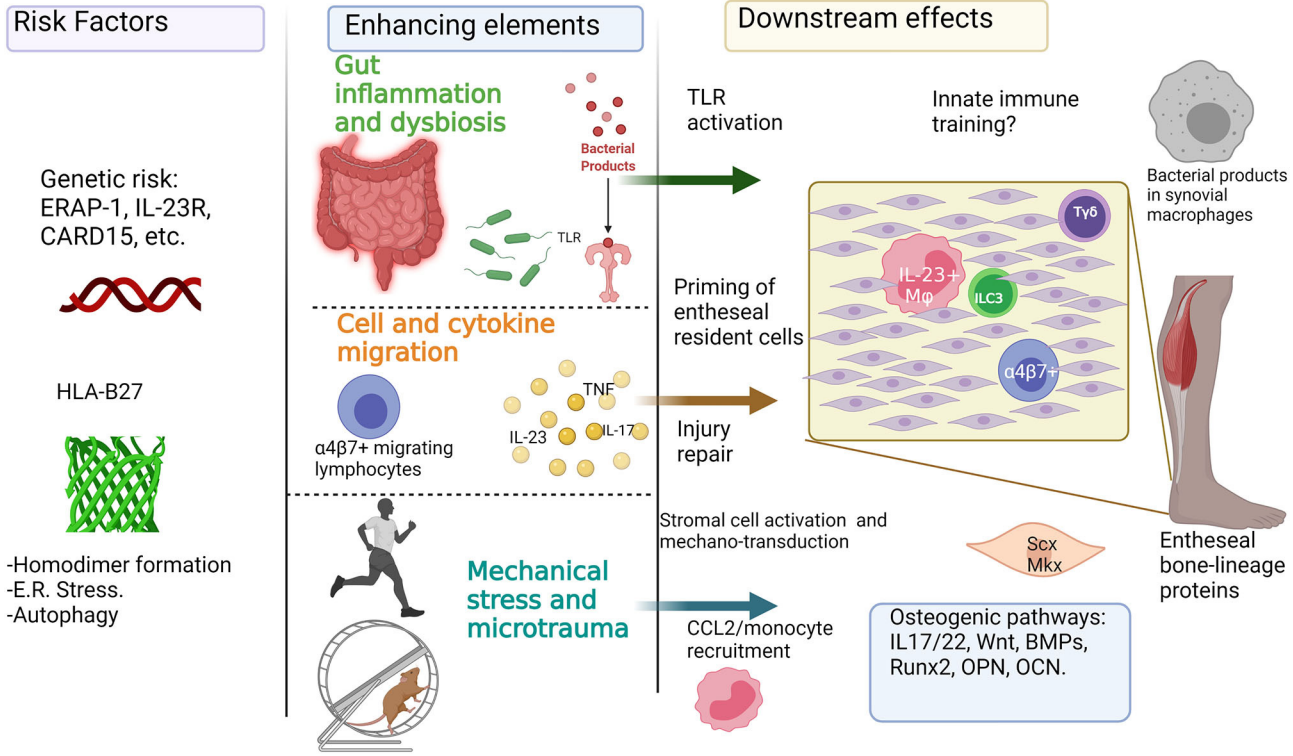


**FIGURE 5 |** Inflammatory and mechanical factors in the onset of enthesitis: Genetic predisposition for enthesitis can be represented by HLA-B27 and its role in the endoplasmic reticulum (E.R.) stress, autophagy, and homodimer formation, and also by other polymorphisms in genes like ERAP-1 and IL-23R. Further enhancing elements involved in the onset of enthesitis include the role of the "leaky gut" syndrome, intestinal dysbiosis, and the activation of gut-homing lymphocytes. Additionally, mechanical stress can play a key role in enhancing inflammation and osteoproliferation through injury repair mechanisms, production of chemokines like CCL2, and the activation of stromal cells like tenocytes. The previous can be added to the potential role of osteogenic pathways like Wnt, BMP, and Runx2. *This figure was done with biorender (Biorender.com).*

gene for SpA susceptibility is the class I histocompatibility molecule HLA-B27 (90, 91), which, previously was considered as a potential link with "arthritogenic peptides" presented to self-reactive lymphocytes; nevertheless, this has not been proven, and current theories postulate other roles like the induction of endoplasmic- reticulum (ER) stress (92) and homodimer formation (93).

Studies in animal models of transgenic HLA-B27 rats and mice (68) revealed that this molecule tends to misfold during its synthesis in the endoplasmic reticulum (ER) (94), causing ER stress, activation of the unfolded protein response (UPR) (95) and the induction of the inflammatory cytokine IL-23 (92). However, UPR activation has not been proven in humans, as UPR markers are not increased in samples of patients of SpA (96), and instead, some reports suggest that IL-23 production could be related to an increase of autophagy markers in the gut (97). Furthermore, killer immunoglobulin receptors (KIR) expressed on NK and Th17 cells can recognize aberrant B27 homodimers (98, 99), and therefore, induce IL-17 production. Remarkably, heavy chain homodimers have been found in patients' gut and vertebral joints (100).

Although most of the cellular and molecular mechanisms related to the initial triggers and the amplification of inflammation in SpA are known for animal models and *in vitro* research, the use of bDMARDs has allowed basic and clinical researchers to study the role of several cytokines in the human SpA. The first and most known biologic target is the pro-inflammatory cytokine TNF, and TNF inhibitors (TNFi) are currently the most used treatment for the disease. The use of TNFi confirmed its therapeutic effect in adult patients with AS (38) and prevented proliferation and structural progression after 8 years (101). Although JoSpA patients can also benefit from TNFi (102), there are no reports of osteoproliferation in these patients' peripheral joints.

Type 3 immunity (mediated by IL-23, IL-17, and IL-22) also plays an essential role in the pathogenesis of SpA. IL-17 is a pro-inflammatory cytokine involved in animal models of SpA (74, 78, 99) and increased in patients' blood and synovial fluid (103–106). The pro-inflammatory and destructive effects of IL-17 have been associated with synovitis, enthesitis, and bowel inflammation (107–109). Also, animal models of IL-23 minicircle injection (78) and SKG mice (74) mainly depend on IL-17. The therapeutic inhibition of this cytokine has significant effects on disease activity and vertebral inflammation (110), although, again, few reports focus on peripheral symptoms, and the existing ones only evaluate PsA patients (111, 112).

IL-23 is a cytokine involved in the differentiation and maintenance of the Th17 phenotype (113). Its role in SpA has become highly relevant for the scientific community since, in 2012, it was published that the over-expression of this cytokine with DNA minicircles in mice could induce a spontaneous model of SpA-like disease (78). In the report by Sherlock *et al.*, this cytokine could act on a previously undescribed population of entheseal resident CD3+, RORγt+, Sca1+, CD4–, CD8–, IL−23R+ T cells. Several groups around the world have tried to identify such entheseal, IL-23 responsive cells, and it has been suggested that invariant-receptor natural-killer T cells (iNKT) (114), Th17 (99), mucosal-associated invariant T (MAIT) cells (107), Tγδ (103), and type 3 innate lymphoid (ILC3) cells (104) could be responsible for the IL-23 role on inflammation and osteoproliferation. Strikingly, when the inhibition of IL-23 was taken to the clinics, it did not result in any therapeutic benefit (115), suggesting that probably, there are IL-17 producing cells independently of the IL-23 status (116, 117) or that IL-23 is involved in very early steps of SpA induction (118).

Almost half of the patients with SpA have microscopical subclinical gut inflammation (119), and there is a strong association of IBD with SpA. Whether or not this relationship is involved in the foot involvement of JoSpA remains unknown. Nonetheless, the increased severity and frequency of tarsitis in developed countries is probably associated with a higher incidence of intestinal infections. The relationship between gut inflammation and arthritis or enthesitis is a challenging research topic that includes intestinal dysbiosis and cell migration (120–122). It has been suggested that some inflammatory mediators like cytokines and leukocytes can be originated in the gut, and such could be the case of ILC3 cells (104) or Tγδ cells (123).

Recently, our group described that a population of Tγδ cells expressing the gut-homing integrin a4β7 is enriched in the peripheral blood of patients with axSpA and that this population has an increased expression of TLR2 and TLR4, which might induce them to a pre-activation state and an enhanced response to pathogenic molecular patterns (123). The characteristics of this population are still unknown; nevertheless, further studies are needed to address a possible migration phenomenon.

SpA has been linked to a strong genetic predisposition (124, 125) and certain micro-organisms interplay with the immune system (126, 127), with reactive arthritis as a prototypic example, although this association is not always that clear in a significant fraction of patients. Similarly, what drives the joint-centered inflammation in this spectrum of diseases has been a longstanding enigma in the field. Previous work from our group has revealed an interplay between infections and SpA (128). Specifically, we described the presence of bacterial DNA in synovial macrophages (129) and antibody and cellular immune response against the enterobacterial heat shock protein-60 (HSP60) in blood and synovial fluid samples (127).

While many residual questions remain to date, some recent concepts have arisen that at least partially address why some joints or joint structures is typically associated with spondyloarthritis. Spondyloarthritides is notoriously known to affect enthesis, particularly those of lower limbs such as Achilles Tendons or *fascia plantaris* (130, 131). This is a feature that at least clinically is considered a hallmark of the spondyloarthritides spectrum. These clinical concepts are supported by a large body of imaging data demonstrating not only soft tissue swelling but also associated osteitis. These observations highlight the importance of the functional unit formed between muscle, tendons, the entheseal part, and the underlying bone. The tissues connecting muscle to bone (tendons) or bone to bone (ligaments) are specialized to permit the transmission of mechanical forces. Despite this, few mechanistic studies have addressed how mechanical forces may drive the onset of joint inflammation.

## MECHANICAL FORCES MIGHT DRIVE ENTHESEAL AND ARTICULAR INFLAMMATION

Healthy tendons and ligaments contain several unique cell types to ensure their homeostasis. They contain stromal cells, referred to as tenocytes, that constitute the majority of cell types within healthy tendons and ligaments. Their primary role is to control the extracellular matrix synthesis by producing collagen or degrade them by releasing proteases (132, 133). Tenocytes are notoriously mechanosensitive cells mediated at least in part by the transcription factors scleraxis (Scx) and mohawk (Mkx), which drive the expression of mechanical stress-activated genes, extracellular matrix genes (e.g., collagen) or adhesion molecules. These elements can interact with the circulating or resident immune system, and although enthesis resident immune cells are scarce, there are rare T cell subsets such as γδT cells and ILC3s, also, IL-23 producing CD14+ myeloid cells have been described (133).

The role of these immune cells in a steady state is relatively poorly understood but is thought to play a role in tendon and ligament repair. Despite the undeniable role of mechanical loading on tendon and ligament homeostasis in health, several observations have indicated that mechanical stress also leads to inflammation. Thus, healthy individuals exposed to intense physical activities (athletes, military recruits) may often develop bone marrow edema on sacroiliac joint imaging with many resemblances to acute inflammatory lesions noted in SpA patients (134, 135). Not surprisingly, mechanical stress has also been linked to inflammatory rheumatic diseases such as RA, PsA, and AS (136). Here, physical trauma has been associated with disease initiation and structural progression (137, 138).

In sum, mechanical forces display a myriad of effects on skin, tendon, and ligaments, reflecting a crosstalk between stromal and immune cells. However, there are still several outstanding questions. The threshold between normal mechanical loading and pathological stress is poorly defined, and whether mechanostress-induced inflammation in PsA reflects altered response to normal or rather exposure to supraphysiological levels of mechanical stress is currently unclear (139). Alternatively, the resolution of inflammation

induced by mechanostress may also be impaired, although the underlying mechanisms are still relatively unclear.

Anatomically, the foot has 28 bones and 31 joints into three large areas: the forefoot (metatarsals and phalanges), the mid-foot (cuboid, navicular, and cuneiforms), and the hindfoot (calcaneus and talus). The foot and the pelvis are the most important weight-bearing structures in spreading loads through the spine, lower limbs, the tarsal areas, and the foot arches. Therefore, mechanical forces could drive structural damage in a similar way to the experimental models described before.

Certainly, future studies are pointing to a potential role of mechanical stress and microtrauma on inflammation and bone formation; However, some questions remain open about the interaction of these elements in the initiation of tissue repair mechanisms and ossification, there is a need to explore if inflammation and mechanostress act as sequential factors, enhancing elements or independent pathways.

## BONE FORMATION IN SpA

Although little is known regarding the specific mechanisms of osteoproliferation in SpA, this phenomenon is probably derived from inflammation, according to radiographic studies. It has been postulated that HLA-B27 homodimers can be recognized by killer immunoglobulin-like receptors (KIR) expressed on Th17 and NK cells and that these cells can produce IL-17 mediated responses that link HLA-B27 with inflammation (91, 140). These homodimers have been found in spinal joints; nevertheless, it has not been explored if they can be found in peripheral joints.

The cytokine IL-22 is a master regulator of epidermal proliferation and barrier integrity, both in the gut and the skin; this capacity to induce proliferation is not restricted to epidermal tissues, as it has shown to interact with joint stromal cells. In the mice model of IL-23 overexpression (78), it has been reported that IL-22 (which can be either produced downstream of the IL-23 effect or independently) can induce the expression of bone growth molecules such as Akp3, Cebpb, Wnt10b, Wnt3a, and Gli1. This cytokine can also induce the mineralization and ossification of mesenchymal stem cells (141) and induce keratinocyte and fibroblast proliferation in psoriasis (142). IL-22 can become a potential therapeutic target to prevent the bone formation in the spondyloarthritides, although its effect on barrier integrity and host defense make this a difficult step.

Remarkably, there are two pathways of bone formation, endochondral and pseudomembranous ossification, and both can participate in the pathogenic bone formation of SpA patients (67, 143–145). Intense research has elucidated some pathways related to bone formation in SpA, such as the Wnt and bone morphogenetic protein (BMP) pathways (84, 146, 147). Results from the animal model of aging DBA/1 mice showed that the injection of the BMP-inhibitor nogging significantly decreases ossification and entheseal cell formation at peripheral joints. The role of BMP in peripheral SpA is also reinforced by the evidence of smad 1/5 activity in Achilles tendon's enthesis samples of SpA patients (84).

The Wnt signaling pathway is a key regulator of bone formation that can be altered in diseases like osteoarthritis (OA), rheumatoid arthritis (RA), and SpA (146). Remarkably, the Wnt inhibitor Dickkopf-1 (Dkk-1) is downregulated in patients with AS (148); therefore, a higher Wnt activity has been related to a pro-osteogenic phenotype as reviewed elsewhere (146, 149).

Sclerostin (encoded by the *sost* gene) is another Wnt inhibitor that can also inhibit BMP function (150) with an important role in SpA pathogenesis. Immunohistochemical analysis of zygapophyseal joints of SpA patients has revealed a very low sclerostin expression compared to samples from OA and RA patients or healthy subjects (151). The levels of sclerostin and anti-sclerostin antibodies can be useful as biomarkers for axial disease (152). However, there are no reports of its involvement in peripheral affection and tarsal ankylosis.

Although the question about the effect of inflammation and biologic therapy in bone formation is a controversial topic (153), preclinical studies revealed that TNF inhibitors could modulate Dkk-1 activity (148) and strikingly, recent studies suggest that the inhibition of the IL-12 and IL-23 pathway with Ustekinumab can increase Wnt activity (154).

The potential role of inflammation and mechanical stress in the onset of peripheral enthesitis is shown in **Figure 5**.

## CONCLUDING REMARKS AND FUTURE DIRECTIONS

Even though peripheral symptoms of SpA and, particularly those of juvenile-onset patients, are widely recognized, there are still many questions regarding the behavior of structural evolution, bDMARD response, association with gut dysbiosis/microbiota, and immune-mediated pathogenesis. There is a current need for more profound studies in all these fields, considering that demographic and clinical characteristics might be recognized and considered as core manifestations of the disease. In this review, we emphasized the critical role of foot affection in JoSpA patients. We attempted to focus on these manifestations as potential early diagnostic elements and on these manifestations as potential early diagnostic elements and prospective sites for translational studies.

The peripheral approach of areas in which physiopathological events occur warrants a potential site for *in-situ* study of the SpA, providing a remarkable opportunity to deepen on the mechanical triggers that influence the proliferation of what can be considered as a dynamic anatomic-functional "foot unit." The relationship between gut dysbiosis and peripheral SpA is still poorly understood. Although many reports analyze these factors separately, there is a lack of integrating elements that explain this interaction. Moreover, the effect of mechanical stress probably acts as an enhancing factor for previously primed immune and environmental elements.

## AUTHOR CONTRIBUTIONS

RB-V conceived the idea and directed the project. JR-L and RB-V revised the final version and prepared the figures. All the authors contributed equally to the writing of the manuscript.

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