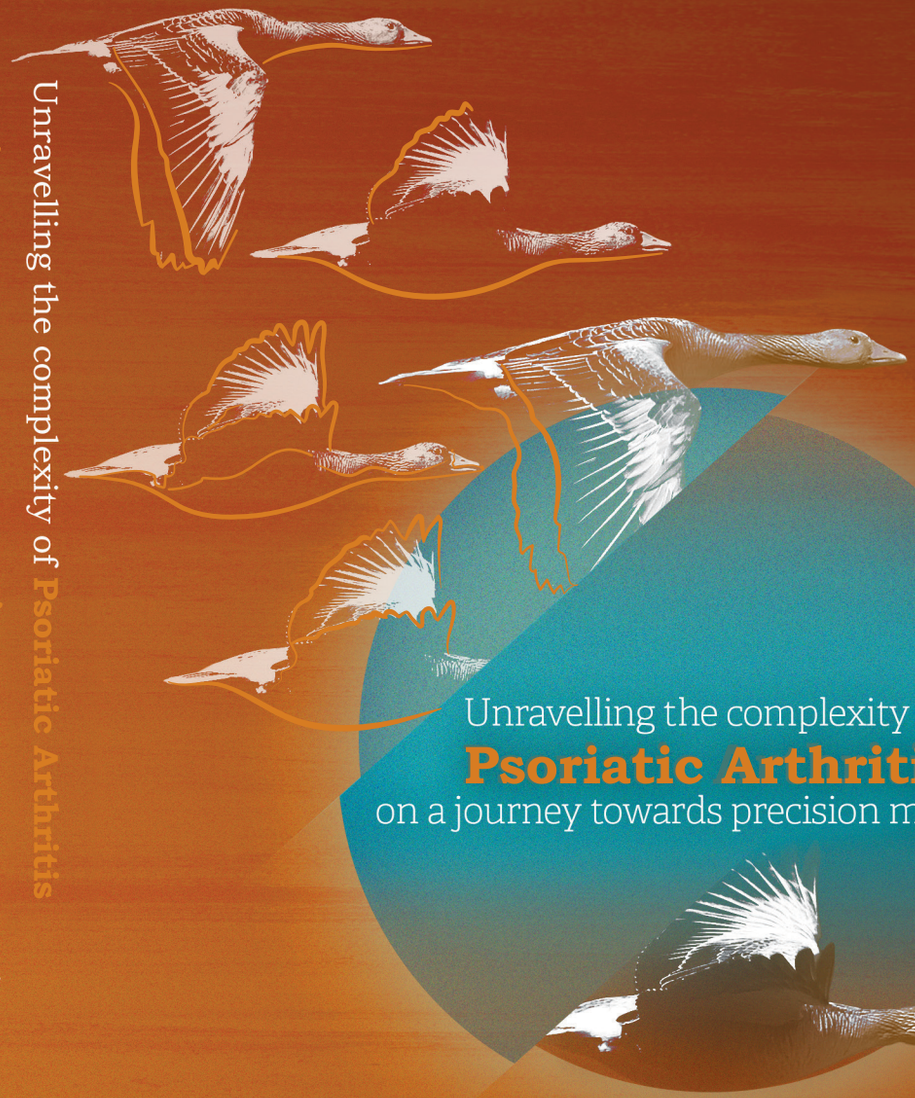
Unravelling the complexity of **Psoriatic Arthritis**

Juliëtte Nadine Pouw



Unravelling the complexity of
Psoriatic Arthritis
on a journey towards precision medicine

Juliëtte Nadine Pouw

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Unravelling the complexity of Psoriatic Arthritis on a journey towards precision medicine

Het ontrafelen van de complexiteit van Artritis Psoriatica op weg naar precisiegeneeskunde

(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
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rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 1 september 2022 des middags te 4:15 uur

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Juliëtte Nadine Pouw

geboren op 8 juli 1991
te Leiden

Promotoren:

dr. M.L. Boes
prof. dr. J.M. van Laar

Copromotoren:

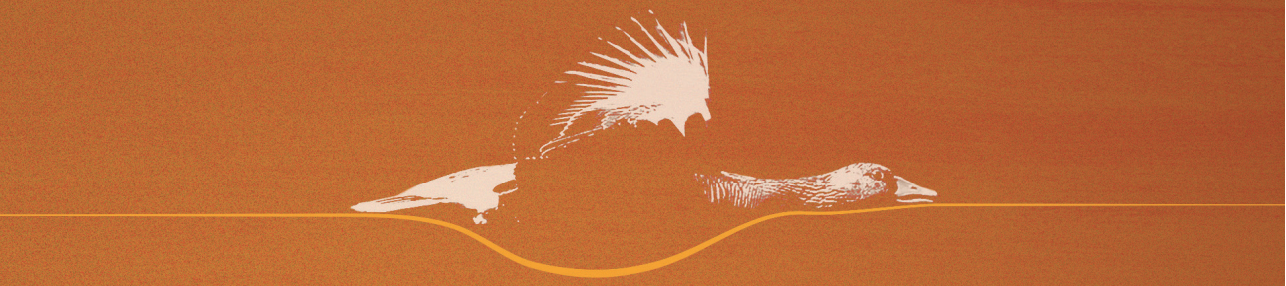
dr. E.F.A. Leijten
dr. J. Spierings

Live neither in the past nor in the future,
but let each day's work absorb your entire energies,
and satisfy your widest ambition.

– *prof. William Osler*

CONTENTS

Appendices



Chapter 1

General introduction and thesis outline

Historical perspective

The first description of psoriatic arthritis (PsA) as a distinct disease entity dates from 1956, when the British rheumatologist Professor Verna Wright described a group of patients with psoriasis and concomitant arthritis.(1) For years after its discovery PsA was considered a relatively mild form of rheumatoid arthritis (RA), with disease strictly limited to the skin and joints. However, over time it became evident that PsA is a potentially debilitating disorder characterized by significant morbidity, systemic inflammation, social stigmatization and multiple comorbidities, which substantially impacts quality of life and lifespan.(2–5) Moreover, being the second-most common form of chronic inflammatory arthritis (global prevalence 0.05-0.25%), PsA is associated with a significant economic burden in terms of psychosocial disability and productivity loss.(5–7)

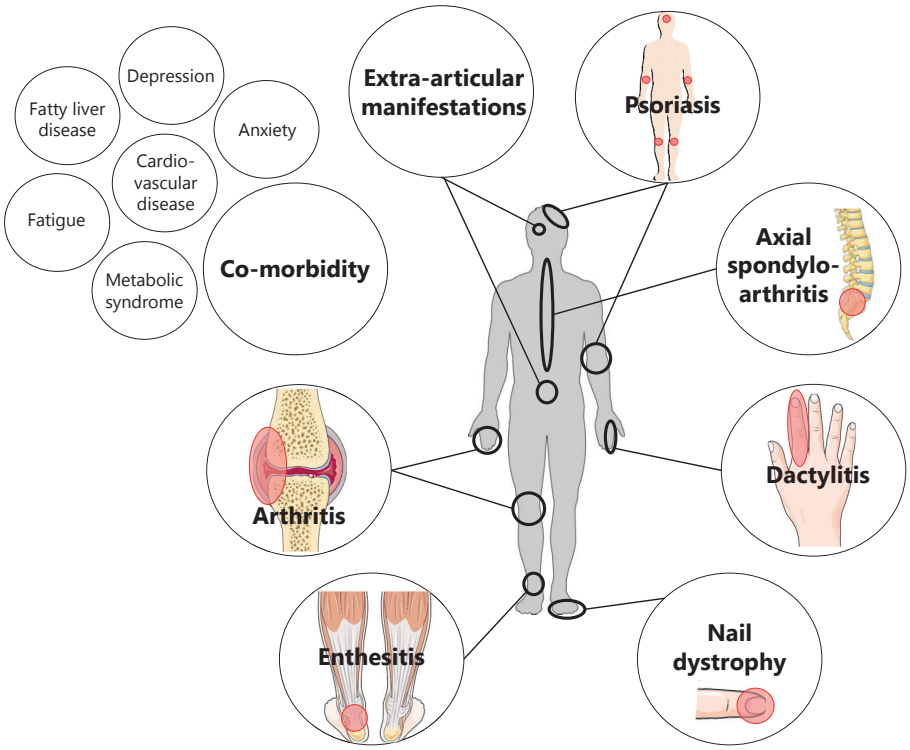


Figure 1. Spectrum of Psoriatic Arthritis. Figure created using images from http://smart.servier.com.

Clinical presentation

PsA is an inflammatory, musculoskeletal disease characterized by a heterogeneous clinical phenotype and variable disease course.(8) Most PsA patients are diagnosed at the age of 35-45.(9) In the course of their life up to 30% of psoriasis patients develop PsA, on average ten years after the onset of skin disease.(9) However, psoriasis is not always present prior to musculoskeletal symptoms: in 30% of PsA patients, arthritis precedes psoriasis, occurs simultaneously or in the absence of psoriasis ('PsA sine psoriasis').(10) The critical genetic, environmental and immunological mechanisms that contribute to transition from psoriasis to PsA remain largely unknown.(8,11–13) Patients with PsA can experience symptoms from six disease domains, multiple co-morbidities (including cardiovascular disease, fatty liver disease, metabolic syndrome, depression, anxiety and fatigue) and extra-articular manifestations (including inflammatory bowel disease and uveitis) (**Figure 1**).

The disease domains include psoriatic skin disease, axial spondyloarthritis (sacroiliitis and/or spondylitis), dactylitis (sausage digit), psoriatic nail dystrophy (discoloration, subungual hyperkeratosis, distal onycholysis, pitting), enthesitis (inflammation at insertion site into bone of tendons, ligaments and joint capsules) and peripheral arthritis affecting small and large joints.(8,13) Illustrations of clinical manifestations are included in **Figure 2**. Due to its heterogeneous phenotype diagnosis is often delayed, resulting in irreversible joint damage and functional impairment.(2) Pain and functional disability as a result of musculoskeletal disease are often thought to primarily affect quality of life. However, the severity of skin disease should not be overlooked, since psoriasis can lead to impaired self-esteem, stigmatization and can negatively affect social interactions and work productivity.(14,15)

Pathophysiology

In a nutshell

The multifactorial pathophysiology of PsA is complex and the exact sequence of events that leads to its development has not been elucidated.(8,16) Nevertheless, there is convincing evidence for PsA being primarily a T cell mediated disorder triggered by an interplay of genetic predisposition and environmental factors, that induce migration and proliferation of innate and adaptive immune cells.(8,13,16) Environmental factors associated with PsA onset include biomechanical stress, obesity and infection.(10,12,17,18)

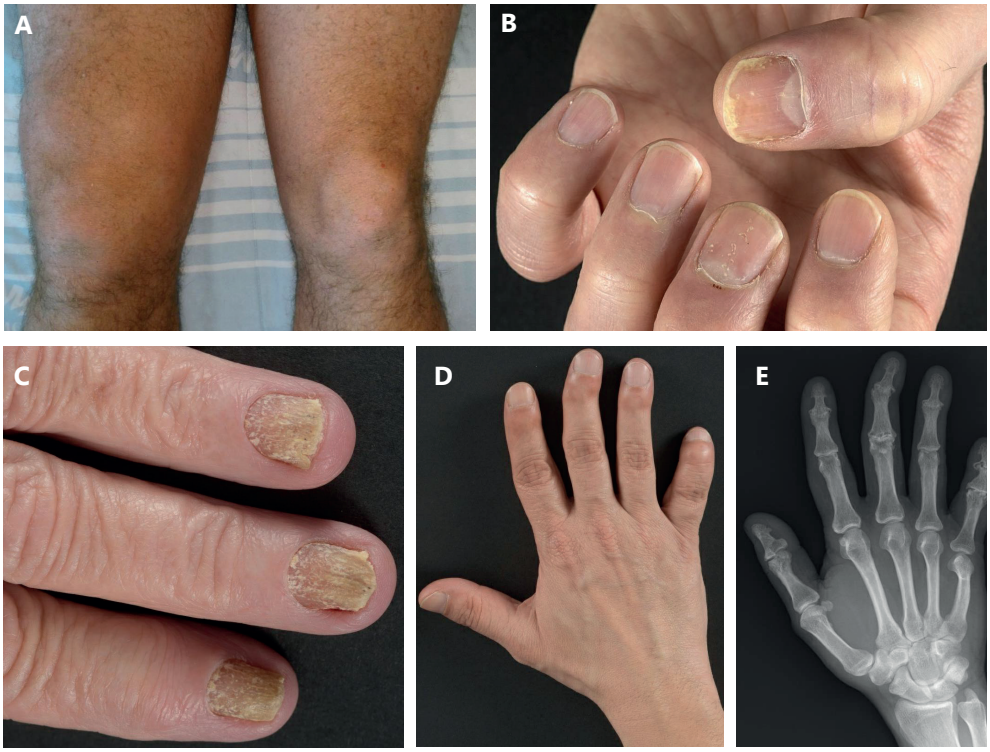


Figure 2. Clinical manifestations of Psoriatic Arthritis. (**A**) Arthritis of the right knee with suprapatellar swelling. (**B**) Subtle psoriatic nail deformities with mild distal onycholysis, oil spots and pitting of digits 1 and 3. (**C**) Severe psoriatic nail dystrophy with discoloration, subungual hyperkeratosis, distal onycholysis, pitting and crumbling of all nails. Photograph (**D**) and conventional radiograph (**E**) of the right hand of a 44 year old male with arthritis mutilans (severe and destructive type of psoriatic arthritis), characterized by erosive changes, osteophytes and joint space narrowing in proximal and distal interphalangeal joints, as well as ulnar and radiar deviation of the distal interphalangeal joints. Written informed consent of all patients was obtained at the University Medical Center Utrecht.

The importance of genetic predisposition is illustrated by the high concordance rate in twins and the strong heritability of PsA: a first-degree relative has a 30-55 times increased risk to develop the disease.(12,17,18) Genetic studies identified strong associations with class I human leukocyte antigen (HLA) susceptibility alleles.(8,19) In addition, associations in genes involved in immune activation have been observed, including interleukin (IL)-17 and interferon (IFN) signaling, the IL-23 receptor, and regulators of nuclear factor kappa B.(8,10,18)

A simplified overview of the pathogenesis is shown in **Figure 3**. PsA may develop after an initial trigger activates stromal cells at articular, peri-articular or extra-articular sites.

These triggers can induce self-reactive mechanisms, that are mediated by the innate (autoinflammation) and adaptive (autoimmunity) immune system.(10,20) Both immune systems can contribute to initiation and perpetuation of inflammation.(20) The innate system is activated through signaling molecules such as damage associated molecular patterns (DAMPs), that are released into the system by stromal cells upon stress. The adaptive system is primed by presentation of (self)antigens by antigen-presenting cells (APCs) to lymphocytes. In this process, co-stimulatory and -inhibitory signals at the contact site of APCs and T cells help orchestrate the adaptive immune response. Subsequently, activation and interaction of innate and adaptive immune systems instigates clonal expansion of T cells (T helper (Th)1, Th17 and CD8+ cytotoxic T cells) and release of pro-inflammatory substances (including the hallmark cytokines tumor necrosis factor alpha (TNF), IL-17 and IL-23). Eventually, stressed stromal cells, infiltrated immune cells and pro-inflammatory mediators all contribute to a self-perpetuating inflammatory loop that causes localized tissue damage and systemic inflammation. In that way, PsA can manifest as synovitis, synovial neogenesis, hyperkeratinization and damage to bone, entheses and cartilage.(8,10,12,13,17)

Disease models

Over recent years, research has significantly improved our understanding of PsA pathophysiology. However, one important question remains to be answered: what is the initial site of inflammation?(8) Three disease models have been proposed, that suggest initial sites of inflammation in skin, entheses and gut.(10) The first model regards dermal plasmacytoid dendritic cells (pDCs) as the key instigators. According to this model, self-nucleotides released by stressed keratinocytes induce pDCs to produce type I IFN and to present autoantigens to CD8+ T cells. pDC-derived IFN activates myeloid dendritic cells (mDCs), that start producing pro-inflammatory cytokines such as TNF, IL-12 and IL-23. These mDC-derived cytokines skew the T helper cell (Th) response in draining lymph nodes towards Th1, Th17 and Th22. Next, primed lymphocytes migrate to dermis and release TNF, IL-12, IL-17 and IL-22, that promote local hyperproliferation of keratinocytes and systemic inflammation by release into the circulation, leading to psoriatic disease.(4,10) The second model, introduced by McGonagle and colleagues, has gained traction over the years. They propose the enthesial site as the initial localization of inflammation, in response to biochemical stress or trauma. This hypothesis was tested in murine models, by administration of IL-23 at entheses, which leads to arthritis development with bone formation and erosions through an enthesial-resident IL-23R+ population of

lymphocytes, that produce TNF, IL-17 and IL-22. From the entheses, activated immune cells reach joints nearby or through circulation, giving rise to PsA disease manifestations.(21–23) The third and last model considers gut dysbiosis key for onset of PsA. Decreased abundance of specific bacteria was shown to result in reduced inhibition of Th17 cells and reduced regulatory T cell (Treg) differentiation, but evidence supporting this hypothesis is limited.(8,24,25) In summary, all models highlight the importance of a misbalanced IL-23/IL-17 axis. Which of these models is closest to the truth continues to be an important topic on the research agenda.

Immune tolerance & T regulatory cells

Although multiple disease models for PsA pathogenesis have been proposed, the exact critical immunological mechanisms that lead to PsA development are not fully understood.(8,11–13) Of particular interest are the factors that lead to reduced immune tolerance. In PsA, loss of tolerance is suggested to play a central role in pathophysiology, as strong associations with class I HLA alleles and ectopic lymphoid neogenesis with T and B cell aggregates in synovial tissues were observed. Moreover, multiple autoantibodies were identified in serum and synovial fluid, in addition to clonal expansion of memory CD8+ T cells in synovial fluid and synovium.(26–29) Thus, in PsA, the immune system somehow fails to control the fragile balance between ensuring protective immunity and preventing immunity against innocuous (self) antigens.(30,31)

Maintenance of tolerance is a continuous process, that requires tight control by central and peripheral mechanisms, including negative selection of autoreactive lymphocytes and subtle feedback regulatory mechanisms involving mutual interactions between (tolerogenic) DCs, effector T cells and Tregs.(32–35) In healthy individuals, Tregs maintain immune homeostasis by controlling DC and T cell-mediated immune responses.(30,31) Tregs are characterized by the transcription factor forkhead box protein P3 (Foxp3) and by production of inhibitory cytokines such as IL-10, IL-35 and transforming growth factor beta (TGF-β). Furthermore, Tregs ensure peripheral tolerance through cytolysis of effectors cells with granzymes and perforins, metabolic disruption of effector T cells and through inhibition of DC maturation and function.(30,31,35) Recent studies showed that in an inflammatory microenvironment Tregs can differentiate into pro-inflammatory immune cells (Treg 'instability' or 'plasticity').(31,35,36) As literature is scarce on the role of Tregs in PsA pathophysiology, the potential pathogenic implications of Treg plasticity in PsA are currently unknown.

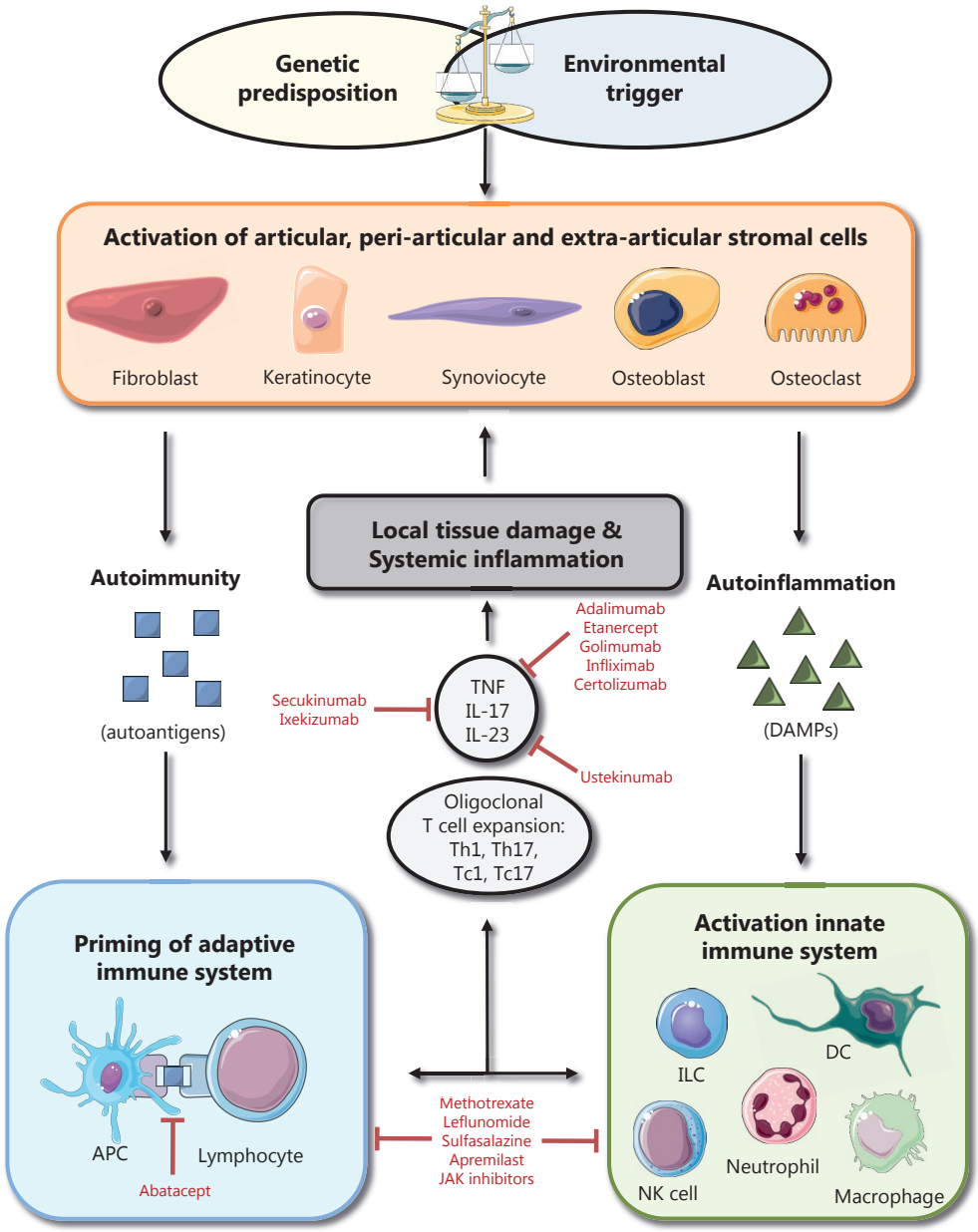


Figure 3. Schematic overview of Psoriatic Arthritis pathophysiology. Abbreviations: APC: antigen-presenting cell; DAMP: damage associated molecular pattern; DC: dendritic cell; IL: interleukin; ILC: innate-like lymphocyte; JAK: janus kinase; NK: natural killer; Tc: cytotoxic T cell; Th: T helper cell; TNF: tumor necrosis factor alpha. Figure created using images from http://smart.servier.com.

Management

Multidisciplinary team

From a dermatological perspective PsA could be considered a comorbidity of psoriasis. However, some state that psoriasis and PsA should be regarded as two phenotypes of one disease entity: psoriatic disease.(11,37–39) The rationale of the umbrella term is that clinical care for psoriatic disease patients requires similar multidisciplinary approaches, given the great overlap in risk factors, pathophysiology, comorbidities and therapeutic options.(11,27,28,40) The concept of 'psoriatic disease' may facilitate bridging the gap between the medical specialties dermatology and rheumatology.(38) In addition, the heterogeneous phenotype of psoriatic disease warrants expertise of additional healthcare professionals, including a general practitioner, clinical nurse specialist, ophthalmologist and gastroenterologist. Integrated multi-disciplinary treatment strategies streamline synchronous determination of formulation and dose of topical therapy, prescription of disease-modifying anti-rheumatic drugs (DMARDs), cardiovascular risk management and coping with psychosocial problems. Successful collaboration is essential to achieve the treatment goals in PsA: to control pain and disease activity, to prevent further joint damage, to enhance quality of life and functioning, and to prevent complications of the disease and its treatment.(5,41) The clinical benefit of multidisciplinary teamwork was confirmed in a recent study, by showing that the most optimal way to prevent delayed PsA diagnosis is to have close collaborations in specialized centers for psoriatic disease. (42) In addition, a Danish trial is ongoing to quantify the benefits of interdisciplinary combined clinical care (NCT04200690).

Therapeutic landscape

The non-pharmacological therapeutic options for PsA patients include education, psychological support, exercise and advice regarding diet and lifestyle.(41) In obese patients, weight reduction is important since multiple studies demonstrate that weight loss associates with improved disease activity, self-reported function and markers for metabolic syndrome.(43–45) Pharmacological interventions for psoriasis include topical treatment with solutions, foams, sprays, shampoos, ointments and creams containing corticosteroids, vitamin D (analogs), retinoids and/or calcineurin inhibitors. For patients with moderate-to-severe psoriasis phototherapy or DMARDs are options too.(4) Treatment of musculoskeletal symptoms is most commonly coordinated by a rheumatologist. Initial therapy consists of non-steroid anti-inflammatory drugs (NSAIDs) and intra-articular glucocorticoids.(46) Systemic glucocorticoids may be used, but only

with caution, for a short period of time, at the lowest effective dose.(46) In daily practice, systemic steroids are not frequently prescribed, which is possibly related to previous reports of dramatic psoriasis flaring upon discontinuation.(8,47) Initiation of DMARDs is indicated when either active disease persists despite the abovementioned measures, or if poor prognostic factors are present: polyarthritis, radiographic damage, structural damage, elevated acute phase reactants and extra-articular manifestations (in particular dactylitis and nail dystrophy).(41,46) Currently, the therapeutic armamentarium for treatment of PsA includes more than fifteen DMARDs, including the therapies listed in **Figure 3**. DMARDs are subdivided in three categories based on their therapeutic target. Conventional synthetics (csDMARDs) inhibit the immune system in a broader sense and are the oldest. From 2002 onwards, biologics (bDMARDs) were used to treat PsA. These are highly specific and target a specific immune pathway. Most recently, targeted synthetics (tsDMARDs) were approved, which are aimed at intracellular signal transduction of immune cells.(38)

Outline thesis

Previous research contributed to the development of innovative treatments that greatly improved quality of life for PsA patients. However, clinical care still faces several challenges that hinder clinicians to tailor medical treatment to the individual patient.(13,37,48) We address three of these challenges in eponymous parts of this thesis, and endeavor to unravel the complexity of PsA on a journey towards precision medicine.

Part I - Pathophysiologic mechanisms underlying psoriatic arthritis

First, full understanding of the diverse pathogenic mechanisms that underlie PsA and its phenotypic diversity is lacking. To that end, **Chapter 2** underlines the importance of reduced B cell tolerance and autoantibodies in PsA, which has long been considered a seronegative autoimmune rheumatic disease. We take a closer look at the loss of peripheral immune tolerance in **Chapter 3**, that focuses on the potential pathogenic role of Treg plasticity at the site of inflammation in psoriatic disease, while investigating a possible link with autoantibody production. Next, in **Chapter 4**, we study orchestration of the adaptive immune response by antigen-presenting cells through CD155/DNAM1/TIGIT signaling in psoriatic disease.

Part II - Transition from psoriasis to psoriatic arthritis

Second, the critical genetic, environmental and immune mechanisms that contribute to transition from psoriasis to PsA remain largely unknown. Moreover, there is an unmet need for prediction of PsA development in psoriasis patients, which would open up an opportunity for early treatment initiation and possibly prevention of PsA. That is why in **Chapter 5** the extent of cutaneous disease - a relatively quick and non-invasive clinical outcome - is studied as a potential predictor for PsA diagnosis and transition. From the clinics we switch to the laboratory in **Chapter 6**, where we describe our research effort to uncover proteomic signatures that drive arthritis development using a high-throughput serum biomarker platform.

Part III - Therapy response and prediction

Third, methods to select the optimal treatment for individual PsA patients are lacking. To guide an early start of effective treatment **Chapter 7** evaluates indirect evidence for csDMARD efficacy by describing first-line csDMARD monotherapy drug retention in daily clinical practice. Next, **Chapter 8** examines new developments within the field of biomarker discovery for prediction of therapeutic response in PsA.

Lastly, in **Chapter 9**, the main findings of this thesis are put in broader perspective by discussion of the results in the context of recent literature.

References

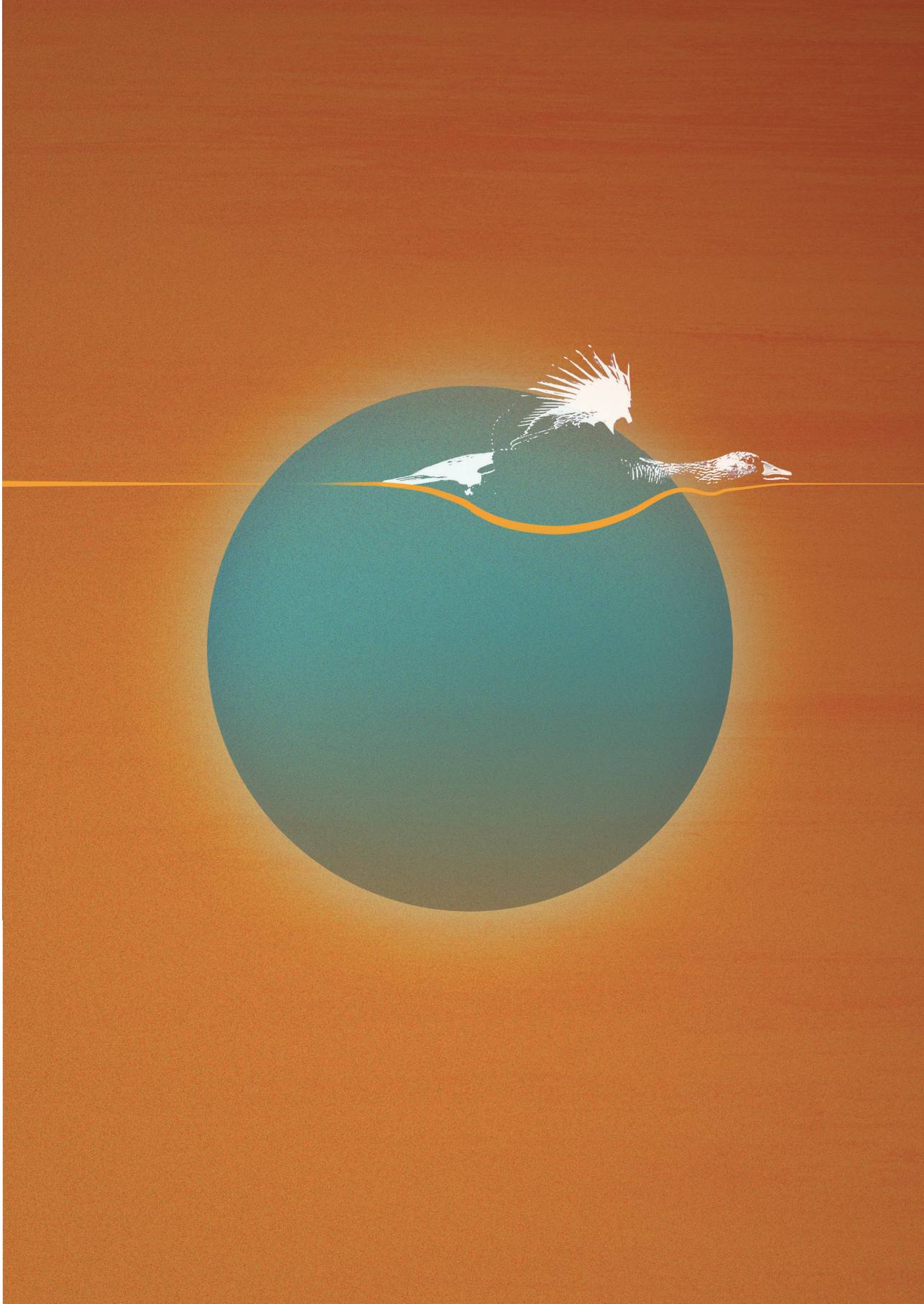
1. Wright V. Psoriasis and arthritis. Ann Rheum Dis. 1956;15:348–56.
2. Haroon M, FitzGerald O. Psoriatic arthritis: complexities, comorbidities and implications for the clinic. Expert Rev Clin Immunol. 2016;12:405–16.
3. Ogdie A, Yu Y, Haynes K, Love TJ, Maliha S, Jiang Y, et al. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. Ann Rheum Dis. 2015;74:326–32.
4. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, et al. Psoriasis. Nat Rev Dis Prim. 2016;2.
5. Haberman R, Perez-Chada LM, Merola JF, Scher J, Ogdie A, Reddy SM. Bridging the Gaps in the Care of Psoriasis and Psoriatic Arthritis: the Role of Combined Clinics. Curr Rheumatol Rep. 2018;20:76.
6. Ogdie A, Weiss P. The Epidemiology of Psoriatic Arthritis. Rheum Dis Clin North Am. 2015;41:545–68.
7. McHugh N, Maguire Á, Handel I, Tillett W, Morris J, Hawkins N, et al. Evaluation of the economic burden of psoriatic arthritis and the relationship between functional status and healthcare costs. J Rheumatol. 2020;47:701–7.
8. FitzGerald O, Ogdie A, Chandran V, Coates LC, Kavanaugh A, Tillett W, et al. Psoriatic arthritis. Nat Rev Dis Prim. 2021;7.
9. Gladman DD, Antoni C, Mease P, Clegg DO, Nash O. Psoriatic arthritis: Epidemiology, clinical features, course, and outcome. Ann Rheum Dis. 2005;64:14–7.
10. Ritchlin C, Colbert R, Gladman D. Psoriatic Arthritis. New Engl J Med. 2017;10:957–70.
11. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. Autoimmun Rev. 2017;16:10–5.
12. Boehncke WH, Qureshi A, Merola JF, Thaçi D, Krueger GG, Walsh J, et al. Diagnosing and treating psoriatic arthritis: An update. Br J Dermatol. 2014;170:772–86.
13. Veale DJ, Fearon U. Psoriatic arthritis 1 The pathogenesis of psoriatic arthritis. Lancet. 2018;391:2273–84.
14. De Korte J, Sprangers MAG, Mombers FMC, Bos JD. Quality of life in patients with psoriasis: A systematic literature review. J Investig Dermatology Symp Proc. 2004;9:140–7.
15. Du Thanh A, Girard C, Morel J, Roubille C. Toward a holistic management of skin and joint disorders in PsA patients. Rheumatology (Oxford). 2021;keab889:[In press].
16. Furst DE, Belasco J, Louie JS. Genetic and inflammatory factors associated with psoriatic arthritis: Relevance to diagnosis and management. Clin Immunol. 2019;202:59–75.
17. Zeng J, Luo S, Huang Y, Lu Q. Critical role of environmental factors in the pathogenesis of psoriasis. J Dermatol. 2017;44:863–72.
18. Zhang A, Kurtzman DJB, Perez-Chada LM, Merola JF. Psoriatic arthritis and the dermatologist: An approach to screening and clinical evaluation. Clin Dermatol. 2018;36:551–60.
19. Winchester R, Fitzgerald O. MHC class i associations beyond HLA-B27: The peptide binding hypothesis of psoriatic arthritis and its implications for disease pathogenesis. Curr Opin Rheumatol. 2020;32:330–6.
20. McGonagle D, McDermott MF. A Proposed Classification of the Immunological Diseases. PLoS Med. 2006;3:e297.

21. McGonagle D, Gibbon W, Emery P. Classification of inflammatory arthritis by enthesitis. Lancet. 1998;352:1137–40.
22. Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J, et al. IL-23 induces spondyloarthropathy by acting on ROR-γt+ CD3+ CD4- CD8- entheseal resident T cells. Nat Med. 2012;18:1069–76.
23. Reinhardt A, Yevsa T, Worbs T, Lienenklaus S, Sandrock I, Oberdörfer L, et al. Interleukin-23–Dependent γ/δ T Cells Produce Interleukin-17 and Accumulate in the Enthesis, Aortic Valve, and Ciliary Body in Mice. Arthritis Rheumatol. 2016;68:2476–86.
24. Gilis E, Mortier C, Venken K, Debusschere K, Vereecke L, Elewaut D. The Role of the Microbiome in Gut and Joint Inflammation in Psoriatic Arthritis and Spondyloarthritis. J Rheumatol Suppl. 2018;94:36–9.
25. Myers B, Brownstone N, Reddy V, Chan S, Thibodeaux Q, Truong A, et al. The gut microbiome in psoriasis and psoriatic arthritis. Best Pract Res Clin Rheumatol. 2019;33:101494.
26. FitzGerald O, Haroon M, Giles JT, Winchester R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. Arthritis Res Ther. 2015;17:115.
27. Chimenti MS, Triggianese P, Nuccetelli M, Terracciano C, Crisanti A, Guarino MD, et al. Auto-reactions, autoimmunity and psoriatic arthritis. Autoimmun Rev. 2015;14:1142–6.
28. Generali E, Bose T, Selmi C, Voncken JW, Damoiseaux JGMC. Nature versus nurture in the spectrum of rheumatic diseases: Classification of spondyloarthritis as autoimmune or autoinflammatory. Autoimmun Rev. 2018;17:935–41.
29. Penkava F, Velasco-Herrera MDC, Young MD, Yager N, Nwosu LN, Pratt AG, et al. Single-cell sequencing reveals clonal expansions of pro-inflammatory synovial CD8 T cells expressing tissue-homing receptors in psoriatic arthritis. Nat Commun. 2020;11:1–11.
30. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008;8:523–32.
31. Guo J, Zhou X. Regulatory T cells turn pathogenic. Cell Mol Immunol. 2015;12:525–32.
32. La Cava A. Putting together the autoimmunity puzzle. J Clin Invest. 2015;125:2184–6.
33. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest. 2015;125:2228–33.
34. Conigliaro P, Chimenti MS, Triggianese P, Sunzini F, Novelli L, Perricone C, et al. Autoantibodies in inflammatory arthritis. Autoimmun Rev. 2016;15:673–83.
35. Shevyrev D, Tereshchenko V. Treg Heterogeneity, Function, and Homeostasis. Front Immunol. 2020;10:1–13.
36. Mijnheer G, Van Wijk F. T-cell compartmentalization and functional adaptation in autoimmune inflammation: Lessons from pediatric rheumatic diseases. Front Immunol. 2019;10:1–11.
37. Van Den Bosch F, Coates L. Psoriatic arthritis 2 Clinical management of psoriatic arthritis. Lancet Rheumatol. 2018;391:2285–94.
38. Jadon DR, Stober C, Pennington SR, FitzGerald O. Applying precision medicine to unmet clinical needs in psoriatic disease. Nat Rev Rheumatol. 2020;16:609–27.
39. Boehncke WH. Psoriasis and psoriatic arthritis: Flip sides of the coin? Acta Derm Venereol. 2016;96:436–41.
40. Chimenti MS, Caso F, Alivernini S, De Martino E, Costa L, Tolusso B, et al. Amplifying the concept of psoriatic arthritis: The role of autoimmunity in systemic psoriatic disease. Autoimmun Rev. 2019;18:565–75.

41. Vivekanantham A, McGagh D, Coates LC. Current treatments and recommendations for Psoriatic Arthritis. Best Pract Res Clin Rheumatol. 2021;35:101680.
42. Ziob J, Behning C, Brossart P, Bieber T, Wilsmann-Theis D, Schäfer VS. Specialized dermatological-rheumatological patient management improves diagnostic outcome and patient journey in psoriasis and psoriatic arthritis: a four-year analysis. BMC Rheumatol. 2021;5:1–8.
43. Klingberg E, Bilberg A, Björkman S, Hedberg M, Jacobsson L, Forsblad-d'Elia H, et al. Weight loss improves disease activity in patients with psoriatic arthritis and obesity: an interventional study. Arthritis Res Ther. 2019;21:17.
44. Klingberg E, Björkman S, Eliasson B, Larsson I, Bilberg A. Weight loss is associated with sustained improvement of disease activity and cardiovascular risk factors in patients with psoriatic arthritis and obesity: a prospective intervention study with two years of follow-up. Arthritis Res Ther. 2020;22:1–13.
45. Weijers JM, Müskens WD, Van Riel PLCM. Effect of significant weight loss on disease activity: Reason to implement this non-pharmaceutical intervention in daily clinical practice. RMD Open. 2021;7:1–4.
46. Gossec L, Baraliakos X, Kerschbaumer A, De Wit M, McInnes I, Dougados M, et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. Ann Rheum Dis. 2020;79:S700–12.
47. Ritchlin CT, Kavanaugh A, Gladman DD, Mease PJ, Helliwell P, Boehncke WH, et al. Treatment recommendations for psoriatic arthritis. Ann Rheum Dis. 2009;68:1387–94.
48. Pitzalis C, Choy EHS, Buch MH. Transforming clinical trials in rheumatology: towards patient-centric precision medicine. Nat Rev Rheumatol. 2020;16:590–9.

PART I

Pathophysiologic mechanisms
underlying Psoriatic Arthritis



Chapter 2

Revisiting B cell tolerance and autoantibodies in seropositive and seronegative autoimmune rheumatic disease (AIRD)

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Abstract

Autoimmune rheumatic diseases (AIRD) are categorized seropositive or seronegative, dependent upon the presence or absence of specific autoreactive antibodies, including rheumatoid factor and anti-citrullinated protein antibodies. Autoantibody-based diagnostics have proved helpful in patient care, not only for diagnosis but also for monitoring of disease activity and prediction of therapy responsiveness. Recent work demonstrates that AIRD patients develop autoantibodies beyond those contained in the original categorization. In this study we discuss key mechanisms that underlie autoantibody development in AIRD: defects in early B cell development, genetic variants involved in regulating B cell and T cell tolerance, environmental triggers and antigen modification. We describe how autoantibodies can directly contribute to AIRD pathogenesis through innate and adaptive immune mechanisms, eventually culminating in systemic inflammation and localized tissue damage. We conclude by discussing recent insights that suggest distinct AIRD have incorrectly been denominated seronegative.

Manuscript

Introduction

Autoimmune rheumatic diseases (AIRD) are heterogeneous musculoskeletal disorders accompanied by substantial morbidity and mortality. AIRD mainly, although not exclusively, affect joints and muscles and are characterized by the presence of specific autoantibodies.(1) Traditionally, AIRD are classified as 'seropositive' or 'seronegative', according to whether or not autoantibodies are a known important feature.(2) Examples of seropositive AIRD include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), primary Sjögren's syndrome (pSS) and idiopathic inflammatory myopathies (IIM).(3) Importantly, although RA is classified within the seropositive category, approximately 30% of patients lack the presence of the classic autoantibodies rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) and have 'seronegative RA'.(4)

Autoantibodies are secreted immunoglobulins (Igs) of isotype IgM, IgG, IgA or IgE.(5) A loss of immune tolerance to self-antigens, including nucleic acids, lipids, proteins and tissue-specific antigens, can elicit autoantibody production.(6) Autoantibodies can be directed against self-antigens inside the nucleus, in the cytoplasm, at the cell surface and extracellular products.(6,7) It has become increasingly evident that autoantibodies contribute to AIRD pathogenesis by promoting systemic inflammation as well as local tissue damage, involving both innate and adaptive immune mechanisms.(6,8,9) Although AIRD share pathophysiological mechanisms through which self-reactive antibodies cause damage, the pathogenicity of autoantibodies varies per antibody isotype, target antigen and clinical disease phase.(6,9)

Extensive investigation is ongoing to improve our understanding of how autoantibodies are generated and through which mechanisms autoantibodies initiate and perpetuate disease. This holds true even for traditionally classified seronegative AIRD, including psoriatic arthritis (PsA), ankylosing spondylitis (AS), seronegative RA and reactive arthritis. (10,12) In this study we provide a literature overview of how loss of B cell tolerance results in autoantibody production in AIRD, and discuss the contribution of genetic predisposition, immune mechanisms and environmental factors. Next, we describe overarching innate and adaptive immune mechanisms that induce systemic inflammation and localized tissue damage. Finally, we revisit the diseases that were previously classified as seronegative AIRD in light of recently discovered autoantibody specificities that allow further differentiation of AIRD groups.

AIRD	Prevalence [a]	Reference	n	Autoantibody	Positive before diagnosis (% patients) [b]	Time from detection to diagnosis (years)	
						mean / median [c]	upper limit
SLE	24/100.000	Arbuckle 2003 (115)	130	ANA	78	3.0	9.2
				Anti-dsDNA	55	2.2	9.3
				Anti-RNP	26	0.9	7.2
RA	860/100.000	Majka 2008 (123)	83	ACPA	61	5.4	13
				RF	57	6.0	14
SSc	4/100.000	Burbelo 2019 (114)	46	≥1 including anti-Topo1, -RNAP III, centromere proteins, -Scl-75, -Scl-100	52	7.4	27.1
pSS	14/100.000	Theander 2015 (120)	117	ANA	n.r.	4.6	18.8
				SS-A	n.r.	4.5-5.1	18.8
				SS-B	n.r.	3.5	16.1
				≥1	75	n.r.	19.5
IIM	5.1/100.000	Miller 1990 (116)	1	Anti-Jo	100	-	0.4
		Abe 2017 (122)	105	Anti-MDA5	2	n.r.	2
		Targoff 1992 (124)	5	Anti-EJ	20	-	0.33
		Vulsteke 2020 (118)	1	Anti-Mi-2	100	-	0.25

Table 1. Autoantibodies can be measured years before AIRD diagnosis (selected studies). [a] Cooper G. S., Stroehla B. C.(3) For IIM pooled prevalence shown of polymyositis and dermatomyositis. [b] Interpret results with care, as most reported studies are case–controls performed in either confirmed patients or patients with risk factors for AIRD. [c] Mean time reported in studies by Arbuckle and Burbelo. Median time reported in studies by Majka and Theander. No mean or median reported because autoantibodies were detected only in one patient before diagnosis in studies by Miller, Targoff and Vulsteke. Abbreviations: ACPA = anti-citrullinated peptide antibodies; AIRD: autoimmune rheumatic disease; anti-EJ: anti-gylcyl antibody; anti-Jo-1 = anti-histidyl-tRNA synthetase antibody; anti-MDA5 = anti-melanoma differentiation-associated gene 5 antibody; anti-RNAP = RNA polymerase; anti-Scl = anti-scleroderma antibody; anti-Sm = Smith antibody; anti-topoI = topoisomerase I antibody = anti-Scl-70 antibody; dsDNA: double-stranded deoxyribonuclease; Ig = immunoglobulin; IIM: idiopathic inflammatory myopathy; Mi-2 = Mi-2 nuclear antigen antibody; *n* = number of patients that participated in the study; n.r. = not reported in published data; pSS = primary Sjögren's syndrome; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erythematosus; SS-A = Sjögren's syndrome antigen A = anti-Ro antibody; SS-B = Sjögren's syndrome antigen B = anti-La antibody; SSc = systemic sclerosis.

History

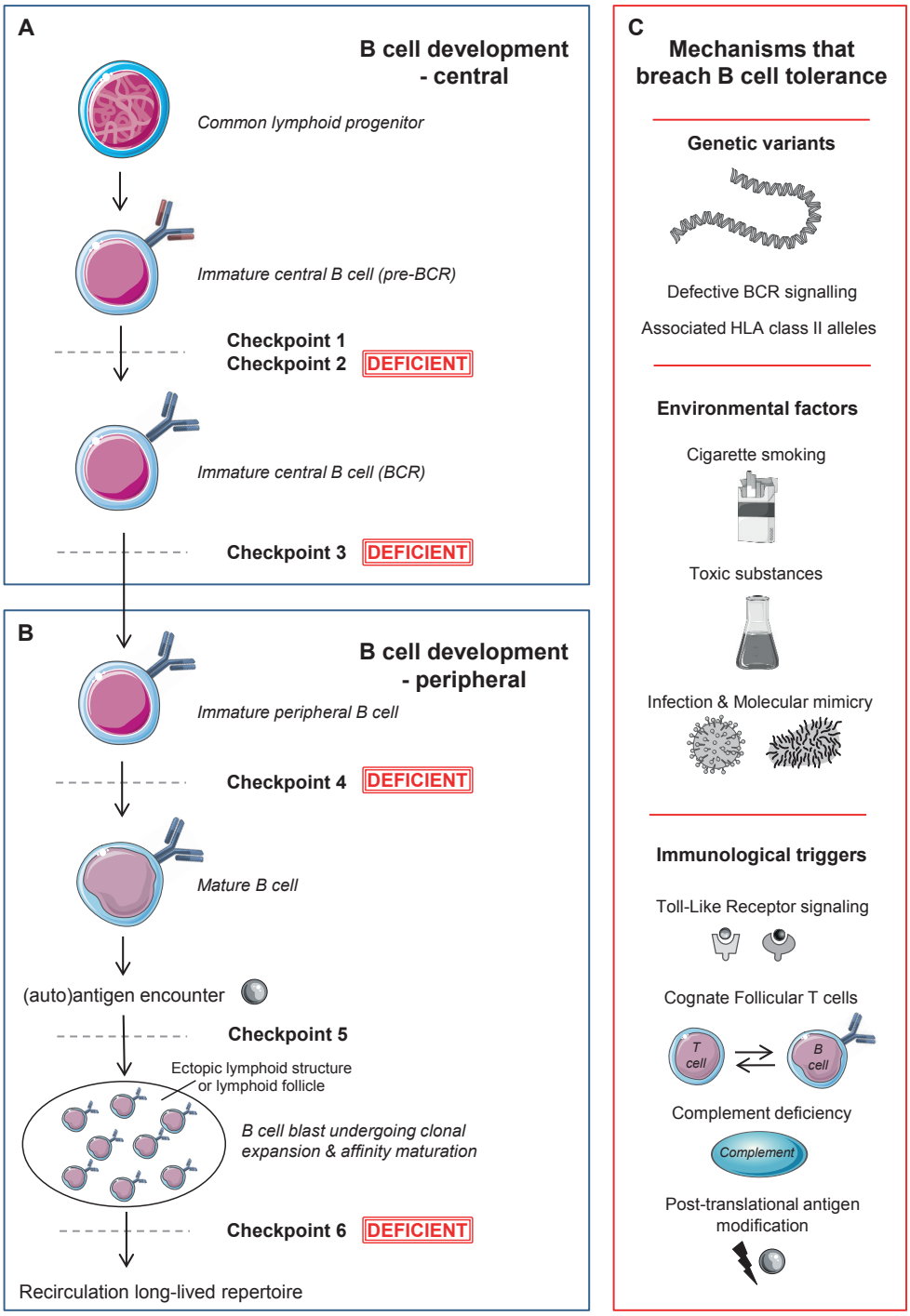
The discovery of antibodies dates back to the 19th century when Emil von Behring and Shibasabura Kitasato, in 1890, used serum from immunized animals to cure animals suffering from diphtheria.(13) Another key insight came from Julius Donath and Karl Landsteiner, who found that under cold circumstances blood components in serum from

paroxysmal cold hemoglobinuria patients could break down their own (or other human) erythrocytes.(14) The concept of autoantibodies was born. The first report on self-reactive antibodies in AIRD was published in 1940, when Erik Waaler discovered the presence of RF in a patient with RA.(15) RF, an immunoglobulin directed towards the Fc part of IgG, would later instigate a major leap in this field of research as a clinically relevant diagnostic and prognostic biomarker. Since then, technical advances have improved the detection rate of disease-specific autoantibodies, resulting in the wide array of AIRD-associated autoantibodies with various clinical implications known today.(8)

Antibodies and B cell tolerance

Effective immune protection requires a broad, diverse and specific antibody repertoire.(16,17) Initially, all naive B cells express an unique IgM-type antigen receptor at the cell surface. After productive encounter of antigen, the naive B cell repertoire is refined by somatic hypermutation of the variable regions of heavy and light chain gene loci of the B cell antigen receptor and by class-switch recombination to produce IgA, IgG and IgE isotypes.(16,17) The resultant refined antibody repertoire enables recognition of a wide range of epitopes with high affinity, but has the inherent risk of recognizing innocuous self-antigens.(16-18) Notably, a substantial part of the antibody repertoire of healthy individuals shows some level of self-reactivity.(19,20) Through incompletely understood mechanisms, the retention of a confined number of proto-autoreactive naive B cells can improve the protective antibody response against foreign antigens. One mechanism was suggested from mouse-based studies.(21) Here, transgenic B cells producing antibodies cross-reactive to a foreign and self-antigen underwent anergy upon encounter of self-antigen, but upon encounter of high-density foreign antigen increased its foreign-specific affinity by directed hypermutation and selection.(21) However, to prevent excessive amounts of autoreactive B cells that may contribute to autoimmune disease, the immune system has incorporated checkpoints during central and peripheral B cell development to ensure that the number of proto-autoreactive B cells gradually decreases during maturation.(6,16) Importantly, this negative selection cannot be too stringent, because this would result in a limited antibody diversity unable to recognize all potential noxious antigens.

Despite the mechanisms of the immune system to ensure B cell tolerance, most AIRD are characterized by high titers of serum autoantibodies.(18) Autoantibody production is explained by a multi-factorial process that involves the failure of the immune system to both eliminate and control autoreactive B cells. Mechanisms that contribute to this loss of tolerance include the persistence of autoreactive B cells through defective central


A
B cell development
- central
Common lymphoid progenitor
Immature central B cell (pre-BCR)
Checkpoint 1
Checkpoint 2
DEFICIENT
Immature central B cell (BCR)
Checkpoint 3
DEFICIENT
B
B cell development
- peripheral
Immature peripheral B cell
Checkpoint 4
DEFICIENT
Mature B cell
(auto)antigen encounter
Checkpoint 5
Ectopic lymphoid structure
or lymphoid follicle
B cell blast undergoing clonal
expansion & affinity maturation
Checkpoint 6
DEFICIENT
Recirculation long-lived repertoire
C
Mechanisms that
breach B cell tolerance
Genetic variants
Defective BCR signalling
Associated HLA class II alleles
Environmental factors
Cigarette smoking
Toxic substances
Infection & Molecular mimicry
Immunological triggers
Toll-Like Receptor signaling
Cognate Follicular T cells
T cell
B cell
Complement deficiency
Complement
Post-translational antigen
modification

◂ **Figure 1. Breaches in B cell tolerance that contribute to autoantibody production in AIRD.** Breaches in B cell tolerance are generated if the immune system fails to eliminate and control (proto)autoreactive B cells. Involved in this multi-factorial process are deficient B cell development checkpoints (A,B) and additional mechanisms that breach B cell tolerance (C). (**A**) Central checkpoints of B cell development in bone marrow include positive selection of cells with a functional pre-B cell receptor (BCR) (checkpoint 1), negative selection of immature B cells with an autoreactive pre-BCR (checkpoint 2) and immunoglobulin (Ig) receptor ligand-mediated apoptosis of immature B cells with an autoreactive BCR (checkpoint 3). (**B**) Peripheral checkpoints include apoptosis of immature proto-autoreactive B cells in the spleen (checkpoint 4), anergy and follicular exclusion of mature proto-autoreactive B cells upon autoantigen encounter (checkpoint 5) and prevention of recirculation of autoreactive B cells that emerged after somatic hypermutation in secondary lymphoid organs (checkpoint 6). (**C**) Additional mechanisms that contribute to a breach in B cell tolerance are the genetic predisposition, environmental factors and immunological triggers. Abbreviations: HLA = human leukocyte antigen. Figure created using images from http://smart.servier.com.

and peripheral B cell development checkpoints (see section below: B cell development: transition through checkpoints), genetic predisposition (see section below: Genetic predisposition), environmental factors (see section below: Environmental factors) and immunological mechanisms, including an important role for cognate T cells (see section below: Immunological triggers) (**Figure 1**).(6,22,23)

B cell development: transition through checkpoints

B cells develop from common lymphoid progenitors in a stepwise fashion to establish a repertoire of cells that is mainly non-self-reactive. For a detailed review of this subject we refer to published work.(16) Defects in the process of B cell development and maturation can contribute to the pathogenesis of autoantibody production in AIRD.(24,25) In AIRD, increased numbers of proto-autoreactive mature naive B cells, that have the potential to produce self-reactive antibodies, persist after key developmental stages (indicated as checkpoints 2, 3 and 4 in **Figure 1**).(18,20,26) A study conducted in SLE patients describes that 20–50% of mature naive B cells produce self-reactive antibodies, compared to 5–20% in healthy controls.(26) In an RA study these percentages were 35–52% in RA versus 20% in healthy controls.(24) These studies, although modest in size, underscore a contribution to autoantibody production of defective central B cell receptor (BCR) signaling in bone marrow and impaired receptor editing.(18,20,26-28)

After checkpoint 4, naive B cell antigen exposure results in activation which requires help from cognate CD4+ T follicular helper (Tfh) cells to mount T cell-dependent high-affinity antibody responses and memory.(29,30) At this developmental stage, proto-autoreactive naive B cells become anergic upon autoantigen encounter and are excluded from migration into lymphoid follicles, which results in rapid cell death.(31,32) These processes comprise checkpoint 5, and depend upon both continuous exposure to self-antigen and competition for the follicle between autoreactive and 'normal' competitor mature B cells,

which have different specificities.(32,33) Next, the activated B cell undergoes class-switch recombination via which the constant region of the antibody is substituted by the class-switched Ig isotype.(34) Subsequently, the activated B cell migrates away from the T–B cell border, becomes a B cell blast and gives rise to a germinal center (GC), supporting clonal proliferation, somatic hypermutation and selection of higher-affinity B cell clones.(29)

An important feature of autoantibody production in AIRD is that it is not strictly limited to GCs in follicular regions of secondary lymphoid tissues. Extrafollicular antibody-forming cells and ectopic lymphoid structures (ELS) were discovered in the synovium in RA, in salivary and lacrimal glands in pSS, in kidneys in SLE and in a small minority of muscles of dermato- and polymyositis patients.(35-39) These ELS are characterized by GC-resembling organized lymphoid aggregates that contain autoantibody-producing plasmablasts and even long-lived plasma cells, normally only present in the bone marrow.(40) Several studies have reported associations of ELS with autoantibody titer, antibody status, circulating inflammatory cytokines and disease severity, suggesting their implication in the perpetuation of disease within target organs.(38)

The sixth and last-known checkpoint ensures that no autoreactive cells emerge after somatic hypermutation. These cells are prevented from recirculation in the long-lived repertoire by undergoing apoptosis.(6,41) Data support that autoreactive B cells in AIRD arise despite the multiple checkpoints discussed.(6,42) In SLE, for example, immunoglobulins produced by memory B cells are highly reactive compared to germline-encoded antibodies. These highly reactive antibodies can only have resulted from affinity maturation after antigen encounter.(43) Furthermore, high-specificity IgG anti-phospholipid antibodies show accumulation of mutations, suggesting affinity maturation.(44) Also, the high-affinity binding of autoantibodies to nucleosomes and anti-dsDNA is acquired by somatic hypermutation.(42) In RA, evidence for the persistence of autoreactive B cells after the last checkpoint comes from highly somatically mutated ACPA-producing IgG secreting cells from synovial fluid, indicative of past encounter with autoantigens.(45) Altogether, failure to eliminate (proto) autoreactive B cells at these B cell developmental checkpoints is suggested to contribute to autoantibody production in AIRD.(16)

Beyond checkpoints

Genetic predisposition

Genetic variants in products regulating B and T cell peripheral immune tolerance can contribute to a breach in B cell tolerance that contributes to generation of autoantibodies

(**Figure 1C**). These variants include molecules of the antigen presentation machinery. (4,6) Autoantibody status and titers in AIRD are associated with specific human leukocyte antigen (HLA) class II haplotypes and several non-HLA genes. The association with HLA class II supports an important role of a T cell-dependent antigen-driven response in autoantibody production. This is because B cells, after internalization and processing of BCR-bound antigen, act as professional antigen-presenting cells for CD4+ T cells. B cells present antigens to T cells as peptide–HLA complexes (class II HLA-DR, -DP, -DQ) on the B cell surface that interact with the α/β T cell receptor on the T cell surface.(46,47) This interaction activates cognate CD4+ T cells that, in turn, allow the furthering of an antigen-specific B cell response to linked epitopes.(48) In RA, HLA-DRB1 alleles that code a 'shared epitope' – an amino acid sequence QKRAA, QRRAA or RRRAA in residues 70-74 of HLA-DRβ chain – are strongly associated with ACPA production.(49,50) Moreover, in SLE and pSS, multiple autoantibodies are strongly correlated with specific DR and DQ haplotypes. (51,52) In myositis, one of the well-known associations is that of the DRB1*03 haplotype with anti-Jo1 production.(53) Lastly, in SSc, DPB1*13:01 and DRB1*07:01 alleles are strongly associated with anti-topoisomerase and -centromere status.(54)

In addition to HLA molecules, additional known gene variants contribute to changes in B cell tolerance. These include molecules involved in BCR downstream signaling, antigen processing, lymphocyte proliferation and differentiation and clearance pathways of apoptotic material.(4,6,37) In SLE, SSc and RA, variants in gene products involved in BCR signaling pathways were shown to associate with autoreactive B cell development.(18,55) Furthermore, abnormalities in genes encoding proteins involved in removal of self-antigens from the extracellular milieu, or sensing the presence of RNA and DNA in endosomes, have been implicated in AIRD development.(56,57) Single nucleotide polymorphisms in genes coding for Toll-like receptors (TLR) – a family of pattern recognition receptors that recognize a wide range of pathogen-associated molecular patterns (PAMPs) and are expressed by stromal cells, B cells, dendritic cells and macrophages – even correlate with the pathogenesis of AIRD, including SLE, RA and SSc (further discussed below, in the Immunological triggers section).(58)

Environmental factors

The association of specific environmental factors with the autoantibody response in AIRD suggests their contribution to loss of B cell tolerance (**Figure 1C**). One example is cigarette smoking, which associates with ACPA positivity in RA, with autoantibody development in myositis and with anti-topoisomerase I positivity in SSc.(53,59,60) Moreover, exposure

to different toxic substances has been associated with autoantibodies, such as the association of silica with significantly higher ANA levels in murine SLE models.(61) Infection with pathogens such as bacteria or viruses should also be included as an environmental factor associated with autoantibody response. Infections can induce a breach in B cell tolerance in at least three ways: first, via direct actions of the invading pathogen. In RA, for example, studies showed that oral P. gingivalis infection induces the citrullination of proteins, thereby generating neoepitopes on self-antigens that trigger autoantibody production.(62) A second mechanism is molecular mimicry: an immune response initially directed towards a pathogen is perpetuated because of cross-reactivity with foreign and self-antigens.(63,64) Molecular mimicry was reported, for example, in SLE for dsDNA with a dominant pneumococcal cell wall hapten, and also for SS-A (anti-Ro) antibodies with a latent viral protein Epstein-Barr virus (EBV) nuclear antigen-1.(63,64) In RA, EBV and human endogenous retrovirus K have been suggested to share multiple epitopes with self-antigens to induce cross-reactivity, resulting in autoantibody production against interleukin (IL)-2 and fibrin.(65,66) Thirdly, autoreactive B cells can be directly prompted to proliferate and produce autoantibodies through innate stimulation, after encounter of PAMPs expressed by pathogens.(67) Ligation of innate receptors by PAMPs is also suggested to stimulate autoantibody production in RA indirectly through synovial fibroblasts.(68) When stimulated with TLR-3 ligand poly(I:C), for example, RA fibroblast-like synoviocytes induced class-switch recombination of RA patient B cells but not B cells from healthy individuals.(68) This supports the role of TLR-stimulated synoviocytes in promoting immunoglobulin class-switch in RA synovium.

Immunological triggers

In addition to genetic variants and environmental factors, immunological triggers may contribute to a breach in B cell tolerance, resulting in autoantibody production in AIRD (**Figure 1C**). The most important triggers are discussed below. A carefully orchestrated collaboration between T and B cells is essential for an effective and rapid affinity-matured protective antibody response. However, T and B cell interaction can also cause harm through triggering autoimmune responses.(69) The important role for cognate CD4+ T cells in AIRD is underlined by the strong association of class II HLA alleles with affinity-matured autoantibodies, supporting a role for antigen presentation via specific HLA alleles by dendritic cells (DCs) and B cells to cognate CD4+ T helper cells in the autoantibody response. In pSS, SSc, SLE and RA increased Tfh frequencies are reported, which are specialized CD4 T cells that control B cell proliferation, isotype-switch and somatic hypermutation.

(70,71) A second follicular CD4 T cell subset, regulatory (Tfreg) cells, exerts further control on (auto)antibody responses and Tfh.(30) In SLE, the ratio of circulating Tfh to Tfreg cells correlates with disease activity and anti-dsDNA antibody level, suggesting their importance in autoantibody production.(72) In pSS, this ratio also strongly correlates with autoantibody production and T cell infiltration in salivary glands.(73,74)

As well as cellular determinants, molecular determinants on cells regulate autoantibody responses in SLE, RA and pSS, such as TLRs. Especially relevant are the endosome-localized TLR-7 and TLR-9 that recognize RNA and DNA.(6,75) Murine lupus models demonstrated that TLR-7 and TLR-9 are required for the generation of RNA and dsDNA-specific autoantibodies, respectively.(76) However, a more complex role for TLR-9 is suggested by autoimmune-prone mouse models.(77) While B cell knock-out experiments support that TLR-7 drives autoantibody production, TLR-9 instead appears protective against systemic autoimmunity through not completely understood mechanisms.(76,78) The encounter of TLR ligands can trigger autoreactive B cell responses in at least two ways. First, co-engagement of BCR and TLR can induce autoantibody production in proto-autoreactive B cells.(6,79,80) Co-engagement is induced by immune complexes that contain nuclear antigens, which elicit synergistic responses that recruit TLRs to internalized BCRs in auto-phagosomes.(67, 81, 82) The presence of these TLRs and their nuclear ligands in auto-phagosomes might explain the preponderance of reactivity of many autoantibodies with nuclear antigens in autoimmune diseases.(67) Secondly, TLR ligation can result in T cell-independent B cell autoreactivity in the presence of B cell-activating factor of the tumor necrosis factor family (BAFF).(83) BAFF is a cytokine essential for maturation, proliferation and survival of peripheral B cells, and increased circulating BAFF is associated with pSS, SLE and RA.(83,84) In the presence of BAFF, TLR ligation promotes B cell activation, class-switch, somatic hypermutation and plasma cell differentiation that can all promote harmful autoantibody generation.(85-87)

As well as T cells and TLRs, deficiency in the complement system is important to include as a factor contributing to the existence of autoantibodies in AIRD. Complement is an essential component of the humoral immune system that plays a role in both innate and adaptive responses. Up-regulation, down-regulation and dysregulation of complement can all contribute to autoimmune disease.(88) Well-recognized anomalies associated with autoantibody production in AIRD are primary deficiencies in complement system pathways and regulators, including C1q, C2, C4, mannose-binding lectin and C1-inhibitor. (88) Furthermore, secondary deficiencies of complement by autoantibodies have been described. In SLE, 30–60% of the patients have anti-C1q autoantibodies which are strongly

associated with severe hypocomplementemia and lupus nephritis.(7,89) In patients with SSc (26%), pSS (14%) and extra-articular RA (> 30%), circulating C1q antibodies are also detected.(89,90) Complement deficiency can reduce B cell tolerance as follows (88,91): first, C1q deficiency specifically intervenes with effective negative selection of autoreactive B cells in bone marrow (89); and secondly, via insufficient elimination of immune complexes, apoptotic and necrotic cell material.(92) Under healthy conditions debris is opsonized by immunoglobulins and complement factors, and then rapidly cleared from the circulation via binding to CR1 on erythrocytes and through engulfment by phagocytes.(7, 93) When complement fails to eliminate debris, including various self-antigens, exposure of the immune system to these antigens can increase the propensity that autoreactive B cells are triggered to be activated and to produce autoantibodies. (6,7,92) In addition to complement serum protein deficiencies, dysfunction of linked and complementary pathways involved in the clearance of apoptotic cells can contribute to AIRD.(94,95) Here we provide one example, which is the deficiency of scavenger receptor type F family member 1, involved in the recognition and engulfment of apoptotic cells via complement component C1q, which was shown to induce lupus-like disease and autoantibody production in mice.(96)

The last important trigger that contributes to autoantibody production in AIRD is antigen modification.(97) Both apoptosis and inflammatory responses can initiate proteolysis of self-proteins, and can cause post-translational protein modifications such as phosphorylation, citrullination, carbamylation and deamination. These changes in protein appearance are relevant to AIRD: antigen modification can alter the immunogenicity of these molecules and thereby result in recognition by autoreactive B cells.(97-99) ACPAs, for example, can recognize various citrullinated proteins including α-enolase, vimentin, fibrinogen and myelin-binding protein.(98)

Pathogenicity

The pathogenicity of autoantibodies varies per antibody type, self-antigen specificity and clinical phase of disease. However, the pathogenic mechanisms through which autoantibodies contribute to localized tissue damage and systemic inflammation overlap and share involvement of both the innate and adaptive immune system (**Figure 2**).(6,9) Collectively, the indicated mechanisms – immune complex deposition, antibody-dependent cellular cytotoxicity, FcγR-mediated cell activation and complement activation – create a proinflammatory environment. In inflamed tissues, both immune and parenchymal cells are damaged through the release of reactive oxygen species, matrix-

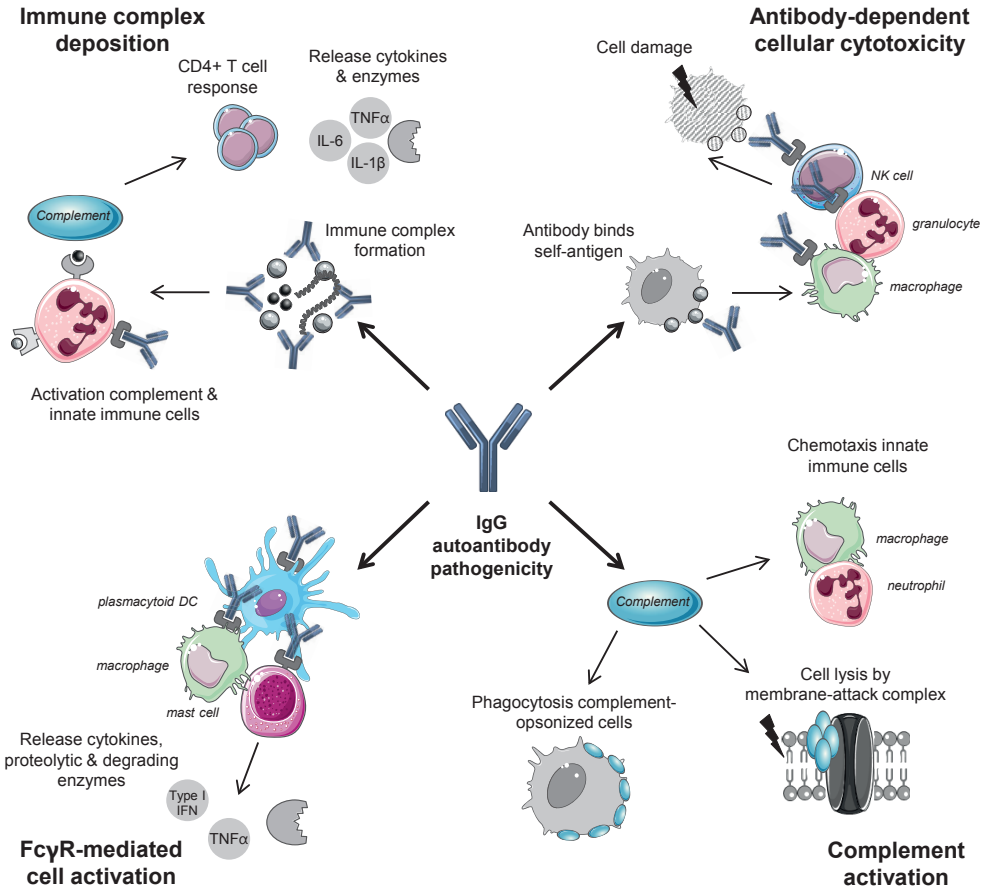


Figure 2. Key pathogenic effects of autoantibodies in autoimmune rheumatic diseases (AIRD). Autoantibodies can induce tissue damage and create a proinflammatory microenvironment through multiple components of the innate and adaptive immune system. Localized tissue damage mediated by autoantibodies involves three mechanisms. First, antibody-dependent cellular cytotoxicity (**top right**): killing of antibody coated target cells by binding of the Fc domain of IgG autoantibody by Fcγ receptor (FcγR)-expressing effector cells, most notably natural killer (NK) cells, granulocytes and macrophages. Secondly, through antibody-induced activation of the complement pathway (**lower right**). Complement activation can cause cell lysis through assembly of the membrane-attack complex, can induce phagocytosis of complement C3 proteolytic fragment-coated (opsonized) damaged cells, and can recruit innate inflammatory cells through release of small complement fragments C3a and C5a (anaphylatoxins). Thirdly, immunpoglobulin (Ig)G autoantibodies can activate various FcγR-expressing innate immune cells (**lower left**). For example, plasmacytoid dendritic cells (DC) that produce type I interferon (IFN), macrophages that produce tumor necrosis factor (TNF)-α and mast cells that release granules with degrading enzymes and produce proinflammatory cytokines. Systemic inflammation is primarily mediated through deposition of circulating immune complexes (IC) (**top left**). These ICs contain autoantibodies bound to self-antigens (such as DNA), Toll-like receptor (TLR) ligands and post-translationally modified proteins. IC deposition induces the systemic and synergistic activation of cells of the innate immune system via FcγR and TLR ligation. As a result, T helper cell responses are amplified and trigger the release of degrading enzymes and production of proinflammatory cytokines such as TNF-α, interleukin (IL)-1β and IL-6. Together, these mechanisms contribute to a proinflammatory microenvironment with cytokines that further enhance inflammation and damage, through activation of parenchymal and immune cells, production of matrix-degrading and proteolytic enzymes and release of reactive oxygen species. References listed in **Supplemental Table A**. Figure created using images from http://smart.servier.com.

degrading enzymes and proteolytic enzymes.(9) Moreover, systemic inflammation further impairs tolerance mechanisms of both B and T cells, which leads to more antibody production and a downward spiral of ongoing autoimmunity.(6)

In SLE the harmful effects of autoantibodies are extensively proven. Numerous data support the potency of immune complexes (ICs) containing ANAs to initiate lupus nephritis.(63,100) However, in other AIRD the pathogenicity of autoantibodies is less well understood.(9,37) In RA, a debate is ongoing regarding whether ACPAs, ACPA-producing B cells or T helper cells are especially responsible for transition from preclinical phases to arthritis.(9,23) A main argument against a major role for autoantibodies in disease onset is the fact that they are present long before disease onset. However, prominent increase of autoantibody titers before onset of symptoms is supportive of such a driver role.(11) Moreover, seropositive RA patients have more severe disease and radiographic damage. Also, ACPAs were shown to promote arthritis in murine models, activate complement, induce cytokine production and activate Fcγ-receptor-expressing immune cells.(9,12,98) With an increasing number of studies reporting a role for ACPAs in RA pathogenesis, we anticipate that the pathogenic role of existing and new autoantibodies will be demonstrated in other AIRD.

Revisiting seronegative AIRD

Some AIRD are traditionally classified as seronegative, because of the low number of patients positive for ACPA, RF, SS-A, SS-B, anti-dsDNA and other prototypical autoantibodies.(2) Common seronegative AIRD include reactive arthritis, undifferentiated spondyloarthritis, PsA, AS and seronegative RA.(4,12,101) We argue that the classification of seronegative AIRD needs revisiting, because increased frequencies of IgM-, IgG- and IgA-producing plasma cells and plasmablasts are detected in the circulation and joints of patients.(102,103) Moreover, increasing numbers of autoantibodies are detected in these 'seronegative' diseases, and emerging evidence suggests that plasma cells and autoantibodies are involved in their disease course.(4,12) Most data supporting the seropositive nature of these diseases are available for AS and PsA, of which we will now discuss recently identified autoantibodies, pathogenicity and breaches in B cell tolerance.

In PsA, several autoantibodies have been identified in plasma, serum and synovial fluid (SF) (**Table 2**). Indicative of the role of autoantibodies in disease pathogenesis is that titers and seropositive status of certain 'new' autoantibodies associate with disease activity.(99) Moreover, their involvement in PsA pathogenesis is suggested by the fact that some antibodies were significantly higher in PsA patients compared to patients with

psoriasis alone.(104) Multiple autoantibodies are also reported in AS, such as anti-CD74 (CLIP), anti-oxidized collagen type II and antibodies against various extracellular matrix proteins. These are discussed in depth elsewhere.(12,105,106) These autoantibodies are suggested to be produced in tertiary organized lymphoid structures – an important source of autoantibodies in seropositive AIRD – as ectopic lymphoid structures were identified in both AS and PsA synovium.(12,107)

Antibody [a)]	Antigen	*n*	Present (% patients)	Clinical association	Reference
Anti-20s proteasome	20s proteasome	36	28	No (tested for disease duration, nail involvement, dactylitis, ANA/ RF/ACPA status, articular phenotype)	Colmegna 2008 (110)
Anti-MCV	MCV	46	24	Association with presence of tender knee joints and nail psoriasis.	Dalmady 2013 (125)
Anti-PsA peptide [b)]	TNRRGRGSPGAL	100	85	n.r.	Dolcino 2014 (113)
Anti-CarP	Carbamylated proteins	30	53	Positive correlation with age, disease duration, ESR and PGA. Negative correlation with functional status.	Chimenti 2015 (99)
Anti-α6-integrin	α6-integrin	46	28	No (tested for early onset PsA)	Gal 2017 (117)
Anti-LL37	Cathelicidin LL37 Native / Carbamylated / Citrullinated	PL: n.r. / 32 / 29 SF: 19 / 17 / 21	PL: 0 / 52 / 32 SF: 37 / 47 / 57	PL anti-carbamylated: positive correlation with DAS44. SF anti-native: positive correlation with CRP, ESR, swollen joints and DAS44.	Frasca 2018 (108)
Anti-ADAMTSL5	ADAMTSL5	22	n.r. [c)]	Positive correlation with skin disease activity.	Yuan 2019 (121)

Table 2. Autoantibodies detected in psoriatic arthritis (selected studies). [a)] Detected in serum, unless otherwise specified. [b)] 'Anti-PsA peptide' (TNRRGRGSPGAL) antibodies recognize epitopes of self-antigens in skin and joints. [c)] No cut-off value for positivity reported, only graphical results of enzyme-linked immunosorbent assay (ELISA) [immunoglobulin (Ig)G levels (μg/ml) for PsO n = 32, PsA n = 22] and autoantigen array [IgG levels (mean fluorescence intensity) for healthy controls (HC) n = 20, systemic lupus erythematosus (SLE) n = 7, PsO n = 73]. Abbreviations: ACPA = anti-citrullinated peptide antibody; ADAMTSL5 = a disintegrin-like and metalloprotease domain with thrombospondin type 1 motifs like 5; ANA = anti-nuclear antibody; CarP = carbamylated protein; CRP = C-reactive protein; DAS44 = disease activity score 44; ESR = erythrocyte sedimentation rate; MCV = mutated citrullinated vimentin; *n* = number of patients with PsA diagnosis that participated in the study; n.r. = not reported in published data; PGA = patient global assessment of disease activity; PL = plasma; PsA = psoriatic arthritis; RF = rheumatoid factor; SF = synovial fluid.

Similar to seropositive AIRD, several breaks of immune tolerance are considered necessary to result in generation of autoantibodies in 'seronegative' disease, and similar mechanisms are identified. For example, post-translational antigen modification is necessary for the generation of antibodies against citrullinated and carbamylated cathelicidin LL37, citrullinated vimentin, oxidized collagen type II and other carbamylated proteins.(12,105,108,109) Also, anti-protease antibodies might alter the cleavage pattern of the proteasome, which potentially results in the generation of immunogenic self-antigens.(110) Next to antigen modification, there is evidence for a role of molecular mimicry leading to autoantibody development.(12,110) Furthermore, neutrophils are important players in breaching tolerance. First, neutrophil extracellular trap (NET)-derived complexes with self-antigens may contribute to autoantibody production by interaction with self-reactive B lymphocytes.(12,110,111) Secondly, neutrophil activation by granulocyte–macrophage colony-stimulating factor (GM-CSF) and complement fragments such as C5a is suggested to initiate autoimmunity through neutrophil degranulation. Degranulation can result in the release and post-translational modification of autoantigens such as LL37, an anti-microbial peptide with immune-modulating properties.(112) This hypothesis is supported by a correlation of GM-CSF and complement factor levels in serum and SF with autoantibody reactivity.(104,108) Notably, not all mechanisms that are described in seropositive AIRD have been identified thus far in seronegative disease, which might be explained by either the absence of these particular mechanisms or by the infancy of this field of research.

The contribution of these newly identified autoantibodies in seronegative AIRD is currently under extensive investigation, and recent reports suggest significant pathogenicity. For example, 'TNRRGRGSPGAL' peptide antibodies, present in 85% of PsA patients, cross-react with epitopes expressed in both skin and entheses.(113) Moreover, these autoantibodies bind TLR-2, which has an important role in activation of the innate immune system.(4,113) Another example concerns the modification of carbamylation, which is suggested to trigger oxidative stress and to contribute to systemic inflammation.(99) It is hypothesized that AS autoantibodies directly damage bony structures by inducing osteoclastogenesis.(12) Neutrophils may play a role in autoantibody-mediated tissue damage in tertiary lymphoid tissues in PsA and AS synovium. Deposited IgG ICs in these ELS co-localize with infiltrating activated neutrophils that mediate inflammatory synovial damage (12) and, as known from seropositive AIRD, the presence of ELS is implicated in the perpetuation of disease.(38)

Overall, these emerging insights support the notion that thus far we may have oversimplified distinct AIRD as being seronegative, by considering only a limited set of autoantibodies. The newly identified autoantibodies and their pathogenic effects support the concept of these disorders as falling within the spectrum of what was previously termed 'seropositive' autoimmune diseases, which opens up new avenues for investigating disease pathogenesis, identification of disease biomarkers or even new therapeutic targets.

Concluding remarks

Autoantibodies in AIRD can develop through sequential antigen-driven events that ultimately cause a loss of B cell tolerance, which include defects in B cell development, genetic variants and specific immunological triggers. In this study we have summarized a current view of the role of autoantibodies in the pathogenesis and perpetuation of AIRD. Especially in recent years, detection technologies have advanced and have now been refined, allowing for the simultaneous assessment of multiple antibody specificities in unbiased non-hypothesis-driven approaches. As prices drop, we now anticipate the implementation of multiplex-based approaches in diagnostic use to allow for simultaneous detection of autoantibody types and improved differentiation of AIRD groups. We believe that the validation of known antibodies and especially identification of relevant autoantibody specificities will provide insights into disease pathogenesis that can be applied in precision medicine. We anticipate that future research will further unravel the role of autoantibodies in AIRD pathogenesis, with the largest gains to be obtained in traditionally classified seronegative AIRD.

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References

1. Goldblatt F, O'Neill SG. Clinical aspects of autoimmune rheumatic diseases. Lancet 2013; 382: 797– 808.
2. Kirino Y, Remmers EF. Genetic architectures of seropositive and seronegative rheumatic diseases. Nat Rev Rheumatol 2015; 11: 401– 414.
3. Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. Autoimmun Rev 2003; 2: 119– 125.
4. Conigliaro P, Chimenti MS, Triggianese P et al. Autoantibodies in inflammatory arthritis. Autoimmun Rev 2016; 15: 673– 683.
5. Kokkonen H, Mullazehi M, Berglin E et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. Arthritis Res Ther 2011; 13: R13.
6. Suurmond J, Diamond B. Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity. J Clin Invest 2015; 125: 2194– 2202.
7. Walport MJ. Complement. N Engl J Med 2001; 344: 1140– 4.
8. Mackay IR. Travels and travails of autoimmunity: a historical journey from discovery to rediscovery. Autoimmun Rev 2010; 9: A251– A258.
9. Saferding V, Blüml S. Innate immunity as the trigger of systemic autoimmune diseases. J Autoimmun 2020; 110: 102382.
10. Ma W-T, Chang C, Gershwin ME, Lian Z-X. Development of autoantibodies precedes clinical manifestations of autoimmune diseases: a comprehensive review. J Autoimmun 2017; 83: 95–112.
11. Tobón GJ, Pers J-O, Cañas CA, Rojas-Villarraga A, Youinou P. Are autoimmune diseases predictable? Autoimmun Rev 2012; 11: 259– 266.
12. Quaden DHF, De Winter LM, Somers V. Detection of novel diagnostic antibodies in ankylosing spondylitis: An overview. Autoimmun Rev 2016; 15: 820– 832.
13. von Behring E, Kitasato S. The mechanism of diphtheria immunity and tetanus immunity in animals. 1890. Mol Immunol 1991; 28: 1317, 1319–1320.
14. Donath J, Landsteiner K. Ueber paroxysmale Hämaglobinurie [About paroxysmal hemaglobinuria]. Munch Med Wochenschr 1904; 51: 1590– 1593.
15. Waaler E. On the occurrence of a factor in human serum activating the specific agglutintion of sheep blood corpuscles. Acta Pathol Microbiol Scand 1940; 17: 172– 188.
16. Melchers F. Checkpoints that control B cell development. J Clin Invest 2015; 125: 2203– 2210.
17. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. Curr Opin Immunol 2008; 20: 632– 638.
18. Wardemann H, Nussenzweig MC. B-cell self-tolerance in humans. Adv Immunol 2007; 95: 83–110.
19. Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. Curr Opin Immunol 1995; 7: 812– 818.
20. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science 2003; 301: 1374– 1377.
21. Burnett DL, Langley DB, Schofield P et al. Germinal center antibody mutation trajectories are determined by rapid self/foreign discrimination. Science 2018; 360: 223– 226.

22. La Cava A. Putting together the autoimmunity puzzle. J Clin Invest 2015; 125: 2184– 2186.
23. Scherer HU, Huizinga TWJ, Krönke G, Schett G, Toes REM. The B cell response to citrullinated antigens in the development of rheumatoid arthritis. Nat Rev Rheumatol 2018; 14: 157– 169.
24. Samuels J, Ng Y-S, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. J Exp Med 2005; 20: 1659– 1667.
25. Yurasov S, Tiller T, Tsuiji M et al. Persistent expression of autoantibodies in SLE patients in remission. J Exp Med 2006; 203: 2255– 2261.
26. Yurasov S, Wardemann H, Hammersen J et al. Defective B cell tolerance checkpoints in systemic lupus erythematosus. J Exp Med 2005; 201: 703– 711.
27. Liu Y, Li L, Kumar KR et al. Lupus susceptibility genes may breach tolerance to DNA by impairing receptor editing of nuclear antigen-reactive B cells. J Immunol 2007; 179: 1340– 1352.
28. Meffre E. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. Ann NY Acad Sci 2011; 1246: 1– 10.
29. MacLennan I. Germinal centers. Annu Rev Immunol 1994; 12: 117– 139.
30. Wing JB, Tekgüç M, Sakaguchi S. Control of germinal center responses by T-follicular regulatory cells. Front Immunol 2018; 9: 1910.
31. Cyster JG, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. Nature 1994; 371: 389– 395.
32. Ekland EH, Forster R, Lipp M, Cyster JG. Requirements for follicular exclusion and competitive elimination of autoantigen-binding B cells. J Immunol 2004; 172: 4700– 4708.
33. Cyster JG, Goodnow CC. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunity 1995; 3: 691– 701.
34. Roco JA, Mesin L, Binder SC et al. Class-switch recombination occurs infrequently in germinal centers. Immunity 2019; 51: 337– 350.e7.
35. Schröder AE, Greiner A, Seyfert C, Berek C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. Proc Natl Acad Sci USA 1996; 93: 221– 225.
36. Salomonsson S, Jonsson MV, Skarstein K et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjögren's syndrome. Arthritis Rheum 2003; 48: 3187– 3201.
37. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjögren syndrome. Nat Rev Rheumatol 2018; 14: 133– 145.
38. Bombardieri M, Lewis M, Pitzalis C. Ectopic lymphoid neogenesis in rheumatic autoimmune diseases. Nat Rev Rheumatol 2017; 13: 141– 154.
39. Soni C, Perez OA, Voss WN et al. Plasmacytoid dendritic cells and type i interferon promote extrafollicular B cell responses to extracellular self-DNA. Immunity 2020; 52: 1022– 1038.e7.
40. Baert L, Manfroi B, Casez O, Sturm N, Huard B. The role of APRIL – a proliferation inducing ligand – in autoimmune diseases and expectations from its targeting. J Autoimmun 2018; 95: 179– 190.
41. Chan TD, Wood K, Hermes JR et al. Elimination of germinal-center-derived self-reactive B cells is governed by the location and concentration of self-antigen. Immunity 2012; 37: 893– 904.
42. Wellmann U, Letz M, Herrmann M, Angermüller S, Kalden JR, Winkler TH. The evolution of human anti-double-stranded DNA autoantibodies. Proc Natl Acad Sci USA 2005; 102: 9258– 9263.

43. Mietzner B, Tsuiji M, Scheid J et al. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. Proc Natl Acad Sci USA 2008; 105: 9727– 32.
44. Giles IP, Haley JD, Nagl S, Isenberg DA, Latchman DS, Rahman A. A systematic analysis of sequences of human antiphospholipid and anti–β2-glycoprotein I antibodies: the importance of somatic mutations and certain sequence motifs. Semin Arthritis Rheum 2003; 32: 246– 265.
45. Steen J, Forsström B, Sahlström P et al. Recognition of amino acid motifs, rather than specific proteins, by human plasma cell-derived monoclonal antibodies to posttranslationally modified proteins in rheumatoid arthritis. Arthritis Rheumatol 2019; 71: 196– 209.
46. Rekvig OP, Moens U, Sundsfjord A et al. Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T-antigen. A molecular basis for induction of antibodies to DNA and eukaryotic transcription factors. J Clin Invest 1997; 99: 2045– 2054.
47. Kaliyaperumal A, Mohan C, Wu W, Datta SK. Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. J Exp Med 1996; 183: 2459– 2469.
48. Adler LN, Jiang W, Bhamidipati K et al. The other function: class II-restricted antigen presentation by B cells. Front Immunol 2017; 8: 319.
49. Huizinga TWJ, Amos CI, Van Der Helm-Van Mil AHM et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 2005; 52: 3433– 3438.
50. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol 2008; 26: 651– 675.
51. Fei HM, Kang H, Peebles C, Fox R, Scharf S, Erlich H. Specific HLA-DQA and HLA-DRB1 alleles confer susceptibility to Sjögren's syndrome and autoantibody production. J Clin Lab Anal 1991; 5: 382– 391.
52. Morris DL, Fernando MMA, Taylor KE et al. MHC associations with clinical and autoantibody manifestations in European SLE. Genes Immun 2014; 15: 210– 217.
53. Chinoy H, Adimulam S, Marriage F et al. Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. Ann Rheum Dis 2012; 71: 961– 965.
54. Gourh P, Safran SA, Alexander T et al. HLA and autoantibodies define scleroderma subtypes and risk in African and European Americans and suggest a role for molecular mimicry. Proc Natl Acad Sci USA 2020; 117: 552.
55. Wu M, Mohan C. B-cells in systemic sclerosis. Curr Opin Rheumatol 2015; 27: 537– 541.
56. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 2006; 312: 1669– 1672.
57. Sharma M, Vignesh P, Tiewsoh K, Rawat A. Revisiting the complement system in systemic lupus erythematosus. Exp Rev Clin Immunol 2020; 16: 397– 408.
58. Gianchecchi E, Fierabracci A. Gene/environment interactions in the pathogenesis of autoimmunity: new insights on the role of Toll-like receptors. Autoimmun Rev 2015; 14: 971– 983.
59. Ciaffi J, van Leeuwen NM, Huizinga TWJ, de Vries-Bouwstra JK. Smoking and systemic sclerosis: influence on microangiopathy and expression of anti-topoisomerase I antibodies in a monocentric cohort. Clin Exp Rheumatol 2020; 38(Suppl 125): 25– 28.

60. Klareskog L, Stolt P, Lundberg K et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA–DR (shared epitope)–restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006; 54: 38– 46.
61. Brown JM, Archer AJ, Pfau JC, Holian A. Silica accelerated systemic autoimmune disease in lupus-prone New Zealand mixed mice. Clin Exp Immunol 2003; 131: 415– 421.
62. Wegner N, Wait R, Sroka A et al. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum 2010; 62: 2662– 2672.
63. Putterman C, Limpanasithikul W, Edelman M, Diamond B. The double edged sword of the immune response: mutational analysis of a murine anti-pneumococcal, anti-DNA antibody. J Clin Invest 1996; 97: 2251– 2259.
64. McClain MT, Heinlen LD, Dennis GJ, Roebuck J, Harley JB, James JA. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. Nat Med 2005; 11: 85– 89.
65. Alspaugh MA, Henle G, Lennette ET, Henle W. Elevated levels of antibodies to Epstein–Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. J Clin Invest 1981; 67: 1134– 1140.
66. Bo M, Niegowska M, Erre GL et al. Rheumatoid arthritis patient antibodies highly recognize IL-2 in the immune response pathway involving IRF5 and EBV antigens. Sci Rep 2018; 8: 1789.
67. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin–IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002; 416: 603– 607.
68. Alsaleh G, François A, Knapp AM et al. Synovial fibroblasts promote immunoglobulin class switching by a mechanism involving BAFF. Eur J Immunol 2011; 41: 2113– 2122.
69. Petersone L, Edner NM, Ovcinnikovs V et al. T cell/B cell collaboration and autoimmunity: an intimate relationship. Front Immunol 2018; 9: 1941.
70. Ricard L, Jachiet V, Malard F et al. Circulating follicular helper T cells are increased in systemic sclerosis and promote plasmablast differentiation through the IL-21 pathway which can be inhibited by ruxolitinib. Ann Rheum Dis 2019; 78: 539– 550.
71. Simpson N, Gatenby PA, Wilson A et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum 2010; 62: 234– 244.
72. Xu B, Wang S, Zhou M et al. The ratio of circulating follicular T helper cell to follicular T regulatory cell is correlated with disease activity in systemic lupus erythematosus. Clin Immunol 2017; 183: 46– 53.
73. Fonseca VR, Romão VC, Agua-Doce A et al. The ratio of blood T follicular regulatory cells to T follicular helper cells marks ectopic lymphoid structure formation while activated follicular helper T cells indicate disease activity in primary Sjögren's syndrome. Arthritis Rheumatol 2018; 70: 774– 784.
74. Fonseca VR, Agua-Doce A, Maceiras AR et al. Human blood Tfr cells are indicators of ongoing humoral activity not fully licensed with suppressive function. Sci Immunol 2017; 2:eaan1487.
75. Kiripolsky J, Kramer JM. Current and emerging evidence for Toll-like receptor activation in Sjögren's syndrome. J Immunol Res 2018; 2018: 1– 12.
76. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RAA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 2006; 25: 417– 428.

77. Suthers AN, Sarantopoulos S. TLR7/TLR9- and B cell receptor-signaling crosstalk: promotion of potentially dangerous B Cells. Front Immunol 2017; 8: 1– 8.
78. Jackson S, Scharping N, Kolhatkar N et al. Opposing impact of B cell intrinsic TLR7 and TLR9 signals on autoantibody repertoire and systemic inflammation. J Immunol 2014; 192: 4525– 4532.
79. Giltiay NV, Chappell CP, Sun X et al. Overexpression of TLR7 promotes cell-intrinsic expansion and autoantibody production by transitional T1 B cells. J Exp Med 2013; 210: 2773– 2789.
80. Azulay-Debby H, Edry E, Melamed D. CpG DNA stimulates autoreactive immature B cells in the bone marrow. Eur J Immunol 2007; 37: 1463– 1475.
81. Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. Immunity 2003; 19: 837– 847.
82. Lau CM, Broughton C, Tabor AS et al. RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. J Exp Med 2005; 202: 1171– 1177.
83. Groom JR, Fletcher CA, Walters SN et al. BAFF and MyD88 signals promote a lupuslike disease independent of T cells. J Exp Med 2007; 204: 1959– 1971.
84. Pers J-O, Daridon C, Devauchelle V et al. BAFF Overexpression is associated with autoantibody production in autoimmune diseases. Ann NY Acad Sci 2005; 1050: 34– 39.
85. Herlands RA, Christensen SR, Sweet RA, Hershberg U, Shlomchik MJ. T cell-independent and Toll-like receptor-dependent antigen-driven activation of autoreactive B cells. Immunity 2008; 29: 249– 260.
86. Genestier L, Taillardet M, Mondiere P, Gheit H, Bella C, Defrance T. TLR agonists selectively promote terminal plasma cell differentiation of B cell subsets specialized in thymus-independent responses. J Immunol 2007; 178: 7779– 7786.
87. Pone EJ, Zhang J, Mai T et al. BCR-signalling synergizes with TLR-signalling for induction of AID and immunoglobulin class-switching through the non-canonical NF-κB pathway. Nat Commun 2012; 3: 767.
88. Conigliaro P, Triggianese P, Ballanti E, Perricone C, Perricone R, Chimenti MS. Complement, infection, and autoimmunity. Curr Opin Rheumatol 2019; 31: 532– 541.
89. Potlukova E, Kralikova P. Complement component C1q and anti-C1q antibodies in theory and in clinical practice. Scand J Immunol 2008; 67: 423– 430.
90. Stojan G, Petri M. Anti-C1q in systemic lupus erythematosus. Lupus 2016; 25: 873– 877.
91. Vignesh P, Rawat A, Sharma M, Singh S. Complement in autoimmune diseases. Clin Chim Acta 2017; 465: 123– 130.
92. Bryan AR, Wu EY. Complement deficiencies in systemic lupus erythematosus. Curr Allergy Asthma Rep 2014; 14: 448.
93. Arora V, Verma J, Dutta R, Marwah V, Kumar A, Das N. Reduced complement receptor 1 (CR1, CD35) transcription in systemic lupus erythematosus. Mol Immunol 2004; 41: 449– 456.
94. Shao W-H, Cohen PL. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. Arthritis Res Ther 2011; 13: 202.
95. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell. 2010; 140: 619– 630.
96. Ramirez-Ortiz ZG, Pendergraft WF III, Prasad A et al. The scavenger receptor SCARF1 mediates apoptotic cell clearance and prevents autoimmunity. Nat Immunol 2013; 14: 917.

97. Rosen A, Casciola-Rosen L. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. Cell Death Diff 1999; 6: 6– 12.

98. Willemze A, Böhringer S, Knevel R et al. The ACPA recognition profile and subgrouping of ACPA-positive RA patients. Ann Rheum Dis 2012; 71: 268– 274.

99. Chimenti MS, Triggianese P, Nuccetelli M et al. Auto-reactions, autoimmunity and psoriatic arthritis. Autoimmun Rev 2015; 14: 1142– 1146.

100. Medina G, Vera-Lastra O, Peralta-Amaro AL et al. Metabolic syndrome, autoimmunity and rheumatic diseases. Pharmacol Res 2018; 133: 277– 288.

101. Koehler L, Kuipers JG, Zeidler H. Managing seronegative spondarthritides. Rheumatology 2000; 39: 360– 368.

102. Eghtedari AA, Davis P, Bacon PA. Immunological reactivity in ankylosing spondylitis. Circulating immunoblasts, autoantibodies, and immunoglobulins. Ann Rheum Dis 1976; 35: 155– 157.

103. Lin Q, Gu JR, Li TW et al. Value of the peripheral blood B-cells subsets in patients with ankylosing spondylitis. Chin Med J 2009; 122: 1784– 9.

104. Chimenti MS, Triggianese P, De Martino E et al. An update on pathogenesis of psoriatic arthritis and potential therapeutic targets. Exp Rev Clin Immunol 2019; 15: 823– 836.

105. Vinci C, Infantino M, Raturi S et al. Immunoglobulin A antibodies to oxidized collagen type II as a potential biomarker for the stratification of spondyloarthritis from rheumatoid arthritis. Scand J Rheumatol 2020; 49: 281– 91.

106. van Kempen TS, Leijten EFA, Lindenbergh MFS et al. Impaired proteolysis by SPPL2a causes CD74 fragment accumulation that can be recognized by anti-CD74 autoantibodies in human ankylosing spondylitis. Eur J Immunol 2020; 50: 1209– 19.

107. Cañete JD, Santiago B, Cantaert T et al. Ectopic lymphoid neogenesis in psoriatic arthritis. Ann Rheum Dis 2007; 66: 720– 726.

108. Frasca L, Palazzo R, Chimenti MS et al. Anti-LL37 antibodies are present in psoriatic arthritis (PsA) patients: new biomarkers in PsA. Front Immunol 2018; 9: 1936.

109. Dalmády S, Kiss M, Képíró L et al. Higher levels of autoantibodies targeting mutated citrullinated vimentin in patients with psoriatic arthritis than in patients with psoriasis vulgaris. Clin Dev Immunol 2013; 2013: 474028.

110. Colmegna I, Sainz B, Citera G, Maldonado-Cocco JA, Garry RF, Espinoza LR. Anti-20S proteasome antibodies in psoriatic arthritis. J Rheumatol 2008; 35: 674– 676.

111. Wigerblad G, Kaplan MJ. NETs spread ever wider in rheumatic diseases. Nat Rev Rheumatol 2020; 16: 73– 74.

112. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. J Immunol 2013; 191: 4895– 4901.

113. Dolcino M, Lunardi C, Ottria A, Tinazzi E, Patuzzo G, Puccetti A. Crossreactive autoantibodies directed against cutaneous and joint antigens are present in psoriatic arthritis. PLOS ONE 2014; 9:e115424.

114. Burbelo Peter D., Gordon Sarah M., Waldman Meryl, et al. Autoantibodies are present before the clinical diagnosis of systemic sclerosis. PLOS ONE. 2019; 14: 3: e0214202.

115. Arbuckle Melissa R., McClain Micah T., Rubertone Mark V., Scofield R. Hal, Dennis Gregory J., James Judith A., Harley John B.. Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus. New England Journal of Medicine. 2003; 349: 16: 1526– 1533.

116. Miller F. W., Waite K. A., Biswas T., Plotz P. H. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proceedings of the National Academy of Sciences. 1990; 87: 24: 9933– 9937.

117. Gál Brigitta, Dulic Sonja, Kiss Mária, Groma Gergely, Kovács László, Kemény Lajos, et al. Increased circulating anti-α6-integrin autoantibodies in psoriasis and psoriatic arthritis but not in rheumatoid arthritis. The Journal of Dermatology. 2017; 44: 4: 370– 374.

118. Vulsteke Jean-Baptiste, Blockmans Daniël, Moons Veronique, Vijgen Sara, Bossuyt Xavier, De Langhe Ellen. Detection of anti-Mi-2 autoantibodies before dermatomyositis-specific manifestations. Rheumatology. 2020; 59: 10: e60– e62.

119. Cooper Glinda S, Stroehla Berrit C. The epidemiology of autoimmune diseases. Autoimmunity Reviews. 2003; 2: 3: 119– 125.

120. Theander Elke, Jonsson Roland, Sjöström Bitte, Brokstad Karl, Olsson Peter, Henriksson Gunnel. Prediction of Sjögren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling. Arthritis & Rheumatology. 2015; 67: 9: 2427– 2436.

121. Yuan Yulin, Qiu Jingyi, Lin Zuan-Tao, et al. Identification of Novel Autoantibodies Associated With Psoriatic Arthritis. Arthritis & Rheumatology. 2019; 71: 6: 941– 951.

122. Abe Yoshiyuki, Matsushita Masakazu, Tada Kurisu, Yamaji Ken, Takasaki Yoshinari, Tamura Naoto. Clinical characteristics and change in the antibody titres of patients with anti-MDA5 antibody–positive inflammatory myositis. Rheumatology. 2017; 56: 9: 1492– 1497.

123. Majka D S, Deane K D, Parrish L A, et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Annals of the Rheumatic Diseases. 2008; 67: 6: 801– 807.

124. Targoff Ira N., Trieu Edward P., Plotz Paul H., Miller Frederick W. Antibodies to glycyl–transfer rna synthetase in patients with myositis and interstitial lung disease. Arthritis & Rheumatism. 1992; 35: 7: 821– 830.

125. Dalmády Szandra, Kiss Mária, Képíró László, Kovács László, Sonkodi Gábor, Kemény Lajos, et al. Higher Levels of Autoantibodies Targeting Mutated Citrullinated Vimentin in Patients with Psoriatic Arthritis Than in Patients with Psoriasis Vulgaris. Clinical and Developmental Immunology. 2013; 2013: 1– 9.

Supplemental Information

Pathogenic effect autoantibody	Reference
Antibody dependent cellular cytotoxicity	Saferding V, Blüml S. Innate immunity as the trigger of systemic autoimmune diseases. J Autoimmun. 2020;110:102382.
	Wallace PK, Howell AL, Fanger MW. Role of Fc gamma receptors in cancer and infectious disease. J Leukoc Biol. 1994;55:816-826.
Complement activation	Ceribelli A, Andreoli L, Cavazzana I, Francheschini F, Radice A, Rimoldi L, et al. Complement cascade in systemic lupus erythematosus: analyses of the three activation pathways. Ann N Y Acad Sci. 2009;1173:427-434.
	Trouw LA, Haisma EM, Levarht EW, van der Woude D, Ioan-Facsinay A, Daha MR, et al. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum. 2009;60:1923-1931.
	Vignesh P, Rawat A, Sharma M, Singh S. Complement in autoimmune diseases. Clin Chim Acta. 2017;465:123-130.
	Shi J, Rose EL, Singh A, Hussain S, Stagliano NE, Parry GC, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. Blood. 2014;123:4015-4022.
FcγR-mediated cell activation	Lövgren T, Eloranta ML, Båve U, Alm GV, Rönnblom L. Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum. 2004;50:1861-1872.
	Clavel C, Nogueira L, Laurent L, Iobagiu C, Vincent C, Sebbag M, et al. Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum. 2008;5:678-688.
	Suurmond J, Rivellese F, Dorjée AL, Bakker AM, Rombouts YJPC, Rispens T, et al. Toll-like receptor triggering augments activation of human mast cells by anti-citrullinated protein antibodies. Ann Rheum Dis. 2015;74:1915-1923.
Immune complex deposition	Sokolove J, Zhao X, Chandra PE, Robinson WH. Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fcγ receptor. Arthritis Rheum. 2011;63:53-62.
	Lövgren T, Eloranta ML, Båve U, Alm GV, Rönnblom L. Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum. 2004;50:1861-1872.
	Suurmond J, Rivellese F, Dorjée AL, Bakker AM, Rombouts YJPC, Rispens T, et al. Toll-like receptor triggering augments activation of human mast cells by anti-citrullinated protein antibodies. Ann Rheum Dis. 2015;74:1915-1923.
	Vogelpoel LT, Hansen IS, Rispens T, Muller FJM, van Capel TMM, Turina MC, et al. Fc gamma receptor-TLR cross-talk elicits pro-inflammatory cytokine production by human M2 macrophages. Nat Commun. 2014;5:5444.
Pro-inflammatory microenvironment	Saferding V, Blüml S. Innate immunity as the trigger of systemic autoimmune diseases. J Autoimmun. 2020;110:102382.

Supplemental Table A. References Figure 2.

Chapter 3

Plasticity of regulatory T cells in psoriatic arthritis: an IL-17A-producing, Foxp3^{int}CD161+RORγt+ICOS+ pro-inflammatory phenotype, that associates with the presence of ADAMTSL5 autoantibodies

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Submitted

Abstract

Objective – In psoriatic arthritis (PsA), predisposing class I HLA alleles, the presence of synovial clonally proliferated CD8+ T cells and autoantibodies all point towards the loss of immune tolerance. However, the key mechanisms that lead to immune dysregulation are not fully understood. In other types of inflammatory arthritis, T regulatory cell (Treg) dysfunction and plasticity at sites of inflammation were suggested to negatively affect peripheral tolerance. We here addressed if Treg plasticity associates with psoriatic disease.

Methods – We collected clinical data, sera and peripheral blood mononuclear cells from 13 healthy controls, 21 psoriasis and 21 PsA patients. In addition, we obtained synovial fluid mononuclear cells from 6 PsA patients. We studied characteristics of CD4+CD25+CD127^{lo}Foxp3+ Tregs by flow cytometry and used ELISA to quantify antibodies against ADAMTSL5, a recently discovered autoantigen in psoriatic disease.

Results – In comparison with their circulating counterparts, Tregs from inflamed joints express increased levels of ICOS, CTLA-4 and TIGIT. Furthermore, synovial fluid-derived Tregs have a pro-inflammatory phenotype, characterized by IL-17A production and upregulation of CD161 and RORγt. We identified a subset of Tregs with intermediate Foxp3 expression as the major cytokine producer. Furthermore, ICOS+ Tregs associate with PsA disease activity as measured by PASDAS. Lastly, we observed that presence of the Foxp3^{int} Tregs associates with an increased abundance of anti-ADAMTSL5 autoantibodies.

Conclusion – Tregs derived from the inflammatory environment of inflamed PsA joints exhibit a potentially pathogenic phenotype, which associates with loss of peripheral immune tolerance in psoriatic disease.

Manuscript

Introduction

Psoriatic arthritis (PsA) is a heterogeneous, inflammatory, musculoskeletal disease characterized by psoriasis, arthritis, enthesitis, dactylitis and nail dystrophy. PsA is the second most common type of inflammatory arthritis and develops in up to 30% of patients with psoriasis.(1) Increasing evidence suggests that autoimmune mechanisms underlie PsA pathogenesis, including strong associations with class I human leukocyte antigen alleles, ectopic lymphoid neogenesis in synovial tissues with T and B cell aggregates, presence of autoantibodies, and clonally proliferated CD8+ T cells in synovial tissue and fluid.(2–4) However, the key immunological factors that decrease immune tolerance and lead to PsA transition in psoriasis patients remain largely unknown.(1,5)

In auto-immune rheumatic diseases, regulatory T cells (Tregs) derived from synovial fluid were shown to effectively suppress effector T cells and thus maintain immune homeostasis.(6,7) However, other studies implicated Tregs in a pathogenic role, showing data that loss of peripheral immune tolerance associated with impaired expression of key immune regulatory molecules and through Treg differentiation.(8–11) Tregs in an inflammatory micro-environment may differentiate under the influence of T cell receptor engagement, IL-2 deprivation and pro-inflammatory cytokines.(10,12,13) Consequently, Tregs can downregulate their key transcription factor forkhead box P3 (Foxp3) and obtain effector T cell (Teff) phenotype and function.(11,13) Moreover, Treg differentiation – or plasticity – can be accompanied by reduced suppressive function, expression of T helper 17 cell features (CD161, retinoic acid-related orphan receptor gamma t (RORγt)) and production of pro-inflammatory cytokines.(8,11,13–17) Hence, these results suggest that differentiated Tregs in inflammatory arthritis may become pathogenic and amplify inflammation, instead of halting disease.(18,19)

With regards to Tregs in patients with psoriatic disease, literature is scarce. Treg abnormalities have been observed, mostly in patients with psoriasis, including decreased expression of CD39 and CD74,(20) increased expression of IL-6Rα,(21) reduced suppressive capacity,(15,22–24) chemotactic deficiency,(25) and the enhanced propensity to differentiate into cells that produce interleukin (IL)-17 – the hallmark cytokine of psoriatic disease.(15) Only few studies investigated the role of Tregs in PsA pathogenesis and in-depth characterization of intra-articular Tregs is lacking.(26) With this study we aimed to study a possible role for Treg plasticity in PsA pathogenesis, and specifically whether Treg differentiation associates with loss of peripheral tolerance.

Methods

Study design

We performed an observational cohort study at the University Medical Center Utrecht, the Netherlands. The study protocol was approved by the medical research ethics committee Utrecht (protocol number 13-696). We obtained written informed consent from all participants. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Subjects

We included patients aged 18 years or older with a diagnosis of psoriasis or PsA. PsA patients met the 'ClASsification for Psoriatic ARthritis' (CASPAR) criteria. We defined psoriasis as a confirmed diagnosis of psoriasis and absence of inflammatory arthritis. The latter was assessed by medical history, physical examination and laboratory parameters. We excluded patients that used disease-modifying anti-rheumatic drugs (DMARDs) in the past three months. In addition, we collected synovial fluid from patients with a clinical diagnosis of PsA, gout and osteoarthritis (OA).

Disease activity

To quantify disease activity we used two validated, disease-specific composite measures for PsA: Disease Activity index for PSA (DAPSA) (range 0-164) and Psoriatic ArthritiS Disease Activity Score (PASDAS) (range 0-10). We used patient-reported outcomes (PROs) to assess disease severity with six questionnaires: dermatology life quality index, health assessment questionnaire, short form-36 physical and mental component score, visual analogue scale for pain and patient global assessment.

Samples

We performed cross-sectional sampling of peripheral blood and synovial fluid. To collect sera, we centrifuged BD Vacutainer™ Plastic Blood Collection serum Tubes (silica and polymer gel) for 10 minutes (1500 g, room temperature). We collected peripheral blood in BD Vacutainer™ Plastic Blood Collection Tubes with Lithium Heparin. For synovial fluid and synovial fluid mononuclear cells (SFMC), we obtained intra-articular fluid of swollen joints in sterile 10-50 mL syringes. We isolated SF by centrifugation for 10 minutes (2300 g). To isolate PBMC and SFMC we performed 25 minutes density centrifugation (400 g, Ficoll-Paque). We stored samples at -80ºC (sera, synovial fluid) and liquid nitrogen (PBMC, SFMC) until measurement.

T cell activation assay

To assess Treg cytokine production upon activation, we cultured PBMC and SFMC in complete medium (RPMI 1640 GlutaMAX (61870044; Thermo Fisher Scientific) + 10% fetal bovine serum + 1% Penicillin-Streptomycin) with 20 ng/mL PMA (P8139-1MG, Sigma) and 1 µg/ml ionomycin (407952, Calbiochem / EMD Chemicals inc.) for 4.5 hours, while inhibiting protein transport with 1:1000 BD GolgiStop (51-2092KZ, BD Bioscience).

Flow Cytometry

We stained samples by incubation with 25 µl antibody mix diluted in buffer (500 ml phosphate-buffered saline + 5 ml 10% sodium azide + 5 g bovine serum albumin) for 25 min at 4°C. Before intracellular stain of IL-10 and IL-17A, we fixed and permeabilized cells with 100 µl Fixation/Permeabilization Concentrate and Diluent (00-5123-43, 00-5223-56, eBioscience). Flow Cytometry antibodies are listed in **Supplemental Table S1**. Using fluorescence minus one controls, we identified viable (assessed by a Fixable Viability Dye) CD3+CD4+CD25+CD127^{lo}Foxp3+ Tregs with intermediate (Foxp3^{int}) and high (Foxp3^{hi}) Foxp3 expression. Of these Foxp3^{int} and Foxp3^{hi} Treg subsets, we assessed median fluorescent intensity (MFI) and proportions of cells that express cytotoxic T-lymphocyte-associated protein 4 (CTLA-4=CD152), CD161, inducible T-cell costimulator (ICOS=CD278), T cell immunoreceptor with Ig and ITIM domains (TIGIT), Ki67 and RORγt. Low expression of RORγt and CD161 required standardization by using a uniform gate based on a representative control sample. We standardized quantification of intracellular IL-10 and IL-17A by applying a cutoff value of <0.5% in the medium control samples. We performed acquisition on the BD LSRFortessa (405, 488, 561, 635 nm lasers) with BD FACSDIVA (version 8.0.1). We used FlowJo (version 10.7.1) for further analyses.

ELISA

We coated 96-well flat-bottom Nunc MaxiSorp™ plates (44-2404-21, ThermoFisher) overnight with 50 µL/well 5 µg/mL recombinant ADAMTSL5 peptide (NBP1-93438PEP, Novus Biologicals), diluted in 2% bovine serum albumin (BSA) (10735094001, Roche) in phosphate buffered saline (PBS). Next, we blocked nonspecific binding sites for 1 hour at room temperature with 100 µL/well 4% BSA in PBS and incubated overnight with 50 µL serially diluted patient serum or SF in duplo in 2% BSA in PBS. For the standard curve, we used 50 µL primary anti-human-ADAMTSL5 antibodies in duplo (HPA044050-100UL, Sigma-Aldrich), serially diluted in 2% BSA in PBS in the following concentrations: 5.00, 1.67, 0.56, 0.1852, 0.0617, 0.0206, 0.0069 µg/mL. After overnight incubation at

4ºC, we incubated patient sample wells with 50 µL/well horseradisch-peroxidase (HRP)-conjugated anti-human IgG (11869130, ThermoFisher Scientific) and standard curve wells with 50 µL HRP-conjugated anti-rabbit IgG (31460, Thermofisher). We developed and stopped the color reaction with 50 µL/well of 3,3', 5,5'-tetramethylbenzidine (TMBW-1000-01, Tebu-Bio) and 2N H2SO4, respectively. We measured absorbance at 450 nm with a reference wavelength of 570 nm. We selected the dilution that best fitted the 5-parameter fit curve using software by Clariostar (version 5.40 R2; firmware version 1.2; serial number 430-1031) and MARS (version 3.31).

Statistical analysis

We applied Wilcoxon Singed Rank tests to compare characteristics between Foxp3^{int} and Foxp3^{hi} Tregs. To compare flow cytometry and ELISA results between patient groups, we used Mann-Whitney U (MWU) tests. Synovial fluid-derived Tregs were only compared with peripheral blood Tregs from PsA patients. To test the association of clinical outcomes, Treg characteristics and autoantibody concentration, we used Spearman's rank correlation. We performed contingency analyses using $\chi 2$ tests for categorical variables, and independent samples T-tests or MWU tests for continuous variables, to analyze patient clinical characteristics. A P-value of <0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS statistics (Version 26 Release 26.0.0.1) and GraphPad Prism 8 (Version 8.3.0).

Results

Cohort

To investigate Treg plasticity in psoriatic disease, we studied characteristics of Tregs in peripheral blood from 13 healthy controls (HC), 21 psoriasis patients and 21 PsA patients, and in synovial fluid of 6 PsA patients. Detailed patient characteristics are shown in **Supplemental Table S2**. We identified Tregs using the best available discriminative markers: CD3+CD4+CD25+CD127^{lo}Foxp3+ (**Figure 1A**).(18,19,27,28)

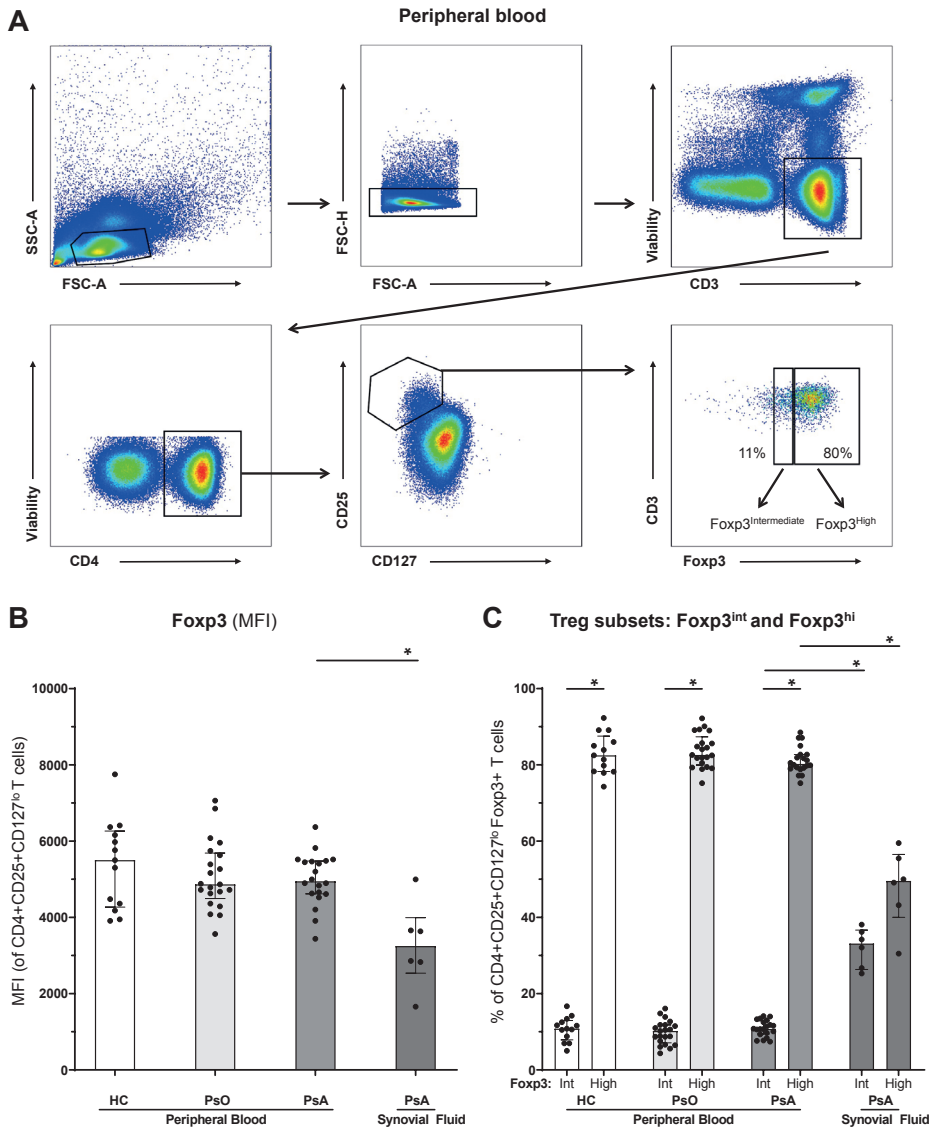


Figure 1. Increase of Tregs with intermediate Foxp3 expression in inflamed PsA joints. Flow cytometry analysis of Foxp3 expression by CD4+CD25+CD127^{lo} T cells derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). (**A**) Gating strategy for CD4+CD25+CD127^{lo}Foxp3+ Tregs. We differentiated between Tregs with intermediate and high expression of Foxp3 (Foxp3^{int} and Foxp3^{hi}, respectively). Percentages in dot plots represent median of PB-derived Tregs in PsA. (**B**) Foxp3 expression by CD4+CD25+CD127^{lo} T cells as measured by MFI. (**C**) Proportion of Foxp3^{int} and Foxp3^{hi} Tregs of CD4+CD25+CD127^{lo} T cells. Abbreviations: Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; FSC-A: forward scatter area; FSC-H: forward scatter height; HC: healthy control; Int: intermediate expression of Foxp3; MFI: median fluorescent intensity; PsA: psoriatic arthritis; PsO: psoriasis; SSC-A: sideward scatter area; Tregs: T regulatory cells.

Increase of CD4+CD25+CD127^{lo}Foxp3+ Tregs with intermediate Foxp3 expression in PsA synovial fluid

We observed that synovial fluid-derived Tregs, as compared to peripheral blood, have lower expression of Foxp3 (MFI 3248 vs. 4948, P=0.002) (**Figure 1B**). As Foxp3 is the key transcription factor of Treg development, maintenance and function, this finding raised our interest in phenotypical and functional properties of Tregs with reduced expression of Foxp3.(29) Therefore, we studied two subset of Tregs: with intermediate (Foxp3^{int}) and high (Foxp3^{hi}) Foxp3 expression (**Figure 1C**). CD4+CD25+CD127^{lo} T cells without Foxp3 were excluded from further analyses. As compared to circulating T cells from PsA patients, in synovial fluid we observed an increase of Foxp3^{int} Tregs of CD3+CD4+CD25+CD127^{lo} lymphocytes from 11% to 33% in SF (P<0.001) and a decrease of Foxp3^{hi} Tregs from 80% to 50% (P<0.001).

Ki67-expressing Tregs are increased in inflamed PsA joints

Subsequently, we focused on differences between Foxp3^{int} and Foxp3^{hi} Tregs in peripheral blood and synovial fluid by assessing their relative frequencies and proliferative capacity. Compared to peripheral blood, synovial fluid was significantly enriched for Foxp3^{int} Tregs (P<0.001) (**Supplemental Figure S1A** and **S1B**). When examining the proliferative capacity, we noted that in general Foxp3^{hi} Tregs had higher proliferative capacity than Foxp^{int} Tregs. Nonetheless, both subsets (Foxp^{int} and Foxp3^{hi}) derived from synovial fluid had higher proliferative capacity compared to their peripheral blood counterparts (**Supplemental Figure S1C-E**).

A subset of Tregs in inflamed joints in PsA upregulate CD161 and RORγt

To further investigate the phenotype of Foxp3^{int} and Foxp3^{hi} synovial fluid-derived Tregs in PsA we studied CD161 and RORγt, as they are associated with arthritis and a pro-inflammatory potential of Tregs.(7,16,30) In PsA patients, we found that the percentage of CD161-expressing Tregs was higher in synovial fluid than in circulation and that more Foxp3^{int} Tregs express CD161 (4.1%), as compared to Foxp3^{hi} Tregs (1.3%) (**Figure 2A-C**). Additionally, in PsA, synovial fluid Tregs express more RORγt than Tregs in circulation (Foxp3^{int} 3.0 vs. 1.0%, P=0.048; Foxp3^{hi} 1.8% vs. 0.5% Treg, P=0.026) (**Figure 2D-F**). The increased expression of CD161 and RORγt by synovial Tregs was most pronounced in the intermediate Foxp3 subset of Tregs (**Figure 2A** and **2D**).

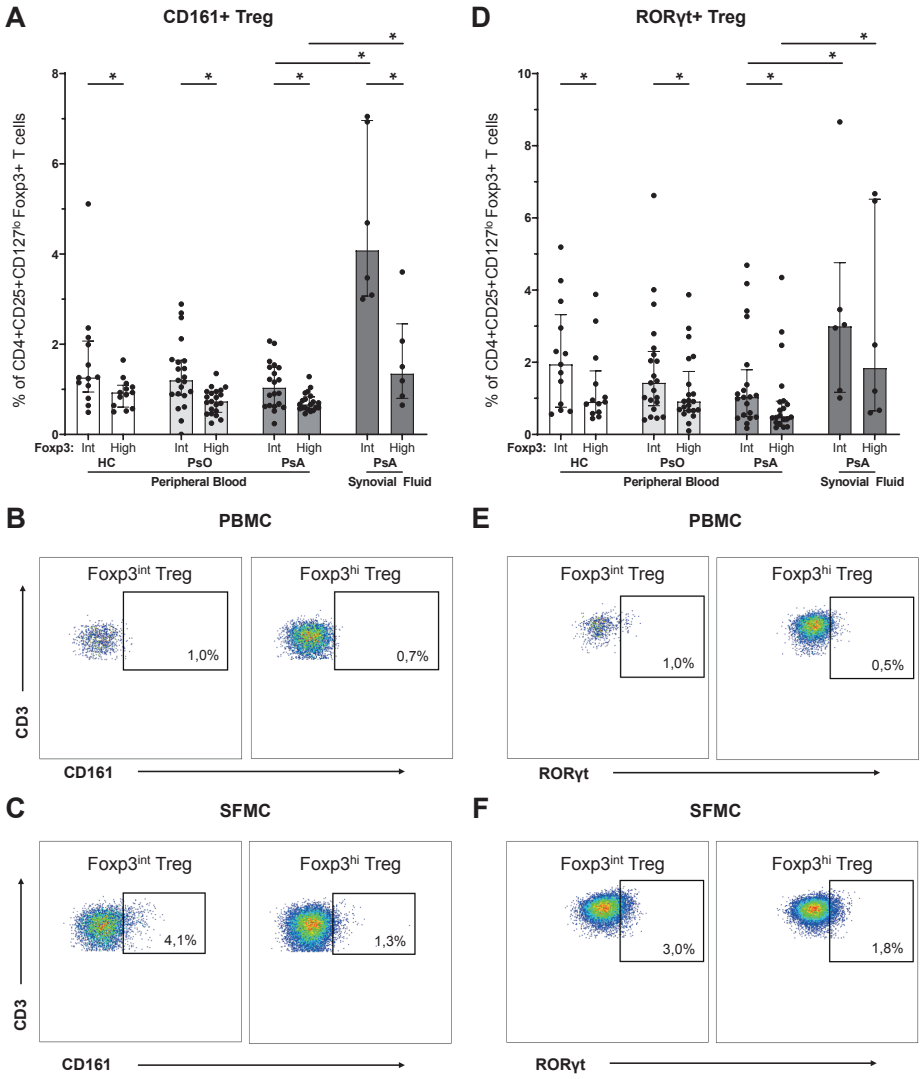


Figure 2. Pro-inflammatory phenotype of synovial fluid-derived Foxp^{int} and Foxp3^{hi} Tregs. Flow cytometry analysis of CD4+CD25+CD127^{lo}Foxp3+ Tregs derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). Dot plots: percentages in PBMC plots represent median of PB-derived Tregs in PsA; percentages in SFMC plots represent median of SF-derived Tregs. (**A**) Proportions of CD161+Foxp3^{int} and CD161+Foxp3^{hi} Tregs. (**B-C**) Representative flow cytometry plots to identify CD161+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (B) and synovial fluid (C). (**D**) Proportions of RORγt+Foxp3^{int} and RORγt+Foxp3^{hi} Tregs. (**E-F**) Representative flow cytometry plots to identify RORγt+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (E) and synovial fluid (F). Abbreviations: Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; Int: intermediate expression of Foxp3; PBMC: peripheral blood mononuclear cells; PsA: psoriatic arthritis; PsO: psoriasis; RORγt: retinoic acid receptor-related orphan receptor gamma; SFMC: synovial fluid mononuclear cells; Tregs: T regulatory cells.

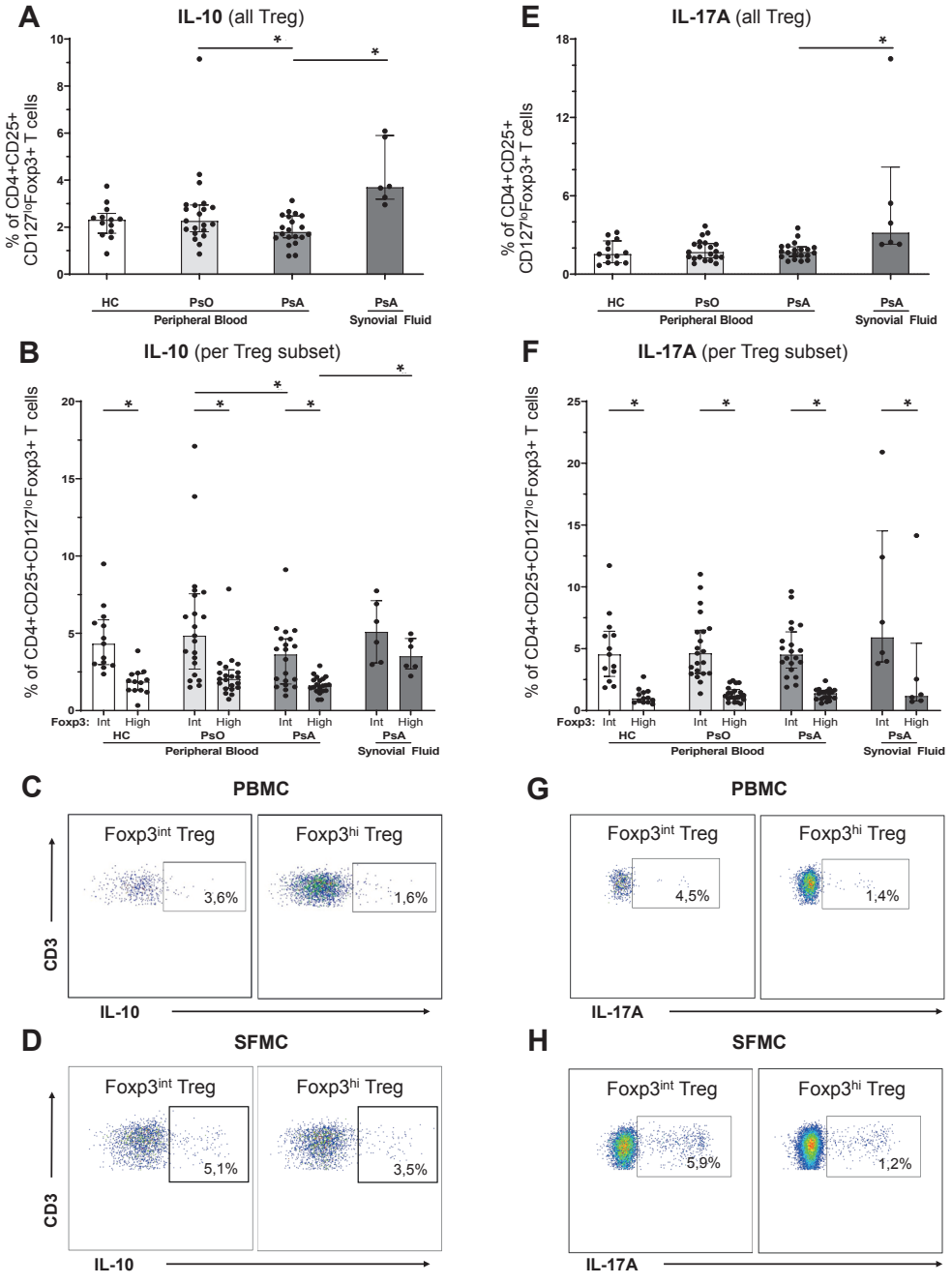
High IL-10 and IL-17A production by synovial fluid-derived Tregs

To study functional differences between Tregs in an inflammatory environment and in circulation of PsA patients, we measured inhibitory and pro-inflammatory cytokine production. As expected, Tregs from synovial fluid showed a modest but increased capacity to produce cytokines, both the anti-inflammatory cytokine IL-10 (3.7% vs. 1.8%, P<0.001) (**Figure 3A**) and the key pro-inflammatory cytokine IL-17A (3.2% vs. 1.7%; P=0.002) (**Figure 3E**). When examining the different subsets of Tregs, we found that the Foxp3^{int} subset was the major cytokine producer, the most notable being the elevated IL-17A producing capacity by Foxp3^{int} synovial fluid Tregs (5.9% vs. 1.2%, P=0.028) (**Figure 3B-D** and **3F-G**).

Synovial fluid-derived Tregs express high CTLA-4, TIGIT and ICOS

Reduced expression of immune receptors by Tregs could contribute to abnormal Treg function in inflammatory arthritis.(8) Therefore, we measured expression of two key inhibitory receptors essential for Treg suppressive function: CTLA-4 and TIGIT (**Figure 4A-F**). Both receptors were expressed more by Foxp3^{hi} Tregs, as compared to the intermediate Treg subset (**Figure 4A** and **4D**). In PsA patients, the proportions Tregs that express CTLA-4 were increased in synovial fluid, as compared to peripheral blood (Foxp3^{int} 23.5% vs. 3.3%, P=0.000; Foxp3^{hi} 28.6% vs. 7.4%, P=0.003) (**Figure 4A-C**). That was similar for TIGIT: proportions of Foxp3^{int} and Foxp3^{hi} Tregs with TIGIT expression were higher in synovial fluid (Foxp3^{int} 85.3% vs. 69.1%, P=0.002; Foxp3^{h}: 90.3% vs. 78.0%, P=0.004) (**Figure 4D-F**).

► **Figure 3. IL-10 and IL-17A production by intra-articular Tregs in PsA patients.** Flow cytometry analysis of CD4+CD25+CD127^{lo}Foxp3+ Tregs derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). PBMC and SFMC were cultured for 4,5 hours with 20 ng/mL PMA, 1 µg/ml ionomycin and 1:1000 BD GolgiStop. Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). Dot plots: percentages in PBMC plots represent median of PB-derived Tregs in PsA; percentages in SFMC plots represent median of SF-derived Tregs. (**A**) Proportion of CD4+CD25+CD127^{lo}Foxp3+ Tregs, that upon activation produce IL-10. (**B**) Proportions of Foxp3^{int} and Foxp3^{hi} Tregs, that upon activation produce IL-10. (**C-D**) Representative flow cytometry plots to identify IL-10 production by Foxp3^{int} and Foxp3^{hi} Tregs derived from peripheral blood (C) and synovial fluid (D). (**E**) Proportion of CD4+CD25+CD127^{lo}Foxp3+ Tregs, that upon activation produce IL-17A. (**F**) Proportions of Foxp3^{int} and Foxp3^{hi} Tregs, that upon activation produce IL-17A. (**G-H**) Representative flow cytometry plots to identify IL-17A production by Foxp3^{int} and Foxp3^{hi} Tregs derived from peripheral blood (G) and synovial fluid (H). Abbreviations: Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; IL: interleukin; Int: intermediate expression of Foxp3; PBMC: peripheral blood mononuclear cells; PsA: psoriatic arthritis; PsO: psoriasis; SFMC: synovial fluid mononuclear cells; Tregs: T regulatory cells.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% of CD4+CD25+ CD127loFoxp3+ T cells
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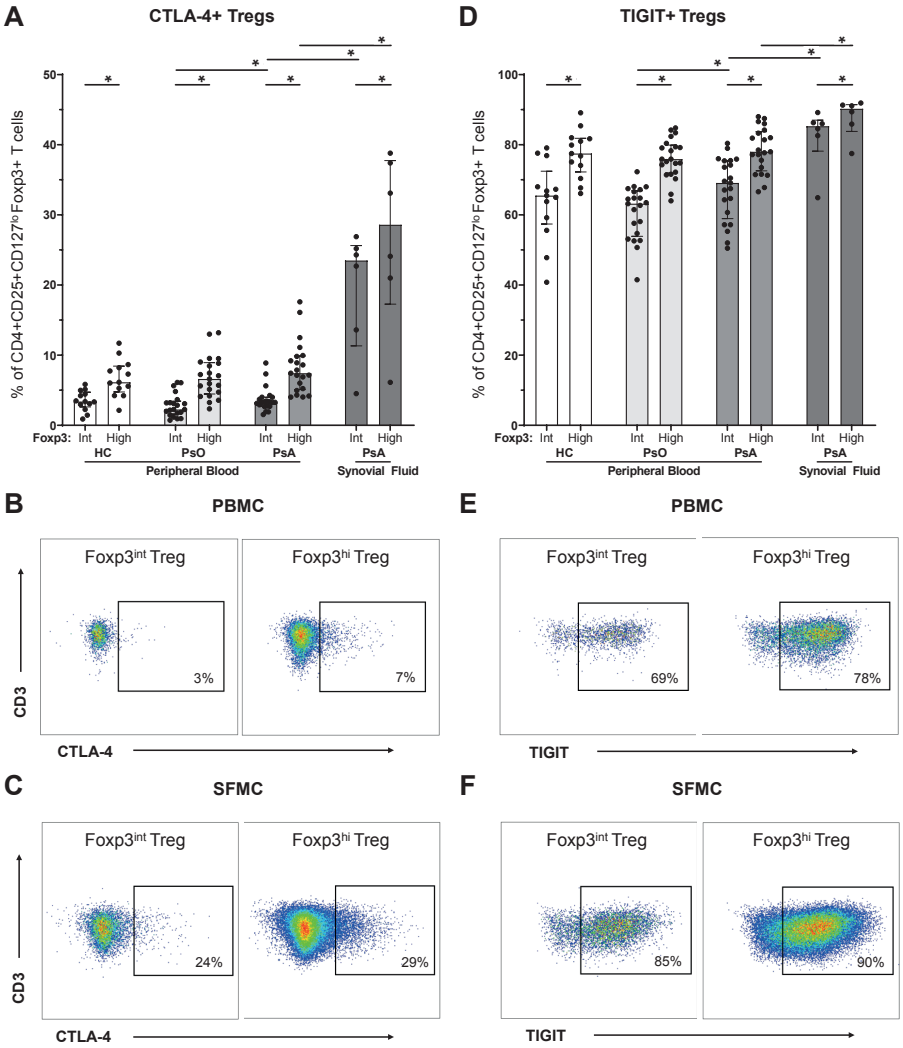

Figure 4. High expression of key regulatory immune receptors by intra-articular Tregs in PsA. Flow cytometry analysis of CD4+CD25+CD127^{lo}Foxp3+ Tregs derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). Dot plots: percentages in PBMC plots represent median of PB-derived Tregs in PsA; percentages in SFMC plots represent median of SF-derived Tregs. (**A**) Proportion of CTLA-4+Foxp3^{int} and CTLA-4+Foxp3^{hi} Tregs. (**B,C**) Representative flow cytometry plots to identify CTLA-4+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (B) and synovial fluid (C). (**D**) Proportions of TIGIT+Foxp3^{int} and TIGIT+Foxp3^{hi} Tregs. (**E,F**) Representative flow cytometry plots to identify TIGIT+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (E) and synovial fluid (F). Abbreviations: CTLA-4: cytotoxic T-lymphocyte-associated protein 4 (CD152); Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; Int: intermediate expression of Foxp3; PBMC: peripheral blood mononuclear cells; PsA: psoriatic arthritis; PsO: psoriasis; SFMC: synovial fluid mononuclear cells; TIGIT: T cell immunoreceptor with Ig and ITIM domains; Tregs: T regulatory cells.

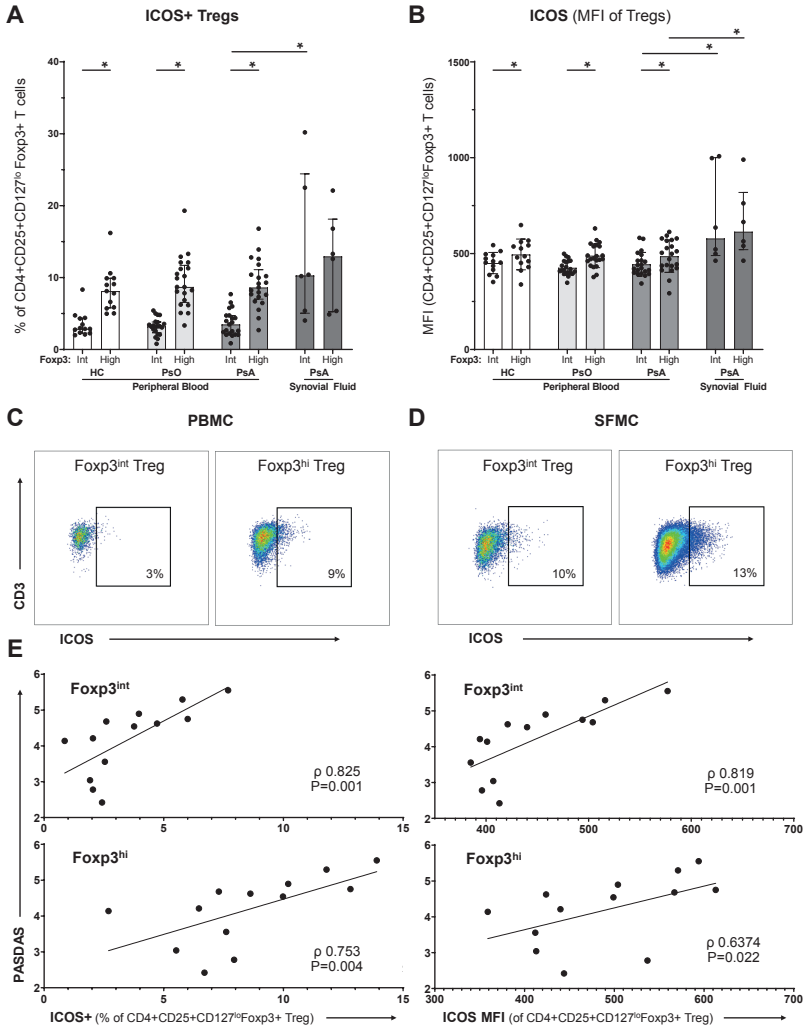

Figure 5. High PsA disease activity associates with ICOS+ Tregs in circulation. Flow cytometry analysis of CD4+CD25+CD127^{lo}Foxp3+ Tregs derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). (**A**) Proportions of ICOS+Foxp3^{int} and ICOS+Foxp3^{hi} Tregs. (**B**) ICOS expression by Foxp3^{int} and Foxp3^{hi} Tregs as measured by MFI. (**C-D**) Representative flow cytometry plots to identify ICOS+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (C) and synovial fluid (D). Percentages in PBMC dot plots represent median of PB-derived Tregs in PsA. Percentages in SFMC dot plots represent median of SF-derived Tregs. (**E**) Scatterplots of significant Spearman's rank correlation of disease activity of PsA (as measured by PASDAS (range 0-10)) with ICOS expression by CD4+CD25+CD127^{lo}Foxp3+ Tregs. Association shown of PASDAS with the proportion of ICOS+Foxp3^{int} Tregs (upper left), proportion of ICOS+Foxp3^{hi} Tregs (lower left), ICOS expression as measured by MFI of Foxp^{int} Tregs (upper right) and ICOS expression as measured by MFI of Foxp^{hi} Tregs (lower right). Abbreviations: CI: confidence interval; Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; ICOS: inducible T-cell costimulator (CD278); Int: intermediate expression of Foxp3; MFI: median fluorescent intensity; PASDAS: psoriatic arthritis disease activity score; PBMC: peripheral blood mononuclear cells; PsA: psoriatic arthritis; PsO: psoriasis; ρ: Spearman's rho; SFMC: synovial fluid mononuclear cells; Tregs: T regulatory cells.

Moreover, we included ICOS in our phenotypical Treg characterization, because ICOS+ Tregs can play a pro-inflammatory, pathogenic role in inflammatory arthritis and immune diseases.(31,32) We observed a comparable expression pattern as for the inhibitory receptors: synovial fluid derived Tregs express more ICOS, as compared to Tregs in circulation (**Figure 5A-D**). Furthermore, we found a difference between the Foxp3^{int} and Foxp3^{hi} Treg subsets: in peripheral blood Foxp3^{int} Tregs express less ICOS as compared to Foxp3^{hi} (3.5% vs. 8.6%, P=0.000), but in synovial fluid both subset express similar levels (10.3% vs. 13.0%, P=0.753) (**Figure 5A**). This is a relevant finding, because we observed an association of ICOS expression on Treg with PsA disease activity as measured by PASDAS (range 0-10), which takes arthritis, enthesitis, dactylitis, C-reactive protein, physician disease activity score and two PROs into account (**Figure 5E**). Both the proportion of ICOS+ Tregs and the MFI of ICOS significantly correlated with PASDAS in the Foxp3^{int} Treg subset and the Foxp3^{hi} Treg subset.

Association of Treg characteristics with disease

In addition, we compared peripheral blood Treg characteristics between psoriasis and PsA patients. Overall, we found few differences, but we did observe higher CTLA-4 and TIGIT expression by Foxp3^{int} Tregs of PsA patients as compared to psoriasis patients (CTLA-4+ Tregs: 2.3% vs. 3.3%, P=0.040) (TIGIT+ Tregs: 63.2% vs. 69.1%, P=0.017) (**Figure 4A** and **4B**). Moreover, Tregs from PsA patients produce less IL-10, as compared to psoriasis patients (**Figure 3A** and **3B**).

ADAMTSL5 autoantibodies associate with Treg Foxp3 expression in psoriatic disease

Lastly, we queried whether Treg plasticity is associated with loss of peripheral tolerance in PsA. To investigate this further, we quantified autoantibodies in serum and synovial fluid against a newly discovered autoantigen specific for psoriatic disease: A Disintegrin And Metalloprotease domain containing ThromboSpondin type 1 motif-Like 5 (ADAMTSL5) (patient characteristics shown in **Supplemental Table S3** and **S4**).(33) ADAMTSL5 is a protein present in extracellular matrix and implicated in microfibril function modulation. (34) We observed higher anti-ADAMTSL5 IgG in PsA serum (575 µg/mL (IQR 321-1523)), as compared to HC serum (205 µg/mL (IQR 28-833), P=0.004), psoriasis serum (319 µg/mL (IQR 103-645), P=0.012) and PsA synovial fluid (138 µg/mL (IQR 77-348), P=<0.0001) (**Figure 6A**, **Supplemental Figure S2A**). ADAMTSL5 autoantibodies discriminated between psoriasis and PsA diagnosis with an AUROC of 0.67 (95%CI 0.543-0.787), P=0.012)

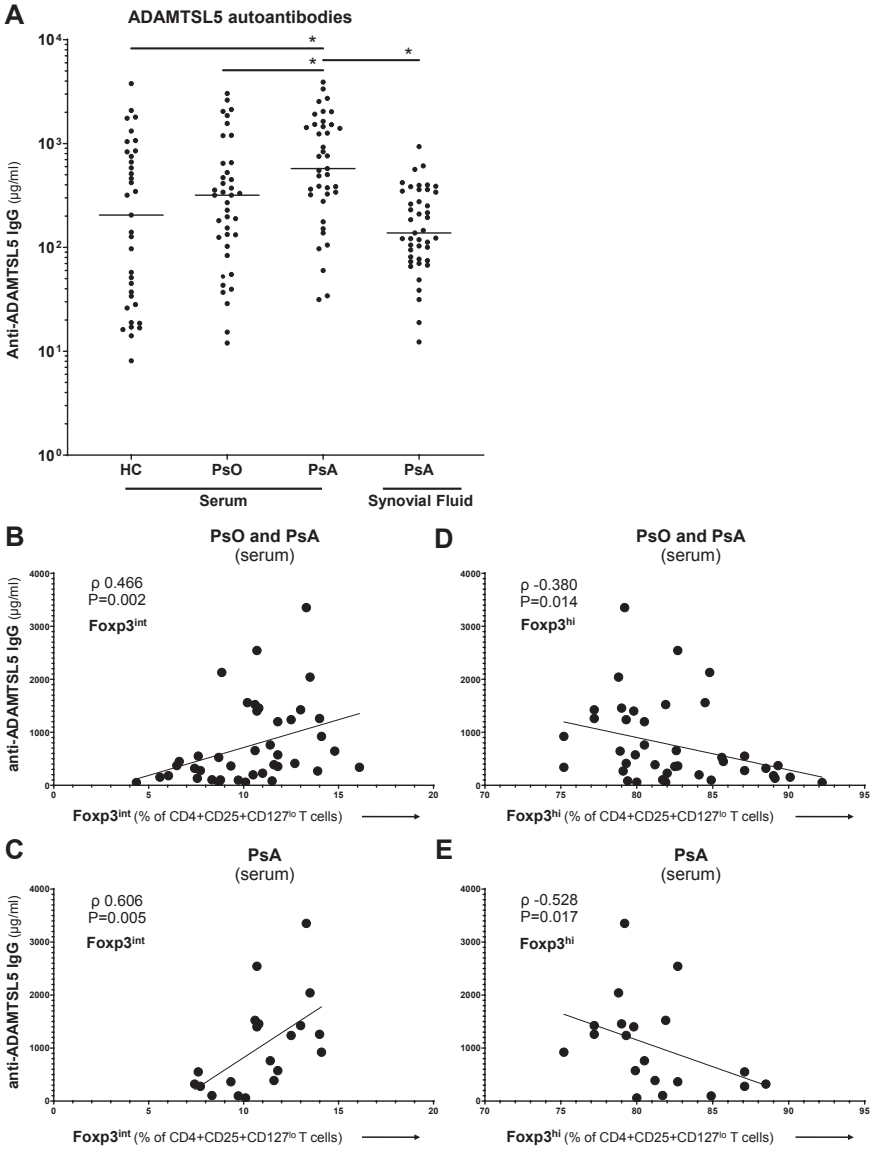

Figure 6. Foxp3 downregulation by Tregs associates with ADAMTSL5 autoantibodies in psoriatic disease. Association of ADAMTSL5 autoantibodies as measured by ELISA with Foxp3 expression by Tregs as measured by flow cytometry. (**A**) ELISA of anti-ADAMTSL5 IgG in sera of HC (n=35), psoriasis patients (n=39), PsA patients (n=39), and synovial fluid of PsA patients (n=43). * P value <0.05. (**B-E**) Scatterplots of significant Spearman's rank correlation of serum anti-ADAMTSL5 autoantibody concentration with Foxp3 expression by CD3+CD4+CD25+CD127^{lo} T cells derived from peripheral blood of psoriasis (n=21) and PsA patients (n=21), as measured by flow cytometry. Differentiation between Foxp3^{int} (B, C) and Foxp3^{hi} (D, E) Tregs in all patients with psoriatic disease (B, D) versus PsA patients only (C, E). Abbreviations: ADAMTSL5: A Disintegrin And Metalloprotease domain containing ThromboSpondin type 1 motif-Like 5; Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; IgG: Immunoglobulin G; P: p value; PsA: psoriatic arthritis; PsA: psoriatic arthritis; PsO: psoriasis; ρ: Spearman's rho.

(**Supplemental Figure S2B**). No associations of anti-ADAMTSL5 IgG with clinical characteristics or disease activity were observed (*data not shown*).

Further, we identified an association of Foxp3 instability with autoantibody production in psoriatic disease. We observed that the Foxp3^{int} Treg subset correlated with the presence of ADAMTSL5 autoantibodies in peripheral blood of patients with psoriatic disease (Spearman's ρ 0.466, P=0.002) (**Figure 6B**). The correlation was even stronger in PsA alone (ρ 0.606, P=0.005) (**Figure 6C**). In contrast, we observed an inverse correlation of Foxp3^{hi} Tregs with anti-ADAMTSL5 IgG in psoriatic disease (ρ -0.380, P=0.014) (**Figure 6D**), again more pronounced in PsA alone (ρ -0.528, P=0.017) (**Figure 6E**).

Discussion

In the inflammatory microenvironment of autoimmune disease, Treg defects and differentiation are suggested to play a role in loss of peripheral immune tolerance. However, the potential pathogenic implications of Treg dysfunction and plasticity have not been clarified in psoriatic disease. To our knowledge, this is the first study to perform in-depth phenotypical characterization of Tregs derived from the inflammatory microenvironment of inflamed joints in patients with psoriatic disease. Here, we provide evidence for Treg plasticity in PsA by showing distinct phenotypical and functional properties of intra-articular Tregs as compared to Tregs in circulation: downregulation of key transcription factor Foxp3, pro-inflammatory cytokine production, upregulation of inhibitory immune receptors, and upregulation of markers associated with a pro-inflammatory potential of Tregs: CD161, RORγt and ICOS.

Foxp3 is the key transcription factor of Tregs and its expression is essential for Treg development, maintenance and function.(29) Our results demonstrate a significant increase of intra-articular Tregs with intermediate Foxp3 expression in PsA patients. Association of Foxp3 with PsA has been previously described by one study, that identified a hemizygous Foxp3 mutation (c.1222G>A) in familial juvenile PsA.(35) Moreover, in psoriasis patients, it was shown that enhanced loss of Foxp3 is linked to Treg differentiation into IL-17A producing cells.(15) In the broader context of autoimmune disease, multiple studies suggested that stability of Foxp3 expression is negatively affected by pro-inflammatory conditions.(13,19) These findings have clinical relevance, because Treg defects – including Foxp3 instability – contribute to disease pathophysiology. This contribution is either through increased escape of autoreactive T cells from Treg regulation or, what has been suggested more recently, by conversion of Tregs into pathogenic, highly autoreactive, memory T cells.(13,15,29)

Furthermore, we show that the intra-articular subset of Tregs with lower Foxp3, as compared to their Foxp3^{hi} counterparts in the same tissue location, have lower expression of the inhibitory receptors CTLA-4 and TIGIT. These Foxp3^{int} Tregs produce even more of the psoriatic disease hallmark cytokine IL-17, and display the highest expression of CD161. These substantial differences between Foxp^{int} and Foxp3^{hi} are relevant, since our results demonstrate that Tregs with decreased Foxp3 expression are present in large numbers in the synovial compartment. In line with the homeostatic importance of Tregs in psoriatic disease, we observed a relation of Foxp3 instability with loss of immune tolerance in psoriatic disease: presence of ADAMTSL5 antibodies in psoriasis and PsA reflected the balance between Foxp^{int} and Foxp3^{hi} Tregs. However, as we did not perform functional experiments and only studied relative Foxp3 expression, we are careful to draw definite conclusions.

Based on their phenotypical characteristics, synovial fluid-derived Tregs – and in particular the Foxp3^{int} Treg subset – are potentially pathogenic. First, because failure to upregulate inhibitory receptors has been shown to contribute to the comprised suppressive function of intra-articular Tregs in inflammatory arthritis.(8) In addition, CD161+ Tregs were previously identified as a subset capable of IL-17A and IFNγ production, and to exhibit a high pro-inflammatory potential.(7,30) In fact, CD161+ Tregs are the predominant IL-17 producing Treg population in inflamed joints of inflammatory arthritis patients.(7) Furthermore, concerning the implications of RORγt+ Tregs, in inflammatory bowel disease it was shown that the capacity of IL-17+RORγt+ Tregs to suppress autologous T cell proliferation is reduced by approximately 60%.(14) Also, RORγt expression associates with IL-17A producing Tregs in psoriasis.(15) These findings are expected, considering that Foxp3 and RORγt transcription factors drive differentiation of T cells towards Tregs or Th17 cells.(36) Hence, the observed phenotypical characteristics of Tregs with intermediate Foxp3 expression all suggest a pathogenic potential in the synovial compartment of PsA patients. Moreover, with regards to functional differences, intra-articular Tregs may contribute to ongoing localized inflammation by production of IL-17A. The latter has previously been associated with unresponsiveness of Teff in the microenvironment of inflammatory arthritis.(18) Taken together, we identified a pro-inflammatory phenotype of synovial fluid-derived Tregs, most pronounced in the Treg subset with downregulated Foxp3.

Further, the association of ICOS+ Tregs with PsA disease activity drew our attention, because ICOS is most commonly associated with a strongly inhibitory Treg subset.(32) However, studies in the last decade have suggested a possibly pathogenic role of

ICOS+ Tregs, that may contribute to autoimmune rheumatic disease. In lupus, RA and spondyloarthritis, associations were found of ICOS expression by Tregs with high disease activity, with non-response to therapy, increased autoantibodies, and pro-inflammatory cytokine production.(31,37–39) Since we show that all intra-articular Tregs upregulate ICOS expression, even independent of Foxp3 expression, further investigation is warranted for ICOS+ Tregs as possible therapeutic target for treatment of psoriatic disease.

Our study has several limitations. First, the sample size of our SFMC cohort was small. However, we deem that the evident observed dissimilarities between Tregs derived from synovial fluid and peripheral blood have enabled us to draw conclusions about Treg plasticity. Second, the use of DMARDs (methotrexate, golimumab) by two patients in our SFMC cohort could have influenced our results, although contradicting results have been published as to whether DMARDs affect Treg phenotype and function.(40–42) Third, we have not assessed the pro-inflammatory potential of Tregs in functional experiments or performed assays to evaluate the suppressive capacity of intra-articular Tregs. Fourth, with flow cytometry analyses we could only assess relative differences. Hence, we can only speculate about the implications of absolute numbers of Tregs with a pro-inflammatory phenotype in PsA pathogenesis. Nevertheless, based on what is known from literature in other types of inflammatory arthritis, we deem to have identified a new – and potentially important - dysregulated mechanism implicated in PsA pathogenesis, that needs further investigation.

Since we have not confirmed our hypothesis by assessing the suppressive capacity of intra-articular Tregs, it must be taken into consideration that even differentiated Tregs may be able to effectively suppress Teff.(6,7,16) If and how differentiated Tregs in inflammatory arthritis can effectively suppress Teff T cells is an increasing topic of interest and contradicting results have been published.(43) Some concluded that impaired expression of immune regulatory molecules or lack of cytokine production are key to defective Teff suppression by Tregs.(8,9,44) Others attributed failure of effective Teff suppression to the factor that Tregs are prone to apoptosis under inflammatory conditions.(43) Moreover, evidence suggested that sustained resistance of local CD4+ and CD8+ Teff in an inflammatory microenvironment could be key to ineffective Treg suppression.(18) Whether these mechanisms play a role in psoriatic disease has yet to be elucidated. Might an important role for Treg differentiation in PsA pathogenesis be indeed confirmed, this may facilitate identification of new treatment targets and therapies, including Treg growth factors, Treg stabilizing factors and therapies that enhance Treg

function.(45) In preclinical models and clinical trials low dose IL-2 and cellular therapy with polyclonal, therapeutic Tregs have already shown promising results.(45–47) Moreover, research to artificially stabilize Treg Foxp3 expression in vitro for clinical applications are ongoing.(48) As treatment options for autoimmune disease are evolving, we deem it essential to further advance our understanding of the role of Treg plasticity in psoriatic disease pathogenesis.

In conclusion, we show that Tregs derived from the inflammatory environment of inflamed joints in PsA patients exhibit a potentially pathogenic phenotype characterized by expression of CD161, RORγt and ICOS. Moreover, we identify the importance of Foxp3 expression by Tregs in PsA pathogenesis, with a novel role for Foxp3^{int} Tregs with a heightened capacity to produce IL-17A.

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Author contributions: JP, EL and MB initiated the study. JP, EL, MO and TvK isolated sera and PBMC samples. EL, MO and AC were responsible for synovial fluid and SFMC collection. JP and MO performed ELISA and flow cytometry experiments. JP, EL, JS and MB primarily wrote the manuscript. All authors contributed to substantial discussion of content, reviewing and revising the manuscript before submission.

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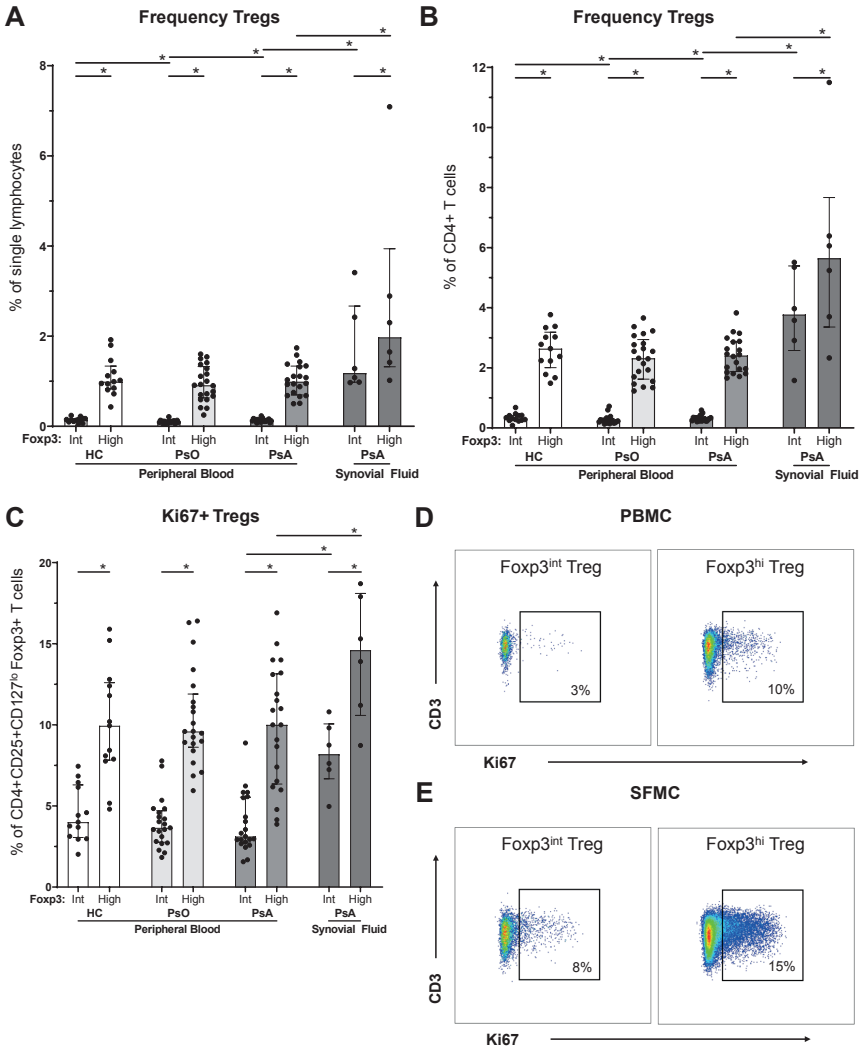
References

1. FitzGerald O, Ogdie A, Chandran V, Coates LC, Kavanaugh A, Tillett W, et al. Psoriatic arthritis. Nat Rev Dis Prim. 2021;7.
2. Chimenti MS, Caso F, Alivernini S, De Martino E, Costa L, Tolusso B, et al. Amplifying the concept of psoriatic arthritis: The role of autoimmunity in systemic psoriatic disease. Autoimmun Rev. 2019;18:565–75.
3. Generali E, Bose T, Selmi C, Voncken JW, Damoiseaux JGMC. Nature versus nurture in the spectrum of rheumatic diseases: Classification of spondyloarthritis as autoimmune or autoinflammatory. Autoimmun Rev. 2018;17:935–41.
4. Penkava F, Velasco-Herrera MDC, Young MD, Yager N, Nwosu LN, Pratt AG, et al. Single-cell sequencing reveals clonal expansions of pro-inflammatory synovial CD8 T cells expressing tissue-homing receptors in psoriatic arthritis. Nat Commun. 2020;11.
5. Veale DJ, Fearon U. Psoriatic arthritis 1 The pathogenesis of psoriatic arthritis. Lancet. 2018;391:2273–84.
6. Mijnheer G, Lutter L, Mokry M, van der Wal M, Scholman R, Fleskens V, et al. Conserved human effector Treg cell transcriptomic and epigenetic signature in arthritic joint inflammation. Nat Commun. 2021;12:2710.
7. Afzali B, Mitchell PJ, Edozie FC, Povoleri GAM, Dowson SE, Demandt L, et al. CD161 expression characterizes a subpopulation of human regulatory T cells that produces IL-17 in a STAT3-dependent manner. Eur J Immunol. 2013;43:2043–54.
8. Flores-Borja F, Jury EC, Mauri C, Ehrenstein MR. Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. Proc Natl Acad Sci U S A. 2008;105:19396–401.
9. Beavis PA, Gregory B, Green P, Cribbs AP, Kennedy A, Amjadi P, et al. Resistance to regulatory T cell-mediated suppression in rheumatoid arthritis can be bypassed by ectopic foxp3 expression in pathogenic synovial T cells. Proc Natl Acad Sci U S A. 2011;108:16717–22.
10. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-Hora M, Kodama T, et al. Pathogenic conversion of Foxp3 + T cells into TH17 cells in autoimmune arthritis. Nat Med. 2014;20:62–8.
11. Macdonald KG, Dawson NAJ, Huang Q, Dunne J V., Levings MK, Broady R. Regulatory T cells produce profibrotic cytokines in the skin of patients with systemic sclerosis. J Allergy Clin Immunol. 2015;135:946-955.e9.
12. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3+ CD25+ CD4+ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. J Exp Med. 2005;201:723–35.
13. Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martínez-Llordella M, Ashby M, et al. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol. 2009;10:1000–7.
14. Ueno A, Jijon H, Chan R, Ford K, Hirota C, Kaplan GG, et al. Increased prevalence of circulating novel IL-17 secreting Foxp3 expressing CD4+ T cells and defective suppressive function of circulating Foxp3+ regulatory cells support plasticity between Th17 and regulatory T cells in inflammatory bowel disease patients. Inflamm Bowel Dis. 2013;19:2522–34.
15. Bovenschen HJ, Van De Kerkhof PC, Van Erp PE, Woestenenk R, Joosten I, Koenen HJPM. Foxp3 regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. J Invest Dermatol. 2011;131:1853–60.

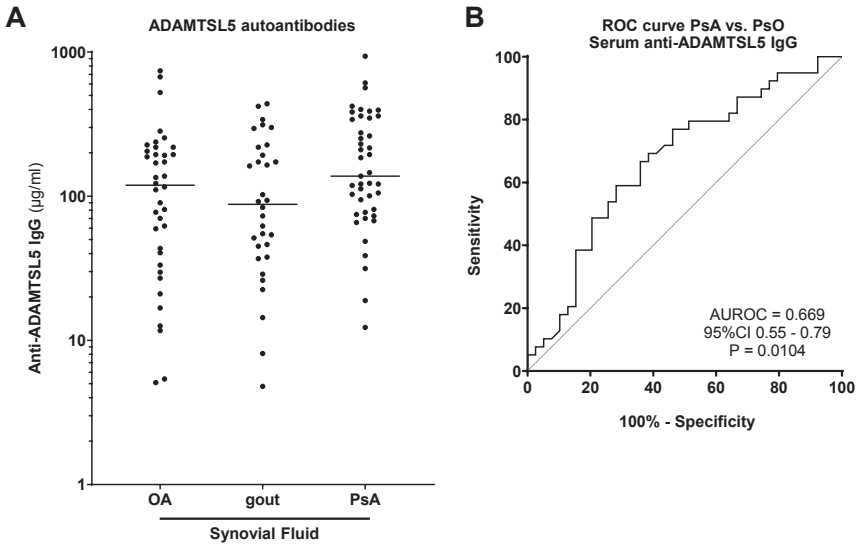
16. Duurland CL, Brown CC, O'Shaughnessy RFL, Wedderburn LR. CD161+ Tconv and CD161+ treg share a transcriptional and functional phenotype despite limited overlap in TCRβ repertoire. Front Immunol. 2017;8:1–18.
17. Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M, et al. Foxp3+ T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal Immunol. 2016;9:444–57.
18. Mijnheer G, Van Wijk F. T-cell compartmentalization and functional adaptation in autoimmune inflammation: Lessons from pediatric rheumatic diseases. Front Immunol. 2019;10:1–11.
19. Shevyrev D, Tereshchenko V. Treg Heterogeneity, Function, and Homeostasis. Front Immunol. 2020;10:1–13.
20. Han L, Sugiyama H, Zhang Q, Yan K, Fang X, McCormick TS, et al. Phenotypical analysis of ectoenzymes CD39/CD73 and adenosine receptor 2A in CD4+CD25highFoxp3+ regulatory T-cells in psoriasis. Australas J Dermatol. 2018;59:e31–8.
21. Fiocco U, Martini V, Accordi B, Caso F, Costa L, Oliviero F, et al. Transcriptional network profile on synovial fluid T cells in psoriatic arthritis. Clin Rheumatol. 2015;34:1571–80.
22. Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, et al. Dysfunctional Blood and Target Tissue CD4 + CD25 high Regulatory T Cells in Psoriasis: Mechanism Underlying Unrestrained Pathogenic Effector T Cell Proliferation. J Immunol. 2005;174:164–73.
23. Yan KX, Fang X, Han L, Zhang ZH, Kang KF, Zheng ZZ, et al. Foxp3+ regulatory T cells and related cytokines differentially expressed in plaque vs. guttate psoriasis vulgaris. Br J Dermatol. 2010;163:48–56.
24. Szentpetery A, Heffernan E, Gogarty M, Mellerick L, McCormack J, Haroon M, et al. Abatacept reduces synovial regulatory T-cell expression in patients with psoriatic arthritis. Arthritis Res Ther. 2017;19:158.
25. Soler DC, Sugiyama H, Young AB, Massari J V., McCormick TS, Cooper KD. Psoriasis patients exhibit impairment of the high potency CCR5+ T regulatory cell subset. Clin Immunol. 2013;149:111–8.
26. Scher JU, Ogdie A, Merola JF, Ritchlin C. Preventing psoriatic arthritis: focusing on patients with psoriasis at increased risk of transition. Nat Rev Rheumatol. 2019;15:153–66.
27. Yu N, Li X, Song W, Li D, Yu D, Zeng X, et al. CD4+CD25+CD127low/- T cells: A more specific treg population in human peripheral blood. Inflammation. 2012;35:1773–80.
28. Liu W, Putnam AL, Xu-yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med. 2006;203:1701–11.
29. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008;8:523–32.
30. Pesenacker AM, Bending D, Ursu S, Wu Q, Nistala K, Wedderburn LR. CD161 defines the subset of FoxP31 T cells capable of producing proinflammatory cytokines. Blood. 2013;121:2647–58.
31. Wang HX, Kang X, Chu S, Li H, Li X, Yin X, et al. Dysregulated ICOS+ proinflammatory and suppressive regulatory T cells in patients with rheumatoid arthritis. Exp Ther Med. 2018;16:3728–34.
32. Li DY, Xiong XZ. ICOS+ Tregs: A Functional Subset of Tregs in Immune Diseases. Front Immunol. 2020;11.
33. Yuan Y, Qiu J, Lin Z, Li W, Haley C, Mui UN, et al. Identification of Novel Autoantibodies Associated With Psoriatic Arthritis. Arthritis Rheumatol. 2019;71:941–51.

34. Bader HL, Wang LW, Ho JC, Tran T, Holden P, Fitzgerald J, et al. A disintegrin-like and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5) is a novel fibrillin-1-, fibrillin-2-, and heparin-binding member of the ADAMTS superfamily containing a netrin-like module. Matrix Biol. 2012;31:398–411.
35. Alzyoud R, Alansari S, Maaitah H, AlDossari H, Monies D, Al-Mayouf SM. Familial Clustering of Juvenile Psoriatic Arthritis Associated with a Hemizygous FOXP3 Mutation. Curr Rheumatol Rep. 2021;23:64.
36. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, et al. Molecular Antagonism and Plasticity of Regulatory and Inflammatory T Cell Programs. Immunity. 2008;29:44–56.
37. Liu Y, Zhu T, Cai G, Qin Y, Wang W, Tang G, et al. Elevated circulating CD4 +ICOS +Foxp3 + T cells contribute to overproduction of IL-10 and are correlated with disease severity in patients with systemic lupus erythematosus. Lupus. 2011;20:620–7.
38. Slauenwhite D, McAlpine SM, Hanly JG, Malik A, Haidl ID, Marshall JS, et al. Association of a Type 2–Polarized T Cell Phenotype With Methotrexate Nonresponse in Patients With Rheumatoid Arthritis. Arthritis Rheumatol. 2020;72:1091–102.
39. Araujo LM, Fert I, Jouhault Q, Labroquère K, Andrieu M, Chiocchia G, et al. Increased production of interleukin-17 over interleukin-10 by treg cells implicates inducible costimulator molecule in experimental spondyloarthritis. Arthritis Rheumatol. 2014;66:2412–22.
40. Cribbs AP, Kennedy A, Penn H, Amjadi P, Green P, Read JE, et al. Methotrexate restores regulatory T cell function through demethylation of the FoxP3 upstream enhancer in patients with rheumatoid arthritis. Arthritis Rheumatol. 2015;67(5):1182–92.
41. Meyer A, Wittekind PS, Kotschenreuther K, Schiller J, Von Tresckow J, Haak TH, et al. Regulatory T cell frequencies in patients with rheumatoid arthritis are increased by conventional and biological DMARDs but not by JAK inhibitors. Ann Rheum Dis. 2019;80:6–7.
42. Oh JS, Kim YG, Lee SG, So MW, Choi SW, Lee CK, et al. The effect of various disease-modifying anti-rheumatic drugs on the suppressive function of CD4+CD25+ regulatory T cells. Rheumatol Int. 2013;33:381–8.
43. Liu Y, Jarjour W, Olsen N, Zheng SG. Traitor or warrior–Treg cells sneaking into the lesions of psoriatic arthritis. Clin Immunol. 2020;215:108425.
44. Appel H, Wu P, Scheer R, Kedor C, Sawitzki B, Thiel A, et al. Synovial and peripheral blood CD4+FoxP3+ T cells in spondyloarthritis. J Rheumatol. 2011;38:2445–51.
45. Bluestone JA, Tang Q. T reg cells—the next frontier of cell therapy. Science (80-). 2018;362:154–5.
46. Rosenzwajg M, Lorenzon R, Cacoub P, Pham HP, Pitoiset F, El Soufi K, et al. Immunological and clinical effects of low-dose interleukin-2 across 11 autoimmune diseases in a single, open clinical trial. Ann Rheum Dis. 2019;78:209–17.
47. Rana J, Biswas M. Regulatory T cell therapy: Current and future design perspectives. Cell Immunol. 2020;356:104193.
48. Someya K, Nakatsukasa H, Ito M, Kondo T, Tateda K ichi, Akanuma T, et al. Improvement of Foxp3 stability through CNS2 demethylation by TET enzyme induction and activation. Int Immunol. 2017;29:365–75.

Supplemental Information



Supplemental Figure S1. Increase of proliferating Tregs in PsA synovial fluid, as compared to circulation. Flow cytometry analysis of CD4+CD25+CD127^{lo}Foxp3+ Tregs derived from peripheral blood of HC (n=13), psoriasis patients (n=21) and PsA patients (n=21), and from synovial fluid of PsA patients (n=6). Bar graphs: symbols represent individual subjects; bars show median with interquartile range; * P value <0.05 (synovial fluid only compared with PsA peripheral blood). (**A,B**) Proportion of Foxp3^{int} and Foxp3^{hi} Tregs of single lymphocytes (A) and CD4+ T cells (B). (**C**) Proportions of Ki67+Foxp3^{int} and Ki67+Foxp3^{hi} Tregs. (**D,E**) Representative flow cytometry plots to identify Ki67+Foxp3^{int} and -Foxp3^{hi} Tregs derived from peripheral blood (D) and synovial fluid (E). Percentages in PBMC dot plots represent median of PB-derived Tregs in PsA. Percentages in SFMC dot plots represent median of SF-derived Tregs. Abbreviations: Foxp3^{int} / ^{hi}: forkhead box P3 expression intermediate / high; HC: healthy control; Int: intermediate expression of Foxp3; Ki67: antigen KI67; MFI: median fluorescent intensity; PBMC: peripheral blood mononuclear cells; PsA: psoriatic arthritis; PsO: psoriasis; SFMC: synovial fluid mononuclear cells; Tregs: T regulatory cells.



Supplemental Figure S2. ADAMTSL5 autoantibodies: discriminating performance of serum concentration, and concentration in synovial fluid. ELISA of anti-ADAMTSL5 IgG. * P value <0.05. (**A**) Autoantibody concentration in synovial fluid of OA (n=38), gout (n=32) and PsA patients (n=43). (**B**) Discriminating performance between PsA and psoriasis of serum autoantibodies against ADAMTSL5 in patients with psoriatic disease (psoriasis patients n=39, PsA patients n=39). Abbreviations: ADAMTSL5: A Disintegrin And Metalloprotease domain containing ThromboSpondin type 1 motif-Like 5; AUROC: area under the receiver operating characteristic curve; CI: confidence interval; IgG: Immunoglobulin G; OA: osteoarthritis; P: p value; PsA: psoriatic arthritis.

Target (Label, Category number, Company)
CD127 (BV421; 562436; BD)
CD127 (BV605; 2356670; Sony Biotechnology)
CD152 (PE; 555853; BD)
CD161 (BV510; 563212; BD)
CD25 (BV711; 563159; BD)
CD25 (PE-Cy7; 557741; BD)
CD278 (APC; 17-9948-42; eBioscience)
CD3 (AF700; 300424; Biolegend)
CD4 (BV785; 300554; Biolegend)
CD4 (PerCP; 300528; Biolegend)
Fixable Viability Dye (eF506; 65-0866-14; eBioscience)
Fixable Viability Dye (eF780; 65-0865-14, eBioscience)
Foxp3 (eF450; 48-4776-42; eBioscience)
Foxp3 (PE-CF594; 562421; BD)
IL-10 (PE; 554706; BD)
IL-17A (FITC; 11-7179-82; eBioscience)
Ki67 (FITC; F7268; DAKO)
RORγt (APC; 17-6988-82; eBioscience)
TIGIT (PerCP-eF710; 46-9500-42; eBioscience)

Supplemental Table S1. Antibodies flow cytometry.

Characteristic	HC (n=13)	Peripheral blood PsO (n=21)	Peripheral blood PsA (n=21)	Synovial Fluid PsA (n=6)
Age (y) *	46 ± 15	39 ± 14	49 ± 11	50 ± 10
Female, n	9 (69%)	10 (48%)	7 (33%)	0 (0%)
DMARD use, n **	-	0 (0%)	0 (0%)	2 (33%) [a)]
PsO duration (y) *	-	5.5 (2.3-20.2)	21.9 (9.5-31.4)	24.0 (1.6-51.8)
PsA duration (y)	-	-	3.0 (0.1-8.6)	6.8 (4.7-12.2)
BMI (kg/m^2)	-	25.7 ± 2.8	26.3 (24.6-30.7)	27.9 (23.5-33.7)
CRP (mg/L)	-	1.6 (1.1-3.7)	2.1 (1.4-3.5)	3 (1.3-.)
ESR (mm/h)	-	5 (2-8)	8 (5-13)	8 (5-15)
ACPA positive, n	-	0 (0%)	0 (0%)	0 (0%)
RF positive, n	-	0 (0%)	1 (7%)	0 (0%)
PASI *	-	5.2 (1.6-8.9)	1.9 (0.9-3.0)	3.7 [d)]
Tender joints (78) *	-	0 (0-0)	4 (1-9)	1 (1-1)
Swollen joints (76) *	-	0 (0-0)	3 (1-7)	1 (1-2)
Nail psoriasis, n	-	10 (48%)	13 (65%)	3 (50%)
Dactylitis, n	-	0 (0%)	2 (11%)	0 (0-0)
Dactylitis ever, n *	-	0 (0%)	6 (32%)	1 (17%)
LEI	-	0 (0-0)	0 (0-0)	0 (0-0)
Enthesitis ever, n	-	5 (24%)	5 (40%)	3 (60%)
Erosions, n *	-	0 (0%)	8 (42%)	2 (40%)
HAQ	-	0.0 (0.0-0.75)	0.38 (0.13-1.00)	0.25 [d)]
DLQI	-	7.5 (3.5-13.3)	3.0 (1.0-5.0)	2.0 [d)]
SF-36 PCS	-	54 (44-58)	48 (45-53)	33.2 [d)]
SF-36 MCS	-	53 (38-56)	54 (46-58)	61.8 [d)]
DAPSA [b)]	-	-	14.3 (12.1-27.1)	16.3 [d)]
PASDAS [c)]	-	-	5.5 (3.3-4.8)	5.4 [d)]

Supplemental Table S2. Baseline characteristics flow cytometry cohort. Presented data are from time of visit, unless otherwise indicated. Categorical data are presented with frequencies (%) and continuous data are presented as mean ± SD (normally distributed variables) or median (IQR) (non-normally distributed variables). * P value PsO vs. PsA <0.05. ** P value PsA PB vs. SF <0.05. [a)] Golimumab (n=1) and methotrexate (n=1). [b)] Formula DAPSA: (TJC68 + SJC66 + VAS PGA (range 0-10) + VAS pain (range 0-10) + CRP (range 0-10 mg/dL))). [c)] Formula PASDAS: (((0.18 √(VAS PhGA (range 0-100)) + (0.159 √(VAS PGA (range 0-100)) – (0.253 * √(SF-36 PCS)) + (0.101 * ln(SJC66 +1)) + (0.048 * ln(TJC68 +1)) + (0.23 * ln(enthesitis count+1)) + (0.37 * ln(dactylitis count+1)) + (0.102 * ln(CRP (mg/L) +1)) +2) * 1.5). [d)] Data available from n=1. Abbreviations: ACPA: anti-citrullinated protein antibodies; BMI: body mass index; CRP: C-reactive protein; DAPSA: disease activity index for PsA (range 0-164); DMARD: disease modifying anti-rheumatic drug use past three months; Erosions: reported by radiologist on conventional radiography and/or magnetic resonance imaging; ESR: erythrocyte sedimentation rate; HAQ: health assessment questionnaire (range 0-3); HC: healthy control; LEI: leeds enthesitis index (range 0-6); PASDAS: psoriatic arthritis disease activity score (range 0-10); PASI: psoriasis severity index (range 0-72); PsA: psoriatic arthritis; PsO: psoriasis; RF: rheumatoid factor.

Characteristic	HC (n=35)	PsO (n=39)	PsA (n=39)
Age (years)	44 ± 13	42 ± 16	44 ± 13
Female, n (%) *	17 (50%)	22 (56%)	12 (31%)
DMARD use, n (%)	0 (0%)	0 (0%)	0 (0%)
PsO duration (y)	-	10.2 (3.7-21.1)	13.8 (7.0-25.8)
PsA duration (y)	-	-	1.0 (0.1-5.4)
BMI (kg/m^2)	-	29.2 ± 7.2	27.5 ± 3.6
CRP (mg/L)	-	2.1 (1.0-5.4)	3.0 (1.9-4.6)
ESR (mm/hour)	-	6 (2-11)	8 (3-13)
ACPA positive, n	-	0 (0%)	1 (4%)
RF positive, n	-	1 (14%)	2 (7%)
PASI	-	3.9 (1.7-8.2)	2.5 (1.0-4.2)
Tender joints (78)	-	0 (0-1)	3 (0-7)
Swollen joints (76)	-	0 (0-0)	2 (0-7)
Nail psoriasis, n		23 (59%)	24 (65%)
Dactylitis, n *	-	0 (0%)	6 (17%)
Dactylitis ever, n	-	1 (3%)	13 (36%)
LEI * [a)]	-	0 (0-0)	0 (0-0)
Enthesitis ever, n	-	8 (21%)	22 (58%)
Erosions, n	-	0 (0%)	12 (32%)
HAQ	-	0.0 (0.0-0.7)	0.5 (0.3-1.0)
DLQI	-	8 (2-12)	3 (1-7)
SF-36 PCS	-	53.0 (41.3-57.2)	47.2 (38.3-51.5)
SF-36 MCS	-	49.9 (36.7-56.6)	55.0 (48.0-58.7)
DAPSA [b)]	-	-	14.3 (11.0-26.9)
PASDAS [c)]	-	-	4.6 (3.6-5.3)

Supplemental Table S3. Baseline characteristics ELISA cohort – serum. Presented data are from time of visit, unless otherwise indicated. Categorical data are presented with frequencies (%) and continuous data are presented as mean ± SD (normally distributed variables) or median (IQR) (non-normally distributed variables). * P value PsO vs. PsA <0.05. [a)] LEI mean: PsO 0.1 ± 0.3; LEI mean PsA 0.4 ± 0.8. [b)] Formula DAPSA: (TJC68 + SJC66 + VAS PGA (range 0-10) + VAS pain (range 0-10) + CRP (range 0-10 mg/dL))). [c)] Formula PASDAS: (((0.18 √(VAS PhGA (range 0-100)) + (0.159 √(VAS PGA (range 0-100)) – (0.253 * √(SF-36 PCS)) + (0.101 * ln(SJC66 +1)) + (0.048 * ln(TJC68 +1)) + (0.23 * ln(enthesitis count+1)) + (0.37 * ln(dactylitis count+1)) + (0.102 * ln(CRP (mg/L) +1)) +2) * 1.5). Abbreviations: ACPA: anti-citrullinated protein antibodies; BMI: body mass index; CRP: C-reactive protein; DAPSA: disease activity index for PsA (range 0-164); DMARD: disease modifying anti-rheumatic drug use past three months; Erosions: reported by radiologist on conventional radiography and/or magnetic resonance imaging; ESR: erythrocyte sedimentation rate; HAQ: health assessment questionnaire (range 0-3); HC: healthy control; LEI: leeds enthesitis index (range 0-6); PASDAS: psoriatic arthritis disease activity score (range 0-10); PASI: psoriasis severity index (range 0-72); PsA: psoriatic arthritis; PsO: psoriasis; RF: rheumatoid factor.

Characteristic	OA (n=38)	Gout (n=32)	PsA (n=43)
Age (years)	54 ± 10	55 ± 12	50 ± 12
Female, n (%) *	17 (45%)	1 (3%)	11 (26%)
DMARD use, n (%)	0 (0%)	0 (0%)	0 (0%)
CRP (mg/L)	NM	NM	9.5 (0.5-21.3)
ESR (mm/hour)	NM	NM	14 (7-24)

Supplemental Table S4. Baseline characteristics ELISA cohort – synovial fluid. SF was collected from 2004 to 2014. Presented data are from time of SF collection, unless otherwise indicated. Categorical data are presented with frequencies (%) and continuous data are presented as mean ± SD (normally distributed variables) or median (IQR) (non-normally distributed variables). * P value PsA vs. Gout <0.05, and P value OA vs. Gout <0.05. Abbreviations: CRP: C-reactive protein; DMARD: disease modifying anti-rheumatic drug use past three months; ESR: erythrocyte sedimentation rate; NM: not measured; OA: osteoarthritis; PsA: psoriatic arthritis.

Chapter 4

DNAM1 and TIGIT balance the T cell response, with low T cell TIGIT expression corresponding to inflammation in psoriatic disease

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Abstract

Objectives – Signals at the contact site of antigen-presenting cells (APCs) and T cells help orchestrate the adaptive immune response. CD155 on APCs can interact with the stimulatory receptor DNAM1 or inhibitory receptor TIGIT on T cells. The CD155/DNAM1/TIGIT axis is under extensive investigation as immunotherapy target in inflammatory diseases including cancer, chronic infection and autoimmune diseases. We investigated a possible role for CD155/DNAM1/TIGIT signaling in psoriatic disease.

Methods – By flow cytometry, we analyzed peripheral blood mononuclear cells of patients with psoriasis (n=20) or psoriatic arthritis (n=21), and healthy individuals (n=7). We measured CD155, TIGIT, and DNAM1 expression on leukocyte subsets and compared activation-induced cytokine production between CD155-positive and CD155-negative APCs. We assessed the effects of TIGIT and DNAM1 blockade on T cell activation, and related the expression of CD155/DNAM1/TIGIT axis molecules to measures of disease activity.

Results – High CD155 expression associates with tumor necrosis factor (TNF) production in myeloid and plasmacytoid dendritic cells (DC). In CD1c+ myeloid DC, activation-induced CD155 expression associates with increased HLA-DR expression. CD8 T cells – but not CD4 T cells – express high levels of TIGIT. DNAM1 blockade decreases T cell pro-inflammatory cytokine production, while TIGIT blockade increased T cell proliferation. Finally, T cell TIGIT expression shows an inverse correlation with inflammation biomarkers in psoriatic disease.

Conclusion – CD155 is increased on pro-inflammatory APCs, while the receptors DNAM1 and TIGIT expressed on T cells balance the inflammatory response by T cells. In psoriatic disease, low TIGIT expression on T cells is associated with systemic inflammation.

Manuscript

Introduction

Integrated signals from antigen-presenting cells (APCs), among those most notably dendritic cells (DCs), can orchestrate effector T cell responses.(1) Accordingly, activated APCs produce cytokines and upregulate the display of peptide/human leukocyte antigen (HLA) complexes and co-stimulatory molecules, to support ensuing adaptive immune responses.(2) Important molecules at the APC-T-cell contact site include co-inhibitory receptor T cell immunoreceptor with Ig and ITIM domains (TIGIT) and co-stimulatory receptor DNAX accessory molecule-1 (DNAM1, also known as CD226) on T cells. These receptors bind the same ligand on APCs, but have opposite effects.(3) Their ligand CD155 (also known as Poliovirus Receptor or NECL5) is mainly expressed by myeloid cells and interacts with effector T cells.(4,5) While DNAM1 binding on T cells induces cytokine release and cytotoxicity, TIGIT evokes an immunosuppressive and non-cytotoxic profile. (6,7) Binding of CD155 in DCs induces a rather tolerogenic profile as part of a negative feedback signal to prevent ongoing inflammation.(6)

Dysregulation of the CD155/DNAM1/TIGIT axis plays an important role in the pathogenesis of various diseases. In cancer CD155 is upregulated in multiple tumor cell types and CD155 can modify tumor mass infiltration by lymphocytes, which may explain the association of CD155 upregulation with an unfavorable prognosis of solid tumors. (8,9) Furthermore, aberrant expression of CD155, DNAM1, and TIGIT are suggested to be involved in the pathogenesis of non-malignant diseases, including primary Sjögren's syndrome,(10) psoriasis,(11) HIV infection,(12–14) and in mouse models for rheumatoid arthritis,(15) and sepsis.(16) In theory, depending on disease- and cell-specific DNAM1 and TIGIT expression, blockade of CD155 could either improve immune response or increase immunosuppression.(6) Moreover, targeting TIGIT with antagonistic monoclonal antibodies (mAbs) appears a logical immunotherapeutic strategy for solid tumors. Therefore, the CD155/DNAM1/TIGIT axis is under investigation as immunotherapy target.

Psoriasis is a common inflammatory disease that mainly affects the skin. Up to 30% of psoriasis patients develop musculoskeletal inflammation, termed psoriatic arthritis (PsA).(17) The pathophysiology of psoriatic disease is characterized by increased release of pro-inflammatory cytokines – such as tumor necrosis factor (TNF), IL-17, and IL-23 – and chronic activation of the innate and adaptive immune system, in which DC and T cells have a central role.(17) In psoriasis, one previous study observed decreased expression of TIGIT on CD4 T cells, which associated with an increased Psoriasis Area

and Severity Index (PASI) – a tool to measure skin disease activity.(11) Stimulation of TIGIT using recombinant CD155/Fc protein inhibited proliferation of these CD4 T cells in vitro.(11) In psoriatic disease, both CD4 and CD8 T cells communicate with APCs to direct the adaptive immune response. CD4 T cells contribute importantly in the chronic phase of psoriatic disease, and CD8 T cells appear particularly important during the initiation of inflammation.(18–20) Variation in CD155/DNAM1/TIGIT expression levels under inflammatory conditions may lead to altered interactions at the APC-T cell contact site in psoriatic disease and offer potential targets for immunotherapy.

We first examined CD155 expression on six APC subsets, and quantified the expression of DNAM1 and TIGIT on CD4 and CD8 T cells. Next, in functional assays, we blocked DNAM1 or TIGIT specifically to monitor the effect of these molecules on T cell responses. Lastly, we examined whether the DNAM1 and TIGIT balance was disturbed in patients with the psoriatic diseases psoriasis and psoriatic arthritis.

Materials and Methods

Subjects

We used blood samples of two cohorts, that included a total of 7 healthy controls (HC), 20 psoriasis patients, and 21 PsA patients. We collected baseline characteristics and disease severity parameters, including disease-modifying anti-rheumatic drug use, PASI, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), swollen joint count, and tender joint count (**Supplemental Tables S1** and **S2**). We used cohort 1 (HC n=7, psoriasis n=7, and PsA n=7) to assess ex vivo CD155, TIGIT and DNAM1 expression, and for DNAM1/TIGIT blocking assays (**Figures 1**, **4-6**; **Supplemental Figures S1A**, **S2**, **S3**, and **S5**). We used cohort 2 (psoriasis n=13 and PsA n=15) to quantify TNF production by CD155-positive and CD155-negative APCs (**Figures 2** and **3**; **Supplemental Figures S1B** and **S4**). We obtained approval of the medical research ethics committee Utrecht for both study cohorts (Cohort 1 source ID 13/696, Trial NL4508; Cohort 2 source ID 15/429, Trial NL53860.041.15). Informed consent was obtained for experimentation with human samples. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Samples

Blood was drawn into BD Vacutainer™ Plastic Blood Collection Tubes with Lithium Heparin. Peripheral blood mononuclear cells (PBMC) were isolated using density centrifugation (Ficoll-Paque).

Ex vivo CD155, TIGIT, and DNAM1 expression

PBMCs ex vivo were used for the quantification of CD155 expression by APC subsets and TIGIT/DNAM1 expression by CD4 and CD8 T cells.

APC activation

To compare activation-induced TNF production between CD155-positive and CD155-negative APCs, we cultured PBMCs in medium (RPMI 1640 + 10% fetal bovine serum). Cells were left untreated (negative control) or stimulated with 100 ng/ml TLR-4 ligand (lipopolysaccharide (LPS)-EB Ultrapure) (tlrl-3pelps, Invivogen) for 4 hours, while inhibiting protein transport with 1:1000 BD GolgiStop (10716676, BD Bioscience).

TIGIT/DNAM1 blocking assays

To assess to assess the effect of TIGIT and DNAM1 blockade on T cell activation and proliferation, we cultured PBMCs in complete medium (RPMI 1640 + 10% fetal bovine serum + 1% Penicillin-Streptomycin) with 10 µg/ml TIGIT blocking antibody (16-9500-82, Invitrogen), 10 µg/ml DNAM1 blocking antibody (559787, BD Pharmingen), or 10 µg/ml isotype control for TIGIT (16-4714-82, Invitrogen) and DNAM1 (555746, BD Pharmingen). To assess proliferation we added 2 µM CellTrace Violet reagent (C34557, Life Technologies). To induce T cell activation and proliferation, we stimulated PBMCs 30 minutes after TIGIT/DNAM1 blockade with CD3/CD28 Dynabeads (11131D, ThermoFisher) in a 10:1 PBMC:Dynabead ratio. After 3 days, PBMCs were re-stimulated with 50 ng/ml phorbol myristate acetate (16561-29-8, Sigma-Aldrich) and 1 µg/ml ionomycin (56092-82-1, Simga-Aldrich) for 4 hours, while inhibiting protein transport with 1:1000 BD GolgiStop (10716676, BD Bioscience).

Antibody panels

Five antibody panels were used for flow cytometry analyses. Panel I was used for ex vivo quantification of APC CD155 expression (**Figure 1**; **Supplemental Figures S1A**, **S3**, and **S5E–J**). We used panel II to quantify CD155 expression and TNF production of LPS-stimulated APCs (**Figures 2 and 3**; **Supplemental Figures S1B** and **S4**). Panel III was used to assess ex vivo TIGIT/DNAM1 expression (**Figure 4**; **Supplemental Figures S2A** and **S5A–D**), panel IV to quantify the effect of TIGIT/DNAM1 blockade on T cell proliferation (**Figure 5**; **Supplemental Figure S2B**) and panel V to quantify the effect of TIGIT/DNAM1 blockade on T cell activation (**Figure 5**; **Supplemental Figure S2C**). Panel I included

antibodies against CD1c (APC; 17-0015-42, eBioscience), CD3 (AF700; 300424, Biolegend), CD19 (AF700; 56-0199-42, eBioscience), CD14 (APC-eFluor 780; 47-0149-42, eBioscience), CD16 (BV510; 563829, BD Horizon), CD56 (PE-CF594; 56228, BD Horizon), CD141 (BV711; 563155, BD Horizon), CD155 (PE; 337609, Biolegend), CD303 (PE-Cy7; 354214, Biolegend), HLA-DR (FITC; 347400, BD), and a Fixable Viability Dye (eF450; 65-0863-14, eBioscience). Panel II comprised antibodies targeting CD1c (APC; 17-0015-42, eBioscience), CD3 (AF700; 300424, Biolegend), CD19 (AF700; 56-0199-42, eBioscience), CD56 (AF700; 557919, BD Pharmingen), CD11c (PE-CF594; 562393, BD Horizon), CD14 (PerCP-Cy5.5; 325622, Biolegend), CD16 (BV510; 302048, Biolegend), CD123 (FITC; 11-1239-42, eBioscience), CD141 (BV711; 563155, BD Horizon), CD155 (PE; 337609, Biolegend), HLA-DR (BV-421; 307636, Biolegend), TNF (PE-Cy7; 25-7349-82 eBioscience), and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience). Panel III consisted of antibodies toward CD3 (AF700; 557919, BD Pharmingen), CD4 (BV711; 2102790, Sony Biotechnology), CD8 (PE-Cy7; 335822, BD), DNAM1 (APC; 338312, Biolegend), TIGIT (PerCP-Cy5-5; 46-9200-42, eBioscience) and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience). Panel IV included CellTrace Violet reagent (C34557, Life Technologies) and antibodies against CD3 (AF700; 300424, Biolegend), CD4 (Pe-Cy7; 25-0049-42, eBioscience), CD8 (V500; 561617, BD horizon), and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience). Panel V included antibodies against CD3 (AF700; 300424, Biolegend), CD4 (Pe-Cy7; 25-0049-42, eBioscience), CD8 (V500; 561617, BD horizon), TNF (BV421; 562783, BD Horizon), IFN-γ (PerCP-Cy5.5; 15599036, Ebioscience), IL-10 (PE; 554706, BD), and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience).

Flow cytometry

We stained samples by incubation with 25 µl antibody mix diluted in buffer (500 ml phosphate-buffered saline + 5 ml 10% sodium azide + 5 g bovine serum albumin) for 25 min at 4°C. Before intracellular stain of TNF and interferon-gamma (IFNγ), we fixed and permeabilized cells with 100 µl Fixation/Permeabilization Concentrate and Diluent (00-5123-43, 00-5223-56, eBioscience). Phenotypical cell surface markers were used to differentiate between PBMC subsets: T cells (CD3, CD4, and CD8), B cells (CD19), monocytes (CD14 and CD16), natural killer (NK) cells (CD56), plasmacytoid (p)DCs (CD123 or CD303), myeloid (m)DC1 and -2 (CD1c, CD11c, and CD141); detailed gating strategies of panels I-V are shown in **Supplemental Figures S1** and **S2**. Based upon the differential expression of CD14 and CD16, we identified classical (cM), intermediate (iM), and non-classical monocytes (ncM) (**Supplemental Figure S1A**). We excluded gated cell

populations of ≤ 30 cells. Acquisition was performed on the BD LSRFortessa with four lasers (405, 488, 561, and 635 nm) with DIVA software version 8.0.1. Compensation for spectral overlap and analysis of FCS files was performed using FlowJo version 10.4.

Statistical analysis

We performed contingency analysis of psoriasis and PsA clinical characteristics using χ^2 tests for categorical variables, and independent samples T or Mann–Whitney U (MWU) tests for continuous variables. We used MWU tests to compare CD155, DNAM1, and TIGIT expression, and TNF production between cell subsets and patient groups. The Wilcoxon-signed rank test was used to compare cytokine production between negative controls and DNAM1/TIGIT blocked T cells. Additionally, we used Spearman's rank correlation to correlate clinical parameters and expression of cell surface markers. We considered a P-value <0.05 statistically significant. Statistical analyses were performed using IBM SPSS version 27 and GraphPad Prism 7.00.

Results

High CD155 expression by monocytes and CD1c+ mDC

We used flow cytometry to explore CD155 expression on six PBMC APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ mDC, and CD303+ plasmacytoid DC (pDC). Nearly all cM, iM, and ncM expressed CD155 (**Figure 1**). In contrast, pDC rarely expressed CD155 and CD141+ mDC showed variable CD155 expression. Overall, CD155 expression among the different APC subsets was comparable in HC, psoriasis, and PsA (**Supplemental Figure S3**). To summarize, irrespective of psoriatic disease, monocyte subsets ubiquitously express CD155, while DCs show variable CD155 expression related to their subset.

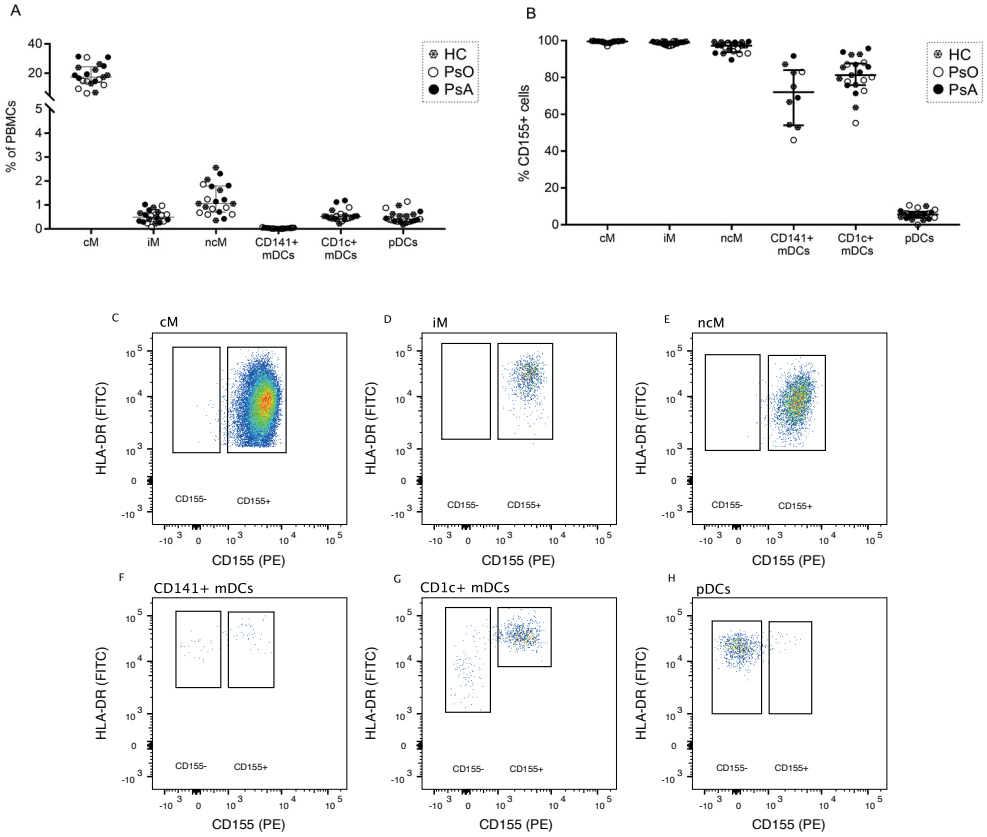


Figure 1. CD155 surface expression by APCs. Pooled flow cytometry analysis of PBMCs ex vivo, of healthy controls (n=7, symbol with cross), psoriasis (n=7, open symbol) and psoriatic arthritis (n=7, filled symbol) patients. Shown data are from six APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ mDC and CD303+ plasmacytoid DC (pDC). (**A**) Proportion of APC subset in PBMCs (gate 'Single Cells' **Supplemental Figure S1A**). (**B**) Proportion of CD155 positive cells in APC subset. (**C-H**) Gating strategy of the selection of CD155 positive cells in cM (C), iM (D), ncM (E), CD141+ mDC (F), CD1c+ mDC (G) and pDC (H). Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, iM: intermediate monocytes, mDC: myeloid dendritic cell, ncM: non-classical monocytes, PBMC: peripheral blood mononuclear cell, pDC: plasmacytoid dendritic cell.

High TNF production in CD155-positive APCs upon activation

Next, we examined whether activation-induced TNF production by monocyte and DC subsets was related to CD155 expression. For both pDC and mDC, their CD155-positive fraction produced significantly more TNF compared to the CD155-negative fraction (**Figure 2**). Considering that all monocyte subsets were ubiquitously positive for CD155, we could not compare TNF production between CD155-negative and CD155-positive fractions. However, the percentage of TNF producing cM correlated positively with the mean

fluorescent intensity (MFI) of CD155 on cM (rs = 0.620 [95% Confidence Interval (CI) 0.370–0.786], P = <0.0001; **Supplemental Figure S4**). Again, no differences between psoriasis and PsA were observed (data not shown). In summary, the capacity for TNF production by cM, CD1c+ mDC, and pDC correlates positively with their expression of CD155.

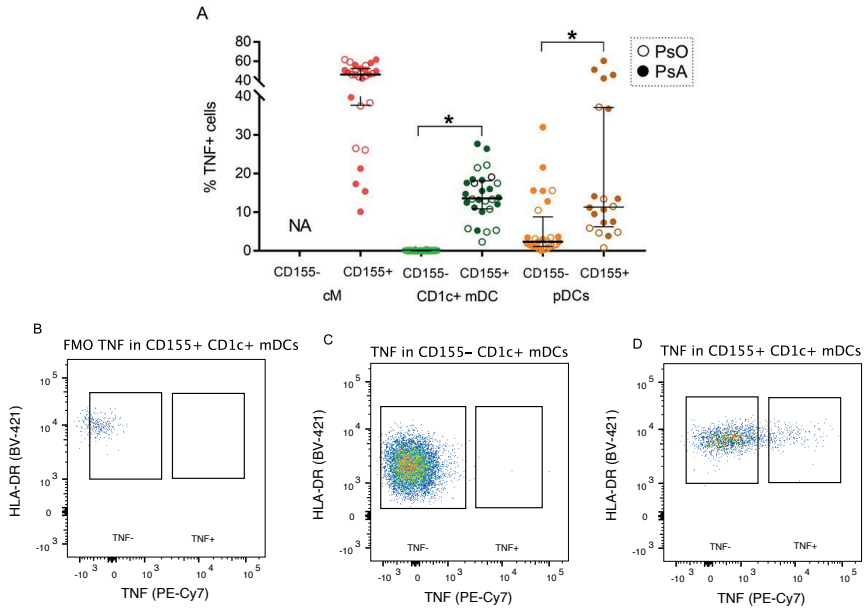


Figure 2. High TNF production by CD155 expressing APCs. Flow cytometry analysis of psoriatic disease patients PBMCs stimulated for 4 hours with LPS (100 ng/mL) in the presence of Brefeldin A (1:1000). (**A**) Proportion of TNF producing cells within CD155-positive and CD155-negative APC subsets of psoriasis patients (n=13, open symbol) and psoriatic arthritis patients (n=15, filled symbol): CD14+CD16- classical monocyte (cM), CD1c+ myeloid dendritic cell (mDC) and CD123+ plasmacytoid dendritic cell (pDC) (detailed gating strategy shown in **Supplemental Figure S1B**). (**B-D**) Representative flow plots of TNF production by CD1c+ mDC: CD155 positive TNF FMO control (B), CD155 negative CD1c+ mDC (C) and CD155 positive CD1c+ mDC (D). * Significant p-value MWU. Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, HLA-DR: human leucocyte antigen DR isotype, FMO: fluorescence minus one, LPS: lipopolysaccharide, mDC: myeloid dendritic cell, MWU: Mann-Whitney U, NA: not applicable, PBMC: peripheral blood mononuclear cell, pDC: plasmacytoid dendritic cell, TNF: tumor necrosis factor.

Correlation of CD155 and HLA-DR expression in CD1c+ mDCs

To further investigate a possible role for CD155 in inflammation, we evaluated whether CD155 expression on CD1c+ mDC associates with HLA-DR expression as a marker for matured, activated DC. To this end, we stimulated PBMCs from psoriatic disease patients with LPS (100 ng/ml) for 4 hours and then analyzed by flow cytometry. We found that in CD1c+ mDC, CD155 expression and HLA-DR expression were significantly correlated (rs = 0.664 [95%CI 0.433–0.813], P < 0.0001; **Figure 3**).

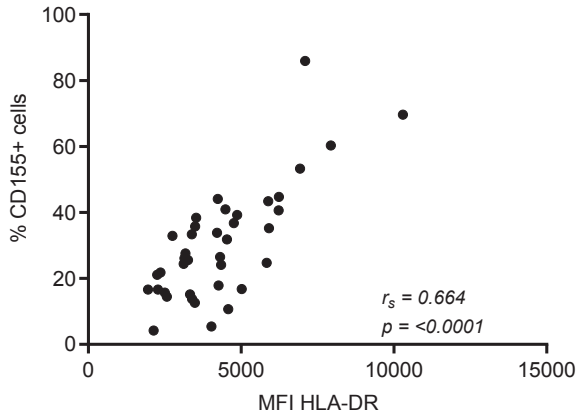
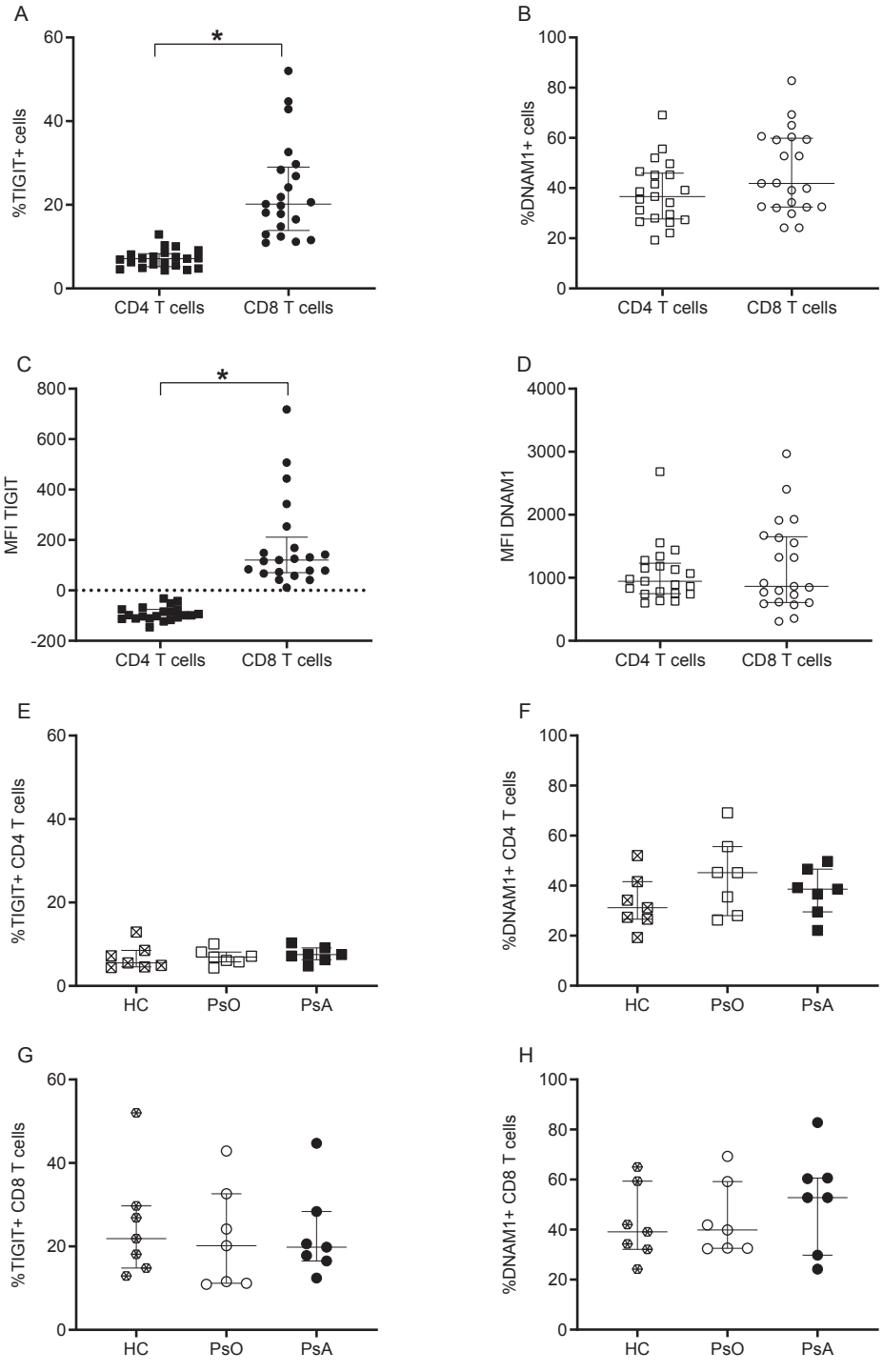


Figure 3. Positive correlation of CD155 and HLA-DR in activated CD1c+ mDC. Flow cytometry analysis of psoriatic disease patients PBMCs stimulated for 4 hours with LPS (100 ng/mL). Shown is the positive correlation of the percentage of CD155 positive CD1c+ mDC and the MFI of HLA-DR expressed by CD1c+ mDC (Spearman's rank correlation coefficient rs = 0.664 [95%CI 0.433-0.813], p = < 0.0001). Abbreviations: CI: confidence interval, DC: dendritic cell, HLA-DR: human leukocyte antigen – DR isotype, LPS: lipopolysaccharide, mDC: myeloid dendritic cell, MFI: median fluorescence intensity, PBMC: peripheral blood mononuclear cell, rs: spearman's rank correlation coefficient.

High TIGIT expression overall on CD8 T cells and low TIGIT expression on CD4 T cells

As CD155 serves as a ligand for DNAM1 and TIGIT on T cells, we quantified baseline expression of these receptors on CD4 and CD8 T cells. TIGIT expression was significantly higher on CD8 T cells, compared to CD4 T cells (P < 0.0001; **Figure 4A** and **4C**), but DNAM1 expression was comparable between CD4 T cells and CD8 T cells (**Figure 4B** and **4D**). We observed no differences in TIGIT and DNAM1 expression between HC, psoriasis and PsA patients (**Figure 4E–H**).

► **Figure 4. Higher TIGIT and comparable DNAM1 expression by CD8 versus CD4 T cells.** Flow cytometry analysis of CD4 (square) and CD8 (circle) T cells ex vivo of healthy controls (HC, n=7, symbol with cross), psoriasis (PsO, n=7, blank symbol) and psoriatic arthritis (PsA, n=7, filled symbol) patients. (A-D) Pooled data of all subjects. (E-F) Data of HC, PsO and PsA patients shown separately. (**A**) Significantly higher proportion of TIGIT positive CD8 T cells compared to CD4 T cells. (**B**) Comparable proportion of DNAM1 positive CD8 and CD4 T cells. (**C**) Significantly higher TIGIT MFI of CD8 T cell compared to CD4 T cells. (**D**) Comparable DNAM1 MFI of CD4 and CD8 T cells. (**E**) Comparable proportion of TIGIT positive CD4 T cells in HC, PsO and PsA. (**F**) Comparable proportion of DNAM1 positive CD4 T cells in HC, PsO and PsA. (**G**) Comparable proportion of TIGIT positive CD8 T cells in HC, PsO and PsA. (**H**) Comparable proportion of DNAM1 positive CD8 T cells in HC, PsO and PsA. ***** Significant p-value MWU. Abbreviations: DNAM1: DNAX-accessory molecule-1, HC: healthy control, MFI: median fluorescence intensity, PsA: psoriatic arthritis, PsO: psoriasis, TIGIT: T-cell immunoglobin and ITIM domain.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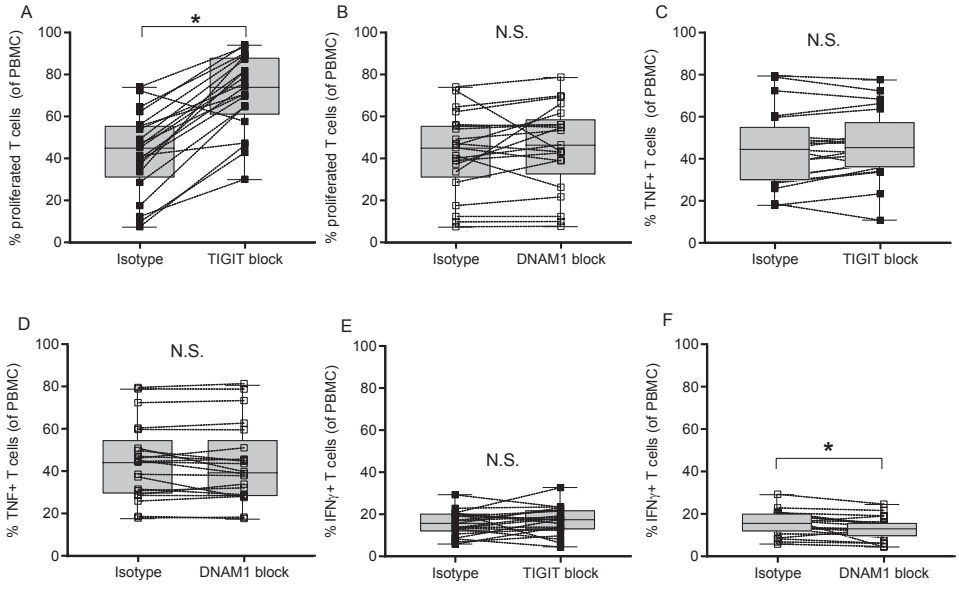
TIGIT blockade increases T cell proliferation and DNAM1 blockade reduces T cell pro-inflammatory cytokine production

Next, we investigated if CD155 ligation can modulate T cell function through selective interaction with DNAM1 or TIGIT. We therefore included anti-TIGIT or anti-DNAM1 blocking antibodies in short-term cultures in which we stimulated PBMCs using anti-CD3/CD28 Dynabeads. Three days blockade of TIGIT caused a significant increase in CD4 and CD8 T cell proliferation compared to the negative control ($P < 0.001$, isotype control antibody) (**Figure 5A** and **5G**). Blockade of DNAM1 did not affect CD8 T cell proliferation (**Figure 5B** and **5H**). We observed no significant differences between psoriasis and psoriatic arthritis patients (data not shown).

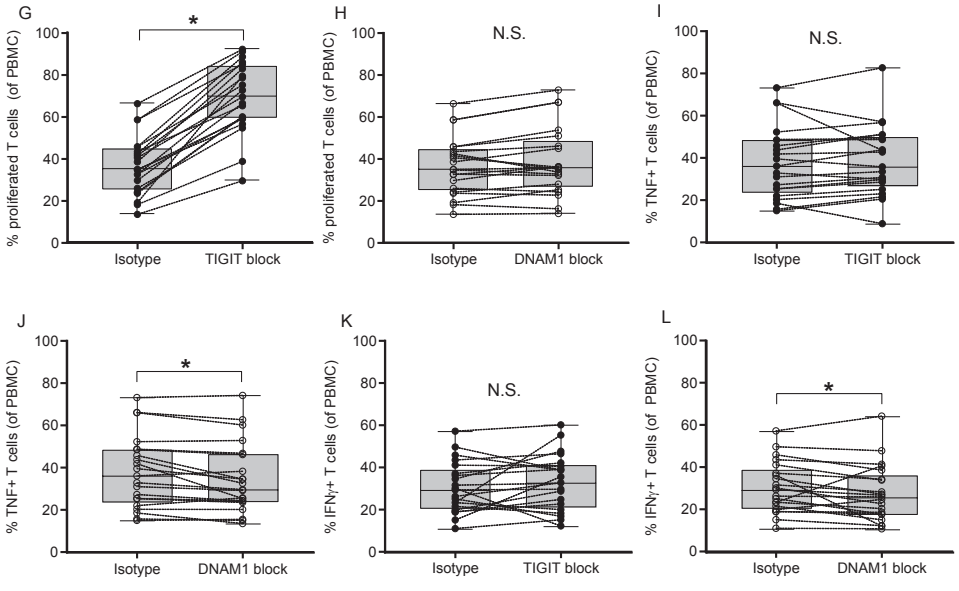
To further explore the effects of TIGIT and DNAM1 on T cell function, we quantified TNF and IFNγ production by CD4 and CD8 T cells after blockade of either TIGIT or DNAM1-receptors. Overall, TIGIT block did not yield a significant increase in cytokine production for IFNγ nor TNF (**Figure 5C,E,I,K**). DNAM1 block, on the other hand, caused a significant decrease in production of IFNγ by CD4 T cells (15.6% vs. 13.0%, P = 0.0015) and decreased both TNF production (36.1% vs. 29.6%, P = 0.0039; **Figure 5J**) and IFNγ production by CD8 T cells (29.4% vs. 25.9%, P = 0.0140; **Figure 5L**). These findings were comparable for T cells from HC, psoriasis and PsA patients (data not shown). Thus, TIGIT blockade caused an increase in CD4 and CD8 T cell proliferation, while DNAM1 blockade resulted in decreased pro-inflammatory cytokine production by T cells.

► **Figure 5. TIGIT blockade increases T cell proliferation, and DNAM1 blockade reduces T cell pro-inflammatory cytokine production.** Flow cytometry analysis of PBMCs stimulated for 3 days with CD3/CD28 Dynabeads (PBMC:Dynabead 10:1), after either 10 µg/mL DNAM1 blocking antibody, 10 µg/mL TIGIT blocking antibody or 10 µg/mL DNAM1 and TIGIT blocking antibody isotypes. Pooled data of healthy controls, psoriasis and psoriatic arthritis patients. Shown are percentages of proliferated T cells, stained with 2 µM CellTrace Violet reagent (A, B, G, H) and percentages of TNF and IFNγ producing T cells after 4 hours re-stimulation with 50 ng/mL PMA, 1 µg/mL ionomycin in the presence of Brefeldin A (1:1000) (C-F, I-L). (**A**) TIGIT block significantly increases CD4 T cell proliferation. (**B**) DNAM1 block has no significant effect on CD4 T cell proliferation. (**C**) No significant difference in CD4 T cell TNF production after TIGIT blockade (44.5% vs. 45.4%, $p > 0.05$). (**D**) No significant decrease in TNF production by CD4 T cells after DNAM1 block (44.5% vs. 39.7%, $p > 0.05$). (**E**) No significant increase in CD4 T cell IFNγ production after TIGIT blockade (15.6% vs. 17.4%, $p > 0.05$). (**F**) Significantly decreased IFNγ production by CD4 T cells after DNAM1 block (15.6% vs. 13.0%, $p = 0.0015$). (**G**) TIGIT block significantly increases CD8 T cell proliferation. (**H**) DNAM1 block has no significant effect on CD8 T cell proliferation. (**I**) No significant difference in CD8 T cell TNF production after TIGIT blockade (35.8% vs. 36.1%, $p > 0.05$). (**J**) Significant decrease in TNF production by CD8 T cells after DNAM1 block (36.1% vs. 29.6%, $p = 0.0039$). (**K**) Trend towards increased CD8 T cell IFNγ production after TIGIT blockade (29.4% vs. 32.0%, $p > 0.05$). (**L**) Significant decrease in IFNγ production by CD8 T cells after DNAM1 block (29.4% vs. 25.9%, $p = 0.0140$). ***** Significant p-value Wilcoxon-signed rank test. Abbreviations: DNAM1: DNAX-accessory molecule-1, HC: healthy control, IFNγ: interferon gamma, PBMC: peripheral blood mononuclear cell, PMA: phorbol myristate acetate, PsA: psoriatic arthritis, TIGIT: T-cell immunoglobin and ITIM domain, TNF: tumor necrosis factor.

CD4+ T cells



CD8+ T cells



TIGIT expression on T cells correlates with APR in psoriatic disease

We finally investigated a possible clinical association of the CD155/DNAM1/TIGIT-axis with psoriatic disease by correlating expression with disease severity measures. We found that TIGIT expression on CD4 T cells negatively correlates with the acute phase reactant (APR) ESR (rs = −0.7918 [95%CI −0.9457 to −0.3476], P = 0.0052) and shows a trend toward correlation with CRP (rs = −0.579 [95%CI −0.8701 to 0.01175], P = 0.0521) in psoriasis and PsA patients (**Figure 6A** and **6B**). Moreover, CD8 T cell TIGIT expression negatively correlates with both ESR (rs = −0.705 [95%CI −0.920 to −0.162], P = 0.0189) as CRP (rs = −0.663 [95%CI −0.900 to −0.125], P = 0.022; **Figure 6C** and **6D**). There was no correlation of CD4 or CD8 T cell TIGIT or DNAM1 expression with psoriatic disease activity measures (**Supplemental Figure S5A–D**). Also, the proportion of CD155-positive APCs did not correlate with the clinical outcomes (**Supplemental Figure S5E–J**).

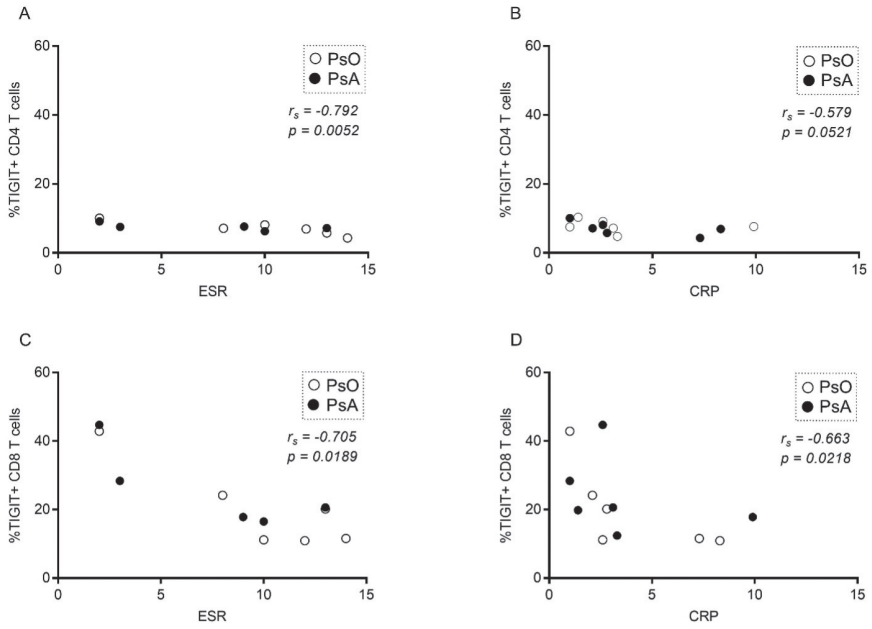


Figure 6. Reduced T cell TIGIT expression associates with increased level of acute phase reactants. Correlation of acute phase reactants in serum and the percentage of TIGIT-expressing T cells analyzed ex vivo in psoriasis (n=7, blank symbol) and psoriatic arthritis (n=7, filled symbol) patients, using flow cytometry. (**A**) Significant correlation of percentage TIGIT-positive CD4 T cells and ESR (rs = -0.7918 [95%CI -0.9457 - -0.3476], p = 0.0052). (**B**) Trend towards correlation of CD4 TIGIT-positive cells and CRP (rs = -0.792 [95%CI -0.8701 – 0.01175], p = 0.0521). (**C**) Significant correlation of percentage TIGIT-positive CD8 T cells and erythrocyte sedimentation rate (ESR) (Spearman's Rank correlation coefficient (rs) = -0.705 [95%CI -0.920 - -0.162], p = 0.0189). (**D**) Significant correlation of CD8 TIGIT-positive CD8 T cells and C-reactive protein (CRP) (rs = -0.663 [95%CI -0.900 - -0.125], p = 0.022). Abbreviations: CI: confidence interval, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, MFI: mean fluorescent intensity, PsA: psoriatic arthritis, PsO: psoriasis, rs: Spearman's rank correlation coefficient, TIGIT: T-cell immunoglobin and ITIM domain.

Discussion

To our knowledge, this study is the first to investigate the role of CD155, DNAM1, and TIGIT in driving inflammation in both psoriasis and psoriatic arthritis. We here confirm that CD155 is highly expressed by human APCs and that CD155 associates with an activated and pro-inflammatory DC phenotype. We confirm a role for TIGIT and DNAM1 in balancing the adaptive inflammatory response. Moreover, our results support the association of low TIGIT expression with systemic inflammation in psoriatic disease.

Our finding that CD155 – the ligand for DNAM1 and TIGIT – is highly expressed on monocytes and mDC is in line with previous research.(21–24) Moreover, our data extend a previously suggested association of CD155 with inflammation, by showing a correlation of CD155 with the production of pro-inflammatory cytokine TNF in classical monocytes, mDC and pDC.(16,25) Additionally, in psoriasis and PsA patients, we found a positive correlation between CD155 expression on CD1c+ mDC and HLA-DR, a DC maturation and activation marker.(26,27) Altogether, these results propose CD155 as key contributor to inflammation.

Our results demonstrate an important role of TIGIT in the preservation of immune homeostasis, as TIGIT blockade results in increased CD4 and CD8 T cell proliferation and low T cell TIGIT expression associates with systemic inflammation in psoriatic disease. The observed increase in CD8 T cell proliferation after TIGIT blockade may be explained by both a direct effect on the CD8 T cell TIGIT receptor for CD155, as by an indirect through reduced inhibition of conventional CD4 T helper (Th) and regulatory T cells (Treg).(28) The observed inhibitory effect of TIGIT on T cell proliferation is in line with literature.(7,8) Previous research in the field of tumor immunology additionally showed that TIGIT blockade enhances T cell-mediated cytokine production, but we did not observe this in our T cell analyses.(11,29) The absence of increased T cell cytokine production after TIGIT blockade in psoriatic disease possibly relates to the co-existence of CD96 (also known as Tactile), which is a second co-inhibitory receptor for CD155 capable of inhibiting T cell cytokine production in vitro, and which might have overruled the effect of our TIGIT block.(4,30) To the best of our knowledge, the association of TIGIT expression with systemic inflammation in psoriatic disease is not yet published, but is in line with previous research that showed a correlation of CD4 T cell TIGIT expression and skin disease severity (PASI) in psoriasis.(11) Altogether, our results suggest that T cell TIGIT expression is important for immune homeostasis.

Furthermore, our results suggest an important role of DNAM1 in the perpetuation of the adaptive immune response because blockade of DNAM1 resulted in decreased T cell

production of pro-inflammatory cytokines. We explain this effect on T cells by both the ceasing of a stimulatory signal, as by more CD155 on APCs available to bind the inhibitory receptors TIGIT and CD96 – both able to reduce effector T cell cytokine production.(4,8) We did not observe an effect of DNAM1 blockade on T cell proliferation, which is not entirely unexpected. Proliferation is likely controlled by additional T cell co-stimulatory receptors besides DNAM1, at least when stimulated by professional APCs in vitro.(31) Moreover, previous research suggests that TIGIT can overrule DNAM1 with regards to T cell proliferation.(32) Explanations for this finding include dose-dependent competition for the ligand CD155 (for which TIGIT has higher affinity), disruption of DNAM1 homo-dimerization by TIGIT, and interference of TIGIT with DNAM1 intracellular signaling cascades.(29,33–35)

We found no direct evidence for disease-specific aberrant expression of the CD155/DNAM1/TIGIT axis relating to T cells in psoriatic disease, although a pathogenic role of TIGIT downregulation on PBMC CD4 T cells had been suggested in psoriasis.(11) Possibly, our relatively small sample size or relatively low disease activity of included patients prevented us from obtaining disease-specific data. However, combining our results from HC and psoriatic patients, we argue that further research is warranted to further elucidate the immunoregulatory role of the CD155/DNAM1/TIGIT axis in psoriatic disease. Indeed, a combination therapy consisting of DNAM1-blocking and TIGIT-stimulating agents might be effective in modulating the adaptive immune response via reduction of T cell proliferation and cytokine production in patient with chronic inflammation.

Our reductionist approach entails an important limitation of our study of the CD155/DNAM1/TIGIT axis. We deem that simplifying this complex network contributed to our step-by-step exploration of its relevance in psoriatic disease. Future studies require additional analyses, such as studying CD155 expression by lymphocytes and non-hematopoietic tissue cells, the effects of TIGIT blockade on NK cell function, the additional CD155 co-inhibitory receptor CD96, and the stimulatory effects of CD112 (an alternative DNAM1 ligand).(4,5,7,36)

In conclusion, we show that CD155 is increased on pro-inflammatory APCs and that the receptors DNAM1 and TIGIT – expressed by T cells – balance the T cell inflammatory response. Moreover, in psoriatic disease, low T cell TIGIT expression is associated with systemic inflammation. Our data supports a contributory role for the CD155/DNAM1/TIGIT axis in a combination therapy, rather than as mono-therapy. Future research exploring how DNAM1 and TIGIT regulate the T cell inflammatory response, could contribute to development of next generation treatments for psoriatic disease.

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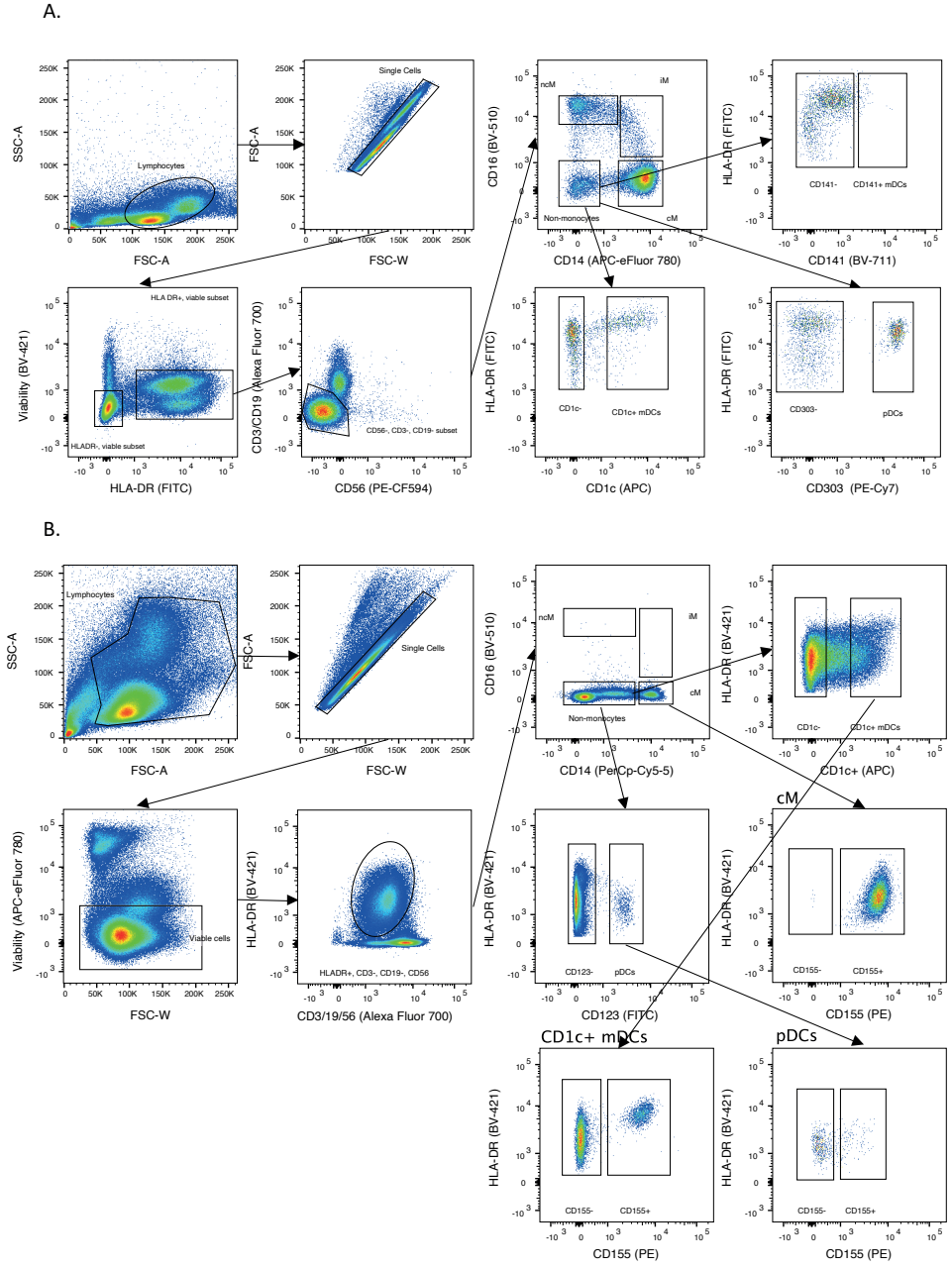
Author contributions: EL, TR and MB were responsible for conceptualization. Funding acquisition was accounted for by TR and EL. EL and MB designed the methodology. MO and EL performed the experiments. MJ, JP, EL and MB were primarily responsible for data analysis and writing the original draft. All authors contributed substantially to reviewing and editing the manuscript before submission.

References

1. Germain RN. T-cell development and the CD4-CD8 lineage decision. Nat Rev Immunol. 2002;2(5):309–22.
2. Kotsias F, Cebrian I, Alloatti A. Antigen processing and presentation. Int Rev Cell Mol Biol. 2019;348:69–121.
3. Gao J, Zheng Q, Xin N, Wang W, Zhao C. CD155, an onco-immunologic molecule in human tumors. Cancer Sci. 2017;108(10):1934–8.
4. Georgiev H, Ravens I, Papadogianni G, Bernhardt G. Coming of age: CD96 emerges as modulator of immune responses. Front Immunol. 2018;9:1072.
5. Martinet L, Smyth MJ. Balancing natural killer cell activation through paired receptors. Nat Rev Immunol. 2015;15(4):243–54.
6. Gorvel L, Olive D. Targeting the "PVR-TIGIT axis" with immune checkpoint therapies. F1000Research. 2020;9.
7. Harjunpää H, Guillerey C. TIGIT as an emerging immune checkpoint. Clin Exp Immunol. 2020;200(2):108–19.
8. Sanchez-Correa B, Valhondo I, Hassouneh F, Lopez-Sejas N, Pera A, Bergua MJ, et al. DNAM-1 and the TIGIT/PVRIG/TACTILE Axis: Novel Immune Checkpoints for Natural Killer Cell-Based Cancer Immunotherapy. Cancers (Basel). 2019;11(6).
9. O'Donnell JS, Madore J, Li X-Y, Smyth MJ. Tumor intrinsic and extrinsic immune functions of CD155. Semin Cancer Biol. 2020;65:189-196.
10. Deng C, Chen Y, Li W, Peng L, Luo X, Peng Y, et al. Alteration of CD226/TIGIT immune checkpoint on T cells in the pathogenesis of primary Sjögren's syndrome. J Autoimmun. 2020;113:102485.
11. Wang FF, Wang Y, Wang L, Wang TS, Bai YP. TIGIT expression levels on CD4+ T cells are correlated with disease severity in patients with psoriasis. Clin Exp Dermatol. 2018;43(6):675–82.
12. Chew GM, Fujita T, Webb GM, Burwitz BJ, Wu HL, Reed JS, et al. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. PLoS Pathog. 2016;12(1):e1005349.
13. Van der Sluis RM, Kumar NA, Pascoe RD, Zerbato JM, Evans VA, Dantanarayana AI, et al. Combination Immune Checkpoint Blockade to Reverse HIV Latency. J Immunol. 2020;204(5):1242–54.
14. Vendrame E, Seiler C, Ranganath T, Zhao NQ, Vergara R, Alary M, et al. TIGIT is upregulated by HIV-1 infection and marks a highly functional adaptive and mature subset of natural killer cells. AIDS. 2020;34(6):801–13.
15. Zhao W, Dong Y, Wu C, Ma Y, Jin Y, Ji Y. TIGIT overexpression diminishes the function of CD4 T cells and ameliorates the severity of rheumatoid arthritis in mouse models. Exp Cell Res. 2016;340(1):132–8.
16. Meng Y, Zhao Z, Zhu W, Yang T, Deng X, Bao R. CD155 blockade improves survival in experimental sepsis by reversing dendritic cell dysfunction. Biochem Biophys Res Commun. 2017;490(2):283–9.
17. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, et al. Psoriasis. Nat Rev Dis Prim. 2016;2.
18. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. Lancet. 2018;391(10136):2273–84.
19. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. N Engl J Med. 2017 Mar;376(10):957–70.

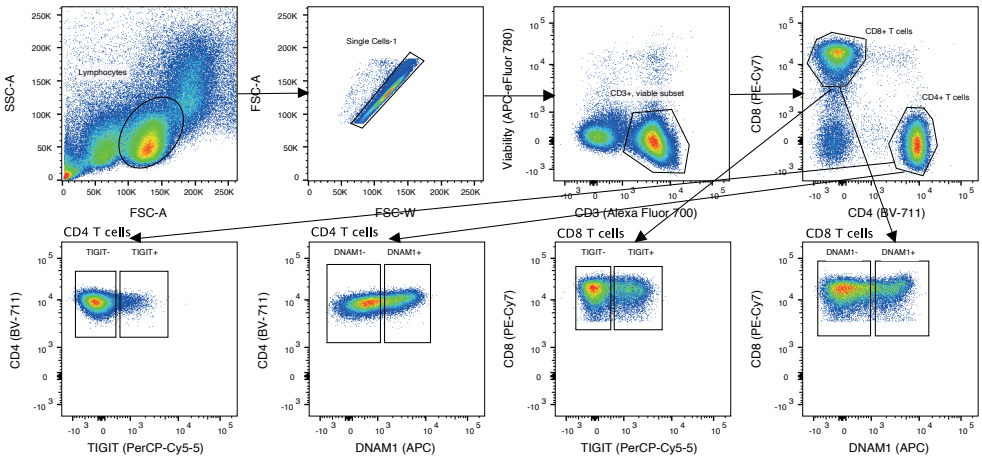
20. Diani M, Altomare G, Reali E. T cell responses in psoriasis and psoriatic arthritis. Autoimmun Rev. 2015;14(4):286–92.
21. Pende D, Castriconi R, Romagnani P, Spaggiari GM, Marcenaro S, Dondero A, et al. Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: Relevance for natural killer-dendritic cell interaction. Blood. 2006;107(5):2030–6.
22. Freistadt MS, Fleit HB, Wimmer E. Poliovirus receptor on human blood cells: a possible extraneural site of poliovirus replication. Virology. 1993;195(2):798–803.
23. Maier MK, Seth S, Czeloth N, Qiu Q, Ravens I, Kremmer E, et al. The adhesion receptor CD155 determines the magnitude of humoral immune responses against orally ingested antigens. Eur J Immunol. 2007;37(8):2214–25.
24. Yamashita-Kanemaru Y, Takahashi Y, Wang Y, Tahara-Hanaoka S, Honda S, Bernhardt G, et al. CD155 (PVR/Necl5) Mediates a Costimulatory Signal in CD4 + T Cells and Regulates Allergic Inflammation. J Immunol. 2015;194(12):5644–53.
25. Kamran N, Takai Y, Miyoshi J, Biswas SK, Wong JSB, Gasser S, et al. Toll-Like Receptor Ligands Induce Expression of the Costimulatory Molecule CD155 on Antigen-Presenting Cells. PLoS One. 2013;8(1):e54406.
26. Kawamura K, Iyonaga K, Ichiyasu H, Nagano J, Suga M, Sasaki Y. Differentiation, maturation, and survival of dendritic cells by osteopontin regulation. Clin Diagn Lab Immunol. 2005;12(1):206–12.
27. Boes M, Cuvillier A, Ploegh H. Membrane specializations and endosome maturation in dendritic cells and B cells. Trends Cell Biol. 2004;14(4):175–83.
28. Kurtulus S, Sakuishi K, Ngiow SF, Joller N, Tan DJ, Teng MWL, et al. TIGIT predominantly regulates the immune response via regulatory T cells. J Clin Invest. 2015;125(11):4053–62.
29. Lozano E, Joller N, Cao Y, Kuchroo VK, Hafler DA, E. L, et al. The CD226/CD155 interaction regulates the proinflammatory (Th1/Th17)/anti-inflammatory (Th2) balance in humans. J Immunol. 2013;191(7):3673–80.
30. Stanko K, Iwert C, Appelt C, Vogt K, Schumann J, Strunk FJ, et al. CD96 expression determines the inflammatory potential of IL-9-producing Th9 cells. Proc Natl Acad Sci U S A. 2018;115(13):E2940–9.
31. Gilfillan S, Chan CJ, Cella M, Haynes NM, Rapaport AS, Boles KS, et al. DNAM-1 promotes activation of cytotoxic lymphocytes by nonprofessional antigen-presenting cells and tumors. J Exp Med. 2008/11/24. 2008;205(13):2965–73.
32. Battella S, Oliva S, Franchitti L, La Scaleia R, Soriani A, Isoldi S, et al. Fine tuning of the DNAM-1/TIGIT/ligand axis in mucosal T cells and its dysregulation in pediatric inflammatory bowel diseases (IBD). Mucosal Immunol. 2019;12(6):1358–69.
33. Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E, Irving B, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009;10(1):48–57.
34. Stanietsky N, Rovis TL, Glasner A, Seidel E, Tsukerman P, Yamin R, et al. Mouse TIGIT inhibits NK-cell cytotoxicity upon interaction with PVR. Eur J Immunol. 2013;43(8):2138–50.
35. Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M, Yang Y, et al. The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8+ T Cell Effector Function. Cancer Cell. 2014;26(6):923–37.
36. Bottino C, Castriconi R, Pende D, Rivera P, Nanni M, Carnemolla B, et al. Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. J Exp Med. 2003;198(4):557–67.

Supplemental Information



◂ **Supplemental Figure S1. Gating strategy for CD155 expression and TNF production by APCs.** (**A**) Gating strategy for CD155 expression by APCs. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF450. Phenotypical cell surface markers were used to exclude non-APCs: T cells (CD3), B cells (CD19), and natural killer (NK) cells (CD56). We differentiated between six APC subsets using the following gates: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD14-CD16-HLA-DR+CD141+ myeloid dendritic cell (mDC), CD14-CD16-HLA-DR+CD1c+ mDC and CD14-CD16-HLA-DR+CD303+ plasmacytoid DC (pDC). Within each APC subset CD155 positive and negative cells were selected, as shown in main **Figure 1C-1H**. (**B**) Gating strategy for TNF production by activated APCs. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. Phenotypical cell surface markers were used to exclude non-APCs: T cells (CD3), B cells (CD19), and natural killer cells (CD56). We differentiated between three APC subsets using the following gates: CD14+ classical monocyte (cM), CD14-HLA-DR+CD1c+ mDC and CD14-HLA-DR+CD123+ plasmacytoid DC (pDC). Within each APC subset CD155 positive and negative cells were selected. Of CD155+ cells, TNF positive cells were gated (representative plots shown in main **Figure 2C** and **2D**). Abbreviations**:** APC: antigen-presenting cell, cM: classical monocytes, DC: dendritic cell, FSC: forward scatter, HLA-DR: human leukocyte antigen – DR isotype, iM: intermediate monocytes, ncM: non-classical monocytes, pDC: plasmacytoid dendritic cell, SSC: sideward scatter, TNF: tumor necrosis factor.

A.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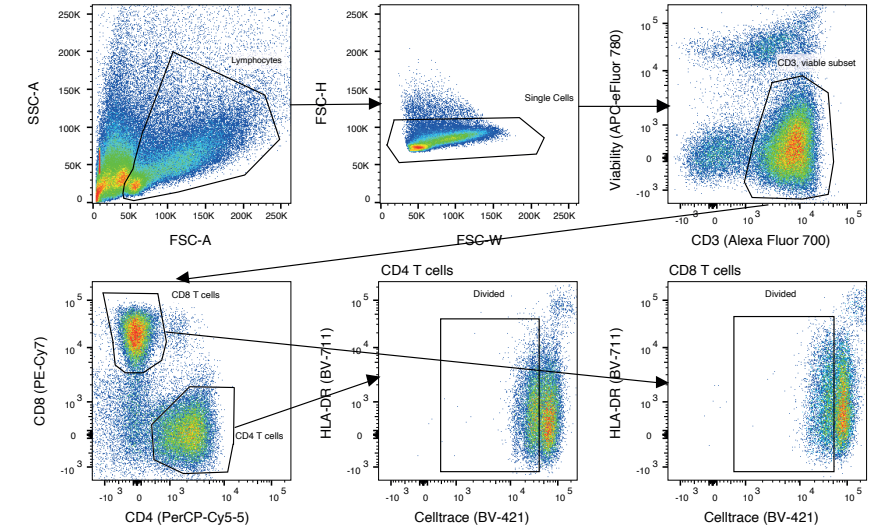
FSC-A
Single Cells-1
FSC-A
FSC-W
Viability (APC-eFluor 780)
CD3+, viable subset
CD3 (Alexa Fluor 700)
CD8+ T cells
CD4+ T cells
CD8 (PE-Cy7)
CD4 (BV-711)
CD4 T cells
TIGIT-
TIGIT+
CD4 (BV-711)
TIGIT (PerCP-Cy5-5)
CD4 T cells
DNAM1-
DNAM1+
CD4 (BV-711)
DNAM1 (APC)
CD8 T cells
TIGIT-
TIGIT+
CD8 (PE-Cy7)
TIGIT (PerCP-Cy5-5)
CD8 T cells
DNAM1-
DNAM1+
CD8 (PE-Cy7)
DNAM1 (APC)

B.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FSC-A
Single Cells
FSC-H
FSC-W
Viability (APC-eFluor 780)
CD3, viable subset
CD3 (Alexa Fluor 700)
CD8 T cells
CD4 T cells
CD8 (PE-Cy7)
CD4 (PerCP-Cy5-5)
CD4 T cells
Divided
HLA-DR (BV-711)
Celltrace (BV-421)
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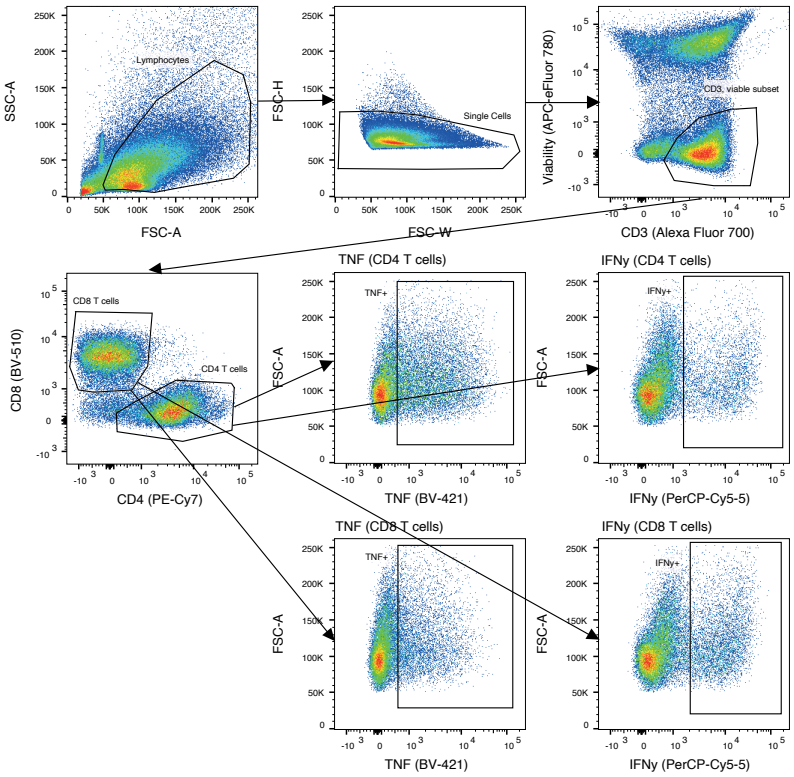
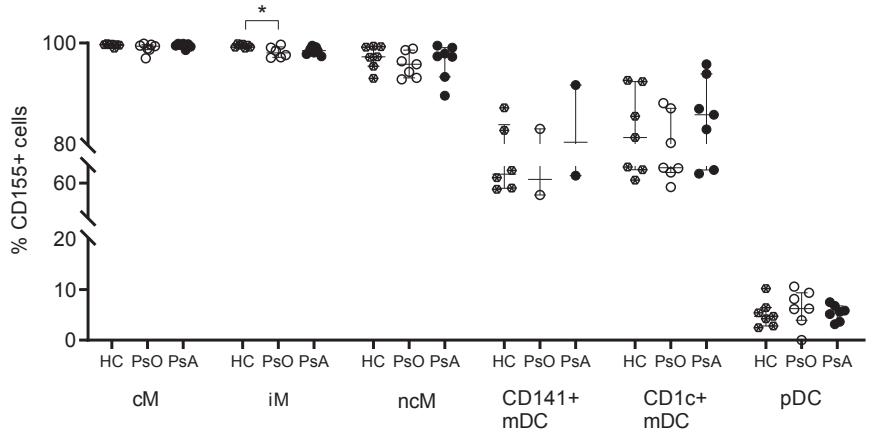
Divided
HLA-DR (BV-711)
Celltrace (BV-421)

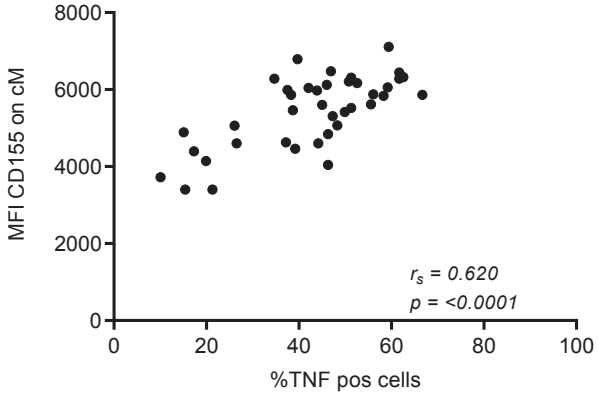
C.



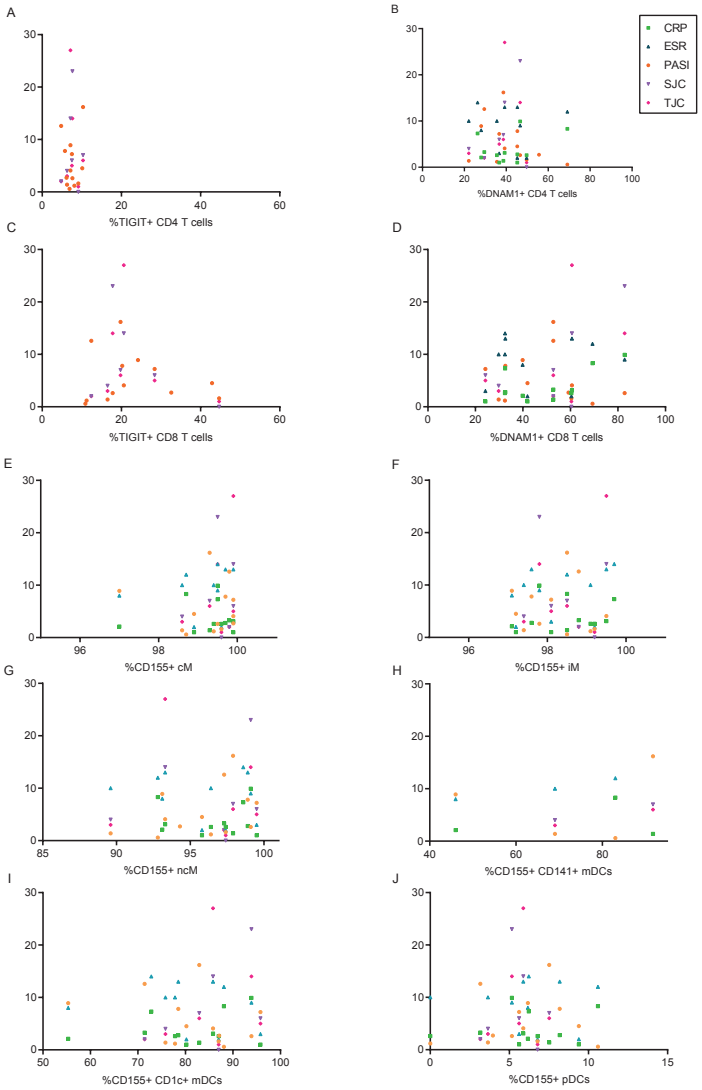
Supplemental Figure S2. Gating strategy for T cell TIGIT and DNAM1 expression, proliferation and cytokine production. (**A**) Gating strategy for *ex vivo* T cell TIGIT and DNAM1 expression. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets T cells positive and negative for TIGIT and DNAM1 were selected. (**B-C**) Gating strategies for T cell proliferation and cytokine production. PBMCs were stimulated for 3 days with CD3/CD28 Dynabeads (PBMC:Dynabead 10:1), after either 10 µg/mL DNAM1 blocking antibody, 10 µg/mL TIGIT blocking antibody or 10 µg/mL DNAM1 and TIGIT blocking antibody isotypes. For proliferation, PBMCs were stained with 2 µM CellTrace Violet reagent. For cytokine production, PBMCs were re-stimulated for 4 hours with 50 ng/mL PMA, 1 µg/mL ionomycin in the presence of Brefeldin A (1:1000). (**B**) Gating strategy for CD4 and CD8 T cell proliferation. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-H and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets the divided population was gated based on the CellTrace Violet stain. (**C**) Gating strategy for CD4 and CD8 T cell cytokine production. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-H and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets T cells positive for TNF and IFNγ were selected. Abbreviations: DNAM1: DNAX-accessory molecule-1, IFNγ: interferon gamma, TIGIT: T-cell immunoglobin and ITIM domain, TNF: tumor necrosis factor.



Supplemental Figure S3. CD155 expression by APC subsets comparable in HC, psoriasis and PsA. Flow cytometry analysis of PBMCs ex vivo of healthy controls, psoriasis and psoriatic arthritis patients. Shown are percentages of CD155 positive cells within six APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ myeloid mDC and CD303+ plasmacytoid DC (pDC). Overall, CD155 expression within the different APC subsets very similar in HC, psoriasis and PsA ($p > 0.05$), except for a marginally lower iM CD155 expression in HC vs. psoriasis ($p = 0.038$). * Significant difference MWU test. Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, DC: dendritic cell, HC: healthy control, iM: intermediate monocytes, mDC: myeloid dendritic cell, MWU: Mann Whitney U test, nCM: non-classical monocytes, pDC: plasmacytoid dendritic cell, PBMC: peripheral blood mononuclear cell, PsA: psoriatic arthritis.



Supplemental Figure S4. Positive correlation of CD155 and TNF presence in activated classical monocytes. Flow cytometry analysis of psoriatic disease patients CD14+ classical monocytes (cM) stimulated for 4 hours with 100 ng/mL LPS in the presence of Brefeldin A (1:1000). Shown is the correlation of the percentage TNF producing cM with cM mean fluorescent intensity (MFI) of CD155. The percentage of TNF producing cM is positively correlated with CD155 MFI (Spearman's Rank correlation coefficient $r_s = 0.620$ (0.370 – 0.786), $p = <0.0001$). Abbreviations: cM: classical monocytes, LPS: lipopolysaccharide, MFI: median fluorescence intensity, r_s: Spearman's rank correlation coefficient, TNF: tumor necrosis factor.



Supplemental Figure S5. Correlation of psoriatic disease activity measures with both T cell TIGIT and DNAM1 expression, as with APC subsets CD155 expression. Shown are scatterplots of psoriatic disease severity measures (PASI, CRP, ESR, SJC, TJC) and the percentage of TIGIT (A,C) or DNAM1 (B,D) expressing CD8 and CD4 T cells, or the percentage of CD155+ cells within six APC subsets (E-J) in psoriasis and psoriatic arthritis patients, measured by flow cytometry. Spearman's rank correlation coefficients (r_s) were calculated to test correlation. (**A**) No correlation of PASI, SJC or TJC with the percentage of TIGIT+ CD8 T cells. (**B**) No correlation of PASI, CRP, ESR, SJC or TJC with the percentage of DNAM1+ CD8 T cells. (**C**) No correlation of PASI, SJC or TJC with the percentage of TIGIT+ CD4 T cells. (**D**) No correlation of PASI, CRP, ESR, SJC or TJC with the percentage of DNAM1+ CD4 T cells. (**E**) No correlation of disease severity measures and the percentage of CD155 positive CD14+CD16- classical monocytes (cM), CD14+CD16+ intermediate monocytes (iM) (**F**), CD14-CD16+ non-classical monocytes (ncM) (**G**), CD141+ myeloid dendritic cells (mDC) (**H**), CD1c+ mDC (**I**) or CD303+ plasmacytoid DC (pDC) (**J**). Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, CRP: C-reactive protein, DC: ESR: erythrocyte sedimentation rate, iM: intermediate monocytes, mDC: myeloid dendritic cell, ncM: non-classical monocytes, PASI: psoriasis area and severity index, pDC: plasmacytoid dendritic cell, SJC: swollen joint count, TJC: tender joint count.

Characteristic	HC (n=7)	Psoriasis (n=7)	PsA (n=7)	(normal) Range
Age (years) *	44 ± 16.2	35 ± 10.2	45 ± 3.7	NA
Female, n (%) *	5 (71%)	5 (71%)	0 (0%)	NA
DMARD use, n (%)	0 (0%)	0 (0%)	0 (0%)	NA
CRP (mg/L)	n.m.	2.7 (1.8-7.6)	2.9 (1.3-3.6)	0 - 10
ESR (mm/hour)	n.m.	11 (7-13)	5 (3-12)	1 – 8
PASI	NA	3.6 (1.1-8.1)	4.1 (1.6-12.6)	0 - 72
SJC, of 76	NA	NA	4 (1-7)	0 - 76
TJC, of 78	NA	NA	3 (2-6)	0 - 78

Supplemental Table S1. Baseline characteristics cohort 1. Categorical data are presented with frequencies (%) and continuous data are shown as mean ± standard deviation (normally distributed variables) or median (interquartile range) (non-normally distributed variables). * Significant difference psoriasis vs PsA (p-value < 0.05). Abbreviations**:** CRP: C-reactive protein, DMARD: disease modifying anti-rheumatic drug use (past three months), ESR: erythrocyte sedimentation rate, HC: healthy control, NA: not applicable, n.m.: not measured, PASI: psoriasis area and severity index, PsA: psoriatic arthritis, SJC: swollen joint count, TJC: tender joint count.

Characteristic	Psoriasis (n=13)	PsA (n=15)
Age (years)	41 ± 13.3	46 ± 9.3
Female, n (%)	7 (54%)	5 (33%)
DMARD use, n (%)	0 (0%)	2 (13%)
CRP (mg/L)	3.0 (1.0-5.1)	2.1 (1.4-5.7)
ESR (mm/hour)	5 (2-11)	5 (2-11)
PASI	4.4 (3.2-9.9)	3.4 (1.5-8.4)
SJC, of 76	NA	1 (0-6)
TJC, of 78	NA	2 (0-6)

Supplemental Table S2. Baseline characteristics cohort 2. Categorical data are presented with frequencies (%) and continuous data are shown as mean ± standard deviation (normally distributed variables) or median (interquartile range) (non-normally distributed variables). No significant differences psoriasis vs PsA (p-values ≥ 0.05). Abbreviations: CRP: C-reactive protein, DMARD: disease modifying anti-rheumatic drug use (past three months), ESR: erythrocyte sedimentation rate, HC: healthy control, NA: not applicable, PASI: psoriasis area and severity index (range 0-72), PsA: psoriatic arthritis, SJC: swollen joint count, TJC: tender joint count.

PART II

Transition from Psoriasis
to Psoriatic Arthritis

Chapter 5

Do patients with psoriatic arthritis have more severe skin disease than patients with psoriasis only? A systematic review and meta-analysis

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In press
Dermatology

Abstract

Background – Early identification of patients at risk of psoriatic arthritis (PsA) is essential to facilitate early diagnosis and improve clinical outcomes. Severe cutaneous psoriasis has been proposed to associate with PsA, but a recent assessment of evidence is lacking. Therefore, in this systematic review we address the association of psoriasis skin severity with the presence and development of PsA.

Summary – We included articles from a review published in 2014 and supplemented these with recent literature by performing an additional systematic search to identify studies published between 01-01-2013 and 11-02-2021. Meta-analysis was performed when sufficient comparable evidence was available. Of 2000 screened articles we included 29 in the analysis, of which 16 were identified by our updated search. 19 Studies reported psoriasis severity as Psoriasis Area and Severity Index (PASI), ten studies as Body Surface Area (BSA), and two studies as 'number of affected sites'. Most studies show that more extensive skin disease associates with the presence of PsA. The quantitative pooled analyses demonstrate higher PASI (mean difference (Δ) 1.59; 95%CI 0.29-2.89) and higher BSA (Δ 5.31; 95%CI 1.78-8.83) in PsA compared to psoriasis. Results from prospective studies – that assess the risk of future development of PsA in psoriasis patients - were inconclusive.

Key messages – In patients with psoriasis, more severe skin involvement is associated with the presence of PsA, underpinning the importance of optimal dermatology-rheumatology collaboration in clinical care. There is insufficient data to support the use of psoriasis skin severity to predict future development of PsA in psoriasis patients.

Manuscript

Introduction

Psoriatic arthritis (PsA) is a musculoskeletal disorder characterized by inflammation of the skin, nail deformities, arthritis, axial spondyloarthritis, enthesitis and dactylitis.(1) PsA develops in 6-41% of psoriasis patients, but it is unknown why only a subset of patients transits to PsA.(1–3) Psoriatic skin disease precedes PsA in 85% of the cases (on average 10 years), which opens a window of opportunity for early recognition, treatment initiation and possibly delaying or even prevention of the onset of PsA.(1,4) Early diagnosis and treatment of PsA are essential, because irreversible joint damage can develop within six months and delayed diagnosis is associated with long-term adverse outcomes.(5–8) Therefore, defining patients at risk of PsA transition has been a topic of interest.(9,10)

Multiple clinical predictors for PsA in psoriasis patients have been suggested, including obesity, trauma, nail dystrophy and psoriasis localization.(9,10) Moreover, a meta-analysis published in 2014 reported a trend for an association between the extent of psoriasis and presence of PsA.(9) The extent of cutaneous disease - commonly expressed as psoriasis area and severity index (PASI; range 0-72) or body surface area (BSA; range 0-100) - is a relatively quick and non-invasive clinical outcome and could therefore function as an useful predictor for transition to PsA in psoriasis patients that can readily be applied in clinical practice.(11) However, a meta-analysis investigating this potential predictor for PsA development is lacking.(10) We aim to update and complement the prior meta-analysis by *Rouzaud et al*. (2014) with current knowledge of the association of psoriatic skin disease severity with PsA by assessing not only the association of psoriasis severity with the *presence* of PsA, but also the association with later *development* of PsA. Furthermore, we postulate that defining the association between skin disease severity and development of PsA may support our understanding of shared pathogenic features within the psoriatic spectrum of disease.(12)

Methods

Search

We conducted a systematic literature search in PubMed and Embase on 11-02-2021 (PICO question: "Is psoriasis skin severity predictive of transition to PsA in psoriasis patients?"). We used a combination of synonym terms in title / abstract and MesH / Emtree terms for 'psoriasis', 'psoriatic arthritis', 'severity', 'PASI' and 'BSA' (**Supplemental Table S1**). We screened studies using pre-defined eligibility criteria in line with *Rouzaud*

et al. (**Supplemental Table S2**). We included original studies published after 01-01-2013, that studied human subjects aged >18 years old and that compared psoriasis severity between psoriasis patients without PsA (Pso-PsA), patients with PsA (PsA) and/or psoriasis patients that developed PsA. We focused on publications after 2012 to supplement the comprehensive meta-analysis by *Rouzaud et al*. (search period 1980 – January 2013).(9)

Data extraction

Eligibility of selected studies for qualitative and quantitative analysis was discussed by two authors (MJ, JP) and quality assessment was reported. After study selection we identified estimators for the association of PsA and psoriasis severity (PASI, BSA, affected sites): mean and standard deviation (±) in (sub)groups, mean difference between groups (Δ), median and interquartile range (IQR) in (sub)groups, odds ratio (OR), risk ratio (RR) and hazard ratio (HR) for association of psoriasis severity and PsA (development) with confidence intervals (95% CI). We calculated missing OR and CI and requested corresponding authors to provide additional data, if information to perform quantitative analyses was lacking.

Differentiation by research question

We differentiated between studies that report the association of cutaneous psoriasis severity with the *presence* of PsA and studies that report the association with later *development* of PsA in patients with psoriasis, because these studies answer different clinical questions. Articles that report the extent of skin disease at a certain baseline and subsequently study conversion to PsA (prospective design), are important to support the potential use of psoriasis severity as biomarker to identify psoriasis patients at risk for PsA transition. On the other hand, studies that compare skin disease severity between Pso-PsA and PsA (cross-sectional design) enable us to study the association of psoriasis severity and the present risk of PsA. Although these studies do not address our PICO, we reckon that they do answer a clinically relevant question and therefore we included these in our analyses.

Meta-analysis

We performed quantitative meta-analyses if ≥3 studies used a homogenous study design, reported similar psoriasis severity measures and used the same association measures.

For quantitative analyses, we used Random effects Models and evaluated heterogeneity with the I^2 statistic. Meta-analyses were performed using Review Manager (Version 5.4) and meta-regression with Comprehensive Meta-Analysis (Version 3). We considered a P-value <0.05 as statistically significant.

Results

Search results

The search yielded 2000 unique studies. One author performed title/abstract screening and thereafter screening of 90 studies full-text (MJ). Selection of 14 studies was discussed by three authors (MJ, JP, EL) (**Figure 1**). Two articles were retrieved via reference and related citations in PubMed and supplemented with 13 studies selected by *Rouzaud et al*.(9) Of the 29 articles included in our final analysis, three studies assessed the extent of skin disease in Pso-PsA patients and later *development* of PsA (PASI n=2, affected sites n=1). The other 26 studies reported psoriasis severity and the *presence* of PsA in Pso-PsA and PsA patients: either the highest value from repeated measures over a period of time (PASI n=1, BSA n=2) or a single measurement (PASI n=14, BSA n=6, PASI and BSA n=2, affected sites n=1) (**Table 1**).

Study quality

Concerning studies that investigated psoriasis severity and presence of PsA, overall quality was low. In seven studies selection bias could have been introduced by patient selection (*Choi et al.* (13)*; Cinar et al.* (14)*; Haroon et al.* (15)*; Henes et al.* (16)*; Jamshidi et al.* (17)*; Leijten et al.* (18)*; Truong et al.* (19)), as they assessed previously undiagnosed PsA in cohorts of psoriasis patients (**Supplemental Table S3:** *Detailed study characteristics*). The majority of studies recruited patients at dermatology departments of hospitals or dedicated dermatology clinics (15 studies). Five studies were performed in combined Dermatology/ Rheumatology clinics, two in Rheumatology departments, and of the remaining studies the setting was unknown or of another category. Concerning the classification of PsA, one third of the studies applied validated criteria (CASPAR, Moll & Wright, ESSG), while 11 studies used either a clinical or self-reported diagnosis (*Salvarani et al.* (20); *Zenke et al.* (21); *Tey et al.* (22); *Christophers et al.* (23); *Gelfand et al.* (24); *Ogdie et al.* (25); *Soltani-Arabshahi et al.* (26); *Stern et al.* (27); *Truong et al.* (19); *Yan et al.* (28); *Thumboo et al.* (29)).

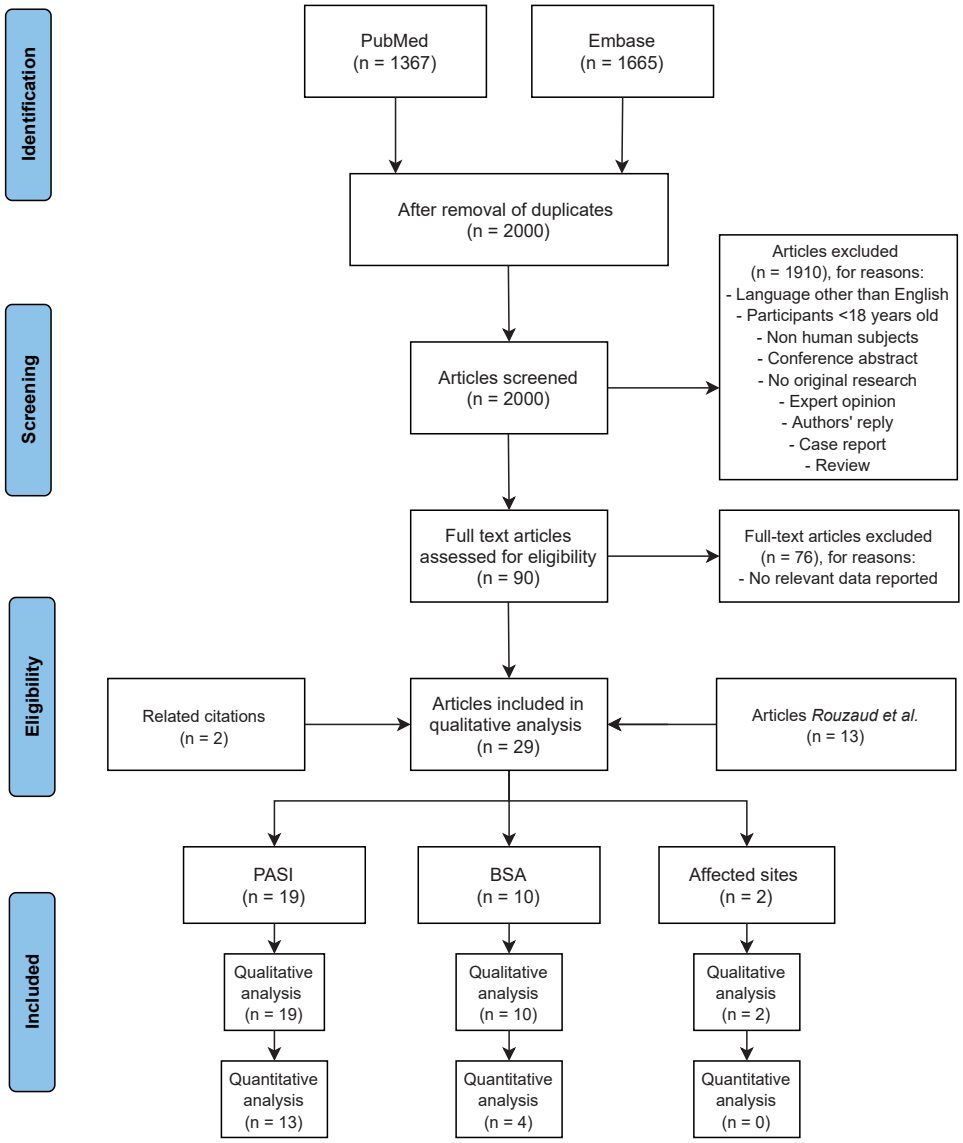

Figure 1. Flowchart. A literature search was conducted to identify original articles that reported psoriasis severity in patients with psoriasis and psoriatic arthritis. PubMed and Embase were searched on 11-02-2021. A combination of synonym terms in title / abstract and MesH / Emtree terms for 'psoriasis', 'psoriatic arthritis', 'severity', 'PASI' and 'BSA' was used (**Supplemental Table S1**). In total 3032 articles were identified. Duplicates were removed and 2000 articles were screened on title and abstract based on pre-defined eligibility criteria. Consequently, 90 selected articles were screened full-text on relevancy to include in the analysis. The search was supplemented with 13 articles by Rouzaud et al. and 2 articles via related citations in PubMed and reference citations of the identified articles in the initial search. In total, 29 studies were included in the qualitative analyses. These studies reported the following outcome measures for skins disease severity: psoriasis area and severity index (PASI) (n=17), PASI and body surface area (BSA) (n=2), BSA (n=8) and number of affected sites (n=2). We included 13, 4 and 0 of these studies in the quantitative analyses, respectively.

Psoriasis measure	Studies [a]	Severity assessment [b]	Patients Pso-PsA / PsA	Severity stratification	Results (Pso-PsA vs. PsA unless otherwise specified)
PASI	**Present PsA**				
	Choi 2017 (13)	Cross	173 / 27	-<10: mild -10-20: moderate ->20: severe	(I) *Mean* - 6.8 ± 4.3 vs. 9.5 ± 6.3; p=0.014 * (II) *Stratified* - Mild: 78.5% vs. 61.9%; p NR - Moderate: 21.5% vs. 33.3%; p NR - Severe: 0.0% vs. 4.8%; p NR (III) *OR* [c] - PASI>10: 2.24 (95%CI 0.86-5.86); p=0.099
	Cinar 2015 (14)	Cross	94 / 32	-<3: mild -3-15: moderate ->15: severe	(I) *Median* - 2.8 (0.3-30.0) vs. 3.6 (0.8-37.7); p=0.032 * (II) *Stratified* - Mild: 59.6% vs. 40.6%; NS - Moderate: 33.0% vs. 53.1%; NS - Severe: 7.4% vs. 6.3%; NS (III) *OR* [c] - PASI>15: 0.83 (95%CI 0.16-4.21); p=0.821
	Dağdelen 2020 (33)	Cross	80 / 40	n.a.	*Mean* - 10.2 ± 9.9 vs. 4.9 ± 4.8; p NR
	Eder 2011 (30)	Cross	159 / 159	Highest during first 3y FU: -<10: non-severe -≥10: severe	(I) *Mean* - 7.1 ± 7.2 vs. 7.3 ± 9.6 (II) *Multivariable logistic regression* - Severe: OR 0.89 (95%CI 0.49-1.61); NS
	El Miedany 2014 (43)	Cross	112 / 126	n.a.	*Mean* - 11.7 ± 11.8 vs. 12.4 ± 10.4; NS
	Gladman 2011 (44)	Cross	438 / 1066	n.a.	*Mean* - 5.7 ± 5.8 vs. 7.0 ± 8.9; p NR
	Haroon 2013 (15)	Cross	71 / 29	n.a.	(I) *Mean* - 1.89 ± 1.14 vs. 2.40 ± 1.13; p=0.04 * (II) *Multivariable logistic regression* - PASI: OR 1.61 (95%CI 1.06-2.44); p=0.02 *
	Henes 2013 (16)	Cross	48 / 50	-0–1: not active -2–10: mild -11–15: moderate ->15: severe	(I) *Median* - 1 (0-3) vs. 1 (0-3); NS (II) *Stratified* - Not active: 26.2% vs. 23.9%; NS - Mild: 42.9% vs. 45.7%; NS - Moderate: 21.4% vs. 28.3%; NS - Severe: 20.8% vs. 10.0%; p NR (III) *OR* [c] - PASI>10: 0.86 (95%CI 0.38-1.94); p=0.715 - PASI>15: 0.42 (95%CI 0.13-1.34); p=0.144

Psoriasis measure	Studies [a]	Severity assessment [b]	Patients Pso-PsA / PsA	Severity stratification	Results (Pso-PsA vs. PsA unless otherwise specified)
	Jamshidi 2008 (17)	Cross	291 / 29	n.a.	*Mean* - 10.70 ± 8.44 vs. 24.33 ± 10.36; p=<0.05 *
	Leijten 2017 (18)	Cross	68 / 18	n.a.	*Mean* - 5.69 ± 4.84 vs. 4.65 ± 5.75; p=0.478
	Maejima 2010 (45)	Cross	23 / 23	n.a.	*Mean* - 9.5 ± 9.4 vs. 9.5 ± 13.3; NS
	Pietrzak 2019 (34)	Cross	62 / 31	n.a.	*Mean* - 26.00 ± 6.54 vs. 28.07 ± 5.87; p=<0.05 *
	Reich 2009 (46)	Cross	1055 / 312	n.a.	*Mean* - 11.5 vs. 14.3; p=<0.0001 *
	Salvarini 1995 (20)	Cross	130 / 75	n.a.	*Mean* - 4.7 ± 3.5 vs. 5.4 ± 5.1; NS
	Schons 2015 (47)	Cross	49 / 16	n.a.	*Mean* - 8.3 ± 7.44 vs. 11.3 ± 9.6; NS
	Soy 2008 (48)	Cross	40 / 49	n.a.	*Mean* - 6.2 ± 8.2 vs. 8.6 ± 12.2; NS
	Yang 2011 (49)	Cross	1816 / 112	n.a.	*Mean* - 6.0 ± 5.6 vs. 9.7 ± 10.4; p=<0.001 *
	Later development of PsA				
	Eder 2016 (31)	Pro	*Baseline*: 464 / 0 *After 8y FU*: 404 / 60	At baseline: -<10: mild -10-20: moderate ->20: severe	(I) *Stratified* - Mild: 88.1% vs. 78.3%; p NR - Moderate: 10.1% vs 15.0%; p NR - Severe: 1.7% vs. 6.7%; p NR (II) *Cox regression* - Moderate vs. mild: RR 1.16 (95%CI 0.50-2.64); NS - Severe vs. mild: RR 5.39 (95%CI 1.64-17.7); p=0.006 *
	Zenke 2017 (21)	Pro	974 / 118	At first visit dermatology clinic: -<10: non-severe -≥10: severe	(I) *Mean* - 4.5 ± 7.5 vs. 9.3 ± 10.2; p=<0.01 * (II) *Multivariable logistic regression* - Severe: OR 1.55 (95%CI 0.89-2.71); NS
BSA	**Present PsA**				
	Choi 2017 (13)	Cross	173 / 27	-<3: mild -3-10: moderate ->10: severe	(I) *Mean* - 6.7 ± 6.6 vs. 11.0 ± 16.4; p=0.029 * (II) *Stratified* - Mild: 27.8% vs. 14.3%; p NR - Moderate: 55.7% vs. 66.7%; p NR - Severe: 16.5% vs. 19.0%; p NR (III) *OR* [c] - BSA>10: 1.19 (95%CI 0.37-3.84); p=0.765

Psoriasis measure	Studies [a]	Severity assessment [b]	Patients Pso-PsA / PsA	Severity stratification	Results (Pso-PsA vs. PsA unless otherwise specified)
	Christophers 2010 (23)	Cross	1434 / 126	n.a.	(I) *Mean* - 17.2 ± 16.9 vs. 26.6 ± 19.9; p=<0.0005 * (II) *Multivariable logistic regression* - BSA: OR 1.020 (95%CI 1.012-1.029); p=<0.0005 *
	Gelfand 2005 (24)	Cross	530 / 71	-<1: no or little -1-2: mild -3-10: moderate ->10: severe	(I) *Stratified* - No or little: 75.7% vs. 30.8%; p NR - Mild: 14.5% vs. 30.8%; p NR - Moderate: 8.0% vs. 21.5%; p NR - Severe: 1.8% vs. 16.9%; p NR (II) *OR* [c] -BSA>10: 11.06 (95%CI 4.11-29.75); p<0.001 *
	Ogdie 2013 (25)	Cross	3699 / 365	-≤2: mild -3-10: moderate ->10: severe	*Multivariable logistic regression* - Moderate vs. mild: OR 1.49 (95%CI 1.1-1.99); p<0.001 * - Severe vs. mild: OR 3.34 (95%CI 2.40-4.65); p<0.001 *
	Pietrzak 2019 (34)	Cross	62 / 31	n.a.	*Mean* - 35.83 ± 15.59 vs. 38.64 ± 13.95; p=0.2438
	Soltani-Arabshahi 2010 (26)	Cross	693 / 250	Highest ever: -<5: mild -5-10: moderate ->10: severe	*Cox regression* - Worst BSA ever: OR 1.01 (95% CI 1.00-1.01); p<0.05
	Stern 1985 (27)	Cross	1019 / 266	n.a.	*Mean* - 31% vs 37%; p=<0.01 *
	Tey 2010 (28)	Cross	266 / 134	Max. in 1y FU: -0-25%: I -26-50%: II -51-75%: III -76-100%: IV	*Multivariable logistic regression* - II vs. I: OR 1.53 (95%CI 0.86-2.71); NS - III vs. I: OR 1.64 (95%CI 0.85-3.19); NS - IV vs. I: OR 2.52 (95%CI 1.33-4.75); p=0.004 *
	Truong 2015 (19)	Cross	399 / 169	n.a.	*Mean* - 13.4 ± 17.4 vs. 16.7 ± 21.3; p=0.05
	Yan 2018 (28)	Cross	497 / 175	-mild -mild-moderate -moderate-severe -severe [c]	(I) *Stratified* - Mild: 6.0% vs 3.6%; NS - Mild-moderate: 21.5% vs 13.1%; p=0.021 * - Moderate-severe: 37.2% vs 30.9%; NS - Severe: 32.0% vs 50.3%; $p=2.39^{-5}$ * (II) *OR* [c] - BSA 'severe': OR 2.15 (95%CI 1.51-3.06); p<0.001 * (III) *Univariate logistic regression* - Severe: OR 2.15 (95%CI 1.51-3.05); p NR (IV) *Multivariable logistic regression* - Severe: OR 1.92 (95%CI 0.88-4.21); NS

Psoriasis measure	Studies [a]	Severity assessment [b]	Patients Pso-PsA / PsA	Severity stratification	Results (Pso-PsA vs. PsA unless otherwise specified)
Affected sites	**Present PsA**				
	Thumboo 2002 (29)	Cross	120 / 60	-≤2: limited ->2: generalized	(I) Generalized - 38.3% vs. 41.7%; p NR (II) *Univariate logistic regression* - Generalized: OR 1.18 (95%CI 0.59-2.34); NS
	Later development of PsA				
	Wilson 2009 (32)	Pro	*Baseline*: 1633 / 0 *End of 20.936 person years FU*: 1593 / 57	At baseline: -Unknown -1 sites -2 sites -≥3	(I) *Cox regression univariate* - 2 vs. 1 sites: HR 0.77 (95%CI 0.37-1.64); p NR - ≥3 vs. 1 sites: HR 2.24 (95%CI 1.23-4.08); p NR * (II) *Cox regression multivariate* - NR (NS)

Table 1. Studies that report the association of cutaneous psoriasis severity and PsA. * Significant (p value <0.05). [a] Differentiation between studies that report the association of cutaneous psoriasis severity with the *presence* of PsA and studies that report the association with future *development* of PsA. [b] Assessment of psoriasis severity in a either a cross sectional (Cross) or prospective (Pro) design. [c] Odds ratios calculated as follows: (a * d) / (b * d), with the standard error (SE) of the log odds ratio being SE{ln(OR)}=√((1/a)+(1/b)+(1/c)+(1/d)), and 95%CI=exp(ln(OR)-1.95*SE{ln(OR)}) to exp(ln(OR)+1.95*SE{ln(OR)}). Abbreviations: BSA: body surface area (1% is equivalent to the size of the palm of the patient's hand); CI: confidence interval; FU: follow-up; n.a.: not applicable; NR: not reported; NS: not significant (p value not reported); OR: odds ratio; PASI: psoriasis area and severity index; PsA: psoriatic arthritis; Pso-PsA: psoriasis without psoriatic arthritis; SD: standard deviation.

Whether psoriasis severity was determined by an experienced dermatologist was not described in more than half of the studies. All studies assessed psoriasis severity at a single time point, except for three studies that measured repeatedly over a period of time and reported the highest value during follow-up (*Eder et al.* (30)*, Soltani-Arabshahi et al.* (26)*; Tey et al.* (22)). Most studies reported psoriasis duration. As expected, because psoriasis precedes PsA in most cases, psoriasis duration was longer in PsA patients compared to Pso-PsA patients (range 0.2 – 9.5 years).(1,4) Details of therapies were not well described in most studies and varied greatly between studies. With regards to confounding, only two studies (*Haroon et al.* (15) and *Eder et al.* (31)) corrected for the use of (topical or systemic) psoriasis therapy. After selection based on our criteria of homogeneity , we included 15 studies in two meta-analyses to compare ∆PASI (n=13) and ∆BSA (n=4) between Pso-PsA and PsA patients (**Figure 2**).

With regards to the three studies that reported psoriasis severity and later development of PsA, the overall risk of bias was low. However, heterogeneity with regards to the reported psoriasis severity measures and estimators impeded pooling of results in quantitative analyses (*Eder et al.* (31); *Zenke et al.* (21); *Wilson et al.* (32)).

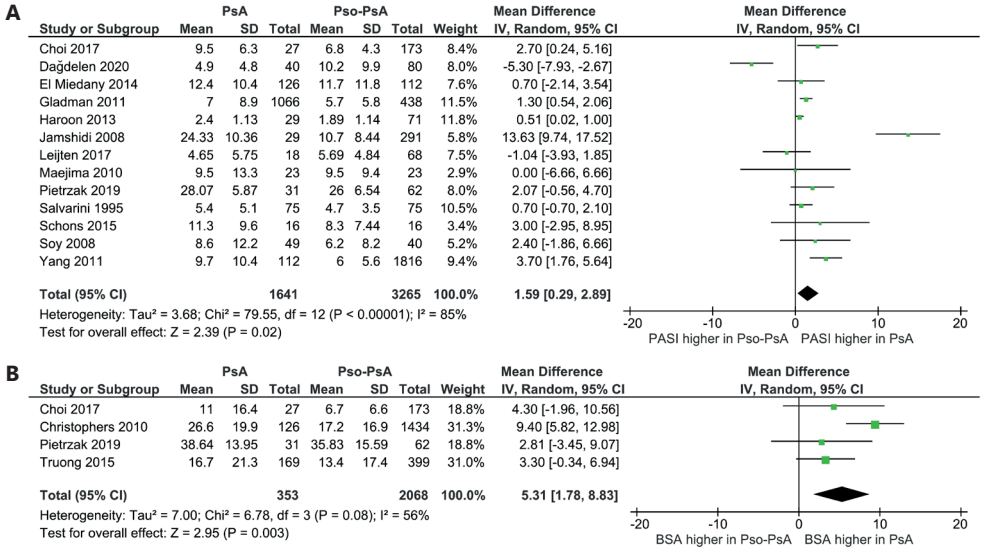

Figure 2. Meta-analysis: psoriasis severity in Pso-PsA and PsA patients. Forrest plots of studies that measure psoriasis severity as PASI (**A**) or BSA (**B**) and compare mean values between Pso-PsA and PsA patients. Abbreviations: BSA: body surface area; CI: confidence interval; PASI: psoriasis area and severity index; PsA: psoriatic arthritis; Pso-PsA: psoriasis without psoriatic arthritis; SD: standard deviation.

Psoriasis severity and presence of PsA

16 Cross-sectional studies reported PASI from one single measurement, of which 12 studies observed a higher mean or median PASI in PsA compared to Pso-PsA. We included 13 studies in our meta-analysis, that showed a significantly higher PASI in PsA (Δ 1.59 (95%CI 0.29-2.89)) with a high level of heterogeneity (I^2 85%) (**Figure 2A**). Given the high heterogeneity and possible publication bias (**Supplemental Figure S1**), we performed a sensitivity analysis by removing the studies by *Dağdelen et al.* (33) and *Jamshidi et al.* (17). The result of the adjusted meta-analysis showed a smaller difference, but still significant (Δ 1.25 (95% CI0.55-1.95)) and with acceptable heterogeneity (I^2 42%) (**Supplemental Figure S2**). Further, three studies (*Choi et al.* (13)*; Cinar et al.* (14)*; Henes et al.* (16)) compared PASI between Pso-PsA and PsA by stratification into mild, moderate or severe psoriasis. Although two studies found that moderate-severe psoriasis was

more prevalent amongst PsA patients, these results were not statistically significant. One study assessed psoriasis severity repeatedly over time and compared highest PASI (dichotomized <10 vs. ≥10) during three years follow-up (*Eder et al. 2012* (30)), but these results too were not significantly different.

Eight cross-sectional studies reported BSA, of which five studies reported mean or median BSA. All studies (*Choi et al.* (13)*; Christophers et al.* (23)*; Pietrzak et al.* (34)*; Stern et al.* (27)*; Truong et al.* (19)) showed that PsA patients have higher BSA compared to psoriasis patients. Our meta-analysis confirms that BSA is significantly higher in PsA patients (Δ 5.31 (95%CI 1.78-8.83)) with an intermediate level of heterogeneity (I^2 56%) (**Figure 2B**). Furthermore, three studies that stratified patients into mild, moderate and severe psoriasis showed that patients with severe skin disease (BSA >10) were more likely to have PsA than those with non-severe psoriasis. This association was significant in two studies (*Gelfand et al.* (24) OR 11.06 P <0.001; *Yan et al.* (28) OR 2.15 P <0.001). Moreover, severe psoriasis was a predictor of present PsA in two studies that performed multivariable regression analysis (*Ogdie et al.* (25) OR 3.34 P <0.001; *Yan et al.* (28) OR 1.92 P = NS). Furthermore, two studies measured BSA severity repeatedly over time and compared the highest value during follow-up between PsA and psoriasis patients. 'Highest BSA ever' (OR 1.01 (95%CI 1.00-1.01); *Soltani-Arabshahi et al.* (26)) and 'very severe skin disease' (as defined by BSA ≥76%) (OR 2.25 (95%CI 1.33-4.75); *Tey et al.* (22)), were significantly associated with PsA diagnosis.

Only one cross-sectional study compared the number of affected psoriasis sites between psoriasis and PsA patients (**Table 1**).(29) The number of patients with generalized psoriasis (>2 affected sites) was higher in PsA (41.4% vs. 38.3%; OR 1.18), but these results were not significant.

Psoriasis severity and future development of PsA

We identified three prospective studies that reported psoriasis severity in Pso-PsA patients and assessed later development of PsA (*Eder et al. 2016* (31); *Zenke et al.* (21); *Wilson et al.* (32)). One study showed with multivariable logistic regression that severe psoriasis (PASI ≥10) at psoriasis onset is not a statistically significantly predictor for PsA transition (OR 1.55, P value not reported), after correction for young age, sex, scalp psoriasis and nail dystrophy.(21) The second study reported that severe psoriasis (PASI ≥20) is significantly associated with PsA transition within 8 years (RR 5.39; P = 0.006).(31) Finally, one study indicated using univariate cox regression that patients with ≥3 affected

sites were significantly more at risk to develop PsA (HR 2.24 (95% CI 1.23-4.08)), but this effect was not sustained in multivariate analysis after correction for age, sex, calendar year, scalp psoriasis, intergluteal psoriasis and nail dystrophy (HR not reported).(32)

Discussion

To our knowledge, this is the first systematic review and meta-analysis in eight years to provide both qualitative and quantitative answers as to whether psoriasis severity is associated with the presence and development of PsA. This is a clinically relevant question, because skin severity measurement could aid in identifying those psoriasis patients at risk for PsA transition and thus serve as an easy implementable clinical measurement to facilitate early PsA diagnosis and improve clinical outcomes. Our results confirm that in patients with psoriasis the presence of slightly more extensive skin disease, as measured by higher PASI and BSA, is associated with concurrent PsA. We were unable to draw a definite conclusion about the association of psoriasis severity and later development of PsA.

The majority of the cross-sectional studies found a positive association between severe psoriasis and the presence of PsA. Moreover, our meta-analyses revealed a statistically significant mean difference of both PASI and BSA between Pso-PsA and PsA patients, although the differences were relatively small. We speculate these results may be an underestimation, because most studies included psoriasis patients that were treated in a hospital and patients with only mild psoriasis are typically less prone to visit a dermatologist. Unfortunately, we were unable to accurately assess the association of psoriasis severity and transition to PsA, as prospective studies were limited and heterogeneous. Although all point estimates were in the direction of a higher risk of developing PsA, the results were not always significant. Therefore, there is currently insufficient evidence to recommend dermatologists to use psoriasis severity as a reliable biomarker for the PsA development.

In the past, particular specific psoriasis localizations have been suggested to associate with PsA, including scalp and intergluteal psoriasis.(9) PASI and BSA capture all anatomically affected sites of psoriasis and therefore may not be the most suitable outcome measures to assess risk for PsA transition. Moreover, a PASI score of severe scalp psoriasis can be numerically comparable with that of only moderate psoriasis on the knees. Therefore, we recommend future studies to include in-depth topographic assessment of psoriasis localization and report individual PASI components.

The difference in psoriasis severity between PsA and Pso-PsA patients could improve our understanding of the pathogenic link between skin and joint disease. From a pathophysiologic perspective, the association between severe psoriasis and PsA may be explained by the important role of the interleukin (IL)-23-IL-17 and tumor necrosis factor alpha (TNF) pathways in inflammation of both the skin and musculoskeletal apparatus.(1) Overlapping cytokines – including IL-17, IL-22, IL-23 and TNF – play a role in immune-mediated inflammation of skin and synovium, that involves infiltration of pathogenic CD8+ T cells, macrophages, dendritic cells, monocytes and B cells.(35) It is hypothesized that local pro-inflammatory cytokine production and activated immune cells in psoriatic skin create a self-perpetuating inflammatory response, that results in systemic inflammation and PsA.(35) However, this does not explain why in 15% of the patients arthritis precedes skin lesions.(1) Moreover, cutaneous psoriasis severity has shown only modest correlation with joint disease.(36) Thus, the exact relation between inflammation of the skin, joints and other domains remains incompletely understood.(35)

This review has several limitations. First, we have not repeated the systematic search performed by *Rouzaud et al.*,(9) but as they employed validated methodology and even broader search methods we assume to have included all relevant publications. Second, our meta-analyses were limited by heterogeneity and a relatively small number of included studies. Third, most studies were conducted in dermatology clinics which may have resulted in an overestimation of psoriasis severity in PsA, since patients with 'PsA sine psoriasis' and limited psoriasis – typically seen by rheumatologists – could have been missed. Fourth, it needs to be taken into account that the meta-analysis did not include high quality studies. Most importantly, the use of therapies could have confounded the results. However, these studies do represent daily clinical practice, as psoriatic patients are frequently treated with topical and/or systemic treatment. Furthermore, we examined the effects of two potential confounders that associate with PsA in psoriasis patients, i.e. the presence of nail psoriasis and psoriasis disease duration. Meta-regression analysis suggested that our results were not explained by confounding by nail psoriasis or psoriasis duration, although we could only analyse the effects in six and eight studies, respectively (**Supplemental Table S4**). Additional subgroup analyses to investigate potential confounders – including psoriasis localization, family history of PsA, obesity, history of trauma of fracture and smoking status - could unfortunately not be performed in consequence of limited reporting of data.(10,37) Overall, we deem that these results are the currently best available answer to a clinically relevant question.

Concluding remarks

In conclusion, our results demonstrate that psoriasis severity is associated with increased likelihood of concurrent PsA. The high extent of psoriasis skin activity in PsA patients reinforces the necessity of multidisciplinary collaboration between rheumatologists and dermatologists in PsA care.

Defining psoriasis patients at risk for PsA transition remains an important topic to facilitate early recognition and prevent irreversible joint damage. Long lasting follow-up studies are necessary to study predictors for the development of PsA in psoriasis patients. Given the complexity of PsA pathogenesis, we deem that prediction models which combine genotypic and phenotypic predictors are the most promising to identify psoriasis patients at risk for PsA transition.(38–42)

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Data availability statement - All data generated or analysed during this study are included in this published article and its supplemental information files.

Statement of Ethics - The paper is exempt from ethical committee approval, because data was collected from published trials in which informed consent had been obtained by the trial investigators.

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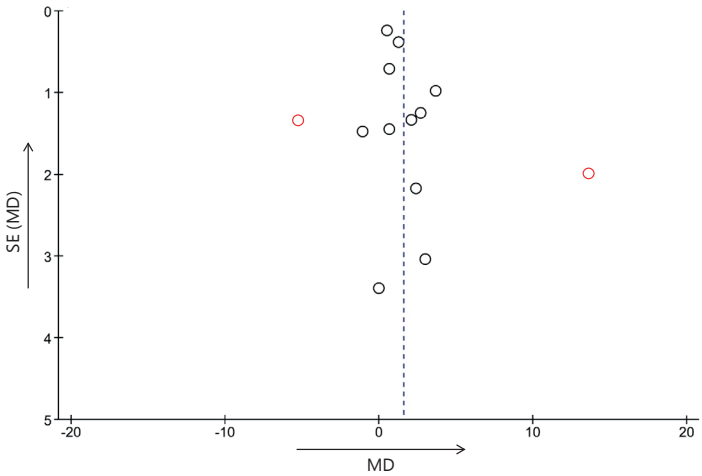
References

1. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. N Engl J Med. 2017 Mar;376(10):957–70.
2. Edson-Heredia E, Zhu B, Guo J, Maeda-Chubachi T, Lebwohl M. Disease burden and quality of life in psoriasis patients with and without comorbid psoriatic arthritis: results from National Psoriasis Foundation panel surveys. Cutis. 2015 Mar;95(3):173–8.
3. Ogdie A, Weiss P. The Epidemiology of Psoriatic Arthritis. Rheum Dis Clin North Am. 2015 Nov 1;41(4):545–68.
4. Raychaudhuri SP, Wilken R, Sukhov AC, Raychaudhuri SK, Maverakis E. Management of psoriatic arthritis: Early diagnosis, monitoring of disease severity and cutting edge therapies. J Autoimmun. 2017;76:21–37.
5. Haroon M, Gallagher P, FitzGerald O. Diagnostic delay of more than 6 months contributes to poor radiographic and functional outcome in psoriatic arthritis. Ann Rheum Dis. 2015 Jun 1;74(6):1045–50.
6. Gladman DD, Thavaneswaran A, Chandran V, Cook RJ. Do patients with psoriatic arthritis who present early fare better than those presenting later in the disease? Ann Rheum Dis. 2011 Dec 1;70(12):2152–4.
7. Tillett W, Jadon D, Shaddick G, Cavill C, Korendowych E, de Vries CS, et al. Smoking and delay to diagnosis are associated with poorer functional outcome in psoriatic arthritis. Ann Rheum Dis. 2013 Aug;72(8):1358–61.
8. Theander E, Husmark T, Alenius G-M, Larsson PT, Teleman A, Geijer M, et al. Early psoriatic arthritis: short symptom duration, male gender and preserved physical functioning at presentation predict favourable outcome at 5-year follow-up. Results from the Swedish Early Psoriatic Arthritis Register (SwePsA). Ann Rheum Dis. 2014 Feb;73(2):407–13.
9. Rouzaud M, Sevrain M, Villani AP, Barnetche T, Paul C, Richard MA, et al. Is there a psoriasis skin phenotype associated with psoriatic arthritis? Systematic literature review. J Eur Acad Dermatology Venereol. 2014;28(SUPPL. 5):17–26.
10. Scher JU, Ogdie A, Merola JF, Ritchlin C. Preventing psoriatic arthritis: focusing on patients with psoriasis at increased risk of transition. Nat Rev Rheumatol. 2019 Mar;15(3):153–66.
11. Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005 Mar;64 Suppl 2:ii65-8; discussion ii69-73.
12. Boehncke WH. Psoriasis and psoriatic arthritis: Flip sides of the coin? Vol. 96, Acta Dermato-Venereologica. Medical Journals/Acta D-V; 2016. p. 436–41.
13. Choi JW, Kim BR, Seo E, Youn SW. Could Psoriatic Arthritis Be Easily Diagnosed from Current Suspicious Physical Findings in the Dermatology Clinic? Ann Dermatol. 2017 Feb;29(1):48–54.
14. Çinar N, Bodur H, Eser F, Gül Ü, Gönül M, Oğuz ID. The prevalence and characteristics of psoriatic arthritis in patients with psoriasis in a Tertiary hospital. Arch Rheumatol. 2015;30(1):23–7.
15. Haroon M, Kirby B, FitzGerald O. High prevalence of psoriatic arthritis in patients with severe psoriasis with suboptimal performance of screening questionnaires. Ann Rheum Dis. 2013 May;72(5):736–40.
16. Henes JC, Ziupa E, Eisfelder M, Adamczyk A, Knaudt B, Jacobs F, et al. High prevalence of psoriatic arthritis in dermatological patients with psoriasis: a cross-sectional study. Rheumatol Int. 2014 Feb;34(2):227–34.
17. Jamshidi F, Bouzari N, Seirafi H, Farnaghi F, Firooz A. The prevalence of psoriatic arthritis in psoriatic patients in Tehran, Iran. Arch Iran Med. 2008;11(2):162–5.

18. Leijten EFA, Sigurdsson V, Wenink MH, Radstake TRDJ. Screening for psoriatic arthritis using the Psoriasis Epidemiology Screening Tool questionnaire: examining the optimal cut-off. Br J Dermatol. 2017;176(5):1357–9.
19. Truong B, Rich-Garg N, Ehst BD, Deodhar AA, Ku JH, Vakil-Gilani K, et al. Demographics, clinical disease characteristics, and quality of life in a large cohort of psoriasis patients with and without psoriatic arthritis. Clin Cosmet Investig Dermatol. 2015;8:563–9.
20. Salvarani C, Lo Scocco G, Macchioni P, Cremonesi T, Rossi F, Mantovani W, et al. Prevalence of psoriatic arthritis in Italian psoriatic patients. J Rheumatol. 1995 Aug;22(8):1499–503.
21. Zenke Y, Ohara Y, Kobayashi D, Arai S, Kishimoto M, Okada M, et al. Nail findings in patients with psoriatic arthritis: A cross-sectional study with special reference to transverse grooves. J Am Acad Dermatol. 2017 Nov;77(5):863–7.
22. Tey HL, Ee HL, Tan AS, Theng TS, Wong SN, Khoo SW. Risk factors associated with having psoriatic arthritis in patients with cutaneous psoriasis. J Dermatol. 2010;37(5):426–30.
23. Christophers E, Barker J, Griffiths C, Daudén E, Milligan G, Molta C, et al. The risk of psoriatic arthritis remains constant following initial diagnosis of psoriasis among patients seen in European dermatology clinics. J Eur Acad Dermatology Venereol. 2010;24(5):548–54.
24. Gelfand JM, Gladman DD, Mease PJ, Smith N, Margolis DJ, Nijsten T, et al. Epidemiology of psoriatic arthritis in the population of the United States. J Am Acad Dermatol. 2005;53(4):573. e1-573.e13.
25. Ogdie A, Langan SS, Love T, Haynes K, Shin D, Seminara N, et al. Prevalence and treatment patterns of psoriatic arthritis in the UK. Rheumatol (United Kingdom). 2013 Mar;52(3):568–75.
26. Soltani-Arabshahi R, Wong B, Feng BJ, Goldgar DE, Callis Duffin K, Krueger GG. Obesity in early adulthood as a risk factor for psoriatic arthritis. Arch Dermatol. 2010;146(7):721–6.
27. Stern RS. The epidemiology of joint complaints in patients with psoriasis. J Rheumatol. 1985 Apr;12(2):315–20.
28. Yan D, Ahn R, Leslie S, Liao W. Clinical and Genetic Risk Factors Associated with Psoriatic Arthritis among Patients with Psoriasis. Dermatol Ther (Heidelb). 2018;8(4):593–604.
29. Thumboo J, Uramoto K, Shbeeb MI, O'Fallon WM, Crowson CS, Gibson LE, et al. Risk factors for the development of psoriatic arthritis: A population based nested case control study. J Rheumatol. 2002;29(4):757–62.
30. Eder L, Law T, Chandran V, Shanmugarajah S, Shen H, Rosen CF, et al. Association between environmental factors and onset of psoriatic arthritis in patients with psoriasis. Arthritis Care Res. 2011;63(8):1091–7.
31. Eder L, Haddad A, Rosen CF, Lee KA, Chandran V, Cook R, et al. The Incidence and Risk Factors for Psoriatic Arthritis in Patients with Psoriasis: A Prospective Cohort Study. Arthritis Rheumatol. 2016;68(4):915–23.
32. Wilson FC, Icen M, Crowson CS, McEvoy MT, Gabriel SE, Kremers HM. Incidence and clinical predictors of psoriatic arthritis in patients with psoriasis: A population-based study. Arthritis Care Res. 2009;61(2):233–9.
33. Dağdelen D, Karadag AS, Kasapoğlu E, Wang J V., Erman H. Correlation of metabolic syndrome with serum omentin-1 and visfatin levels and disease severity in psoriasis and psoriatic arthritis. Dermatol Ther. 2020;33(6):1–7.
34. Pietrzak A, Chabros P, Grywalska E, Kicinski P, Pietrzak-Franciszkiewicz K, Krasowska D, et al. Serum lipid metabolism in psoriasis and psoriatic arthritis - an update. Arch Med Sci. 2019 Mar;15(2):369–75.

35. Veale DJ, Fearon U. Psoriatic arthritis 1 The pathogenesis of psoriatic arthritis. Lancet. 2018;391:2273–84.
36. Mease PJ, Etzel CJ, Huster WJ, Muram TM, Armstrong AW, Lisse JR, et al. Understanding the association between skin involvement and joint activity in patients with psoriatic arthritis: Experience from the Corrona Registry. RMD Open. 2019;5(1).
37. Zabotti A, De Lucia O, Sakellariou G, Batticciotto A, Cincinelli G, Giovannini I, et al. Predictors, Risk Factors, and Incidence Rates of Psoriatic Arthritis Development in Psoriasis Patients: A Systematic Literature Review and Meta-Analysis. Rheumatol Ther. 2021;8(4):1519–34.
38. van Muijen ME, van Hal TW, Groenewoud HMM, van den Reek JMPA, de Jong EMGJ. The Skin May Clear But the Arthritis Won't Disappear: Focusing on Concomitant and New-Onset Psoriatic Arthritis in a Daily Practice Cohort of Psoriasis Patients on Biologic Therapy. Psoriasis (Auckland, NZ). 2020 Oct;10:29–37.
39. Winchester R, FitzGerald O. The many faces of psoriatic arthritis: their genetic determinism. Rheumatology (Oxford). 2020 Mar;59(Supplement_1):i4–9.
40. Winchester R, FitzGerald O. MHC class I associations beyond HLA-B27: the peptide binding hypothesis of psoriatic arthritis and its implications for disease pathogenesis. Curr Opin Rheumatol. 2020 Jul;32(4):330–6.
41. Hile G, Kahlenberg JM, Gudjonsson JE. Recent genetic advances in innate immunity of psoriatic arthritis. Clin Immunol. 2020;214(March):108405.
42. Patrick MT, Stuart PE, Raja K, Gudjonsson JE, Tejasvi T, Yang J, et al. Genetic signature to provide robust risk assessment of psoriatic arthritis development in psoriasis patients. Nat Commun. 2018;9:4178.

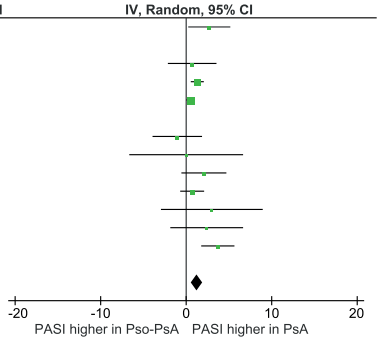
Supplemental Information



Supplemental Figure S1. Assessment of publication bias in PASI meta-analysis. Funnel plot of studies included in meta-analysis of PASI (**Figure 2A**). Y-axis: standard error of mean difference in PASI. X-axis: mean difference PASI. Red studies: *Dağdelen et al.* (left) and *Jamshidi et al.* (right). Abbreviations: MD: mean difference; PASI: psoriasis area and severity index; SE: standard error.

	PsA			Pso-PsA				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Choi 2017	9.5	6.3	27	6.8	4.3	173	6.4%	2.70 [0.24, 5.16]
Dağdelen 2020	4.9	4.8	40	10.2	9.9	80	0.0%	-5.30 [-7.93, -2.67]
El Miedany 2014	12.4	10.4	126	11.7	11.8	112	5.1%	0.70 [-2.14, 3.54]
Gladman 2011	7	8.9	1066	5.7	5.8	438	22.7%	1.30 [0.54, 2.06]
Haroon 2013	2.4	1.13	29	1.89	1.14	71	27.0%	0.51 [0.02, 1.00]
Jamshidi 2008	24.33	10.36	29	10.7	8.44	291	0.0%	13.63 [9.74, 17.52]
Leijten 2017	4.65	5.75	18	5.69	4.84	68	4.9%	-1.04 [-3.93, 1.85]
Maejima 2010	9.5	13.3	23	9.5	9.4	23	1.1%	0.00 [-6.66, 6.66]
Pietrzak 2019	28.07	5.87	31	26	6.54	62	5.8%	2.07 [-0.56, 4.70]
Salvarini 1995	5.4	5.1	75	4.7	3.5	75	13.9%	0.70 [-0.70, 2.10]
Schons 2015	11.3	9.6	16	8.3	7.44	16	1.3%	3.00 [-2.95, 8.95]
Soy 2008	8.6	12.2	49	6.2	8.2	40	2.5%	2.40 [-1.86, 6.66]
Yang 2011	9.7	10.4	112	6	5.6	1816	9.2%	3.70 [1.76, 5.64]
Total (95% CI)			**1572**			**2894**	**100.0%**	**1.25 [0.55, 1.95]**

Heterogeneity: Tau² = 0.41; Chi² = 17.14, df = 10 (P = 0.07); I² = 42%
Test for overall effect: Z = 3.49 (P = 0.0005)



Supplemental Figure S2. Sensitivity analysis PASI – Dağdelen et al. and Jamshidi et al. excluded. Forrest plot of studies that measure psoriasis severity as PASI (**Figure 2A**), after exclusion of the study results by *Dağdelen et al.* and *Jamshidi et al*. Abbreviations: CI: confidence interval; PASI: psoriasis area and severity index; PsA: psoriatic arthritis; Pso-PsA: psoriasis without psoriatic arthritis; SD: standard deviation.

Database	Search terms	Results
PubMed	((((((((((((((Arthritis, Psoriatic[MeSH Major Topic]) OR Arthritic psoriasis[Title/Abstract]) OR Arthropathic psoriasis[Title/Abstract]) OR Psoriasis arthropathica[Title/Abstract]) OR Psoriatic arthritis[Title/Abstract]) OR Psoriatic arthropathies[Title/Abstract]) OR Psoriatic arthropathy[Title/Abstract])))) AND (((psoriasis[MeSH Major Topic]) OR psoriasis[Title/Abstract]) OR psoriatic skin disease[Title/Abstract]))))) AND (((((sever*[Title/Abstract]) OR PASI[Title/Abstract]) OR BSA[Title/Abstract]) OR psoriasis area severity index[Title/Abstract]) OR body surface area[Title/Abstract])) AND (("2013/01/01"[PDat]:"3000/12/31"[PDat]))) AND ((English[lang] OR Dutch[lang])))	1367
Embase	('psoriatic arthritis'/exp OR 'psoriatic arthritis' OR 'arthritic psoriasis':ab,ti OR 'arthropathic psoriasis':ab,ti OR 'psorasis arthopathica':ab,ti OR 'psoriatic arthritis':ab,ti OR 'psoriatic arthropathies':ab,ti OR 'psoriatic arthopathy':ab,ti) AND ('psoriasis'/exp OR 'psoriasis' OR 'psoriasis':ab,ti OR 'psoriatic skin disease':ab,ti) AND ('sever*':ab,ti OR 'pasi':ab,ti OR 'bsa':ab,ti OR 'psoriasis area severity index':ab,ti OR 'body surface area':ab,ti) AND [2013-2020]/py AND ([article]/lim OR [article in press]/lim OR [conference paper]/lim OR [letter]/lim) AND ([dutch]/lim OR [english]/lim)	1665

Supplemental Table S1. Literature search.

Inclusion criteria	Exclusion criteria
- Published after 01-01-2013 - Study participants aged >18 years old - Human subjects - Original studies	- Meta-analysis - Review - Case-report - Expert opinion - Authors' reply - Conference abstracts - Language other than English - No relevant data report

Supplemental Table S2. Eligibility criteria.

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
PASI									
Choi 2017	Cross-sectional	173 27	S-Korea; Seoul National University Bundang Hospital; Dermatology	CASPAR	Psoriasis Pso-PsA 8.7 ± 10.0 PsA 9.8 ± 9.6	(i) Investigate prevalence of undiagnosed PsA in cohort of Pso-PsA patients, (ii) Study association PsA with psoriasis severity and other medical conditions	Topical (100 / 100), phototherapy (50 / 52), systemic (28 / 30; of which biologics 3 / 0)	Cross-sectional by 1 highly experienced dermatologist	-<10: mild -10-20: moderate ->20: severe
Cinar 2015	Cross-sectional	94 32	Turkey; Ankara Numune Training and Research Hospital; Dermatology	CASPAR	Psoriasis Pso-PsA 121.9 mo ± 97.7 PsA 205.4 mo ± 199.4	(i) Investigate prevalence of undiagnosed PsA in a cohort of Pso-PsA patients, (ii) Compare clinical and laboratory characteristics	Not specified	Cross-sectional by 1 dermatologist	-<3: mild -3-15: moderate ->15: severe
Dağdelen 2020	Case-control	80 40	Turkey; Istanbul Medeniyet University; Dermatology & Rheumatology	CASPAR	Psoriasis Pso-PsA 13.8 ± 13.0 PsA 17.0 ± 15.2 PsA 8.2 ± 7.1	Assess prevalence om metabolic syndrome in patients with PsA, Pso-PsA vs. HC	No systemic treatment <1 mo	Cross-sectional, assessor not specified	n.a.
Eder 2011	Case-control	159 159	Canada; University of Toronto PsA and psoriasis cohorts; Dermatology & Rheumatology	CASPAR	Psoriasis Pso-PsA 18.6 ± 14.5 PsA 17.2 ± 13 PsA 3.1 ± 2.2	Investigate association environmental exposure with development PsA in patients with psoriasis	MTX (3 / 14), biologics (1 / 6)	During FU, assessor not specified (data from cohort database)	Highest during first 3y FU: -<10: non-severe -≥10: severe
El Miedany 2014	Prospective case-control (FU 1 y)	112 126	Egypt; center not specified; Early inflammatory arthritis clinic for MSK symptoms	CASPAR	Psoriasis Pso-PsA 4.6 ± 3.6 PsA 4.8 ± 3.1 PsA 4.3 ± 1.6 (at baseline)	(i) Identify predictors of arthritis in Pso-PsA patients (ii) Evaluate US as predictor for structural progression	No systemic treatment <3 mo	At baseline, assessor not specified	n.a.

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
Gladman 2011	Observational cohort	438 1066	Canada; University of Toronto PsA and psoriasis cohort; Dermatology & Rheumatology	Modified Moll & Wright criteria	Psoriasis Pso-PsA 16.1 ± 14.1 PsA 15.2 ± 12.3 PsA 7.1 ± 8.3 (at baseline)	Describe PsA disease manifestations, course and prognosis	Not specified	At entry in cohort, assessor not specified	n.a.
Haroon 2013	Cross-sectional	71 29	Ireland; St. Vincent's University Hospital; Dermatology & Rheumatology	CASPAR	Psoriasis Pso-PsA 28.82 ± 14.29 PsA 29.10 ± 15.08	(i) Assess prevalence of undiagnosed PsA among Pso-PsA patients (ii) Identify predictors of PsA, (iii) compare performance of PsA screening tools	TNFi (41 / 34), fumaric acid (34 / 38), photo (13 / 10), UST (3 / 3), ciclosporin (3 / 0)	Cross-sectional, assessor not specified	n.a.
Henes 2013	Cross-sectional	48 50	Germany; University hospital Tuebingen; Dermatology	CASPAR	Psoriasis Pso-PsA 16 (IQR 1-57) PsA 19.5 (IQR 1-56)	Assess prevalence of PsA among patients with psoriasis, that have suspected PsA based on the GEPARD screening tool	Pooled: TNFi (4), MTX (18)	Cross-sectional, one physician	-0–1: not active -2–10: mild -11–15: moderate ->15: severe
Jamshidi 2008	Cross-sectional	291 29	Iran; Razi Hospital Tehran; Dermatology	Moll & Wright criteria	Psoriasis Pso-PsA 10.6 PsA 10.2	Assess prevalence of undiagnosed PsA among Pso-PsA patients	Not specified	Cross-sectional, a dermatologist	n.a.
Leijten 2017	Cross-sectional	68 18	The Netherlands; University Medical Center Utrecht; Dermatology	CASPAR	Psoriasis Pso-PsA 16 ± 13 PsA 23 ± 13	Assess prevalence of undiagnosed PsA among Pso-PsA patients using the PEST screening tool	Topical only (62 / 56), photo (13 / 17), systemic (12 / 22)	Cross-sectional, a dermatologist	n.a.
Maejima 2010	Case-control	23 23	Japan; Department of Dermatology Kitasato University; Rheumatology	CASPAR	Not specified	Clarify clinical importance of nail disease in PsA	Not specified	Cross-sectional, assessor not specified	n.a.

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
Pietrzak 2019	Case-control	62 31	Poland; Medical University of Lublin; not specified	CASPAR	Psoriasis Pso-PsA 9.3 ± 10.1 PsA 16.8 ± 13.2 PsA 10.4 ± 12.9	Assess blood parameters of lipid metabolism and markers of oxidative stress in Pso-PsA and PsA patients	No topical retinoids, systemic therapy not specified	Cross-sectional, assessor not specified	n.a.
Reich 2009	Cross-sectional	1055 312	Germany; 48 community and academic centers; Dermatology	Moll & Wright criteria	Psoriasis Pso-PsA 16.0 PsA 21.0 PsA Not specified	Assess prevalence and clinical patterns of PsA among Pso-PsA and PsA patients	Not specified	Cross-sectional, 'dermatological assessment'	n.a.
Salvarani 1995	Cross-sectional	130 75	Italy; University of Bologna; unknown	Clinical	Unknown	(i) evaluate prevalence of PsA in Pso-PsA patients, (ii) compare ESSG and Amor classification criteria	Unknown	Unknown	n.a.
Schons 2015	Cross-sectional	49 16	Brazil; University Hospital of Santa Maria; Dermatology	CASPAR	Psoriasis Pso-PsA 10.0 (IQR 1-41) PsA 19.5 (IQR 1-40)	Study nail changes - and their clinical implications – in Pso-PsA and PsA patients	Pooled: topical (75), systemic (45)	Cross-sectional. one researcher	n.a.
Soy 2008	Case-control	40 49	Turkey; Trakya University School of Medicine; Dermatology & Rheumatology	ESSG criteria	Psoriasis Pso-PsA 17 ± 11 PsA 19 ± 23 PsA 4.6 ± 3.5	Explore characteristics of joint and nail involvement in PsA	Not specified	Cross-sectional, an experienced dermatologist	n.a.
Yang 2011	Cross-sectional	1816 112	China; Shandong Provincial Institute of Dermatology and Venereology; Dermatology	CASPAR	Psoriasis Pso-PsA 7.8 ± 8.9 PsA 14.1 ± 11.7 PsA Not specified	Assess prevalence of PsA among Pso-PsA and PsA patients	Not specified	Cross-sectional, multiple dermatologists	n.a.
Eder 2016	Prospective cohort	Baseline 464 0 8y FU 404 60	Canada; University of Toronto psoriasis cohort; Dermatology clinics & advertisement	CASPAR	Psoriasis All Pso 16.4 ± 14.4 PsA 17 ± 15.2 (at baseline)	In cohort of Pso-PsA patients (i) Estimate annual incidence of PsA, (ii) Identify markers for high risk of PsA	Ever use of retinoids (9 / 13), MTX (9 / 15), TNFi (6 / 10) at baseline	At baseline, assessor not described (data from cohort database)	At baseline: -<10: mild -10-20: moderate ->20: severe

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
Zenke 2017	Retro-spective cohort	974 118	Japan; St. Luke's International Hospital Tokyo; Dermatology	Clinical by board-certified rheumatologists	Psoriasis Pso-PsA 8.6 ± 9.5 PsA 11.8 ± 10.6	Investigate whether nail findings discriminate between PsA and Pso-PsA	Not specified	At first visit, by multiple dermatologists	At first visit at the dermatology clinic: -<10: non-severe -≥10: severe
BSA									
Tey 2010	Case-control	266 134	Singapore; National Skin Center; Dermatology	Clinical diagnosis by rheumatologist	Not reported (median age of psoriasis onset Pso-PsA 1 (mean age 44); PsA 30 (mean age 46))	Determine characteristics associated with PsA in a sample of Pso-PsA patients	Not specified	Two designated dermatologists	Max. in 1y FU: -0-25%: I -26-50%: II -51-75%: III -76-100%: IV
Choi 2017	Cross-sectional	173 27	S-Korea; dermatology clinic Seoul National University Bundang Hospital; Dermatology	CASPAR	Psoriasis Pso-PsA 8.7 ± 10.0 PsA 9.8 ± 9.6 PsA Not specified	(i) Investigate PsA prevalence in cohort of Pso-PsA patients, (ii) Study association PsA with psoriasis severity and other medical conditions	Topical (100 / 100), photo (50 / 52), systemic (28 / 30; of which biologics (3 / 0))	Cross-sectional by 1 highly experienced dermatologist	-<3: mild -3-10: moderate ->10: severe
Cristhophers 2010	Cross-sectional	1434 126	UK, Italy, France, Spain, Germany; Dermatology	Clinical diagnosis	Psoriasis Pso-PsA 11.0 ± 11.3 PsA 17.3 ± 11.3 PsA Not specified	(i) Assess whether time since PsO diagnosis affects risk of developing PsA in a cohort of Pso-PsA patients, (ii) compare Pso-PsA vs. PsA differences in QOL, comorbidities and healthcare resource utilization	Not specified	Multiple dermatologists	n.a.

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
Gelfand 2005	Cross-sectional	530 71	USA (48 states); random digital dialing technique	Self-report patient	Not specified	(i) Determine the prevalence of PsA in a cohort Pso-PsA patients, (ii) Determine impact on QOL of PsA	Not specified	self-report patient	-<1: no or little -1-2: mild -3-10: moderate ->10: severe
Ogdie 2013	Cross-sectional	3699 365	UK; The Health Improvement Network; electronic primary care medical record database	≥ 1 read code consistent diagnosis [b)]	Psoriasis Pso-PsA range 10-19 PsA range 5-9 PsA Not specified	(i) Determine PsA prevalence in Pso-PsA patients in a population-based medical records database, (ii) Examine PsA-associated factors, (iii) Describe PsA patients DMARD use	Topical (nr / 72), DMARD (nr / 46; of which biologics nr / 0.3)	General practitioners	-≤2: mild -3-10: moderate ->10: severe
Pietrzak 2019	Case-control	62 31	Poland; Medical University of Lublin; not specified	CASPAR	Psoriasis Pso-PsA 9.3 ± 10.1 PsA 16.8 ± 13.2 PsA 10.4 ± 12.9	Assess blood parameters of lipid metabolism and markers of oxidative stress in Pso-PsA and PSA	No topical retinoids, systemic therapy not specified	Cross-sectional, assessor not specified	n.a.
Soltani-Arabshahi 2010	Cross-sectional	693 250	USA; Utah Psoriasis Initiative; Dermatology	Physician diagnosis from self-reported questionnaire	Not specified (age PsA patients 47 ± 17, age at Pso onset 27 ± 17)	Study whether obesity increases the risk of PsA	Not specified	2 faculty dermatologists	Highest ever: -<5: mild -5-10: moderate ->10: severe
Stern 1985	Cross-sectional	1019 266	Unknown	Clinical diagnosis	Unknown	Better define the epidemiology of arthritis among Pso patients	Unknown	Unknown	n.a.
Truong 2015	Observational cohort	399 169	USA (Oregon, Washington); CEPPA clinic; Dermatology	Clinical diagnosis by rheumatologist	Psoriasis Pso-PsA 17.7 ± 14 PsA 20.2 ± 14.2	Identify and compare demographics, clinical characteristics and QOL in cohort of Pso-PsA and undiagnosed PsA	Systemic (10 / 12)	Multiple dermatologists	n.a.

Study	Reported design	Patients, n Pso-PsA PsA	Origin; Center; Department / study setting [a)]	PsA classification	Duration disease (y)	Objective(s)	Therapy (% Pso-PsA / PsA)	Assessor psoriasis severity	Psoriasis severity stratification
Yan 2018	Cross-sectional	497 175	USA (California); University of California, San Francisco; Dermatology	Clinical diagnosis by dermatologist or rheumatologist	Psoriasis Pso-PsA 17 PsA 22 PsA Not specified	Identify clinical and genetic factors that discriminate PsA from Pso-PsA	Naïve, topical, systemic and phototherapy (numbers not specified)	Not specified	-mild -mild-moderate -moderate-severe -severe [c)]
Sites									
Thumboo 2002	Case-control	120 60	USA (Minnesota); Rochester Epidemiology Project; residents seeking medical care	Clinical diagnosis by physician	Psoriasis Pso-PsA 6.2 PsA 5.8 PsA Not specified	Identify factors influencing development of PsA in Pso-PsA	Coal tar (49 / 45), MTX (1 / 0), photo (10 / 3)	Multiple dermatologists	-≤2: limited ->2: generalized
Wilson 2009	Prospective cohort (FU 30 years)	Baseline 1633 0 20.936 person y FU 1593 57	USA (Minnesota); Rochester Epidemiology Project medical; residents seeking medical care	CASPAR	Not specified	Identify predictors of PsA in a large cohort of Pso-PsA only patients	Not specified	Multiple dermatologists	At baseline: -Unknown -1 sites -2 sites -≥3

Supplemental Table S3. Detailed study characteristics. [a)] Study setting in which patients were recruited. Dermatology / Rheumatology stands for outpatient clinics. [b)] Read code: comprehensive hierarchical alphanumeric clinical language developed in the UK to record diagnoses, symp- toms and tests, similar to International Classification of Diseases codes (Chishom J. The Read clinical classification. BMJ 1990; 300:1092). Abbreviations: BSA: body surface area; CASPAR: classification criteria for psoriatic arthritis; DMARD: disease-modifying antirheumatic drug; ESSG: European Spondyloarthropathy Study Group; FU: follow-up; GEPARD: GErman Psoriasis ARthritis Diagnostic questionnaire; HR: hazards ratio; HRCU: healthcare resource utilization; MSK: musculoskeletal; MTX: methotrexate; NR: not reported; NS: not significant; OR: odds ratio; PASI: psoriasis area and severity index; PEST: Psoriasis Epidemiology Screening Tool; PsA: psoriatic arthritis; Pso-PsA: psoriasis without psoriatic arthritis; QOL: quality of life; RR: risk ratio; SD: standard deviation; TNFi: tumour necrosis factor α inhibitor; US: ultrasound; UST: ustekinumab (IL-12/23 inhibitor).

Nail psoriasis	Psoriasis duration
Correction of the association between severity of cutaneous psoriasis and PsA for nail psoriasis and psoriasis duration marginally changed our results: of six studies that reported prevalence of nail psoriasis, the crude association showed a mean difference in PASI between PsA and Pso-PsA of 3.5495 (95%CI 1.2606-5.8384; p=0.0024). After correction for difference in nail psoriasis prevalence between PsA and Pso-PsA this effect was 2.7418 (95%CI -1.6255-7.1091; p=0.2185) (effect nail psoriasis: 0.0366 (95%CI -0.1157-0.189; p=0.6375)).	Of eight studies that described psoriasis duration, the crude association showed a PASI mean difference of 1.2471 (95%CI -0.0694-2.5636; p=0.0634). The effect after correction for psoriasis duration was 0.8397 (95%CI -0.6912-2.3706; p=0.2823) (effect psoriasis duration: 0.1957 (95%CI -0.2184-0.6098; p=0.3543). Even though the results of mean difference PASI were no longer statistically significant, possibly due to the low number of patients, this suggests that our results are not (fully) explained by confounding by psoriasis duration.

Supplemental Table S4. Meta-regression analysis. Abbreviations: CI: confidence interval; PASI: psoriasis area and severity index; PsA: psoriatic arthritis; Pso-PsA: psoriasis without psoriatic arthritis.

Chapter 6

Broad proteomic screen reveals shared serum proteomic signature in patients with psoriatic arthritis and psoriasis without arthritis

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Abstract

Objective – To identify novel serum proteins involved in the pathogenesis of PsA as compared with healthy controls, psoriasis (Pso) and AS, and to explore which proteins best correlated to major clinical features of the disease.

Methods – A high-throughput serum biomarker platform (Olink) was used to assess the level of 951 unique proteins in serum of patients with PsA (n=20), Pso (n=18) and AS (n=19), as well as healthy controls (HC, n=20). Pso and PsA were matched for Psoriasis Area and Severity Index (PASI) and other clinical parameters.

Results – We found 68 differentially expressed proteins (DEPs) in PsA as compared with HC. Of those DEPs, 48 proteins (71%) were also dysregulated in Pso and/or AS. Strikingly, there were no DEPs when comparing PsA with Pso directly. On the contrary, hierarchical cluster analysis and multidimensional scaling revealed that HC clustered distinctly from all patients, and that PsA and Pso grouped together. The number of swollen joints had the strongest positive correlation to ICAM-1 ($r = 0.81$, $P < 0.001$) and CCL18 (0.76, $P < 0.001$). PASI score was best correlated to PI3 ($r = 0.54$, $P < 0.001$) and IL-17 receptor A ($r = –0.51$, $P < 0.01$). There were more proteins correlated to PASI score when analysing Pso and PsA patients separately, as compared with analysing Pso and PsA patients pooled together.

Conclusion – PsA and Pso patients share a serum proteomic signature, which supports the concept of a single psoriatic spectrum of disease. Future studies should target skin and synovial tissues to uncover differences in local factors driving arthritis development in Pso.

Manuscript

Introduction

Psoriasis (Pso) is a common autoimmune disease that causes excessive scaling, redness and itchiness of skin at prototypical sites of the body. Approximately 20% of patients with Pso will at some point in their life develop PsA.(1) A clinical diagnosis of PsA is typically made in a patient with Pso or psoriatic nail disease with concomitant arthritis. PsA is clinically heterogeneous and other manifestations include those of the SpA spectrum, such as enthesitis, dactylitis and SpA. Adding to this heterogeneity is that in ~15% of the cases of PsA, arthritis manifests prior to Pso.(1) Both cutaneous and rheumatic manifestations of Pso negatively impact quality of life and should be treated appropriately.(2)

Tremendous advances have been made in the treatment options available for Pso. The current and emerging therapeutics can almost completely reverse skin inflammation in a majority of patients, but their capacity to halt arthritis is less impressive.(3) This discrepancy is well-illustrated by examining the current gold standard of trial outcome measures: a 90% improvement for Pso disease severity (Psoriasis Area and Severity Index, PASI90), compared with a 20% improvement for arthritis severity (ACR20). Numerous factors could explain the trailing treatment response in arthritis, including drug bioavailability, the cellular target and cellular turnover at the target tissue, as well as (still unidentified) differences in tissue-specific drivers of pathogenesis.(4–6)

It is unknown whether the immunologic drivers in Pso vs PsA patients are different. (7,8) This raises the question of whether these diseases are part of the same spectrum or distinct entities.(8,9) Pso is one of the strongest known clinical risk factors for the development of arthritis, thus providing a unique opportunity to better understand arthritis development and improve treatment. It has historically been difficult to identify early PsA in Pso patients in daily clinical practice and there are currently no serum diagnostic biomarkers used in care. This impedes clarification of the presence or absence of a window of opportunity for treating early PsA. To overcome these important open questions, Pso and PsA should be studied head-to-head to uncover potential differences in pathogenesis that could serve as therapeutic targets, as well as to identify possible biomarkers to be used in early diagnosis.

Genetic studies reveal vast overlap between Pso and PsA, in which the few differences found were variants related to chromatin marks on a subset of T lymphocytes and CD8 T cells, and to variants in the IL-23 receptor.(10,11) In comparative studies from

peripheral blood mononuclear cells, Pso patients with PsA have higher expression of genes associated with the IFN signature in their monocytes,(12,13) and their T cells more readily produce IL-2 and IL-22 upon re-stimulation.(14,15) Recent work has also shown that patients with PsA have higher levels of auto-antibodies directed against two previously identified putative auto-antigens of Pso, namely (carbamylated) LL37 and ADAMTSL5.(16,17) So far, serum-based biomarker studies have revealed elevated levels of high-sensitivity CRP, pro-inflammatory cytokines (e.g. IL-6, IL-33, TNF-α), adipokines and changes in markers of bone/cartilage damage in the Pso patients with PsA.(18–27)

Overall, there is a scarcity of head-to-head serum biomarker comparisons in well-defined cohorts of Pso and PsA. The current study measured serum biomarkers in the early stage of PsA as compared with Pso matched for skin disease severity. We used a novel high-throughput proteomic platform capable of screening over 950 proteins in a small volume of serum. Previously, this technology proved valuable in providing new mechanistic insights into the pathogenesis of immune-mediated diseases of skin,(28,29) but results have not yet been reported in patients with rheumatic disease. The goal was to determine whether this biomarker platform could identify novel serum protein disturbances in PsA as compared with HC, Pso and AS (non-psoriatic reference group), and to specify which proteins best reflected major skin and joint manifestations.

Methods

Study design

This study was performed at the University Medical Center Utrecht and conducted in compliance with the Helsinki principles. Ethical approval was obtained from the institutional review board and all patients signed written informed consent before participation. Clinical parameters and serum samples were collected from a cohort of patients with Pso, PsA and AS as part of larger prospective observational study performed at the outpatient clinic of the Department of Rheumatology and Clinical Immunology.

For this study 79 patients were recruited. The Pso cohort (Pso, n=20) included patients with a dermatologist-confirmed diagnosis of Pso in whom concomitant PsA was clinically excluded by a rheumatologist (in training). Patients with PsA (n=20) fulfilled ClASsification of Psoriatic ARthritis (CASPAR) criteria.(30) Patients with a clinical diagnosis of AS (n=19), all without a history of Pso, were included as a non-psoriatic reference group. Serum samples were collected from healthy controls (HC, n=20) from the University Medical Center Utrecht.

Serum proteomic analysis

Serum samples were collected, centrifuged at 1700g for 10 min at 4°C and stored directly at −80°C. Frozen serum aliquots were shipped on dry ice to the Olink Facility (Uppsala, Sweden) without prior thawing and measured according to manufacturer's instructions as previously published.(31) The Olink high-throughput proteomic platform employs a proximity extension-assay technology, in which oligonucleotide-labelled antibody pairs bind to a protein target. DNA reporter molecules bind to these antibodies, and are amplified to provide relative protein concentrations. One serum aliquot of 250 µl was used to run 11 different Olink platform 'panels' encompassing 1012 proteins, some of which were run in more than one panel (panels: CARDIOMETABOLIC, CARDIOVASCULAR II, CARDIOVASCULAR III, CELL REGULATION, DEVELOPMENT, IMMUNE RESPONSE, INFLAMMATION, METABOLISM, NEUROLOGY, ONCOLOGY II and ORGAN DAMAGE). Only data that passed Olink internal quality control were used for analysis. We removed samples entirely if they did not pass Olink internal quality control in >80% of the data. We removed proteins entirely if they were below the limit of assay detection in >40% of the samples. Some proteins were measured in multiple panels, in which case the protein data with the fewest missing values after quality control were used for analysis.

Statistical approach

For analysis of clinical characteristics, contingency analysis of two groups were performed using Chi-squared tests for categorical variables, and independent samples T-tests or Mann–Whitney U tests for continuous variables. Contingency analysis of more than two groups were conducted with one-way independent analysis of variance or Kruskal–Wallis for continuous variables, and with χ^2 test for categorical variables. Spearman's rank correlation was used to correlate disease activity parameters to protein levels. Unless otherwise stated, a P-value of <0.05 was considered statistically significant.

The statistical analysis of proteomic data was performed on protein data received by Olink without further normalization (quantile normalization did not impact the overall results, data not shown). Olink protein data are expressed as an arbitrary unit (Normalized Protein eXpression, 'NPX') representing the relative protein concentration based on a log2 scale (i.e. absolute protein quantity cannot be compared across different proteins). Protein levels were compared between groups based on the likelihood ratio test and considered statistically significant at a false discovery rate (FDR)-corrected P-value of <0.05, referred to as differentially expressed proteins (DEPs). Analysis was performed to compare two groups (e.g. HC vs PsA) or to compare multiple groups (HC, Pso, PsA, AS), as

specified in the text. Hierarchical cluster analysis was based on Ward's method to create heatmaps (R pheatmap package, version 1.0.12). Classical multidimensional scaling was performed with the R built-in 'stats' package (cmdscale function), using the Euclidean distance matrix between samples based on protein data. The hierarchical cluster analysis and multidimensional scaling were performed using DEPs between groups based on a nominal P-value <0.05. The protein data shown in figures of hierarchical cluster analysis underwent Z-score normalization for the sake of visualization in heatmaps. Venn diagrams were modified from web-based BioVenn tool.(32) Reactome pathway and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis for DEPs was performed based on hypergeometric test using ReactomePA package (version 1.28.0) and clusterProfiler package (version 3.12.0), respectively. Statistical analysis was performed in R (version 3.6) and SPSS (version 25, SPSS Inc., Chicago IL, USA).

Results

Cohort description

Clinical characteristics of the study participants are shown in **Table 1**. The Pso and PsA groups were matched for age, gender and PASI score. The PsA cohort was recruited early after disease onset, typically with <1 year of disease duration. Except for two patients with PsA, none of the study participants was being treated with DMARDs. Following quality control (see Methods), a total of 951 unique proteins and 77 samples (18 Pso, 20 PsA, 19 AS, 20 HC) were retained for further analysis.

Major proteins changes in PsA serum compared with HC serum

We first set out to specifically compare the serum of PsA to HC and found 68 differentially expressed proteins (DEPs) (FDR-corrected P < 0.05) (**Supplemental Table S1**). Most of the top DEPs between PsA and HC have not previously been implicated in the pathogenesis of PsA, which included proteins such as ANXA1, ADAM23 and VIM (**Supplemental Figure S1A**). Hierarchical cluster analysis revealed that the serum proteomic profile of PsA patients could be clearly distinguished from the serum proteomic profile of HC (**Supplemental Figure S1B**).

	HC (N=20)	Pso (N=18)	PsA (N=20)	AS (N=19)
Age (years)	43 ± 13	37 ± 15	41 ± 9	40 ± 12
Female, n (%)	7 (35)	7 (39)	7 (35)	5 (26)
BMI (kg/m^2)	-	29.9 ± 7.9	27.7 ± 4.5	24.2 ± 3.4*
Smoker, n (%)	-	6 (40)	7 (35)	2 (11)
Disease duration (years)				
- Psoriasis	-	12.4 (6.1-18.6)	20.0 (7.2-31.4)	-
- Psoriatic arthritis	-	-	0.7 (0.1-8.3)	-
- Ankylosing spondylitis	-	-	-	5.6 (0.4-13.4)
NSAID use, n (%)	-	1 (6)*	11 (55)	11 (58)
DMARD use, n (%)	-	0 (0)	2 (10)	0 (0)
CRP (mg/L)	-	2.8 (1-6)	2.8 (2-4)	3.2 (1-7)
ESR (mm/hour)	-	5 (2-8)	5 (2-13)	5 (3-14)
Psoriasis indices				
- PASI	-	2.7 (2-7)	3.0 (1-6)	-
- Nail involvement, n (%)	-	9 (56.3)	13 (72)	-
- Vulgaris type only, n (%)	-	12 (67)	14 (78)	-
SpA manifestations				
- Swollen joint count, of 76	-	-	3 (1-9)*	0 (0-0)
- Tender joint count, of 78	-	-	3 (1-10)*	0 (0-0)
- Dactylitis ever, n (%)	-	-	7 (35)*	0 (0)
- Enthesitis, n (%)	-	-	9 (47)*	3 (16)
- Inflammatory back pain, n (%)	-	-	3 (15)*	19 (100)
- BASDAI	-	-	-	4.2 (2-5)

Table 1. Baseline characteristics. Presented data are from time of baseline visit, unless otherwise indicated. Categorical data are presented with frequencies (%) and continuous data are shown as mean ± standard deviation (normally distributed variables) or median (interquartile range) (non-normally distributed variables). * Significant (P value < 0.05). Abbreviations: AS: ankylosing spondylitis; BMI: body mass index; CRP: C-reactive protein; BASDAI: Bath ankylosing spondylitis disease activity index (range 0-10); DMARD: disease modifying anti-rheumatic drug use (past three months); ESR: erythrocyte sedimentation rate; HC: healthy control, NSAID use: non-steroidal anti-inflammatory drug use daily on stable dose; PASI: psoriasis area and severity index (range 0-72); PsA: psoriatic arthritis, Pso: psoriasis; SpA: spondyloarthritis.

Common and unique protein disturbances in serum of PsA

We first examined whether those serum proteins changes were unique to PsA, or if they were also dysregulated in Pso and/or AS. Of the 68 DEPs between PsA and HC, 48 proteins (71%) were also dysregulated in Pso and/or AS (**Figure 1A**). The most significant DEPs between the groups were proteins that all had higher serum levels in patient groups as compared with HC (**Figure 1B**). This list again included the proteins ANXA1, VIM and TOP2B. In total, 20 proteins (29%) were dysregulated in PsA as compared with HC, which were not dysregulated in AS or Pso as compared with HC (**Figure 1C**). This list

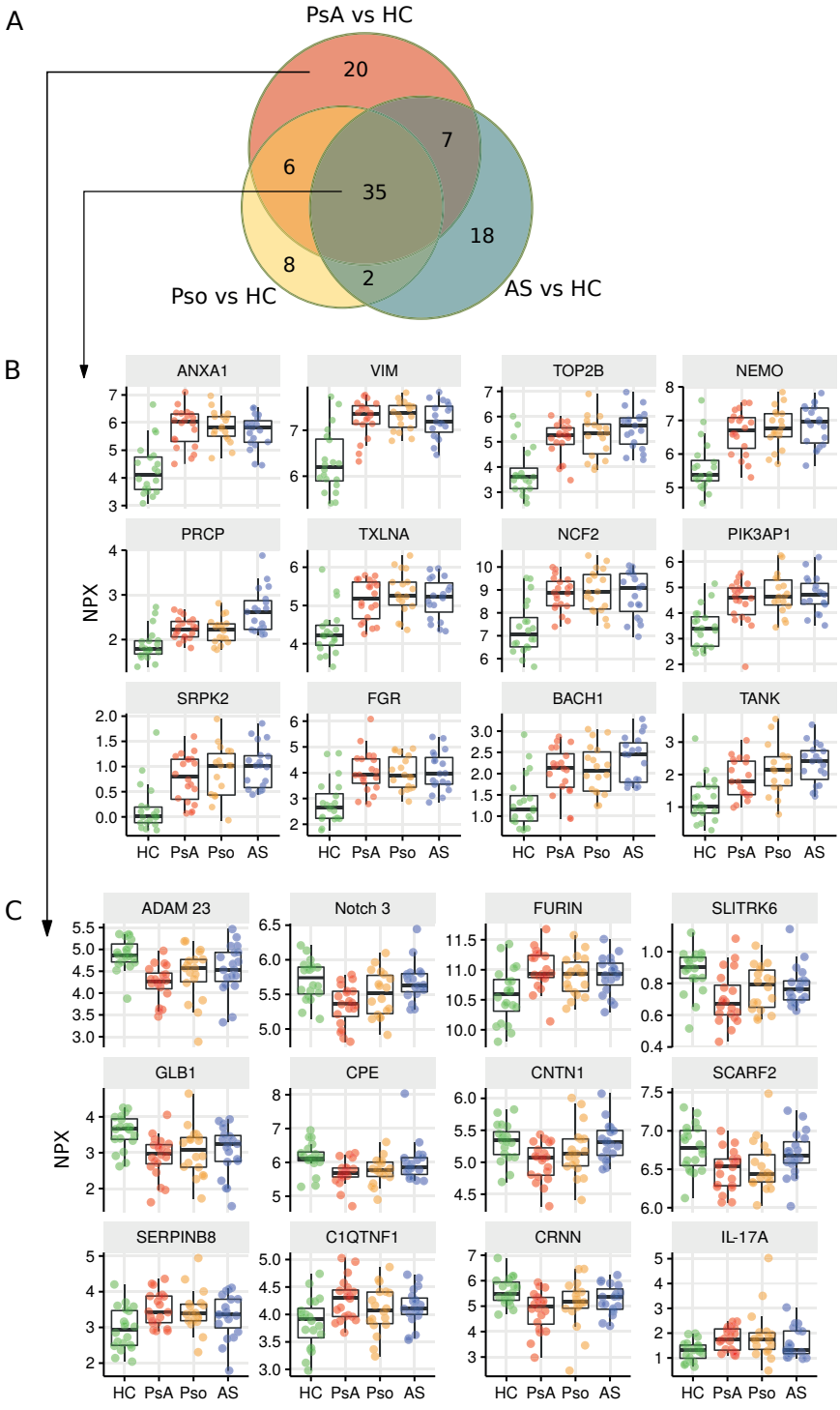
included proteins ADAM23, Neurogenic locus notch homologue protein 3 (Notch 3) and SLITRK6. Interestingly, many of the proteins in this list were lower in the serum of PsA as compared with that from HC.

We next compared patient groups directly. Importantly, there were no DEPs when directly comparing PsA with Pso based on FDR-corrected $P < 0.05$. An exploratory analysis (based on nominal P-value) comparing PsA with Pso can be found in **Supplemental Figure S2A** and **B**. We found that CLEC4A and SOD1 were the only proteins significantly different between patient groups, being elevated in AS (**Supplemental Figure S3**). Some specific proteins that have previously been implicated in the pathogenesis of these disease are displayed in **Supplemental Figure S4**. The list of DEPs can be found in **Supplemental Tables S1–S4**. Taken together, we identified 20 proteins uniquely dysregulated in PsA, while the majority of protein disturbances were also dysregulated in Pso and/or AS.

Overall serum proteomic signature is similar in PsA and Pso

Hierarchical cluster analysis showed that most patients, regardless of diagnosis, clustered separately from HC. The serum proteomic profile of PsA patients grouped closer to the Pso patients than to the AS patients (**Figure 2A**). Using an alternative method of analysing the data, namely multidimensional scaling analysis, we also found that HC grouped separately from patients, and that PsA and Pso grouped close together (**Figure 2B**). Finally, pathway enrichment analysis on the sets of DEPs between patient groups vs HC similarly revealed that very similar pathways were enriched in PsA and Pso (**Supplemental Figure S5**).

► **Figure 1. Common and unique protein disturbances in serum of PsA.** (**A**) Overlap in DEPs between patient group versus HC. This Venn diagram shows the number of DEPs between each patient group as compared to healthy controls. For example, there were 68 DEPs when comparing PsA to HC, of which 35 proteins were also differentially expressed when comparing Pso to HC or comparing AS to HC. Results are based on FDR-corrected p value <0.05. (**B**) Common DEPs in all patient groups. 35 proteins were differentially expressed in all patient groups as compared to HC. The twelve most significant proteins are displayed as boxplots (DEPs with the lowest FDR-corrected p value). (**C**) DEPs only found in PsA versus HC. 20 proteins were differentially expressed in PsA compared to HC, but not dysregulated in other patient groups compared to HC. The twelve most significant proteins are displayed in boxplots (DEPs with the lowest FDR-corrected p value). Abbreviations: healthy control (HC); psoriatic arthritis (PsA); psoriasis (Pso); ankylosing spondylitis (AS); Normalized Protein eXpression (NPX); differentially expressed proteins (DEPs).


A
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Pso vs HC
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ANXA1
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TXLNA
NCF2
PIK3AP1
SRPK2
FGR
BACH1
TANK
NPX
HC PsA Pso AS
C
ADAM 23
Notch 3
FURIN
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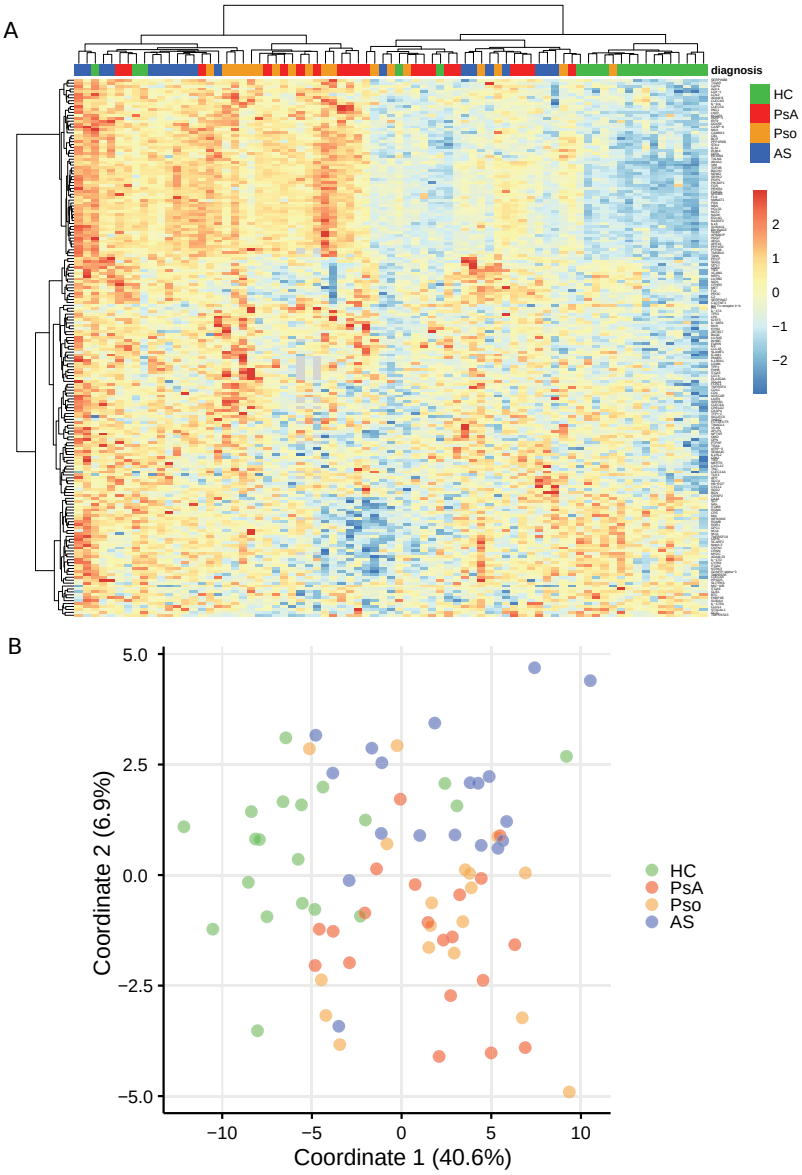

Figure 2. Overall serum proteomic signature is similar in PsA and Pso. (**A**) Serum in Pso and PsA overlap based on hierarchical clustering analysis. Hierarchical clustering shown in the heatmap reveals HC cluster separately from the different patient groups. The clustering also reveals that most Pso and PsA patients cluster separately from AS patients. This analysis is based on DEPs with nominal p value <0.05 when comparing all groups. (**B**) Serum in Pso and PsA overlap based on multidimensional scaling. Multidimensional scaling (MDS) plot reveals HC cluster separately from the different patient groups. Similar to the hierarchical clustering, Pso and PsA tend to cluster separately from AS. This analysis is based on DEPs with nominal p value <0.05 when comparing all groups. Abbreviations: healthy control (HC); psoriatic arthritis (PsA); psoriasis (Pso); ankylosing spondylitis (AS); differentially expressed proteins (DEPs).

Proteins reflecting joint and skin disease activity

We next examined which serum proteomic changes best reflected the major disease manifestations with respect to joint and skin disease activity in patients with PsA and Pso. The number of swollen joints had the strongest positive correlation to intracellular adhesion molecule 1 (ICAM-1; r = 0.81, P < 0.001), C-C motif chemokine 18 (CCL18; r = 0.76, P < 0.001) and dipeptidyl peptidase 4 (DPP4) (r = 0.75, P < 0.001), whereas swollen joint count had the strongest negative correlation to VEGFD (r = −0.73, P < 0.001) (**Figure 3**).

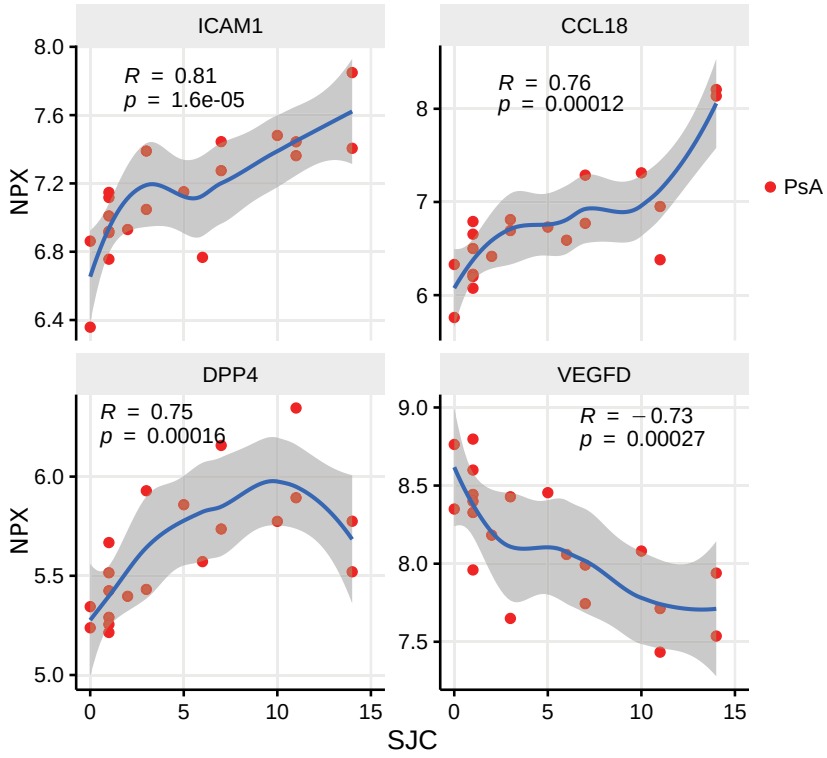

Figure 3. Top proteins related to arthritis activity. The swollen joint count (SJC) versus relative protein levels of ICAM1, CCL18, DPP4 and VEGFD. Spearman's rank correlation (R) and p value in PsA are displayed in the figure. Locally estimated scatterplot smoothing (loess) curve is shown. Abbreviations: Normalized Protein eXpression (NPX); Intracellular adhesion molecule 1 (ICAM1); C-C motif chemokine 18 (CCL18); Dipeptidyl peptidase 4 (DPP4); Vascular endothelial growth factor D (VEGFD).

When PsA and Pso patients were considered as one group (data pooled together), PASI scores had the strongest correlation to the proteins PI3 (r = 0.54, P < 0.001), IL-17 receptor A (r = −0.51, P < 0.01), MMP-1 (r = 0.47, P = 0.01) and SERPINB8 (r = 0.46, P < 0.01). Surprisingly,

there were more proteins that correlated to PASI score when analysing the Pso and PsA cohorts separately as compared with analysing the Pso and PsA patients pooled together (**Figure 4**). PASI score was correlated to Gal-4 ($r = -0.72$, $P < 0.001$) and IGFBPL1 ($r = -0.65$, $P < 0.01$), but only in patients with PsA. PASI score was correlated to PD-L2 ($r = 0.68$, $P < 0.01$) and MSR1 ($r = 0.67$, $P < 0.01$), but only in patients with Pso (**Figure 4**).

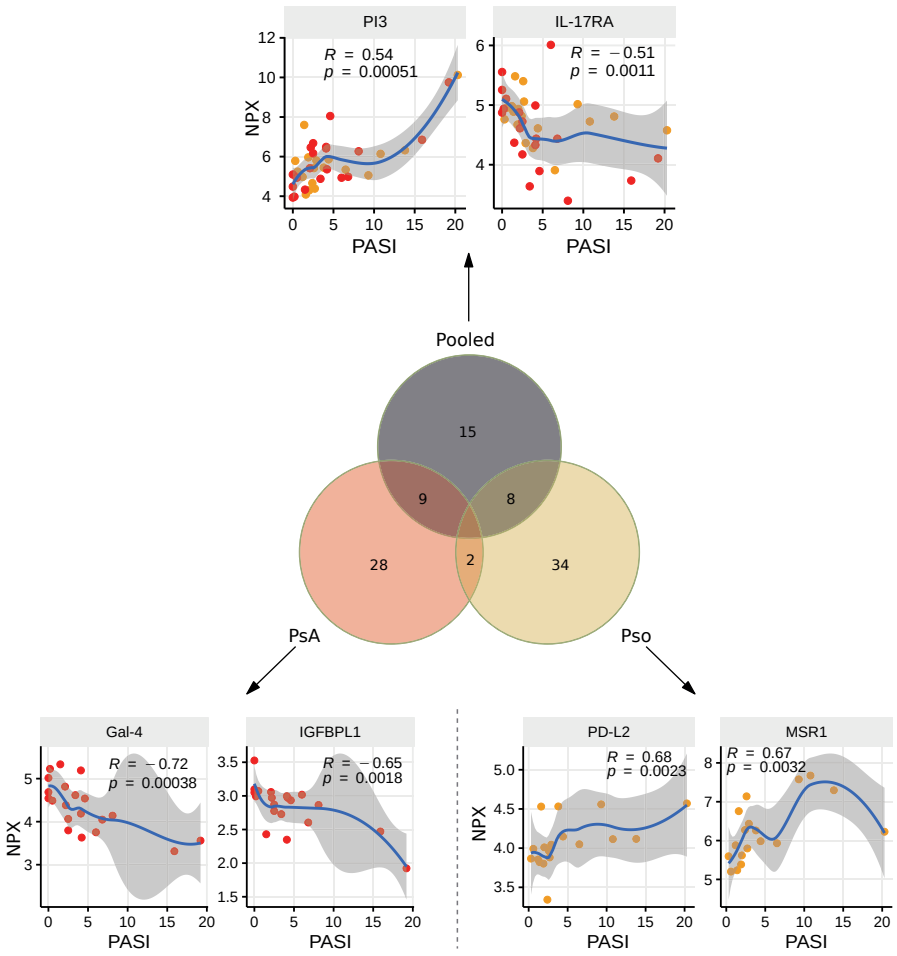

Figure 4. Top proteins related to psoriasis activity. The Venn diagram shows the total number of proteins that significantly correlate to PASI score (nominal p value <0.05). The analysis was performed when pooling PsA and Pso patients (grey circle), taking PsA patients only (red circle), or taking Pso patients only (orange circle). The PASI was correlated to relative protein levels. Spearman's rank correlation (R) and p value are shown in the figure. Locally estimated scatterplot smoothing (loess) curve is shown. Abbreviations: psoriatic arthritis (PsA); psoriasis (Pso); Normalized Protein eXpression (NPX); Elafin (PI3); Interleukin-17 receptor A (IL-17RA); Galectin-4 (Gal-4); Insulin-like growth factor-binding protein-like 1 (IGFBPL1); Programmed cell death 1 ligand 2 (PD-L2); Macrophage scavenger receptor types I and II (MSR1).

Discussion

This study found large proteomic disturbances in the serum of patients with PsA and revealed that the strongest proteomic changes occurred in novel proteins not yet linked to the pathogenesis of PsA. Importantly, the majority of protein changes in serum of patients with PsA were similarly disturbed in patients with Pso in whom PsA was excluded. From over 950 proteins screened, we were able to narrow down specific proteins of interest correlating to the major clinical manifestations of these diseases.

This is one of few head-to-head serum proteomic comparisons in a well-characterized cohort of patients with PsA and Pso. Our PsA cohort consisted of patients with early disease onset and was carefully matched to have similar clinical characteristics (including PASI score) to the Pso patients. From a clinical perspective, our results indicate that none of the evaluated serum proteins (singularly) is a likely candidate for a simple diagnostic biomarker capable of discriminating early PsA from Pso. In other words, a simple blood test to differentiate PsA from Pso may not be a feasible goal for daily clinical practice, at least not based on the proteins we evaluated. Instead, our results primarily contribute to the understanding of the pathogenesis of PsA, which includes specifying potential drug targets. From a pathophysiological perspective, our data support the 'two phenotypes of one disease' hypothesis.(8,9)

Our study adds important insight into the question as to which type of tissue sample is best suited to unravel the pathogenesis of PsA. PsA and Pso fall within a spectrum of diseases with shared genetic background and presumably shared immunologic drivers. From a clinician point of view, however, they are distinct: some patients develop (poly) arthritis, which requires specific clinical intervention. Therefore, there must be specific drivers (local and/or systemic) within this overlapping psoriatic spectrum that enable the development of overt arthritis manifestations. Our broad analysis reveals that PsA and Pso are extremely difficult to discriminate based on serum proteomic changes, underscoring that other sites of the body, such as synovial tissue, should be an important target of future research. It will still be important to find methods of incorporating appropriate control groups, ideally Pso patients in whom PsA is excluded by a rheumatologist, even when studying tissue sites such as synovial tissue. Surprisingly, we found that many serum proteins were related to PASI score when dichotomizing the analysis for Pso only and PsA only. This may indicate there are different primary drivers of cutaneous inflammation and/or secondary systemic responses upon inflammation occurring in PsA compared with Pso. A comparison of the skin in PsA compared with Pso as tissue site has

only been addressed in a small number of studies and therefore warrants specific tissue comparisons.(33,34)

We here identified specific proteins strongly associated with joint disease activity. ICAM-1 is a molecule important for trans-endothelial migration of leucocytes via interaction with LFA-1. ICAM-1 has previously been identified in the pathogenesis of Pso and PsA.(35,36) In RA synovial tissue it was shown that ICAM-1 expression marked a specific myeloid synovial tissue phenotype.(37) Interestingly, previous attempts to target LFA-1 with mAbs for the treatment of Pso lead to the new-onset arthritis in many patients enrolling in the trials,(38) supporting the notion that the balance of leucocyte extravasation mediated by ICAM-1 could be important in arthritis development. VEGFD is one of the members of the endothelial growth factors involved in angiogenesis and lymphangiogenesis in cancer, and while this specific family member has not been described in rheumatic disease,(39) VEGF has been implicated in the pathogenesis of arthritis.(40) Considering that we performed a broad, unbiased serologic screening, our data again highlight the importance of angiogenesis in PsA, which is in agreement with existing histologic data in PsA showing increased angiogenesis to be an important feature of PsA synovial tissue.(7,35,41) Two additional proteins were strongly correlated to arthritis activity: CCL18 and DPP4. DPP4 is currently a target for type 2 diabetes mellitus, and the role of DPP4 in development of arthritis is still unclear.(42) CCL18 is expressed by endothelial cells in the synovial tissue of RA and has been identified as a disease activity marker in RA and other diseases.(43)

A strength of our study is the broad set of protein panels we have measured. We hence observed that the strongest protein disturbances were not well-known cytokines and chemokines, but rather proteins not previously implicated in the pathogenesis of rheumatic disease, including ADAM23 and Notch 3. ADAM23 is a non-proteolytic member of the 'A disintegrin and metalloproteases' (ADAM) family known for high expression in brain and roles in neuronal differentiation, but also shown to inhibit cell adhesion and cell migration in cancer cells, possibly via interaction with integrin $\alpha v\beta 3$.(44,45) Notch 3 has very broad functions and is aberrantly expression in psoriatic skin, and was shown to modulate Th cell phenotypes function.(46,47) Our patient cohorts have an expected overlapping pathogenic spectrum (Pso, PsA, AS). Future studies should consider including other rheumatic diseases with more distinct clinical features and pathogenesis (e.g. gout and OA) in order to further address the specificity of the protein changes. While the protein disturbances were not specific to PsA, this per se does not preclude their importance in pathogenesis or their role as potential therapeutic target: many of the

current therapeutics (e.g. TNF-α inhibitors) are effective across a range of distinct clinical entities considered to be driven by different pathways.

Some of the more familiar proteins changes included IL-6 and IL-17A, which are known drug targets for rheumatologic diseases. Studies in RA highlight that serum levels of cytokines are unlikely to predict clinical response to mAbs targeting that respective cytokine.(48,49) Nevertheless, we detected elevated levels of IL-6 in PsA and also found a positive correlation between IL-6 levels and joint disease activity measures, which supports current efforts examining IL-6 as a potential therapeutic target for patients with PsA.

Our study was designed to recruit PsA patients without DMARDs use and early after disease onset, resulting in PsA patients with mostly oligoarthritis. The serum proteomic results best represent the oligoarthritis pattern in PsA, but our cohort does not represent the entire spectrum of PsA patients, i.e. those with very severe polyarticular disease. Our choice to avoid patients with DMARDs is underscored by recent data using the same proteomic platform in Pso patients confirming that most proteins undergo vast changes upon initiation of immunomodulatory drugs.(29)

A limitation of the current study is the relatively small cohort size, which means that we may have underestimated the number of proteins that are different between Pso and PsA groups due to stringent FDR-correction. Realistically, it is challenging to include large numbers of patients in basic science studies with very severe disease that are not (yet) treated with immunomodulatory drugs. Clearly, it will be necessary to (i) replicate the major protein disturbances identified by our screening and (ii) determine whether the proteins are downstream biomarkers of the disease or directly involved in the pathogenesis. Functional validation will be necessary to determine which of these specific factors or combination of factors contribute to the pathogenesis of PsA.

To overcome some of the aforementioned challenges we recommend that, similar to sharing gene expression data, these proteomic datasets can be publicly shared (e.g. repositories). Firstly, this provides additional scientific transparency of the results. Secondly, by sharing datasets the proteins can be compared across diseases (determine specificity) and allow for rapid validation and identification of those proteins worth pursuing for in vitro experiments. These collaborative efforts should maximize the yield of costly scientific endeavours, whilst ensuring acknowledgement of data in a competitive scientific landscape.

In summary, we have identified novel serum protein disturbances in PsA and furthermore establish that both Pso patients and PsA patients with oligoarthritis have an overall shared serum proteomic signature.

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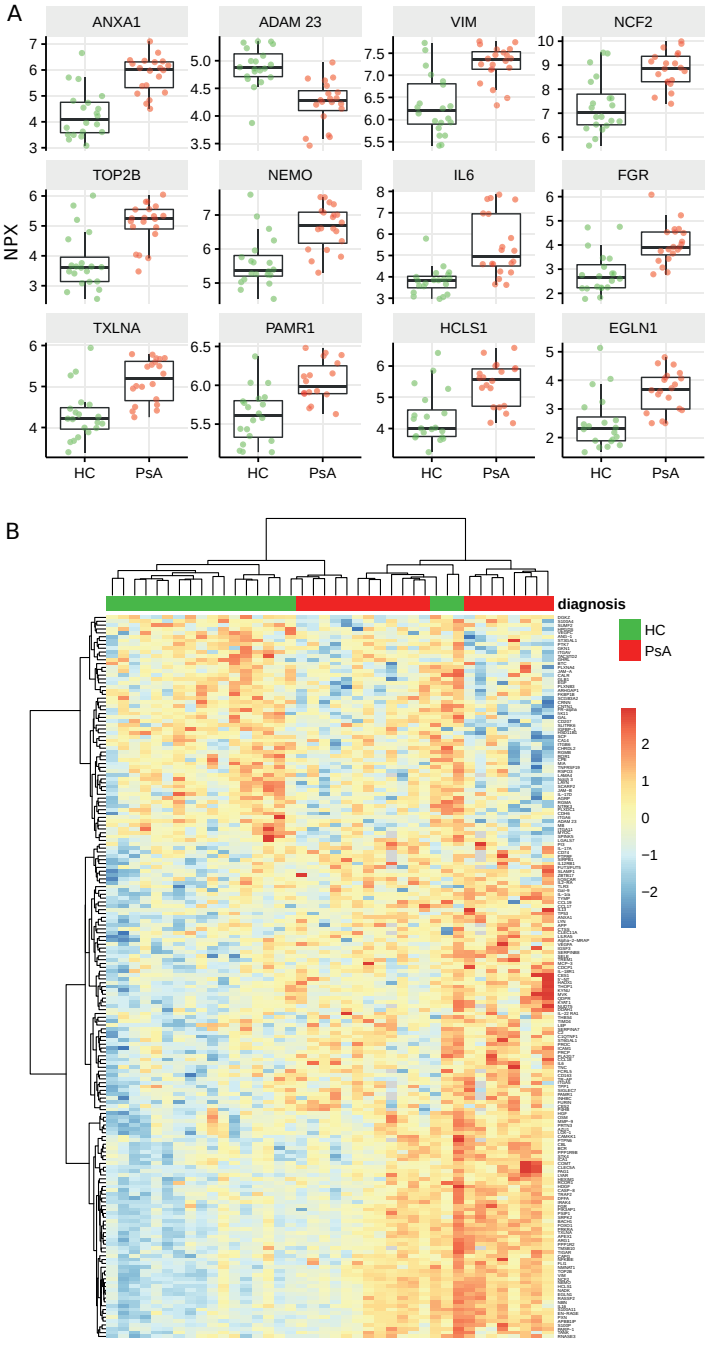
References

1. Ritchlin CT , Colbert RA , Gladman DD. Psoriatic arthritis. N Engl J Med 2017;376:957–70.
2. Kavanaugh A , Gottlieb A , Morita A et al. The contribution of joint and skin improvements to the health-related quality of life of patients with psoriatic arthritis: a post hoc analysis of two randomised controlled studies. Ann Rheum Dis 2019;78:1215–9.
3. Furue K , Ito T , Furue M. Differential efficacy of biologic treatments targeting the TNF-α/IL-23/IL-17 axis in psoriasis and psoriatic arthritis. Cytokine 2018;111:182–8.
4. Belasco J , Louie JS , Gulati N et al. Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis. Arthritis Rheumatol 2015;67:934–44.
5. Krueger JG , Kirkham B , Ritchlin CT. Basic and translational science: a report from the GRAPPA 2016 Annual Meeting. J Rheumatol 2017;44:679–83.
6. Ospelt C , Frank-Bertoncelj M. Why location matters—site-specific factors in rheumatic diseases. Nat Rev Rheumatol 2017;13:433–42.
7. Veale DJ , Fearon U. The pathogenesis of psoriatic arthritis. Lancet 2018;391:2273–84.
8. Boehncke W-H. Psoriasis and psoriatic arthritis: flip sides of the coin? Acta Derm Venereol 2016;96:436–41.
9. Sakkas LI , Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. Autoimmun Rev 2017;16:10–5.
10. Bowes J , Budu-Aggrey A , Huffmeier U et al. Dense genotyping of immune-related susceptibility loci reveals new insights into the genetics of psoriatic arthritis. Nat Commun 2015;6:6046.
11. Stuart PE , Nair RP , Tsoi LC et al. Genome-wide association analysis of psoriatic arthritis and cutaneous psoriasis reveals differences in their genetic architecture. Am J Hum Genet 2015;97:816–36.
12. Pollock RA , Abji F , Liang K et al. Gene expression differences between psoriasis patients with and without inflammatory arthritis. J Invest Dermatol 2015;135:620–3.
13. Abji F , Pollock RA , Liang K , Chandran V , Gladman DD. Brief report: CXCL10 is a possible biomarker for the development of psoriatic arthritis among patients with psoriasis. Arthritis Rheumatol 2016;68:2911–6.
14. Capsoni F , Molteni S , Raeli L et al. Differential expression of interleukin-2 by anti-CD3-stimulated peripheral blood mononuclear cells in patients with psoriatic arthritis and patients with cutaneous psoriasis. Clin Exp Dermatol 2014;39:385–90.
15. Benham H , Norris P , Goodall J et al. Th17 and Th22 cells in psoriatic arthritis and psoriasis. Arthritis Res Ther 2013;15:R136.
16. Yuan Y , Qiu J , Lin Z-T et al. Identification of novel autoantibodies associated with psoriatic arthritis. Arthritis Rheumatol 2019;71:941–51.
17. Frasca L , Palazzo R , Chimenti MS et al. Anti-LL37 antibodies are present in psoriatic arthritis (PsA) patients: new biomarkers in PsA. Front Immunol 2018 2018;9:1936.
18. Chandran V , Cook RJ , Edwin J et al. Soluble biomarkers differentiate patients with psoriatic arthritis from those with psoriasis without arthritis. Rheumatology (Oxford) 2010;49:1399–405.
19. Alenius G-M , Eriksson C , Dahlqvist R S. Interleukin-6 and soluble interleukin-2 receptor alpha-markers of inflammation in patients with psoriatic arthritis? Clin Exp Rheumatol 2009;27:120–3.
20. Xue Y , Jiang L , Cheng Q et al. Adipokines in psoriatic arthritis patients: the correlations with osteoclast precursors and bone erosions. PLoS One 2012;7:e46740.

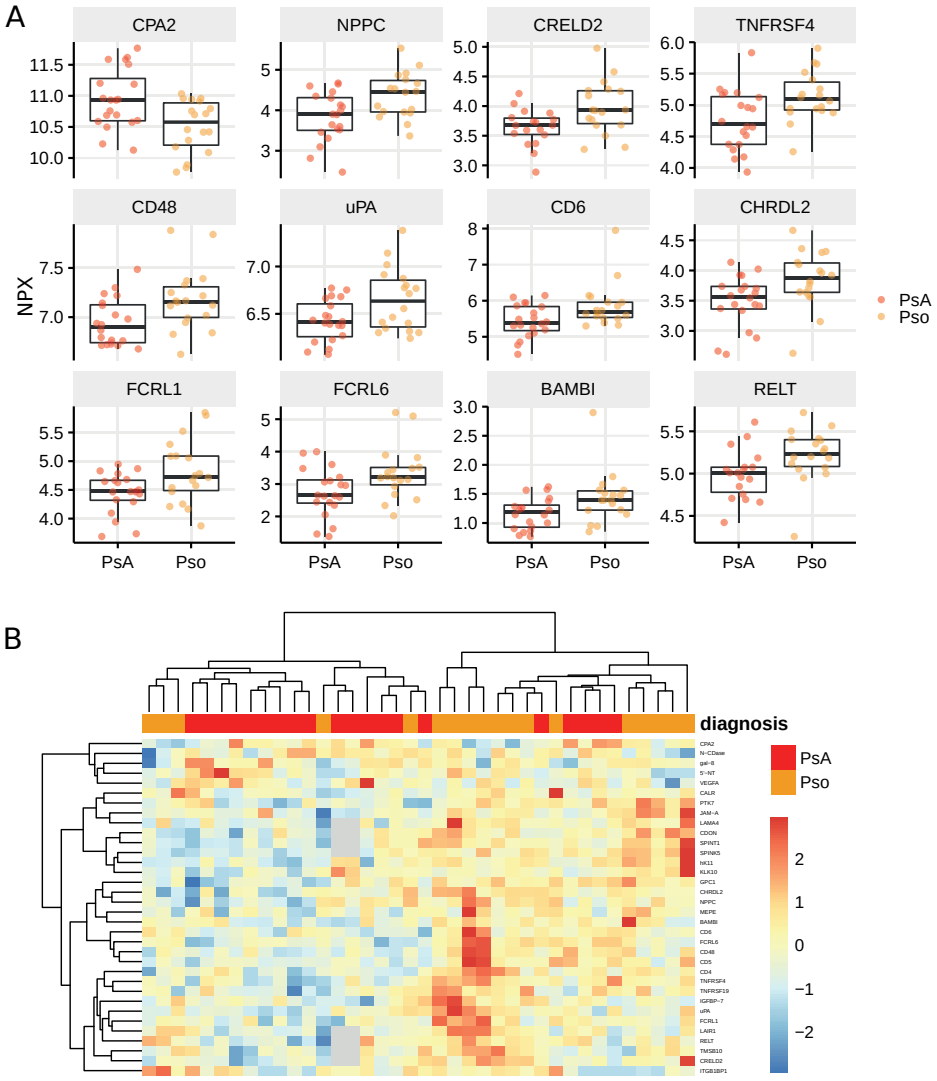
21. Li J , Liu L , Rui W et al. New interleukins in psoriasis and psoriatic arthritis patients: the possible roles of interleukin-33 to interleukin-38 in disease activities and bone erosions. Dermatology 2017;233:37–46.
22. Dalbeth N , Pool B , Smith T et al. Circulating mediators of bone remodeling in psoriatic arthritis: implications for disordered osteoclastogenesis and bone erosion. Arthritis Res Ther 2010;12:R164.
23. Bartosińska J , Michalak-Stoma A , Juszkiewicz-Borowiec M , Kowal M , Chodorowska G. The assessment of selected bone and cartilage biomarkers in psoriatic patients from Poland. Mediators Inflamm 2015;2015:1–8.
24. Cubillos S , Krieg N , Norgauer J. Effect of vitamin D on peripheral blood mononuclear cells from patients with psoriasis vulgaris and psoriatic arthritis. PLoS One 2016;11:e0153094.
25. Amin TE , ElFar NN , Ghaly NR et al. Serum level of receptor activator of nuclear factor kappa-B ligand in patients with psoriasis. Int J Dermatol 2016;55:e227–33.
26. Kim DS , Shin D , Lee MS et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. J Dermatol 2016;43:305–10.
27. Mitsui A , Tada Y , Takahashi T et al. Serum IL-33 levels are increased in patients with psoriasis. Clin Exp Dermatol 2016;41:183–9.
28. Brunner PM , Suárez-Fariñas M , He H et al. The atopic dermatitis blood signature is characterized by increases in inflammatory and cardiovascular risk proteins. Sci Rep 2017;7:8707.
29. Kim J , Tomalin L , Lee J et al. Reduction of inflammatory and cardiovascular proteins in the blood of patients with psoriasis: differential responses between tofacitinib and etanercept after 4 weeks of treatment. J Invest Dermatol 2018;138:273–81.
30. Taylor W , Gladman D , Helliwell P et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006;54:2665–73.
31. Assarsson E , Lundberg M , Holmquist G et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 2014;9:e95192.
32. Hulsen T , Vlieg J , de , Alkema W. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 2008;9:488.
33. Veale DJ , Barnes L , Rogers S , FitzGerald O. Immunohistochemical markers for arthritis in psoriasis. Ann Rheum Dis 1994;53:450–4.
34. Šahmatova L , Sügis E , Šunina M et al. Signs of innate immune activation and premature immunosenescence in psoriasis patients. Sci Rep 2017;7:7553.
35. Veale D , Yanni G , Rogers S et al. Reduced synovial membrane macrophage numbers, ELAM-1 expression, and lining layer hyperplasia in psoriatic arthritis as compared with rheumatoid arthritis. Arthritis Rheum 1993;36:893–900.
36. Veale D , Rogers S , Fitzgerald O. Immunolocalization of adhesion molecules in psoriatic arthritis, psoriatic and normal skin. Br J Dermatol 1995;132:32–8.
37. Dennis G , Holweg CTJ , Kummerfeld SK et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. Arthritis Res Ther 2014;16:R90.
38. Viguier M , Richette P , Aubin F et al. Onset of psoriatic arthritis in patients treated with efalizumab for moderate to severe psoriasis. Arthritis Rheum 2008;58:1796–802.
39. Stacker SA , Achen MG. Emerging roles for VEGF-D in human disease. Biomolecules 2018;8:1.

40. Balogh E , Biniecka M , Fearon U , Veale DJ , Szekanecz Z. Angiogenesis in inflammatory arthritis. Isr Med Assoc J 2019;5:345–52.
41. Kruithof E , Baeten D , Rycke L , De et al. Synovial histopathology of psoriatic arthritis, both oligo- and polyarticular, resembles spondyloarthropathy more than it does rheumatoid arthritis. Arthritis Res Ther 2005;7:R569–80.
42. Kim SC , Schneeweiss S , Glynn RJ et al. Dipeptidyl peptidase-4 inhibitors in type 2 diabetes may reduce the risk of autoimmune diseases: a population-based cohort study. Ann Rheum Dis 2015;74:1968–75.
43. Lieshout AWT , van Fransen J , Flendrie M et al. Circulating levels of the chemokine CCL18 but not CXCL16 are elevated and correlate with disease activity in rheumatoid arthritis. Ann Rheum Dis 2007;66:1334–8.
44. Hsia H-E , Tüshaus J , Brummer T et al. Functions of "A disintegrin and metalloproteases (ADAMs)" in the mammalian nervous system. Cell Mol Life Sci 2019;76:3055–81.
45. Ota M , Mochizuki S , Shimoda M et al. ADAM23 is downregulated in side population and suppresses lung metastasis of lung carcinoma cells. Cancer Sci 2016;107:433–43.
46. Ota T , Takekoshi S , Takagi T et al. Notch signaling may be involved in the abnormal differentiation of epidermal keratinocytes in psoriasis. Acta Histochem Cytochem 2014;47:175–83.
47. Jiao Z , Wang W , Xu H et al. Engagement of activated Notch signalling in collagen II-specific T helper type 1 (Th1)- and Th17-type expansion involving Notch3 and Delta-like1. Clin Exp Immunol 2011;164:66–71.
48. Cuppen BVJ , Welsing PMJ , Sprengers JJ et al. Personalized biological treatment for rheumatoid arthritis: a systematic review with a focus on clinical applicability. Rheumatology (Oxford) 2016;55:826–39.
49. Romão VC , Fonseca JE. Major challenges in rheumatology: will we ever treat smarter, instead of just harder? Front Med 2019;6:144.

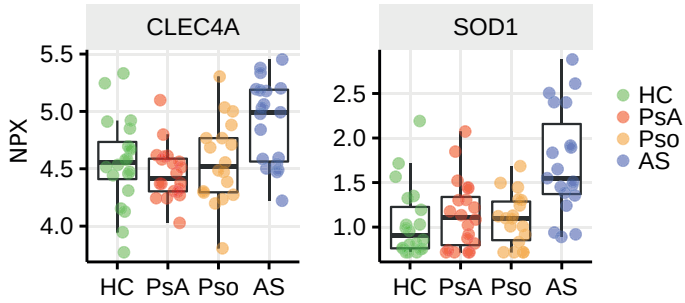
Supplemental Information



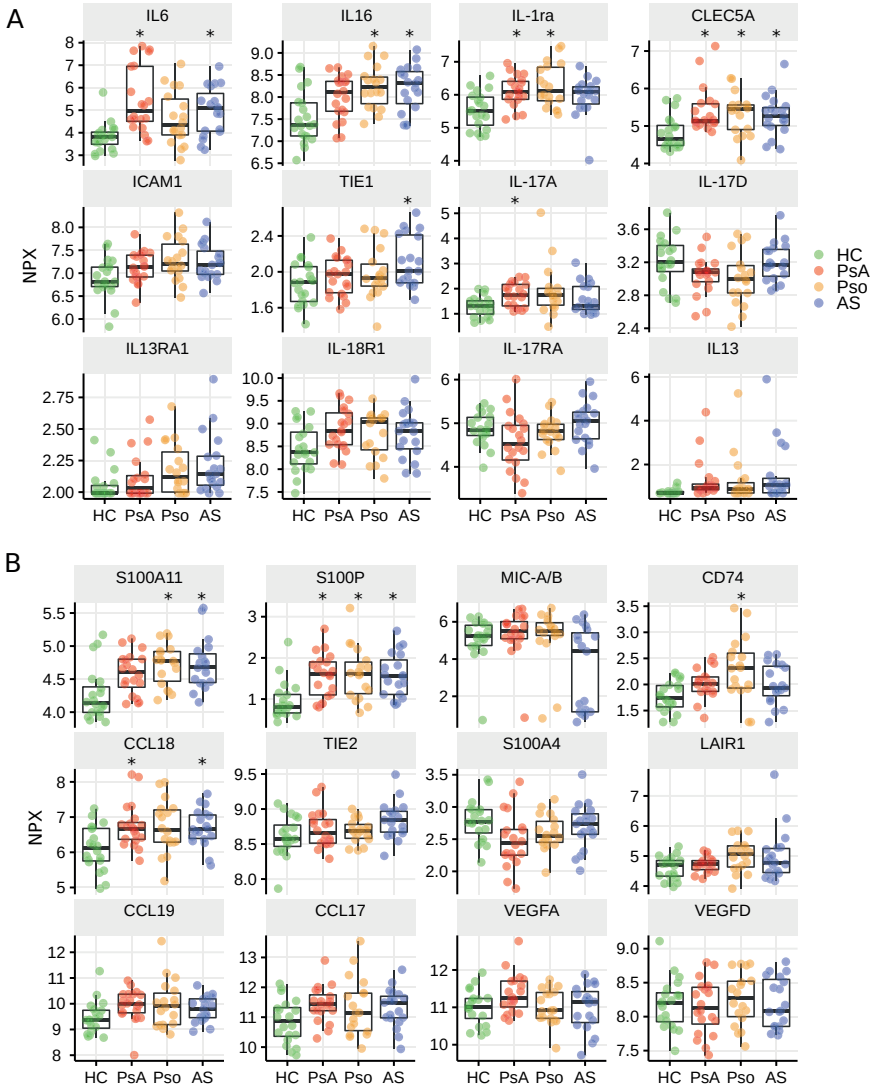
◂ **Supplemental Figure S1. Top proteins differentiating PsA from HC.** (**A**) Differentially expressed proteins (DEPs) between PsA and HC. Boxplots show the twelve most significant proteins differentiating PsA from HC (DEPs with the lowest FDR-corrected P value). (**B**) Hierarchical clustering differentiates HC from PsA. Clustering is shown based on all of the DEPs when comparing PsA to HC (nominal P value <0.05). Abbreviations: healthy control (HC); psoriatic arthritis (PsA); Normalized Protein eXpression (NPX); differentially expressed proteins (DEPs); Annexin A1 (ANXA1); Disintegrin and metalloproteinase domain-containing protein 23 (ADAM 23); Vimentin (VIM); Neutrophil cytosol factor 2 (NCF2); DNA topoisomerase 2-beta (TOP2B); NF-kappa-B essential modulator (NEMO); Interleukin-6 (IL6); Tyrosine-protein kinase Fgr (FGR); Alpha-taxilin (TXLNA); Inactive serine protease PAMR1 (PAMR1); Hematopoietic lineage cell-specific protein (HCLS1); Egl nine homolog 1 (EGLN1).



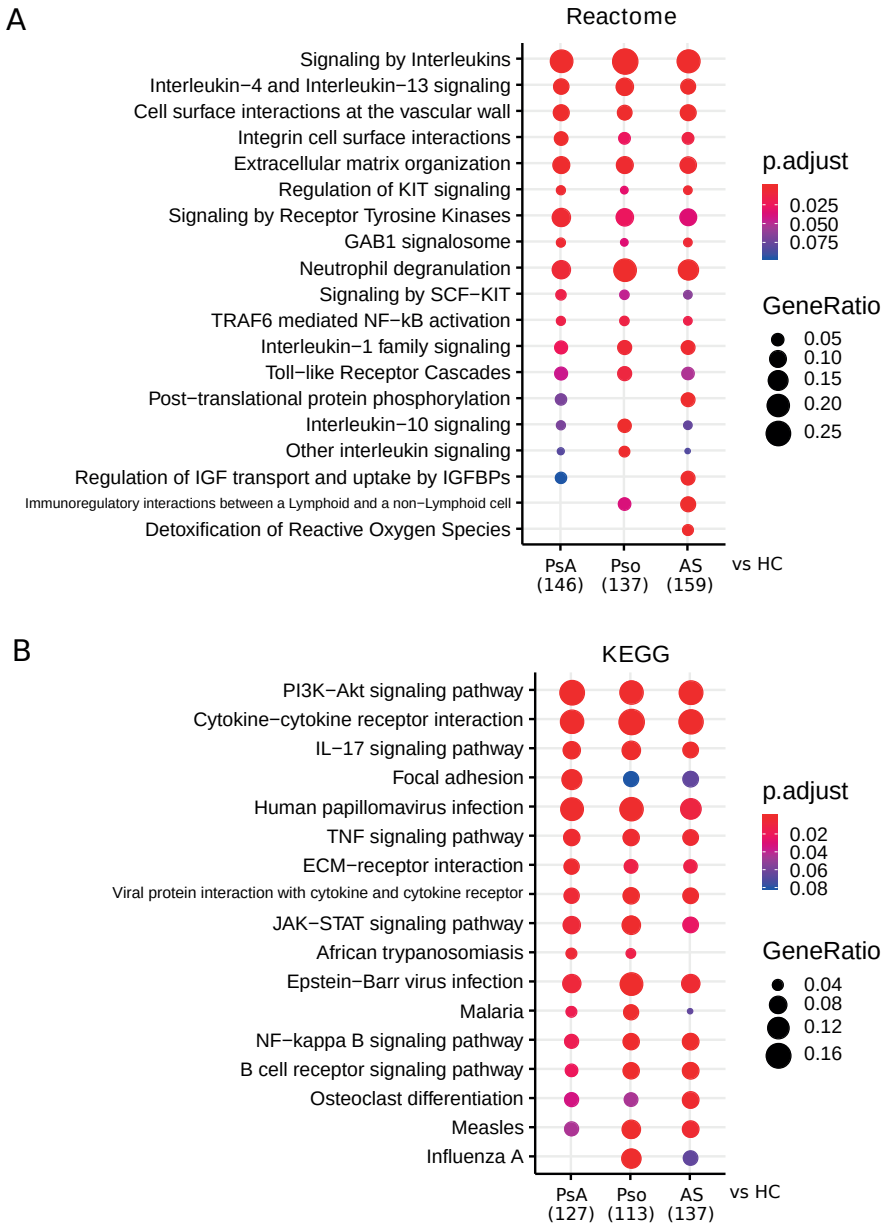
Supplemental Figure S2. Top proteins differentiating PsA from Pso. (**A**) Differentially expressed proteins (DEPs) between PsA and Pso. This is an exploratory analysis, showing differentially expressed proteins (DEPs) between PsA and Pso based on nominal P value <0.05. The boxplots show the twelve most significant proteins differentiating PsA from HC. (**B**) Hierarchical clustering between PsA and Pso shown in the heatmap shows moderate potential to differentiate PsA from Pso. This analysis is based on DEPs with nominal P value <0.05 when comparing PsA to Pso. Abbreviations: psoriatic arthritis (PsA); Psoriasis (Pso); Normalized Protein eXpression (NPX); differentially expressed proteins (DEPs); Carboxypeptidase A2 (CPA2); C-type natriuretic peptide (NPPC); Cysteine-rich with EGF-like domain protein 2 (CRELD2); Tumor necrosis factor receptor superfamily member 4 (TNFRSF4); CD48 antigen (CD48); Urokinase-type plasminogen activator (uPA); T cell surface glycoprotein CD6 isoform (CD6); Chordin-like protein 2 (CHRDL2); Fc receptor-like protein 1 (FCRL1); Fc receptor-like protein 6 (FCRL6); BMP and activin membrane-bound inhibitor homolog (BAMBI); Tumor necrosis factor receptor superfamily member 19L (RELT).



Supplemental Figure S3. CLEC4A and SOD1 elevated in AS. CLEC4A was significantly elevated in AS as compared to PsA (FDR-corrected, p<0.05). SOD1 was significantly elevated in AS as compared to Pso and in AS as compared to HC (FDR-corrected, p<0.05). Abbreviations: healthy control (HC); psoriatic arthritis (PsA); Psoriasis (Pso); Ankylosing spondylitis (AS); Normalized Protein eXpression (NPX); differentially expressed proteins (DEPs); C-type lectin domain family 4 member A (CLEC4A); Superoxide dismutase [Cu-Zn] (SOD1).



Supplemental Figure S4. Proteins previously implicated in PsA pathogenesis. A list of literature based proteins of interest is displayed. An asterisk (*) indicates significant difference between HC and the respective patient group (FDR-corrected, p<0.05). Abbreviations: healthy control (HC); psoriatic arthritis (PsA); Psoriasis (Pso); Ankylosing spondylitis (AS); Normalized Protein eXpression (NPX); differentially expressed proteins (DEPs); Interleukin-6 (IL6); Pro-interleukin-16 (IL16); Interleukin-1 receptor antagonist protein (IL-1ra); C-type lectin domain family 5 member A (CLEC5A); Intercellular adhesion molecule 1 (ICAM1); Tyrosine-protein kinase receptor Tie-1 (TIE1); Interleukin-17A (IL-17A); Interleukin-17D (IL-17D); Interleukin-13 receptor subunit alpha-1 (IL13RA1); Interleukin-18 receptor 1 (IL-18R1); Interleukin-17 receptor A (IL-17RA); Interleukin-13 (IL-13); Protein S100-A11 (S100A11); Protein S100-P (S100P); MHC class I polypeptide-related sequence A/B (MIC-A/B); HLA class II histocompatibility antigen gamma chain (CD74); C-C motif chemokine 18 (CCL18); Angiopoietin-1 receptor (TIE2); Protein S100-A4 (S100A4); Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); C-C motif chemokine 19 (CCL19); C-C motif chemokine 17 (CCL17); Vascular endothelial growth factor A (VEGF-A); Vascular endothelial growth factor D (VEGFD).



Supplemental Figure S5. Pso and PsA overlap based on pathway enrichment analysis. Pathway enrichment analysis was performed based on the differentially expressed proteins (DEPs) with nominal P value <0.05 when comparing HC versus each patient groups. Abbreviations: healthy control (HC); psoriatic arthritis (PsA); Psoriasis (Pso); Ankylosing spondylitis (AS); Kyoto Encyclopedia of Genes and Genomes (KEGG).

Olink ID	Protein	groupmean.HC	groupmean.PsA	log2FC	P value
P04083	ANXA1	4.293	5.823	0.440	3.70E-06
O75077	ADAM 23	4.893	4.233	-0.209	1.64E-05
P08670	VIM	6.314	7.257	0.201	3.22E-05
P19878	NCF2	7.323	8.821	0.268	1.91E-04
Q02880	TOP2B	3.806	5.062	0.411	3.81E-04
Q9Y6K9	NEMO	5.608	6.643	0.244	4.38E-04
P05231	IL6	3.807	5.460	0.520	4.38E-04
P09769	FGR	2.833	4.038	0.511	4.65E-04
P40222	TXLNA	4.307	5.142	0.256	4.65E-04
Q6UXH9	PAMR1	5.604	6.048	0.110	6.42E-04
P14317	HCLS1	4.351	5.406	0.313	1.31E-03
Q9GZT9	EGLN1	2.521	3.634	0.528	1.80E-03
P50749	RASSF2	2.770	3.981	0.523	2.37E-03
P78362	SRPK2	0.163	0.773	2.246	2.37E-03
O95544	NADK	5.290	6.379	0.270	2.37E-03
O00221	NFKBIE	1.990	2.584	0.376	2.40E-03
Q6ZUJ8	PIK3AP1	3.414	4.456	0.384	2.56E-03
P16278	GLB1	3.578	2.902	-0.302	2.57E-03
Q7Z5R6	APBB1IP	4.147	4.459	0.105	2.90E-03
O14867	BACH1	1.311	2.060	0.651	3.01E-03
O75475	PSIP1	2.842	3.738	0.395	3.03E-03
Q01543	FLI1	1.312	2.119	0.692	3.21E-03
Q9UM47	Notch 3	5.705	5.346	-0.094	3.52E-03
P27695	APEX1	2.094	3.134	0.582	3.54E-03
Q9NX58	LYAR	0.264	0.849	1.684	3.55E-03
P25815	S100P	0.954	1.561	0.711	3.70E-03
P42785	PRCP	1.881	2.237	0.250	6.31E-03
P55103	INHBC	3.727	4.294	0.204	6.31E-03
P49023	PXN	3.712	4.650	0.325	6.96E-03
Q9H5Y7	SLITRK6	0.880	0.705	-0.319	8.26E-03
Q9NWQ8	PAG1	1.826	2.362	0.371	8.26E-03
Q9HAN9	NMNAT1	5.797	6.869	0.245	8.28E-03
O75569	PRKRA	1.031	1.523	0.562	8.74E-03
O60934	NBN	3.092	4.012	0.376	9.05E-03
Q9UBG3	CRNN	5.582	4.820	-0.212	9.05E-03
P80511	EN-RAGE	5.541	6.540	0.239	9.25E-03
P18510	IL-1ra	5.531	6.108	0.143	9.41E-03
Q9NY25	CLEC5A	4.818	5.409	0.167	9.50E-03
P16870	CPE	6.145	5.693	-0.110	1.03E-02
P05089	ARG1	1.620	2.454	0.599	1.05E-02
P07948	LYN	2.812	3.069	0.126	1.09E-02
P22466	GAL	6.654	5.867	-0.182	1.22E-02
Q16552	IL-17A	1.286	1.756	0.450	1.40E-02
P09958	FURIN	10.576	11.001	0.057	1.69E-02

Olink ID	Protein	groupmean.HC	groupmean.PsA	log2FC	P value
Q12860	CNTN1	5.318	5.007	-0.087	1.72E-02
P06681	C2	5.631	5.994	0.090	1.84E-02
P50452	SERPINB8	2.955	3.512	0.249	1.84E-02
P41236	PPP1R2	2.523	3.017	0.258	1.90E-02
Q92844	TANK	1.212	1.874	0.629	1.93E-02
A6NI73	LILRA5	4.470	4.793	0.100	2.10E-02
Q96GP6	SCARF2	6.776	6.486	-0.063	2.13E-02
P22681	CBL	1.591	2.281	0.520	2.18E-02
Q9BXJ1	C1QTNF1	3.849	4.272	0.150	2.60E-02
P55774	CCL18	6.114	6.741	0.141	2.64E-02
Q9NS68	TNFRSF19	5.204	4.881	-0.093	3.16E-02
P11274	BCR	2.487	3.070	0.303	3.16E-02
Q99972	MYOC	6.212	5.695	-0.125	3.20E-02
P63313	TMSB10	2.962	3.540	0.257	3.20E-02
Q13105	ZBTB17	2.547	2.952	0.213	3.20E-02
P24821	TNC	2.369	2.925	0.304	3.25E-02
P06756	ITGAV	5.405	5.222	-0.050	3.25E-02
O00241	SIRPB1	3.887	4.279	0.139	3.37E-02
P08648	ITGA5	3.214	3.508	0.126	3.37E-02
O00273	DFFA	3.796	4.216	0.151	3.92E-02
Q6UX15	LAYN	5.742	5.424	-0.082	3.94E-02
Q12778	FOXO1	1.708	2.139	0.325	3.95E-02
Q16674	MIA	11.533	11.290	-0.031	4.45E-02
P18564	ITGB6	2.708	2.362	-0.198	4.45E-02

Supplemental Table S1. List of the 68 differentially expressed proteins when comparing PsA to HC. Abbreviations: Log2FC = log2 fold change. P value shown is the FDR-corrected P value.

Olink ID	Protein	groupmean.HC	groupmean.Pso	log2FC	P value
P04083	ANXA1	4.293	5.853	0.447	8.61E-07
P08670	VIM	6.314	7.321	0.213	3.96E-06
P40222	TXLNA	4.307	5.324	0.306	3.65E-05
Q9Y6K9	NEMO	5.608	6.779	0.274	3.85E-05
Q6ZUJ8	PIK3AP1	3.414	4.742	0.474	3.94E-05
P19878	NCF2	7.323	8.985	0.295	1.34E-04
Q02880	TOP2B	3.806	5.208	0.453	1.89E-04
P63313	TMSB10	2.962	3.917	0.403	2.83E-04
P09769	FGR	2.833	4.001	0.498	2.83E-04
P07948	LYN	2.812	3.171	0.173	4.51E-04
P78362	SRPK2	0.163	0.885	2.441	5.38E-04
P14317	HCLS1	4.351	5.415	0.315	1.19E-03
Q01543	FLI1	1.312	2.211	0.753	1.29E-03
O00221	NFKBIE	1.990	2.557	0.361	1.94E-03
P50749	RASSF2	2.770	4.083	0.560	1.94E-03
O75475	PSIP1	2.842	3.765	0.406	2.12E-03
Q9NWQ8	PAG1	1.826	2.355	0.367	2.12E-03
Q92844	TANK	1.212	2.159	0.833	2.23E-03

Olink ID	Protein	groupmean.HC	groupmean.Pso	log2FC	P value
Q12778	FOXO1	1.708	2.301	0.429	2.48E-03
O95544	NADK	5.290	6.422	0.280	2.51E-03
O14867	BACH1	1.311	2.098	0.678	2.51E-03
Q14005	IL16	7.529	8.236	0.129	2.84E-03
Q7Z5R6	APBB1IP	4.147	4.515	0.123	2.84E-03
Q9GZT9	EGLN1	2.521	3.698	0.553	3.19E-03
P29350	PTPN6	1.262	1.679	0.411	3.37E-03
P31949	S100A11	4.265	4.717	0.146	3.37E-03
P11274	BCR	2.487	3.229	0.377	4.53E-03
P18510	IL-1ra	5.531	6.284	0.184	4.53E-03
Q9NX58	LYAR	0.264	0.773	1.549	5.91E-03
P25815	S100P	0.954	1.620	0.764	6.18E-03
P01275	GCG	4.337	3.107	-0.481	6.32E-03
P49023	PXN	3.712	4.741	0.353	6.32E-03
P41236	PPP1R2	2.523	3.160	0.325	7.48E-03
P04233	CD74	1.768	2.307	0.384	9.37E-03
P04637	TP53	1.746	2.056	0.236	9.37E-03
P08648	ITGA5	3.214	3.695	0.201	1.04E-02
P27695	APEX1	2.094	3.141	0.585	1.05E-02
O60934	NBN	3.092	4.041	0.386	1.12E-02
Q8N5S9	CAMKK1	0.015	0.356	4.614	1.71E-02
Q9HAN9	NMNAT1	5.797	6.888	0.249	1.73E-02
P22466	GAL	6.654	5.792	-0.200	1.73E-02
O75569	PRKRA	1.031	1.536	0.575	1.75E-02
P55103	INHBC	3.727	4.260	0.193	1.94E-02
O00241	SIRPB1	3.887	4.412	0.183	1.94E-02
Q9NQ88	TIGAR	1.474	1.890	0.358	2.71E-02
P22681	CBL	1.591	2.292	0.527	3.63E-02
Q9NY25	CLEC5A	4.818	5.323	0.144	4.21E-02
Q00978	IRF9	2.083	2.521	0.276	4.63E-02
P80511	EN-RAGE	5.541	6.410	0.210	4.63E-02
P42785	PRCP	1.881	2.200	0.226	4.63E-02
P78380	LOX-1	9.070	9.601	0.082	4.76E-02

Supplemental Table S2. List of the 51 differentially expressed proteins when comparing Pso to HC. Abbreviations: Log2FC = log2 fold change. P value shown is the FDR-corrected P value.

Olink ID	Protein	groupmean.HC	groupmean.AS	log2FC	P value
Q02880	TOP2B	3.806	5.518	0.536	6.66E-07
P78362	SRPK2	0.163	0.995	2.609	4.75E-06
Q9Y6K9	NEMO	5.608	6.850	0.288	4.94E-06
P42785	PRCP	1.881	2.637	0.488	5.75E-06
P04083	ANXA1	4.293	5.677	0.403	8.23E-06
Q6ZUJ8	PIK3AP1	3.414	4.709	0.464	8.96E-06
O14867	BACH1	1.311	2.318	0.822	1.07E-05
Q92844	TANK	1.212	2.319	0.936	1.08E-05
P08670	VIM	6.314	7.227	0.195	3.34E-05
P40222	TXLNA	4.307	5.185	0.268	1.77E-04
O00221	NFKBIE	1.990	2.604	0.388	2.25E-04
P09769	FGR	2.833	4.038	0.511	2.81E-04
Q9GZT9	EGLN1	2.521	3.802	0.593	2.81E-04
O75569	PRKRA	1.031	1.676	0.701	3.11E-04

Olink ID	Protein	groupmean.HC	groupmean.AS	log2FC	P value
O75475	PSIP1	2.842	3.901	0.457	3.11E-04
Q12778	FOXO1	1.708	2.381	0.479	3.16E-04
P27695	APEX1	2.094	3.305	0.659	4.20E-04
Q9NX58	LYAR	0.264	0.922	1.803	4.35E-04
Q01543	FLI1	1.312	2.295	0.807	6.00E-04
P19878	NCF2	7.323	8.804	0.266	7.83E-04
P14317	HCLS1	4.351	5.398	0.311	9.84E-04
P50749	RASSF2	2.770	4.060	0.552	9.84E-04
P00441	SOD1	1.050	1.727	0.717	1.20E-03
O95544	NADK	5.290	6.481	0.293	1.40E-03
O60934	NBN	3.092	4.175	0.433	1.60E-03
P05231	IL6	3.807	4.948	0.378	1.74E-03
P80511	EN-RAGE	5.541	6.800	0.295	1.95E-03
P08648	ITGA5	3.214	3.600	0.163	2.28E-03
Q9NWQ8	PAG1	1.826	2.361	0.371	2.89E-03
P14543	NID1	4.491	4.913	0.130	2.89E-03
Q96SB3	PPP1R9B	2.954	3.721	0.333	2.89E-03
P25815	S100P	0.954	1.571	0.720	2.89E-03
P49023	PXN	3.712	4.752	0.356	3.42E-03
P11274	BCR	2.487	3.215	0.370	3.78E-03
P05067	APP	4.349	4.963	0.190	4.44E-03
Q14005	IL16	7.529	8.197	0.123	4.49E-03
P31949	S100A11	4.265	4.726	0.148	4.78E-03
P06681	C2	5.631	5.992	0.090	4.93E-03
P51693	APLP1	6.241	6.934	0.152	4.93E-03
P41236	PPP1R2	2.523	3.160	0.325	5.24E-03
Q9NWZ3	IRAK4	0.826	1.428	0.791	6.12E-03
Q9C0C4	SEMA4C	1.238	1.454	0.232	8.08E-03
A6NI73	LILRA5	4.470	4.957	0.149	8.08E-03
Q92765	sFRP-3	2.651	3.238	0.288	8.08E-03
P21217	FUT3/FUT5	3.249	3.689	0.183	8.44E-03
Q05084	ICA1	1.417	1.907	0.428	8.90E-03
P10586	PTPRF	4.692	5.054	0.107	1.10E-02
Q07065	CKAP4	5.060	5.383	0.089	1.19E-02
Q9HAN9	NMNAT1	5.797	6.901	0.252	1.43E-02
O00273	DFFA	3.796	4.329	0.189	1.56E-02
Q8N423	LILRB2	3.527	3.910	0.149	1.65E-02
P04179	SOD2	9.964	10.154	0.027	2.22E-02
P05089	ARG1	1.620	2.460	0.603	2.44E-02
P63313	TMSB10	2.962	3.586	0.276	2.91E-02
P05543	SERPINA7	4.643	5.073	0.128	3.34E-02
P55774	CCL18	6.114	6.689	0.130	3.65E-02
P07948	LYN	2.812	3.056	0.120	4.04E-02
Q16769	QPCT	2.632	2.997	0.187	4.31E-02
P19320	VCAM1	4.817	5.191	0.108	4.39E-02
Q6UXH9	PAMR1	5.604	5.922	0.079	4.80E-02
P35590	TIE1	1.868	2.117	0.181	4.81E-02
Q9NY25	CLEC5A	4.818	5.282	0.133	4.91E-02

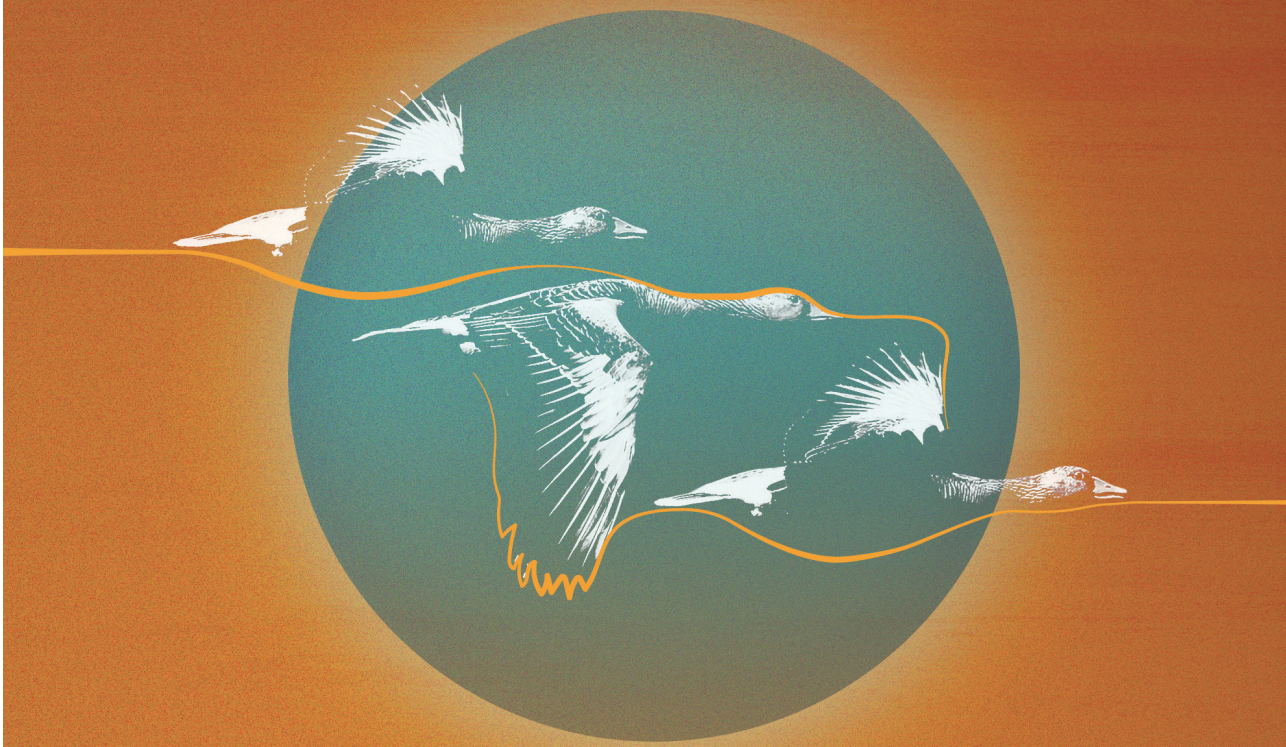
Supplemental Table S3. List of the 62 differentially expressed proteins when comparing AS to HC. Abbreviations: Log2FC = log2 fold change. P value shown is the FDR-corrected P value.

Olink ID	Protein	groupmean.PsA	groupmean.Pso	log2FC	P value
P48052	CPA2	10.949	10.507	-0.059	2.51E-03
P23582	NPPC	3.824	4.370	0.193	4.22E-03
Q6UXH1	CRELD2	3.639	3.980	0.129	7.21E-03
P43489	TNFRSF4	4.755	5.135	0.111	7.47E-03
P09326	CD48	6.943	7.180	0.049	8.55E-03
P00749	uPA	6.425	6.652	0.050	1.16E-02
Q8WWJ7	CD6	5.430	5.869	0.112	1.21E-02
Q6WN34	CHRDL2	3.487	3.848	0.142	1.31E-02
Q96LA6	FCRL1	4.447	4.816	0.115	1.34E-02
Q6DN72	FCRL6	2.730	3.342	0.292	1.56E-02
Q13145	BAMBI	1.161	1.435	0.306	2.01E-02
Q969Z4	RELT	4.990	5.219	0.065	2.40E-02
Q9NR71	N-CDase	5.126	4.610	-0.153	2.48E-02
O43278	SPINT1	2.629	2.868	0.125	2.64E-02
P06127	CD5	4.668	4.930	0.079	2.77E-02
Q9Y624	JAM-A	6.207	6.510	0.069	3.37E-02
P01730	CD4	5.888	6.071	0.044	3.46E-02
Q9NS68	TNFRSF19	4.881	5.130	0.072	3.55E-02
Q6GTX8	LAIR1	4.722	5.013	0.086	3.81E-02
Q13308	PTK7	1.912	2.132	0.157	3.89E-02
O00214	gal-8	8.220	7.904	-0.057	4.01E-02
Q4KMG0	CDON	4.470	4.741	0.085	4.05E-02
Q16363	LAMA4	4.434	4.660	0.072	4.22E-02
P15692	VEGFA	11.354	11.023	-0.043	4.25E-02
P21589	5'-NT	11.868	11.547	-0.040	4.36E-02
P63313	TMSB10	3.540	3.917	0.146	4.45E-02
P27797	CALR	0.198	0.393	0.990	4.57E-02
Q16270	IGFBP-7	8.473	8.686	0.036	4.63E-02
Q9NQ76	MEPE	6.704	7.021	0.067	4.64E-02
O14713	ITGB1BP1	0.121	0.236	0.963	4.66E-02
Q9NQ38	SPINK5	1.318	1.551	0.235	4.68E-02
P35052	GPC1	6.115	6.306	0.044	4.94E-02
O43240	KLK10	3.473	3.932	0.179	4.97E-02
Q9UBX7	hK11	7.677	7.981	0.056	4.98E-02

Supplemental Table S4. List of the 34 differentially expressed proteins when comparing PsA to Pso. Analysis based on nominal P value <0.05. Abbreviations: Log2FC = log2 fold change. P value shown is the nominal P value.

PART III

Therapy response and prediction



Chapter 7

First-line csDMARD monotherapy drug retention in psoriatic arthritis: methotrexate outperforms sulfasalazine

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Abstract

Background – Conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) are the first-line treatment for psoriatic arthritis (PsA), but there is conflicting data regarding their efficacy and scarce reports describing the duration of use (drug retention) of csDMARD in this population. Their position in treatment recommendations is a matter of growing debate due to the availability of alternative treatment options with higher levels of evidence.

Objective – To study drug retention and predictors for drug retention among PsA patients receiving first-line csDMARD monotherapy.

Methods – Retrospective cohort study in DMARD-naïve adult PsA patients in whom a first csDMARD was prescribed as monotherapy primarily to treat PsA-related symptoms. Main outcome was time to failure of the csDMARD (i.e stopping the csDMARD or adding another DMARD).

Results – 187 Patients were included, who were mainly prescribed methotrexate (MTX) (n=163) or sulfasalazine (SSZ) (n=21). The pooled median drug retention time was 31.8 months (IQR 9.04-110). Drug retention was significantly higher in MTX (median 34.5 months; IQR 9.60-123) as compared to SSZ treated patients (median 12.0 months; IQR 4.80- 55.7) ($p=0.016$, log-rank test). In multivariable cox-regression the use of MTX and older age were associated with increased retention. The main reasons for treatment failure were inefficacy (52%) and side-effects (28%). Upon failure, MTX treated patients were more commonly, subsequently treated with a biologic DMARD compared to SSZ ($p<0.05$).

Conclusion – MTX outperforms SSZ as first-line csDMARD in DMARD-naïve PsA patients with respect to monotherapy drug retention in daily clinical practice.

Manuscript

Introduction

Psoriatic arthritis (PsA) is a chronic, inflammatory musculoskeletal disorder, which develops in approximately one in ten patients with psoriasis and often leads to a decreased quality of life and impaired function.(1) Currently, conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) are the most commonly prescribed drugs as first-line treatment for peripheral arthritis in PsA, as recommended by the international EULAR and GRAPPA guidelines.(2,3) These guidelines refer to methotrexate (MTX), sulfasalazine (SSZ), leflunomide (LEF) and cyclosporin A as possible treatment options. However, previous studies found little or no effect of MTX on psoriatic synovitis, and higher effectiveness of tumour necrosis factor (TNF) inhibitors compared to MTX in reducing radiographic progression in PsA patients.(4–9) Efficacy of LEF in PsA has been shown in one randomized controlled trial.(10) Regarding SSZ, clinical trials have found a modest favourable effect on musculoskeletal symptoms.(11–13)

While there is a lack of high level evidence to support the use of csDMARD efficacy in PsA, csDMARD drug retention rates can provide indirect evidence. One large study found a two-year retention rate of MTX therapy of ~65% in both rheumatoid arthritis (RA) and PsA, suggestive of a beneficial effect of MTX in PsA.(14) Another comparable study observed mean MTX and LEF drug retention of 13 and 6 years, respectively.(15) More indirect evidence comes from the fact that PsA has historically been treated similar to RA, where high-level evidence supports the use of csDMARDs, and justifies this treatment in PsA patients. Other arguments to consider csDMARD therapy include well described long-term safety outcomes and low costs; a factor taken into account by some (inter) national guidelines.

Despite these reasons to treat PsA with csDMARDs, their position is under pressure due to alternative treatment options with higher levels of evidence.(1) In line, a recent guideline recommends the use of TNF-inhibitors as a first-line treatment.(16) Furthermore, in daily practice csDMARD side effects are commonly reported which negatively impact drug retention.

This study aimed to evaluate the level of indirect evidence for csDMARD efficacy by describing first-line csDMARD monotherapy drug retention for treating PsA in daily clinical practice, comparing the retention rate of different csDMARDs and investigating possible predictors of drug retention.

Methods

Study design

This retrospective cohort study was performed at the University Medical Center Utrecht, the Netherlands, and approved by the local institutional review board. The first selection of eligible patients was performed via electronic search based on diagnosis and diagnosis related groups (DRGs). Manual screening was performed twice to ensure eligibility. Inclusion criteria were (1) clinical diagnosis of PsA and (2) DMARD-naïve (no prior DMARD therapy for any cause, including psoriasis), and (3) initiated DMARD as monotherapy after January 1st 2000. Patients were excluded if csDMARD therapy was primarily initiated for treating extra-articular manifestations (e.g. to treat psoriasis). All patient data was encrypted and saved using the online database CastorEDC.

Outcome measures

The main outcome was defined as the first-line csDMARD monotherapy drug retention time. The first-line csDMARD monotherapy cessation date (abbreviated "csDMARD monotherapy failure") was set at the last recorded date during which the first-line csDMARD was prescribed as monotherapy. Thus, discontinuation of csDMARD monotherapy occurred upon (i) cessation of the first-line csDMARD therapy or (ii) continuing the first-line csDMARD but adding a bDMARD or second csDMARD. Observations were considered "censored data" if the patient was still on therapy at the last known medical record observation point or if the patient was lost to follow-up.

Using pre-defined categories the research team retrospectively identified the main reason for first-line treatment cessation, as based on the reason recorded by the treating physician in the medical record. Categories included remission, inefficacy, side-effects, (planned) pregnancy and other reasons. We registered the subsequent treatment prescribed within a window of six months after csDMARD monotherapy failure. A maximum tolerated "drug holiday" of three months was allowed to mimic clinical care. Demographic, clinical and radiographic parameters were collected to identify predictors of treatment response.

Statistical analysis

Data analyses were performed using SPSS software (version 25.0). Data were represented as mean and standard deviation (SD) for normally distributed data and median and interquartile range (IQR) for non-normally distributed data. Baseline characteristics

between MTX and SSZ groups were compared using the independent samples T-test (normally distributed data), Mann-Whitney U test (non-normally distributed data) or Chi-square test as appropriate. A P-value of less than 0.05 was considered statistically significant. Drug retention was described using Kaplan-Meier plots and statistically compared using the log-rank test. Potential predictors were of drug retention were studied using a multivariable cox model (described in **Supplemental Data S1**).

Results

Cohort characteristics

In total, 187 patients with PsA met the inclusion criteria. Main demographics and disease activity characteristics are shown in **Supplemental Table S1**. The cohort consisted of 68% males with mean age 48 years (SD 13.3). The duration of disease was 0.4 years (IQR 0.1–1.0) and 7.5 years (IQR 2.1–18.1) for PsA and psoriasis, respectively. The most commonly prescribed first-line csDMARD was MTX (87%), followed by SSZ (11%) and LEF (2%). As compared to SSZ, patients initiating MTX had significantly higher age, body mass index and swollen and tender joint count. Also, there was a trend for erosive disease to be more common in the MTX than SSZ group.

csDMARD monotherapy drug retention

In total, 132 patients (71%) failed their first line therapy during follow-up, while 55 patients (29%) had censored observation. The monotherapy drug retention showed a large drop in retention early after treatment initiation. In the entire study population, the median monotherapy drug retention was 31.8 months (95% CI 18.9-44.6; IQR 9.04-110). At 12 months after treatment initiation, 70% of patients were still using the first-line csDMARD as monotherapy.

We next compared the different csDMARDs initiated, excluding LEF from further analysis due to low numbers. MTX had significantly higher monotherapy drug retention as compared to SSZ (p=0.016) (**Figure 1**; **Supplemental Figure S1**). For MTX the median monotherapy drug retention was 34.5 months (95% CI 22.2-46.8; IQR 9.60-123). For SSZ the median monotherapy drug retention was 12.0 months (95% CI 4.32-19.8; IQR 4.80-55.7). At 12 months, 72% of patients that initiated MTX were still using MTX as monotherapy, whereas 52% of patients that initiated SSZ were still using SSZ as monotherapy.

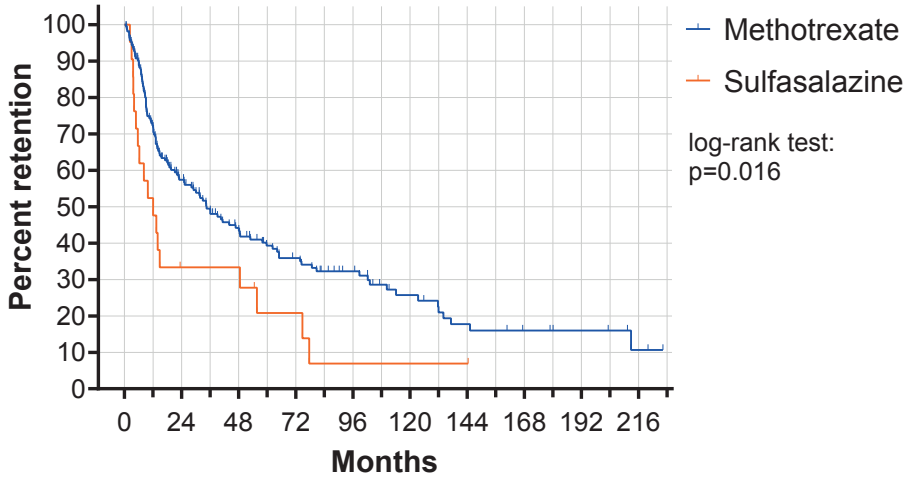

Figure 1. csDMARD monotherapy drug retention in PsA. Kaplan-Meier plot shows monotherapy drug retention rate of methotrexate or sulfasalazine prescribed as first-line treatment in DMARD-naïve psoriatic arthritis patients. Ticks indicate censored data. Methotrexate showed significantly higher monotherapy drug retention as compared to sulfasalazine.

Based on univariable cox regression analysis the DMARD initiated was significantly associated with DMARD retention, where MTX-initiated patients had better retention as compared to SSZ-initiated patients (HR 0.545 (95% CI 0.330-0.899), p=0.017). In addition, older age increased csDMARD monotherapy retention (HR 0.985 per year age increase (95% CI 0.971-0.998), p=0.026). When incorporating age and csDMARD initiated into multivariable cox regression model there was a non-significant trend for longer drug retention in MTX group [HR 0.630 ((CI 0.372-1.069), p=0.087] and older patients (per year age increase HR 0.988 [(CI 0.974-1.002), p=0.095]. We next screened for potential predictors of drug retention in a multivariable cox model: the final model included csDMARD-initiated and age as the only predictors of drug retention (same HR as above).

Cause of csDMARD monotherapy failure

The main reason for csDMARD monotherapy treatment cessation was treatment inefficacy (52%), followed by side-effects (28%) (**Figure 2**). The main reasons for treatment cessation between MTX and SSZ were slightly different, with more patients that stopped MTX due to (planned) pregnancy and more patients that stopped SSZ due to inefficacy (**Supplemental Table S2**). Remission occurred in 11 patients that initiated MTX and 2 patients that initiated SSZ. Retention analysis remained similar when remission cases were excluded or considered censored. The most important side effects were

gastrointestinal complaints (32%) and general malaise (24%) (**Figure 2**). Patients treated with MTX reported more side effects than patients treated with SSZ (**Supplemental Table S3** and **S4**). At time of csDMARD monotherapy failure, the patients that failed due to inefficacy had more active disease than the patients that failed due to other reasons (**Supplemental Table S5**).

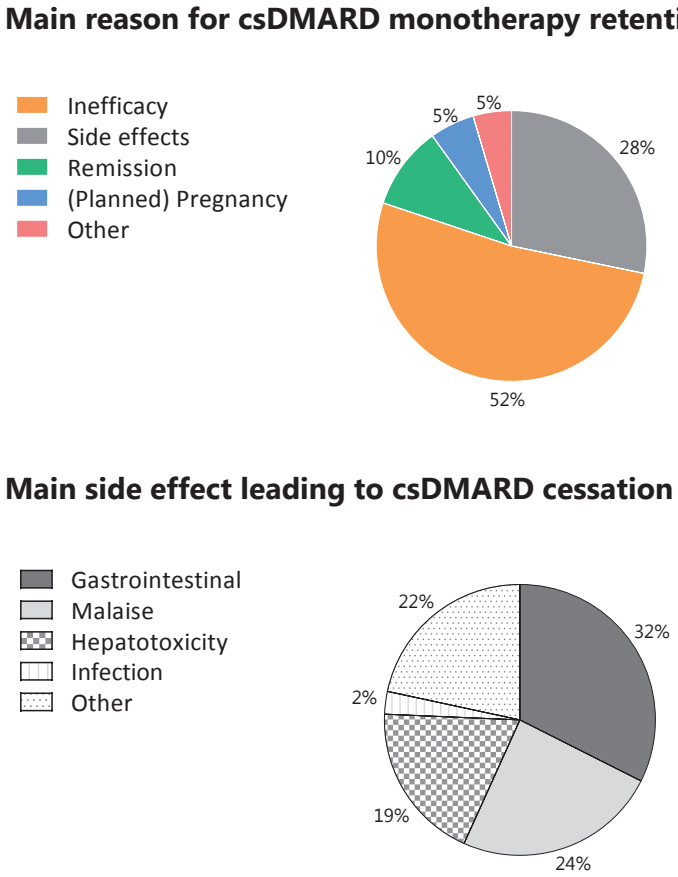

Figure 2. csDMARD monotherapy retention failure. Top: Main reasons for csDMARD monotherapy retention failure (non-censored cases, n=132). Bottom: Main side effect reported at stop date for patients in whom the primary reason for csDMARD cessation was side effects (non-censored data, n=37).

Follow-up treatment upon csDMARD monotherapy failure

Upon csDMARD monotherapy failure, the first-line csDMARD was most commonly switched to a different csDMARD (27%) or a bDMARD was added (25%). However, the

follow-up treatment regimen was significantly different between the MTX and SSZ groups: MTX treated patients were more commonly prescribed a bDMARD upon failure ($p<0.05$). In addition, failure due to side effects versus inefficacy resulted in different follow-up treatment strategies: a bDMARD was prescribed in 55% of patients that failed due to inefficacy as compared to 11% of patients that failed due to side effects ($p<0.05$) (**Supplemental Figure S2; Supplemental Table S6-S8**).

Discussion

This study shows that MTX as a first-line csDMARD for treating peripheral arthritis in PsA has higher monotherapy drug survival than SSZ. For all csDMARDs, monotherapy drug retention shows a large drop in the first year of treatment. Inefficacy is most commonly seen as reason for drug cessation, followed by side-effects. The results from this study are derived from a real-world setting and display a realistic clinical scenario of the first-line csDMARD monotherapy retention in csDMARD-naïve PsA patients.

A limited number of previous studies have evaluated csDMARD monotherapy drug retention.(14,15,17) We found a median csDMARD monotherapy retention of approximately 2.5 years, but witnessed a large drop in drug retention within the first year of treatment. One previous study found a 10-year MTX retention rate of more than 50%, which largely exceeds our 10-year retention rate of around 25%. This difference may be partly explained by the concomitant steroid use.(15) Overall, the validity of our results are strengthened by those of other studies that found a similar drug retention rate.(14,17) Our data also reveal that – even in the presence of potential efficacy – side effects were reported in more than 50% of patients at the moment of csDMARD monotherapy failure. Although not all of these side effects were deemed the principle cause of failure, they may have contributed to the modification of the treatment regimen.

With regards to predictors of csDMARD survival, one study described a larger PsA study cohort treated with MTX, but a shorter follow-up period with a maximum of 2 years. Their regression analysis showed age, disease duration, and patient reported outcomes to be significant predictors of MTX drug retention.(14) We also found that older age was a predictor of longer drug retention, but did not identify other clinical parameters to be associated with drug retention. Additionally, we didn't find sex or C-reactive protein (CRP) levels to be significant predictors of drug retention, as proposed in earlier cohort studies.(15,18)

This study has a number of limitations. An important limitation is the retrospective nature of the study. Another factor that needs to be taken into account is the small number of subjects in the SSZ group compared to the MTX group. Also, drug retention is an assumed indirect measure of treatment efficacy, while drug adherence and treatment modifications are dependent on multiple factors in daily practice. Nonetheless, the use of real-world data also contains advantages over trial data by better portraying the setting in which DMARDs are initiated, as exemplified by the relative low joint count in our study cohort as compared to patients enlisted in PsA trials.

Overall our results support the use of MTX as first-line therapy in treating peripheral arthritis in PsA, as recommended by current EULAR and GRAPPA guidelines.(2,3) These data show that, at least compared to SSZ, MTX performs better with respect to monotherapy drug retention. Considering the emergence of numerous novel drugs for treating PsA, prospective studies (e.g. pragmatic randomized clinical trials (19)) are required to further elucidate the differential efficacy of specific csDMARDs as first line treatment in PsA. The future research agenda should continue to focus on treatment challenges faced in the real-world setting, where the largest group of patients with PsA present with early, mono- or oligoarticular disease.

In conclusion, we found that MTX outperforms SSZ as first-line csDMARD in DMARD-naïve PsA patients with respect to monotherapy drug retention in daily clinical practice. Future prospective studies should further elucidate the efficacy of csDMARDs as first-line treatment for PsA.

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Disclosure statement: TR is currently an employee of AbbVie, with no conflicts of interest regarding the work of this manuscript. The other authors have declared no conflicts of interest.

References

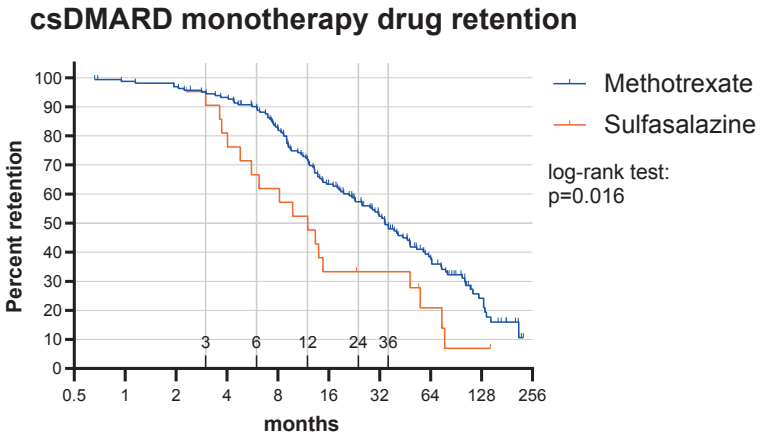
1. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. N Engl J Med. 2017 Mar;376(10):957–70.
2. Gossec L, Smolen JS, Ramiro S, de Wit M, Cutolo M, Dougados M, et al. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. Ann Rheum Dis. 2016 Mar 1;75(3):499–510.
3. Coates LC, Kavanaugh A, Mease PJ, Soriano ER, Laura Acosta-Felquer M, Armstrong AW, et al. Group for Research and Assessment of Psoriasis and Psoriatic Arthritis 2015 Treatment Recommendations for Psoriatic Arthritis. Arthritis Rheumatol. 2016 Mar 1 ;68(5): 1060-1071
4. Mahmood F, Coates LC, Helliwell PS. Current concepts and unmet needs in psoriatic arthritis. Clin Rheumatol. 2018;37(2):297–305.
5. Eder L, Thavaneswaran A, Chandran V, Gladman DD. Tumour necrosis factor α blockers are more effective than methotrexate in the inhibition of radiographic joint damage progression among patients with psoriatic arthritis. Ann Rheum Dis. 2014 Jun;73(6):1007 LP – 1011.
6. Marchesoni A, Lubrano E, Cauli A, Ricci M, Manara M. Psoriatic Disease: Update on Traditional Disease-modifying Antirheumatic Drugs. J Rheumatol. 2015 Nov;93:61 LP – 64.
7. Kingsley GH, Kowalczyk A, Taylor H, Ibrahim F, Packham JC, McHugh NJ, et al. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. Rheumatology. 2012 Feb;51(8):1368–77.
8. Mease PJ, Gladman DD, Collier DH, Ritchlin CT, Helliwell PS, Liu L, et al. Etanercept and Methotrexate as Monotherapy or in Combination for Psoriatic Arthritis: Primary Results From a Randomized, Controlled Phase III Trial. Arthritis Rheumatol. 2019 Jul;71(7):1112–24.
9. Wilsdon TD, Whittle SL, Thynne TR, Mangoni AA. Methotrexate for psoriatic arthritis. Cochrane Database Syst Rev. 2019 Jan 18;(1).
10. Kaltwasser JP, Nash P, Gladman D, Rosen CF, Behrens F, Jones P, et al. Efficacy and safety of leflunomide in the treatment of psoriatic arthritis and psoriasis: A multinational, double-blind, randomized, placebo-controlled clinical trial. Arthritis Rheum. 2004 Jun;50(6):1939–50.
11. Clegg DO, Reda DJ, Mejias E, Cannon GW, Weisman MH, Taylor T, et al. Comparison of sulfasalazine and placebo in the treatment of psoriatic arthritis. A Department of Veterans Affairs Cooperative Study. Arthritis Rheum. 1996 Dec;39(12):2013–20.
12. Gupta AK, Grober JS, Hamilton TA, Ellis CN, Siegel MT, Voorhees JJ, et al. Sulfasalazine therapy for psoriatic arthritis: a double blind, placebo controlled trial. J Rheumatol. 1995 May;22(5):894–8.
13. Dougados M, vam der Linden S, Leirisalo-Repo M, Huitfeldt B, Juhlin R, Veys E, et al. Sulfasalazine in the treatment of spondylarthropathy. A randomized, multicenter, double-blind, placebo-controlled study. Arthritis Rheum. 1995 May;38(5):618–27.
14. Lie E, van der Heijde D, Uhlig T, Heiberg MS, Koldingsnes W, Rødevand E, et al. Effectiveness and retention rates of methotrexate in psoriatic arthritis in comparison with methotrexate-treated patients with rheumatoid arthritis. Ann Rheum Dis. 2010 Apr;69(4):671 LP – 676.
15. Zaffarana CA, Cerda O, Schneeberger EE, Carrillo I, Gonzalez MC, Citera G. Methotrexate and leflunomide survival in patients with psoriatic arthritis. Int J Clin Rheumtol. 2018;13(2):102.
16. Singh JA, Guyatt G, Ogdie A, Gladman DD, Deal C, Deodhar A, et al. Special Article: 2018 American College of Rheumatology/National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis. Arthritis Rheum. 2019 Jan;71(1):5-32
17. Ricci M, De Marco G, Desiati F, Mazzocchi D, Rotunno L, Battafarano N, et al. Long-term survival of methotrexate in psoriatic arthritis. Reumatismo. 2009;61(2):125–31.

18. Glintborg B, Østergaard M, Dreyer L, Krogh NS, Tarp U, Hansen MS, et al. Treatment response, drug survival, and predictors thereof in 764 patients with psoriatic arthritis treated with anti–tumor necrosis factor α therapy: Results from the nationwide Danish DANBIO registry. Arthritis Rheum. 2011 Feb;63(2):382–90.
19. Gamerman V, Cai T, Elsäßer A. Pragmatic randomized clinical trials: best practices and statistical guidance. Heal Serv Outcomes Res Methodol. 2019;19(1):23–35.

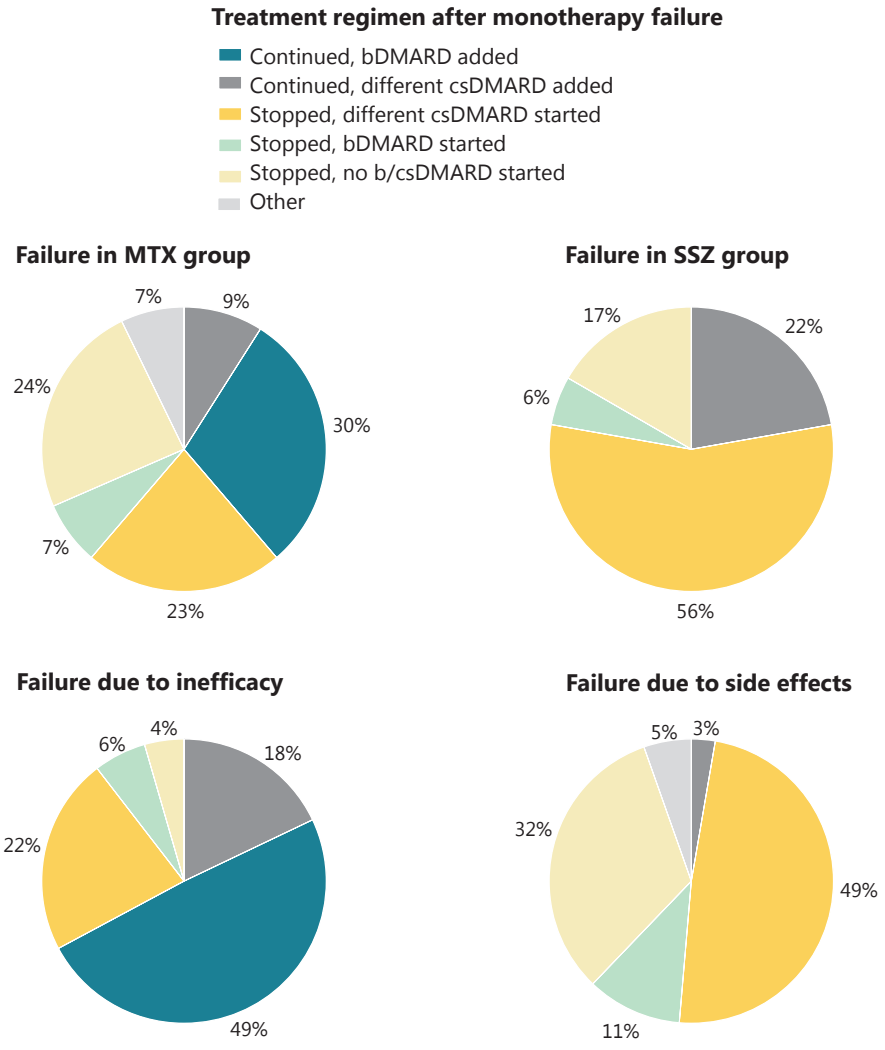
Supplemental Information

Supplemental Data S1. Multivariable cox regression in a multivariable cox model. As this was not a randomized study, correction for possible confounders influencing the difference in retention between MTX and SSZ was necessary. Therefore, any clinical factor considered potentially different between MTX and SSZ groups and related to drug retention (variables: sex, age, smoker, disease duration, joint count, dactylitis, psoriasis phenotype, erythrocyte sedimentation rate, erosive disease) were evaluated in the multivariable Cox regression. To optimally correct for confounding while keeping a parsimonious model, potential confounders were added to the model one by one. At each step, the necessity to control for the variable was determined. A change in the regression coefficient for treatment effect of more than 10% led to retention of the variable in the model.

In a separate analysis, we screened for potential predictors of drug retention using a multivariable cox model, by entering blocks of variables, starting with DMARD group, followed by demographic factors, followed by clinical factors. Per block the least significant variables were removed while retaining variables with a nominal p value of < 0.15. The influence of variables in the final cox models are described as hazard ratio (HR) with 95% confidence interval (CI) and p value.



Supplemental Figure S1. csDMARD monotherapy drug retention in PsA. Same data is shown as in **Figure 1**, but the graph is displayed with the X-axis as logarithmic scale to visualize the early retention rate. Kaplan-Meier plot is shown for methotrexate (MTX) or sulfasalazine (SSZ) as first-line csDMARD to treat psoriatic arthritis. Ticks indicate censored data. MTX had significantly higher monotherapy drug survival as compared to SSZ (p=0.016, log-rank test).



Supplemental Figure S2. Follow-up treatment. Follow-up treatment regimen the first six months after the monotherapy csDMARD treatment failure date, in all non-censored cases. Top Left: In patients that initiated MTX (n=111). Top Right: In patients that initiated SSZ (n=18). Bottom left: In patients with inefficacy as main treatment failure cause (regardless of treatment) (n=68). Bottom right: In patients with side effects as main treatment failure cause (regardless of treatment (n=37).

		All. N = 187	MTX. N = 163	SSZ. N = 21
Male, N (%)		128 (68)	115 (71)	12 (57)
Age (years), mean±SD*		48.3±13.3	49.1±12.7	39.5±13.4
Body mass index, mean±SD*		26.7±4.5	27.4±4.4	25.3±3.5
Smoker, N (%)		34/150 (18.2)	31/129 (19.0)	3/18 (14.3)
Disease duration (years), median (IQR)				
	PsA	0.4 (0.1 – 3.0)	0.4 (0.1 – 2.9)	0.3 (0.1 – 2.0)
	Psoriasis	7.5 (2.1 – 18.4)	7.5 (2.0 – 15.7)	10.8 (3.1 – 26.7)
Psoriasis phenotype, N (%)*				
	Vulgaris only	130/185 (69.5)	117/161 (71.8)	10/21 (47.6)
	Sine psoriasis	31/185 (16.6)	21/161 (12.9)	10/21 (47.6)
	Other types [a)]	24/185 (12.8)	23/161 (14.1)	1/21 (4.8)
Nail disease present, N (%)		81/121 (43.3)	72/104 (44.2)	9/17 (42.9)
Swollen joint count, median (IQR)*		4 (2 – 6)	4 (2 – 6)	2 (1 – 3)
Tender joint count, median (IQR)*		4 (1 – 6)	4 (2 – 6)	1 (0 – 3)
Dactylitis present, N (%)		18/161 (9.6)	17/142 (10.4)	1/17 (4.8)
Axial disease present, N (%) [b)]		27/185 (14.4)	24/161 (14.7)	3/21 (14.3)
CRP, median (IQR)		8.0 (2.9 – 16.0)	8.0 (3.0 – 15.0)	10.0 (1.0 – 26.0)
ESR, median (IQR)		14.0 (6.0 – 27.5)	14.0 (7.0 – 27.0)	16.0 (6.0 – 31.0)
Erosive disease, N (%)		40/156 (21.4)	37/140 (22.7)	2/15 (9.5)

Supplemental Table S1. Study Cohort. Main demographic and clinical characteristics of the study cohort at time of csDMARD therapy initiation. The total cohort (n=187) included 3 patients treated with leflunomide (data not shown separately). Psoriasis area and severity scores were unavailable for most cases and not shown. Descriptive data show the mean±SD, median (IQR) or N (%). * Clinical parameter is significantly different (P <0.05) between MTX and SSZ. [a)] Other psoriasis types included guttate, palmoplantaris, inverse, and mixed types. [b)] Presence/absence of axial disease was based on the clinical diagnosis from the treating physician. Abbreviations: MTX = methotrexate. SSZ = sulfasalazine. CRP = C-reactive protein. ESR = erythrocyte sedimentation rate. PsA = psoriatic arthritis.

	All. N = 132	MTX. N = 111	SSZ. N = 18
Inefficacy, N (%)	68 (51.5)	55 (49.5)	12 (66.7)
Side effects, N (%)	37 (28.0)	31 (27.9)	4 (22.2)
Remission, N (%)	13 (9.8)	11 (9.9)	2 (11.1)
(Planned) Pregnancy, N (%)	7 (5.3)	7 (6.3)	0 (0.0)
Other reason, N (%)	7 (5.3)	7 (6.3)	0 (0.0)

Supplemental Table S2. Main reasons for csDMARD monotherapy retention failure for all non-censored cases. The total cohort (n=132) included cases from 3 patients treated with leflunomide (data not shown separately).

	All. N = 37	MTX. N = 31	SSZ. N = 4
Gastrointestinal, N (%)	12 (32.4)	11 (35.5)	1 (25.0)
Hepatotoxicity, N (%)	7 (18.9)	7 (22.6)	0 (0.0)
Malaise, N (%)	9 (24.3)	8 (25.8)	1 (25.0)
Infection, N (%)	1 (1.7)	0 (0.0)	0 (0.0)
Other or unspecified side effect, N (%)	8 (21.6)	5 (14.1)	2 (50.0)

Supplemental Table S3. Major side effect leading to csDMARD monotherapy retention failure. Major side effect reported at stop date for patients in whom the primary reason for cessation was side-effects (non-censored cases). The total cohort (n=37) included 2 patients treated with leflunomide (data not shown separately).

	All. N = 132	MTX. N = 111	SSZ. N = 18
No side effect, N (%)	56 (42.4)	45 (40.5)	10 (55.6)
Gastrointestinal, N (%)	26 (19.7)	24 (21.6)	2 (11.1)
Hepatotoxicity, N (%)	15 (11.4)	15 (13.5)	0 (0.0)
Malaise, N (%)	13 (9.8)	11 (9.9)	2 (11.1)
Infection, N (%)	4 (3.0)	3 (2.7)	0 (0.0)
Other or unspecified side effect, N (%)	11 (8.4)	9 (8.1)	2 (11.1)
Missing data, N (%)	6 (4.5)	4 (3.6)	2 (11.1)

Supplemental Table S4. Major side effect reported at stop date for all non-censored cases. The total cohort (n=132) included cases from 3 patients treated with leflunomide (data not shown separately). The major side effects are shown for both patients in whom the primary reason for cessation was side-effects as well as for patients in whom the primary reason for cessation was another reason (e.g. treatment failure due to inefficacy, but where side-effects were also reported at the treatment failure date).

	Reason for csDMARD monotherapy retention failure	
	Inefficacy. N = 68	Other. N = 64
Swollen joint count, median (IQR)*	3.00 (1.00-5.00)	Swollen joint count, median (IQR)*
Tender joint count, median (IQR)*	4.00 (0.75-8.00)	Tender joint count, median (IQR)*
CRP, median (IQR)	8.00 (3.00-14.00)	CRP, median (IQR)
ESR, median (IQR)*	8.00 (5.00-15.75)	ESR, median (IQR)*

Supplemental Table S5. Indicators of disease activity at time of csDMARD monotherapy failure date in all non-censored cases. Descriptive data show the median (IQR). Data of swollen joint count, tender joint count, CRP and ESR were available for 43 (63%), 42 (62%), 27 (40%), 56 (82%) patients in the inefficacy group, and for 33 (52%), 35 (55%), 13 (20%) and 42 (66%) patients in the 'other' group, respectively. * Clinical parameter is significantly different (P <0.05) between patients that stopped csDMARD treatment because of inefficacy compared to patients that stopped because of other reasons.

	All. N = 132	MTX. N = 111	SSZ. N = 18
Continued, different csDMARD added, N (%)	14 (10.6)	10 (9.0)	4 (22.2)
Continued, bDMARD added, N (%)	33 (25.0)	33 (29.7)	0 (0.0)
Stopped, different csDMARD started, N (%)	36 (27.3)	25 (22.5)	10 (55.6)
Stopped, bDMARD started, N (%)	9 (6.8)	8 (7.2)	1 (5.6)
Stopped, no b/csDMARD started, N (%)	32 (24.2)	27 (24.3)	3 (16.7)
Stopped, no b/csDMARD started, with less than 6 months follow up data, N (%)	4 (3.0)	4 (3.6)	0 (0.0)
Other, N (%)	2 (1.5)	2 (1.8)	0 (0.0)
Missing data, N (%)	2 (1.5)	2 (1.8)	0 (0.0)

Supplemental Table S6. Follow-up treatment regimen the first six months after the monotherapy csDMARD treatment failure date. The total cohort (n=132) included 3 patients treated with leflunomide (data not shown separately). The rows "continued" refer to the patients in whom the first-line csDMARD monotherapy was continued and an additional DMARD was prescribed as combination therapy. The rows "stopped" refer to the patients in which the first-line csDMARD was stopped entirely. The follow-up treatment or lack thereof within a window of six months after monotherapy treatment failure date was recorded for non-censored cases.

	All. N = 68	MTX. N = 55	SSZ. N = 12
Continued, different csDMARD added, N (%)	12 (17.6)	8 (14.5)	4 (33.3)
Continued, bDMARD added, N (%)	33 (48.5)	33 (60.0)	0 (0.0)
Stopped, different csDMARD started, N (%)	15 (22.1)	6 (10.9)	8 (66.7)
Stopped, bDMARD started, N (%)	4 (5.9)	4 (7.3)	0 (0.0)
Stopped, no b/csDMARD started, N (%)	3 (4.4)	3 (5.5)	0 (0.0)
Stopped, no b/csDMARD started, with less than 6 months follow up data, N (%)	0 (0.0)	0 (0.0)	0 (0.0)
Missing data, N (%)	1 (1.5)	1 (1.8)	0 (0.0)

Supplemental Table S7. Follow-up treatment regimen upon inefficacy. Follow-up treatment regimen the first six months after the monotherapy csDMARD treatment failure date, in all non-censored cases with "inefficacy" as main treatment failure cause. The total cohort (n=68) included 1 patient treated with leflunomide (data not shown separately). The rows "continued" refer to the patients in whom the first-line csDMARD monotherapy was continued and an additional DMARD was prescribed as combination therapy. The rows "stopped" refer to the patients in which the first-line csDMARD was stopped entirely. The follow-up treatment or lack thereof within a window of six months after monotherapy treatment failure date was recorded for non-censored cases.

	All. N = 37	MTX. N = 31	SSZ. N = 4
Continued, different csDMARD added, N (%)	1 (2.7)	1 (3.2)	0 (0.0)
Continued, bDMARD added, N (%)	0 (0.0)	0 (0.0)	0 (0.0)
Stopped, different csDMARD started, N (%)	18 (48.6)	16 (51.6)	2 (50.0)
Stopped, bDMARD started, N (%)	4 (10.8)	3 (9.7)	1 (25.0)
Stopped, no b/csDMARD started, N (%)	12 (32.4)	9 (29.0)	1 (25.0)
Stopped, no b/csDMARD started, with less than 6 months follow up data, N (%)	1 (2.7)	1 (3.2)	0 (0.0)
Other, N (%)	1 (2.7)	1 (3.2)	0 (0.0)

Supplemental Table S8. Follow-up treatment regimen upon side-effects. Follow-up treatment regimen the first six months after the monotherapy csDMARD treatment failure date, in all non-censored cases with "side-effects" as main treatment failure cause. The total cohort (n=37) included 2 patients treated with leflunomide (data not shown separately). The rows "continued" refer to the patients in whom the first-line csDMARD monotherapy was continued and an additional DMARD was prescribed as combination therapy. The rows "stopped" refer to the patients in which the first-line csDMARD was stopped entirely. The follow-up treatment or lack thereof within a window of six months after monotherapy treatment failure date was recorded for non-censored cases.

Chapter 8

Emerging molecular biomarkers for predicting therapy response in psoriatic arthritis: A review of literature

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Abstract

Psoriatic arthritis (PsA) is a heterogeneous, chronic inflammatory musculoskeletal disorder that affects ~0.1% of the population. PsA may severely impact quality-of-life and constitutes a significant economic burden on our health care system. While early effective treatment is deemed essential to prevent irreversible joint damage and functional impairment, not all patients respond to the same disease modifying anti-rheumatic drugs (DMARDs). DMARD options for PsA are rapidly evolving, yet only 50–60% of patients show a satisfactory response to their first-line DMARD therapy. Hence, there is an urgent medical need to predict which patients benefit from a particular treatment. To this end, molecular biomarkers capable of predicting therapeutic response are currently being scrutinized in clinical studies, that together should build a framework for clinical guidelines that improve personalized targeted treatment. In this review new developments within the field of biomarker discovery for predicting therapeutic response to DMARDs in PsA are examined.

Manuscript

Introduction

The identification of predictors of treatment response in psoriatic arthritis (PsA) is one of the candidate flagship research areas to "*permit personalized and stratified medicine approaches*", stated at the 2017 Collaborative Research Network Meeting of the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA).(1) This perspective from an international consortium of rheumatologists and dermatologists highlights the importance of identifying predictors to disease modifying anti-rheumatic drugs (DMARDs) before treatment initiation.(2) Furthermore, the European League Against Rheumatism (EULAR) - an organization representing European health care professionals, patients and scientific societies of rheumatology - addressed this matter in their 2015 research agenda.(3)

PsA is a chronic inflammatory musculoskeletal disorder that affects ~0.1% of the global population.(4) It can severely impact quality of life and it contributes to a significant economic burden on our health care system.(5,6) Characterized by a heterogeneous disease presentation,(7) PsA patients may suffer from diverse musculoskeletal and extra-articular manifestations including peripheral arthritis, axial spondyloarthritis, enthesitis, dactylitis, psoriasis and nail disease.(5,8) Therapies include non-steroidal anti-inflammatory drugs (NSAIDs), intra-articular glucocorticoids and DMARDs,(9) which have significantly improved quality-of-life of many patients.(10) The repertoire of DMARDs approved for PsA treatment consist of 15 options and is expanding (**Table 1**).(11,12) Still, up to 40-50% of patients fail to show a partial or complete response.(8,12) This response deficit can have major implications. Firstly, early effective treatment is essential to prevent irreversible joint damage and functional impairment.(7,13) Secondly, DMARDs can be accompanied by serious adverse effects that should be avoided, particularly if there is no (expected) treatment benefit.(14) Lastly, the medications place tremendous strain on the healthcare system due to increasing costs.(13) All these factors underscore potential benefits of treating patients directly with the right drug of choice.

Thus far, no evidence-based strategies are available for rheumatologists that guide the decision as to which DMARD best suits the individual PsA patient.(11) The presence of certain disease phenotype or adverse prognostic factors – being polyarthritis, extra-articular manifestations, elevated acute phase reactants and radiographic damage – may somewhat guide clinicians in their therapeutic decision-making, as based on the international PsA management recommendations.(9,13) However, selection of a specific

treatment based on an accurate prediction of the disease course is not possible,(8) and it is unknown if and how the differential response to the available DMARDs could be predicted.(8,9,13)

DMARD group	Generic name		Mechanism of action
Conventional synthetic	Methotrexate	MTX	Induce adenosine accumulation, alter pro-inflammatory cytokine production & modulate humoral / cellular immunity
	Cyclosporin	CSA	Reduces proliferation of activated T cells
	Leflunomide	LEF	Inhibits T cell activation and proliferation
	Sulfasalazine	SSZ	Inhibits NFκB, inhibits osteoclast formation & reduces secretion of pro-inflammatory cytokines
Biologic	Adalimumab	ADA	Anti-TNF-α monoclonal antibody
	Certolizumab pegol	CZP	Anti-TNF-α Fab fragment of monoclonal antibody
	Etanercept	ETN	Anti-TNF-α dimeric TNF receptor p75-IgG I fusion protein
	Golimumab	GOL	Anti-TNF-α monoclonal antibody
	Infliximab	IFX	Anti-TNF-α chimeric monoclonal antibody
	Ustekinumab	UST	Anti-IL-12 and -23 monoclonal antibody to shared p40 subunit
	Ixekizumab	IXE	Anti-IL-17 monoclonal antibody
	Secukinumab	SEC	Anti-IL-17 monoclonal antibody
	Abatacept	ABT	Selectively inhibits T cell co-stimulation
Targeted synthetic	Tofacitinib	TOF	JAK1, -2 and 3 inhibitor
	Apremilast	APR	Intracellular PDE-4 inhibitor

Table 1. DMARDs approved for treatment of psoriatic arthritis. DMARD: disease modifying anti-rheumatic drug; IL: interleukin; JAK: janus kinase; NFκB: nuclear factor kappa B; PDE: phosphodiesterase; TNF: tumor necrosis factor;

This calls for accurate predictors of a favorable drug response to identify patients who will benefit from particular DMARDs. The perfect predictor would be an objective, quantifiable, accurate and reproducible measurable indicator: a biomarker.(15) Biomarkers are an important clinical need to improve personalized medicine in care for patients with PsA.(7,16–19) Currently there is much progress in biomarkers discovery on this topic, which we will summarize here.(16–18,20,21) Moreover, we will highlight their practical clinical use, review ongoing research, discuss future perspectives, and suggest recommendations for future research. The identification of biomarkers for other purposes, including diagnosis, disease onset and disease activity, are discussed elsewhere.(5,17,21–23) The scope of this review concerns predictive molecular biomarkers of drug response.

Methods

A literature search was conducted to identify articles discussing molecular biomarkers predictive of therapeutic response in PsA. PubMed and Embase were searched in September 2019 for combinations of synonyms, MeSH and Emtree terms for 'biomarkers' and 'psoriatic arthritis' (**Supplemental Tables A-C**). In total 1119 articles were identified. Duplicates were removed and 849 articles were screened on title and abstract, based on pre-defined eligibility criteria (**Supplemental Table D**). Consequently, 74 selected articles were screened full-text on relevancy to include in the analysis. The search was supplemented by related citations in PubMed and reference citations of the identified articles in the initial search.

Results

Search

Nine studies identified molecular biomarkers that predict therapy response in PsA (**Figure 1**, **Table 2**). All studies included patients using a tumor necrosis factor-α inhibitor (TNFi): adalimumab, certolizumab pegol, etanercept, golimumab and/or infliximab. Only one study included patients that were administered a non-TNFi.(24) The results are discussed below, subdivided by genetic, circulating and tissue biomarkers.

Genetic biomarkers

PsA is known to harbor a strong genetic inheritable component.(25) The risk ratio for first-degree relatives is up to 40, mainly explained by genetic variants within the human leukocyte antigen (HLA) region.(22,25) MicroRNAs, long non-coding RNAs, gene expression levels, human leukocyte antigen (HLA) variants and single nucleotide polymorphisms (SNPs) have been studied extensively in the search for biomarkers associated with the onset of PsA, its severity and comorbidities.(22) Considering predicting therapeutic response, two polymorphisms (s6920220 and rs610604 (TNFAIP3)) were associated with improved quality-of-life at 3 and 6 months after initiation of TNFi based on the European Quality Of Life (EQ) – Visual Analogue Scale (VAS).(26) However, no associations with other outcomes were observed (Psoriasis Area and Severity Index (PASI) and Numeric Rating Scale (NRS) for pain).

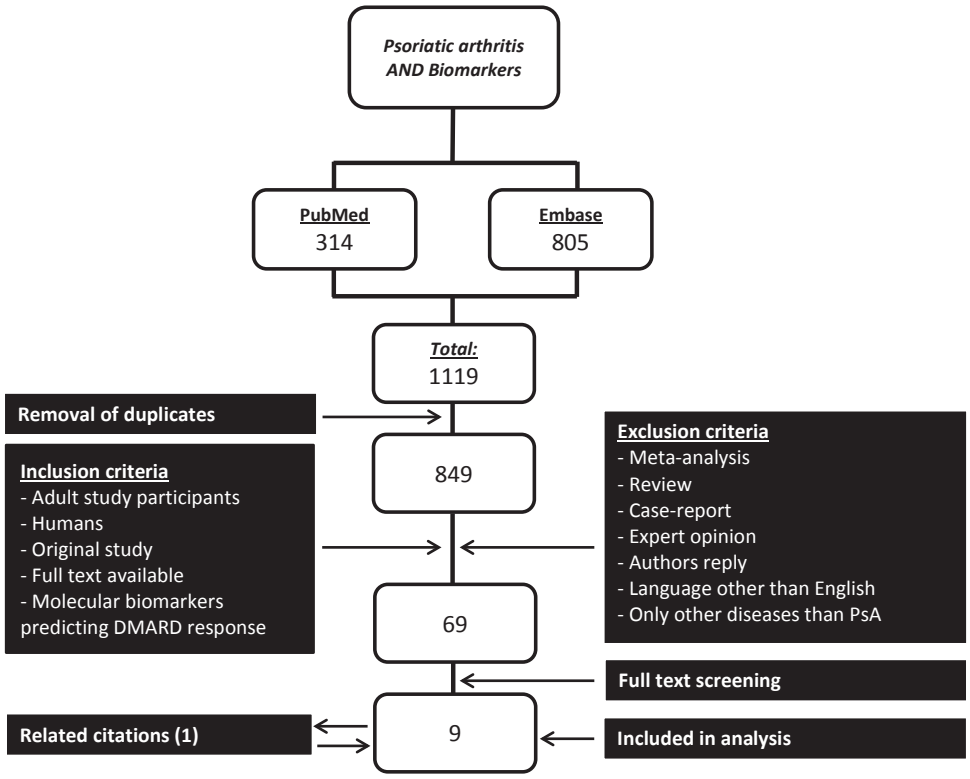

Figure 1. Flowchart. The search yielded 314 articles in PubMed and 805 in Embase. After removal of duplicates 849 articles remained for screening on title and abstract. 69 Articles were screened full-text for relevancy, of which 7 articles were included in the final analysis. One relevant articles was retrieved by assessing reference citations of the selected articles and related citations in PubMed. Abbreviations: DMARD: disease modifying anti-rheumatic drug; PsA: psoriatic arthritis.

Article	Origin Cohort	N	Previous treatment	Therapy (No. patients)	Assessment response (timing)	Material	Biomarker	Analysis	Statistics	Result
Ademowo (2014)	NL (discover)	10	n/a	ADA (10)	DAS28-CRP ≤3.2 and improved >1.2	Synovium	107 proteins	MS	ANOVA (DEPs)	Panel of 57 proteins predictive of response (AUROC 0.76) (including: S100-A8, S100-A10, Ig κ chain C
Ann Rheum Dis	NL (confirm)	18	n/a	ADA (18)	(12 wk)		Panel 57 proteins	MRM-MS assay	Random forest	fibrinogen-α and -γ, haptoglobin, annexin A1 and A2, collagen α-2, vitronectin, α-1 acid glycoprotein)
	IE (validate)	7	n/a	ABT (7)			Panel 57 proteins	MRM-MS assay	Random forest	
Chandran (2013) *J Rheumatol*	CA	40	csDMARD NR	ADA (6), ETN (28), GOL (4), IFX (2) +csDMARD (26)	TJC <3, SJC <1, PASI <4 (11 mo)	Serum	MMP-3	ELISA	Multivariate logistic regression	Higher MMP-3 (36.3±23.8 vs 19.8±6.6 ng/ml) associated with response (OR 1.07, p=0.045)
Chimenti (2012) *Clin Exp Rheumatol*	IT	55	csDMARD NR or contra-indicated	ADA (28), ETN (27) +csDMARD (44)	EULAR response criteria [a] (22 wk)	Plasma	Complement C3	Nephelometry	Multivariate logistic regression	Lower C3 (116.1±25.2 vs 135.5±19.6 mg/dl) associated with response (p=0.011)
Collins (2016) *Proteomics Clin Appl*	IE (pilot 1)	12	csDMARD NR or naïve	ETN (12)	ACR70 and good response EULAR criteria [a] (12 wk)	Synovium	7 proteins	MS	ANOVA (DEPs)	Single proteins associated with response: serum albumin, collagen α 3, annexin A1/2, Ig κ chain C, BTB/POZ domain-containing protein, tryptase
	NL (pilot 2)	10	csDMARD NR or naïve	ADA (10) +MTX (6)	DAS28-CRP ≤3.2 & improved >1.2, and good response EULAR criteria [a] (12 wk)	Synovium	14 proteins	MS	ANOVA (DEPs)	Single proteins associated with response: annexin A1/2, serum albumin, haptoglobin, ApoA1, collagen α3, actin, ρ-GDP-dissociation inhibitor 2, α-1B-glycoprotein, 78kDa glucose-related protein, replication protein A, PK-M1/2, HSP 70/71kDa, vimentin, lamin-B2
Gratacós (2007) *Ann Rheum Dis*	ES	69	MTX NR or contra-indicated	IFX (69)	ACR50 (38 wk)	Serum	CRP	n/a	Multivariate logistic regression	Higher CRP (>10 mg/L) associated with response (OR 18.7, 95% CI 1.8-181.6, p=0.011)
Hellman (2019) *Scand J Rheumatol*	SE	18	TNFi-naïve	ADA (10)	ACR criteria (12 wk)	Serum	Hyaluronan	ELISA	Independent-samples Kruskal Wallis test	Hyaluronan serum levels within reference range (healthy controls 21.7±11.4 ng/mL) associated with better response

Article	Origin Cohort	N	Previous treatment	Therapy (No. patients)	Assessment response (timing)	Material	Biomarker	Analysis	Statistics	Result
Ovejero – Benito (2019) *J Eur Acad Derm Ven*	ES	20	n/a	TNFi (20)	EQ-VAS, NRS-pain50, PASI75 (3 and 6 mo)	Peripheral blood	rs6920220 rs610604	Genotyping	Multivariate logistic regression	SNPs rs6920220 and -610604 associated with improved quality of life at 3 (both) and 6 months (only rs610604) based upon EQ-VAS
Wagner (2013) *Ann Rheum Dis*	US	74	n/a	GOL (74) +csDMARD (26)	ACR20, good or moderate DAS28 response, PASI75 (14 wk)	Serum	2 panels consisting of 4 and 5 proteins	ELISA LUMINEX	Multivariate logistic regression	Two panels predictive: panel A (pyridinoline, adiponectin, PAP, factor VII) for ACR20 (specificity 65%, sensitivity 85%); and panel B (adiponectin, factor VII, SGOT, IgA, leptin) for DAS28 (specificity 71%, sensitivity 90%).

Table 2. Molecular biomarkers predictive of treatment response in psoriatic arthritis. [a)] Van Gestel, *et al.* (30). Abbreviations: ACPA: Anti-Citrullinated Peptide Antibodies; ACR(x): (x% improvement of) ACR response criterion: American College of Rheumatology response criterion based on x% improvement of swollen and tender joints, quality of life questionnaires and three visual analogue scales scoring pain, patients and physicians global assessment of disease activity; ADA: adalimumab; ANOVA: analysis of variance; Apo: apolipoprotein; AUROC: area under the receiver operating characteristic curve; CI: confidence interval; CRP: c-reactive protein; csDMARD: conventional synthetic disease modifying anti-rheumatic drug (e.g. MTX, LEF, SSZ); DAS28: disease activity score, including joint count, visual analogue scale scoring patient's global assessment of disease activity, and acute phase reactants; DEP: differentially expressed protein; ELISA: enzyme-linked immunosorbent assay; EQ-VAS: European quality of life visual analog scale; ESR: erythrocyte sedimentation rate; ETN: etanercept; EULAR: European League Against Rheumatism; GOL: golimumab; GPA: patients global assessment of disease activity; HSP: heat shock protein; IFX: infliximab; IL: interleukin; MMP: matrix metalloproteinase; MRM: multiple reaction monitoring; MS: mass spectrometry; N: number of PsA patients included in the biomarker analysis; NR: non-responder to previous therapy; NRS-pain50: 50% improvement of numeric rating scale for pain; OPG: osteoprotegerin; OR: odds ratio; PASI(75): (75% improvement of) psoriasis area and severity index; PK: pyruvate kinase; PsA: psoriatic arthritis; RA: rheumatoid arthritis; SJC: swollen joint count; SNP: single nucleotide polymorphism; TJC: tender joint count; TNFi: tumor necrosis factor alpha inhibitor (ADA, CZP, ETN, GOL, IFZ); VAS: visual analogue scale.

Circulating biomarkers

Throughout the years several potential circulating biomarkers have been studied, using peripheral blood measurements.(18) Examples include acute phase reactants such as C-reactive protein (CRP), auto-antibodies, cytokines and peripheral blood mononuclear cell subsets. Already by 2007, CRP was suggested as a biomarker predictive of treatment response in refractory PsA.(27) Elevated baseline levels of CRP were associated with good therapeutic response to infliximab with multivariate regression analysis. Response was defined using the American College of Rheumatology (ACR)50 response criterion. CRP is a mediator of the innate immune mechanism of complement activation, and considering that high CRP levels associates with disease progression, CRP levels are widely used to monitor infection, inflammation, chronic disease and tissue injuries.(28)

Lowered baseline levels of the complement component C3 was found to associate with response to adalimumab and etanercept after 22 weeks of treatment,(29) based on the EULAR response criteria.(30) C3 is part of the complement cascade of the innate immune system and disturbances in complement activation might contribute to tissue damage. (31) However, in this study, no significant associations were found with CRP, erythrocyte sedimentation rate or other (activation-induced) complement cleavage products and therapy response.

As potential biomarkers for joint destruction and inflammation, increased baseline levels of matrix metalloproteinase (MMP)-3 were found to associate with response to TNFi therapy defined as PASI <4, tender joint count (TJC) <3 and swollen joint count (SJC) <1.(32) No associations were found for TNF superfamily member 14, receptor activator of NFκB ligand, osteoprotegerin, cartilage oligomeric matrix protein, c-propeptide of type II collagen, type II collagen neoepitopes Col2-3/4C$_{\text{long mono}}$ and C1-2C, aggrecan 846 epitope or CRP. MMP-3 is implicated in cartilage destruction in rheumatic inflammatory diseases,(33) and has shown to be predictive of structural progression in ankylosing spondylitis.(34)

Two protein panels predictive of response to the TNFi golimumab have been published. (35) Both panels revealed adiponectin, which is known to reduce inflammation in various cell types,(36) and factor VII, a blood coagulation factor and antibacterial zymogen,(37) as being predictive for response rate. Response was based upon the ACR20 response criterion and Disease Activity Score (DAS)28 for the first and second panel, respectively.

Finally, low-molecular mass hyaluronan (LMHA) was found predictive: normal serum levels are associated with better response to adalimumab.(38) Response was evaluated with ACR response criteria. LMHA is a polysaccharide present on the surface of epithelial

cells and a known regulator of inflammation and tissue repair by recruiting immune cells and initiating secretion of cytokines.(39) For example, LMHA fragments can activate Toll Like Receptors.(40)

Tissue biomarkers

Inflammation in PsA prototypically occurs at the site of both skin and joint. Some biomarker-finding research has therefore focused on the discovery of tissue biomarkers in the synovium, the synovial lining of joints.(41) In a landmark study on synovial biomarkers in PsA, a panel of 57 proteins was shown to predict response to biologicals assessed by DAS28.(24) Here, an unbiased high throughput approach was used to identify proteins with multiple reaction monitoring mass-spectrometry assays. This was the only study to also include a T cell inhibitor next to TNFi as therapy of interest. The most predictive protein was S100-A8, a known damage-associated molecular pattern and regulator of inflammatory processes and immune response.(42) S100-A8 does so via stimulation of leukocyte recruitment and induction of cytokine secretion.(43) Many other proteins of the panel are also known to be implicated in inflammation.(24)

In another proteomics study using synovial tissue, two panels in two separate cohorts of 7 and 14 proteins were found predictive of TNFi response measured with ACR70, DAS28 and EULAR response criteria.(44) Proteins that changed in both cohorts were haptoglobin, actin, serum albumin, annexin A2, serum amyloid P, Collagen α3 and fibrinogen. These are involved in various pro- and anti-inflammatory processes.(44) However, not all proteins overlapped and validation of these panels was not performed.

Of note, extensive research on synovial fluid in PsA has resulted in various new insights into the molecular basis of the disease, next to identification of both diagnostic as prognostic soluble biomarkers.(45) Yet our search revealed no studies on synovial fluid biomarkers predictive for therapy response. In addition, there have been no studies examining the skin of PsA patients as predictor of therapy response, whereas this is currently being explored for predicting PASI response in psoriasis patients.(46,47)

Discussion

Challenges of implementation

Altogether the abovementioned studies provide experimental support for the predictive value of biomarkers for therapeutic response. However, none are currently implemented

in routine practical clinical care.(8,22,48,49) Here we discuss possible explanations for the obstruction between biomarker discovery and the following steps of validation, clinical implementation and evaluation.(50)

To find a predictor of therapy response, the definition of response should be unequivocal. However, in PsA this is not straightforward. Firstly because PsA - although now known as a clinical entity characterized by a distinct pathogenesis, phenotype and course (7,16,51) - has long been considered a relatively mild form of rheumatoid arthritis (RA). (7) This led to initially copying outcome measures like DAS28,(7,30,51–53) which do not include PsA-specific manifestations.(5,52) This reduces the clinical applicability of some previously published work. Secondly, the heterogeneous disease manifestations of PsA make it difficult to define response.(5,7,51,52) For example, ACR response criteria are useful to assess peripheral arthritis. However, for disease activity of skin, dactylitis, axial spondyloarthritis and enthesitis other outcome measures are required. Since patients exhibit different disease phenotypes, treatment goals vary based upon their individual needs and complaints. It might thus be relevant to identify predictive biomarkers for specific clinical manifestations, next to pooling response to all disease manifestations as a whole.

Furthermore, the hypothesis that differences in immune pathogenesis underlie the heterogeneous disease manifestations,(54) raises the question which tissue site should be studied for biomarker discovery: skin, synovium, synovial fluid or peripheral blood? For example, skin biomarkers may predict psoriasis remission, but not reduction in dactylitis or enthesitis. In this respect it is further important to acknowledge that even the same broad type of "tissue" (e.g. skin) shows site-specific differences in steady state across the human body.(55,56) The ideal biomarker is also practical and safe to obtain and this should be taken into account.(24)

Finally, there are overarching challenges with respect to biomarker implementation in clinical practice.(50) Analysis methods used for discovery are frequently costly, technically difficult and labor-intensive.(17,50) Importantly, external validation is often lacking in studies that report on discovery of a new biomarker (50) and thus validation of candidate biomarkers has been difficult.(18,57) For example, a predictive value of CRP for TNFi responders (27) was validated by a single study,(58) but not confirmed by three others.(29,32,35) Generally speaking, a biomarker for clinical use needs to demonstrate excellent sensitivity and specificity, thus a single biomarkers (e.g. protein) may lack the ability to singularly predict a certain outcome or definitive diagnosis.(18,59)

Taking all these challenges into account, it might not be surprising that no biomarkers predictive of treatment response are implemented yet. It has becomes increasingly evident that it is a challenge to develop robust, reproducible, cheap and fast assays that are validated in representative PsA patient cohorts.(60)

Emerging tools & approaches for biomarker research

In the past decade, advances in research have led to improved understanding of PsA etiology.(8,22) The current consensus is that the pathogenesis is multifactorial,(8) and this awareness has resulted in expanding the field of biomarker discovery to include (epi)genetics, proteins, metabolites, microbiome and environmental factors.(20) In the few years technical advances have made it possible to extensively study all these 'multi-omics' with unbiased approaches, using various next-generation, high-throughput technologies.(61) For example in the field of epigenomics, multiple players in disease pathogenesis – including DNA methylation sites, histone modifications and microRNAs - were discovered with pan-genomic microarrays.(62,63) Also in other omics field – like proteomics, transcriptomics, exposomics, metabolomics and microbiomics - next-generation techniques are increasingly applied for biomarker discovery.(18,44,45,63–66) These evolving technologies result in large amounts of data, requiring computational modeling for advanced analyses and integration of multiple omics datasets to produce composite panels of biomarkers.(18,64,67) These advances may help drive future biomarker research, with a critical role for bioinformatics to analyze and integrate large omics datasets.(16,68)

Future perspectives

Currently, biomarkers make up a notable part of the research agenda in rheumatology in the search for tools to improve personalized medicine.(21) Also within the PsA field researchers have made great strides. Recently, a trial was conducted that evaluated treatment efficacy of different drugs based on standard care versus strategically selected bDMARD choice, the latter guided by phenotypes of peripheral T helper cell characteristics.(69) They found significantly more low disease activity after six months in the patients that received strategically selected drugs, showing the potential benefits of personalized medicine. Further trials to explore this concept are mandatory.(70) Furthermore, **Table 3** highlights some promising research on the topic of biomarkers predictive of therapy response in PsA.(58,71–75) Our own group has initiated the TOFA-PREDICT study (EudraCT number 2017-003900-28), which is a multicenter randomized clinical trial in the

Netherlands, integrating multiple data layers to predict treatment response to cs-, b- and tsDMARDs. Moreover, important data are expected from the OUTPASS study, a United Kingdom prospective observational cohort of 300 patients to investigate serological, clinical, genetic and psychological factors influencing PsA response to biologics (UKCRN number 13910).(76)

Abstract	N	Drug	Definition response	Biomarker	Result
Conti Ceccarelli (2019) *Ann Rheum Dis*	17	APR	EULAR criteria	Treg	Higher proportion of Tregs within CD4+ T cell population associated with response
David (2019) *Rheumatology*	50	Biologics	EULAR criteria, DAS28	HLA-B27	No association of HLA-B27 with response
Mascia (2019) *J Psoriasis PsA*	70	TNFi	PsARC, ACR20	SNP TNF-α genomic region	Significant association of SNP-29 with response
Scrivo (2019) *Clin Exp Rheumatol*	151	GOL	MDA	hsCRP	Higher hsCRP predictive of response

Table 3. Promising research on molecular biomarkers predictive of treatment response in PsA. Abbreviations: ACR response: American College of Rheumatology response criterion; APR: apremilast; DAS28: disease activity score; GOL: golimumab; HLA: human leukocyte antigen; hsCRP: high sensitive C-reactive protein; MDA: minimal disease activity score; PsA: psoriatic arthritis; PsARC: Psoriatic Arthritis Response Criteria; SNP: single nucleotide polymorphism; TNF: tumor necrosis factor; TNFi: tumor necrosis factor alpha inhibitor (ADA, CZP, ETN, GOL, IFZ); Treg: T regulatory cell.

Recommendations

Research initiatives in the field of biomarker discovery towards prediction of PsA therapy response are ongoing, and the discovery and validation of these biomarkers is internationally considered an important and urgent clinical need and therefore a recurring topic on international research agendas.(1,16) Since no biomarkers have been implemented in clinical care, we formulate specific points of consideration to improve the clinical utility of future biomarker research results (**Table 4**).

Firstly, it is important to include DMARDs other than only TNFi.(3) Data on biomarkers predictive of response to targeted synthetic DMARDs, IL-17 inhibitors and IL-12/23 inhibitors are lacking to date. Since these treatment options are currently recommended in international guidelines and increasingly selected by clinicians,(9) research on predictive biomarkers for these therapies is warranted.

Difficulty	Recommendation
• Large repertoire of available DMARDs	• Include other therapies than TNF-α inhibitors
• Defining therapy response	• Careful selection of the most appropriate outcome measure based on treatment and research goals
• High false-positive rates in biomarker discovery	• Validation in independent external cohorts of patients • Exploring not single, but combinations of biomarkers
• Invasive procedure for obtaining tissue material, followed by costly, labor-intense and technically difficult analyses methods	• Translation and adaption of discovery assays into less-invasive, affordable and technically simple assays for clinical implementation
• Relatively low disease prevalence	• Sharing of clinical, imaging, molecular data • International collaborations of patient representatives, health care professionals, non-profit organizations and pharmaceutical companies

Table 4. Recommendations for research on biomarkers predicting therapy response in PsA. Abbreviations: DMARD: disease modifying anti-rheumatic drug; PsA: psoriatic arthritis; TNF: tumor necrosis factor.

Secondly, future research needs accurate and robust composite responder indices, that take into account the core domains of this heterogeneous disease. To answer to this need, the Outcome Measures in Rheumatology (OMERACT) group published in 2017 an updated core outcome set for research.(77) Since one scoring system might not be attainable for all patients,(53) outcome measures should be carefully selected dependent on both the treatment goals, as well as the research goals.

Thirdly, it is critical for researchers to not only discover, but also confirm and validate their findings in independent external cohorts of patients, since false-positive biomarker leads are unfortunately quite common in large datasets.(48) Preferably, after validation the assays would be translated and adapted into non-invasive, affordable and technically simple assays.

Fourthly, it is plausible that single biomarkers might not exceed the thresholds for accurate and robust prediction of clinical outcomes.(18) Rather, we recommend first computationally exploring a broad range of biomarkers (based on different -omics approaches) in large cohorts of PsA patients followed throughout time, before and after treatment. In doing so, it may be possible to develop panels of biomarkers that reflect changes in clinical manifestations and response (or lack thereof) to treatment.(16,18)

This brings us to our last recommendation: the sharing of data. Considering that PsA is a relatively uncommon disease, the sharing of patient data - clinical characteristics, demographics, imaging and omics – would enable higher patient numbers to discover and validate new findings. Improved collaboration with computational biologists will be critical for success.(78) The fruitful result of such a collaboration has already been described by Ademowo and collegues.(24) They used not only Irish patients for their biomarkers discovery, but also included a cohort of Dutch patients for validation of their findings. Another illustration of this line of thinking is the 'ArthroMark' project.(79) These German researchers created a cooperation between several national institutions that share their resources in a consortium for biomarker analysis in rheumatic diseases. Patient data is expected to be of more value if clinicians would accurately record clinical characteristics and collaborate with fundamental researchers and experts in genetics. (22) Another example in the field of collaborative projects is the Accelerating Medicines Partnership (AMP), an American partnership between the National Institutes of Health, Food and Drug Administration, pharmaceutical companies and non-profit organizations. They have already conducted some interesting research in the field of RA.(80,81) Projects like these are likely to optimize the process of discovery and validation of not only molecular biomarkers in this field, but also clinical and imaging biomarkers.(79)

Conclusion

PsA is a potentially aggressive inflammatory musculoskeletal disorder, which may severely impact physical function and quality-of-life. Currently it is not possible to predict which patients respond to which particular treatment, which underlines the urgent clinical need for biomarkers predictive of drug response to optimize personalized medicine. Biomarker discovery has shown to be a time-consuming and difficult process, in which discovery has to be followed by confirmation, validation, clinical implement and evaluation. Promising new tools and approaches are emerging to identify new molecular biomarkers in omics datasets with computational modeling analyses. As several research groups are working on identification and validation of such markers in PsA, we anticipate that this urgent clinical need will be answered in the future to reduce health care costs and improve patient care.

Limitations

We are aware that our review has limitations. One important limitation is the reporting bias that is inherent to literature reviews,(82) through which we might have missed

research that studied biomarkers and did not find any predictive capacities of the biomarkers listed here. Moreover, given that we only included articles with (synonyms of) 'psoriatic arthritis' in their title or abstract we might have missed articles primarily describing biomarkers in the context of psoriasis or spondylarthropathies.

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References

1. Jadon, D. R. *et al.* Proceedings of the 2017 GRAPPA Collaborative Research Network Meeting. *J. Rheumatol. Suppl.* 94, 54–61 (2018).
2. Menegatti, S. *et al.* Anti-TNF therapy in spondyloarthritis and related diseases, impact on the immune system and prediction of treatment responses. *Front. Immunol.* 10, 382 (2019).
3. Gossec, L. *et al.* European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann. Rheum. Dis.* 75, 499–510 (2016).
4. Scotti, L., Franchi, M., Marchesoni, A. & Corrao, G. Prevalence and incidence of psoriatic arthritis: A systematic review and meta-analysis. *Semin. Arthritis Rheum.* 48, 28–34 (2018).
5. Ritchlin CT, Colbert RA, G. D. Psoriatic Arthritis. *NEJM* 10, (2017).
6. McHugh, N. *et al.* Evaluation of the Economic Burden of Psoriatic Arthritis and the Relationship Between Functional Status and Healthcare Costs. *J. Rheumatol.* 47, 701-707 (2020).
7. Haroon, M. & FitzGerald, O. Psoriatic arthritis: complexities, comorbidities and implications for the clinic. *Expert Rev. Clin. Immunol.* 12, 405–416 (2016).
8. Veale, D. J. & Fearon, U. Psoriatic arthritis 1 The pathogenesis of psoriatic arthritis. *Lancet.* 391, 2273–2284 (2018).
9. Gossec, L. *et al.* Management of psoriatic arthritis in 2016: a comparison of EULAR and GRAPPA recommendations. *Nat. Rev. Rheumatol.* 12, 743–750 (2016).
10. Kang, E. J. & Kavanaugh, A. Psoriatic arthritis: latest treatments and their place in therapy. *Ther. Adv. Chronic Dis.* 6, 194–203 (2015).
11. D'Angelo, S., Tramontano, G., Gilio, M., Leccese, P. & Olivieri, I. Review of the treatment of psoriatic arthritis with biological agents: choice of drug for initial therapy and switch therapy for non-responders. *Open Access Rheumatol. Res. Rev.* 9, 21–28 (2017).
12. FitzGerald, O. & Ritchlin, C. Opportunities and challenges in the treatment of psoriatic arthritis. *Best Pract. Res. Clin. Rheumatol.* 32, 440–452 (2018).
13. Van Den Bosch, F. & Coates, L. Clinical management of psoriatic arthritis. *Lancet.* 391, 2285-2294 (2018).
14. Singh, J. A. *et al.* 2018 American College of Rheumatology/National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis. *Arthritis Care Res. (Hoboken).* 71, 2-29 (2019).
15. Strimbu, K. & Tavel, J. A. What are Biomarkers? *Curr. Opin. HIV AIDS* 5, 463 (2010).
16. Villanova, F., Meglio, P. Di & Nestle, F. O. Biomarkers in psoriasis and psoriatic arthritis. *Ann. Rheum. Dis.* 72, ii104-ii110 (2013).
17. Berekmeri, A., Mahmood, F., Wittmann, M. & Helliwell, P. Expert Review of Clinical Immunology Tofacitinib for the treatment of psoriasis and psoriatic arthritis. *Expert Rev. Clin. Immunol.* 0, (2018).
18. Verheul, M. K., Fearon, U., Trouw, L. A. & Veale, D. J. Biomarkers for rheumatoid and psoriatic arthritis. *Clin. Immunol.* 161, 2–10 (2015).
19. Paek, S. Y. *et al.* Emerging biomarkers in psoriatic arthritis. *IUBMB Life* 67, 923–927 (2015).
20. Generali, E., Scirè, C. A., Favalli, E. G. & Selmi, C. Expert Review of Clinical Immunology Biomarkers in psoriatic arthritis: a systematic literature review Biomarkers in psoriatic arthritis: a systematic literature review. *Expert Rev Clin Immunol.* 12, 651-60 (2016).

21. Chandran, V. & Scher, J. U. Biomarkers in psoriatic arthritis: recent progress. *Curr. Rheumatol. Rep.* 16, 453 (2014).
22. Furst, D. E., Belasco, J. & Louie, J. S. Genetic and inflammatory factors associated with psoriatic arthritis: Relevance to diagnosis and management. *Clin. Immunol.* 202, 59–75 (2019).
23. Iragorri, N., Hazlewood, G., Manns, B., Danthurebandara, V. & Spackman, E. Psoriatic arthritis screening: a systematic review and meta-analysis. *Rheumatology (Oxford).* 58, 692-707 (2019).
24. Ademowo, O. S. *et al.* Discovery and confirmation of a protein biomarker panel with potential to predict response to biological therapy in psoriatic arthritis. *Ann. Rheum. Dis.* 75, 234–241 (2016).
25. Karason, A., Love, T. J. & Gudbjornsson, B. A strong heritability of psoriatic arthritis over four generations--the Reykjavik Psoriatic Arthritis Study. *Rheumatology.* 48, 1424–1428 (2009).
26. Ovejero-Benito, M. C. *et al.* Polymorphisms associated with anti-TNF drugs response in patients with psoriasis and psoriatic arthritis. *J. Eur. Acad. Dermatology Venereol.* 33, e175–e177 (2019).
27. Gratacós, J., Casado, E., Real, J. & Torre-Alonso, J. C. Prediction of major clinical response (ACR50) to infliximab in psoriatic arthritis refractory to methotrexate. *Ann. Rheum. Dis.* 66, 493–7 (2007).
28. Wu, Y., Potempa, L. A., Kebir, D. El & Filep, J. G. C-reactive protein and inflammation: conformational changes affect function. *Biol. Chem* 396, 1181–1197 (2015).
29. Chimenti, M. et al. Complement system in psoriatic arthritis: a useful marker in response prediction and monitoring of anti-TNF treatment. *Clinical and Experimental Rheumatology* 30, (2012).
30. van Gestel, A. M. *et al.* Development and validation of the european league against rheumatism response criteria for rheumatoid arthritis: Comparison with the preliminary american college of rheumatology and the world health organization/international league against rheumatism criteria. *Arthritis Rheum.* 39, 34–40 (1996).
31. Walport, M. J. Complement. *N. Engl. J. Med.* 344, 1058–1066 (2001).
32. Chandran, V. *et al.* Soluble Biomarkers Associated with Response to Treatment with Tumor Necrosis Factor Inhibitors in Psoriatic Arthritis. *J. Rheumatol.* 40, 866–871 (2013).
33. Ribbens, C. *et al.* Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment. *Ann. Rheum. Dis.* 61, 161–6 (2002).
34. Maksymowych, W. P. *et al.* Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum.* 56, 1846–1853 (2007).
35. Wagner, C. L. *et al.* Markers of inflammation and bone remodelling associated with improvement in clinical response measures in psoriatic arthritis patients treated with golimumab. *Ann. Rheum. Dis.* 72, 83–88 (2013).
36. Wang, Z. V & Scherer, P. E. Adiponectin, the past two decades. *J. Mol. Cell Biol.* 8, 93–100 (2016).
37. Chen, J. *et al.* Coagulation factors VII, IX and X are effective antibacterial proteins against drug-resistant Gram-negative bacteria. *Cell Res.* 29, 711–724 (2019).
38. Hellman, U., Engström-Laurent, A., Larsson, A. & Lindqvist, & U. Hyaluronan concentration and molecular mass in psoriatic arthritis: biomarkers of disease severity, resistance to treatment, and outcome. *Scand J Rheumatol*. 48, 284-293 (2019).
39. Jiang, D., Liang, J. & Noble, P. W. Hyaluronan as an immune regulator in human diseases. *Physiol. Rev.* 91, 221–64 (2011).
40. Marshak-Rothstein, A. Toll-like receptors in systemic autoimmune disease. *Nat. Rev. Immunol.* 6, 823–835 (2006).

41. Kuijk, A. W. R. van *et al.* A prospective, randomised, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue. *Ann. Rheum. Dis.* 68, 1303–1309 (2009).
42. Cesaro, A. *et al.* An Inflammation Loop Orchestrated by S100A9 and Calprotectin Is Critical for Development of Arthritis. *PLoS One* 7, e45478 (2012).
43. Wang, S. *et al.* S100A8/A9 in Inflammation. *Front. Immunol.* 9, 1298 (2018).
44. Collins, E. S. *et al.* A clinically based protein discovery strategy to identify potential biomarkers of response to anti-TNF-α treatment of psoriatic arthritis. *Proteomics - Clin. Appl.* 10, 645–662 (2016).
45. Mahendran, S. M. & Chandran, V. Exploring the Psoriatic Arthritis Proteome in Search of Novel Biomarkers. *Proteomes* 6, (2018).
46. Krueger, J. G. *et al.* IL-17A inhibition by secukinumab induces early clinical, histopathologic, and molecular resolution of psoriasis. *J. Allergy Clin. Immunol.* 144, 750–763 (2019).
47. Visvanathan, S. *et al.* Psoriatic skin molecular and histopathologic profiles after treatment with risankizumab versus ustekinumab. *J. Allergy Clin. Immunol.* 143, 2158–2169 (2019).
48. Gibson, D. S. *et al.* Biomarkers in rheumatology, now and in the future. *Rheumatology* 51, 423–433 (2012).
49. Winthrop, K. L. *et al.* Unmet need in rheumatology: reports from the Targeted Therapies meeting 2018. *Ann. Rheum. Dis.* 78, 872–878 (2019).
50. Ioannidis, J. P. A. & Bossuyt, P. M. M. Waste, Leaks, and Failures in the Biomarker Pipeline. *Clin. Chem.* 63, 963–972 (2017).
51. Lancet, T. Psoriatic arthritis: classification and holistic management. *Lancet* 391, 2185 (2018).
52. Solmaz, D., Eder, L. & Aydin, S. Z. Update on the epidemiology, risk factors, and disease outcomes of psoriatic arthritis. *Best Pract. Res. Clin. Rheumatol.* 32, 295–311 (2018).
53. Orbai, A.-M. Content validity of psoriatic arthritis composite indices: anchoring with the patient perspective and the core domain set. *Rheumatology.* 59,1-4 (2020).
54. FitzGerald, O., Haroon, M., Giles, J. T. & Winchester, R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. *Arthritis Res. Ther.* 17, 115 (2015).
55. Ospelt, C. & Frank-Bertoncelj, M. Why location matters-site-specific factors in rheumatic diseases. *Nat. Rev. Rheumatol.* 13, 433–442 (2017).
56. Del Duca, E. *et al.* Major Differences in Expression of Inflammatory Pathways in Skin from Different Body Sites of Healthy Individuals. *J. Invest. Dermatol.* 139, (2019).
57. Brower, V. Biomarkers: Portents of malignancy. *Nature* 471, S19–S20 (2011).
58. Scrivo, R. *et al.* An observational prospective study on predictors of clinical response at six months in patients with active psoriatic arthritis treated with golimumab. *Clin. Exp. Rheumatol.* 38, 107-114 (2019).
59. Chambers, A. G., Percy, A. J., Yang, J., Camenzind, A. G. & Borchers, C. H. Multiplexed quantitation of endogenous proteins in dried blood spots by multiple reaction monitoring-mass spectrometry. *Mol. Cell. Proteomics* 12, 781–91 (2013).
60. Mc Ardle, A. *et al.* Early biomarkers of joint damage in rheumatoid and psoriatic arthritis. *Arthritis Res. Ther.* 17, 141 (2015).
61. Hasin, Y., Seldin, M. & Lusis, A. Multi-omics approaches to disease. *Genome Biol.* 18, 83 (2017).
62. Robinson, W. H. & Mao, R. Biomarkers to guide clinical therapeutics in rheumatology? *Curr. Opin. Rheumatol.* 28, 168–75 (2016).

63. Celis, R. *et al.* Psoriatic synovitis: Singularity and potential clinical implications. *Front. Med.* 6, 14 (2019).

64. Lorenzon, R. *et al.* Clinical and multi-omics cross-phenotyping of patients with autoimmune and autoinflammatory diseases: The observational TRANSIMMUNOM protocol. *BMJ Open* 8, 1–8 (2018).

65. Lambrecht, S., Deforce, D. & Elewaut, D. Entering the era of proteomics in rheumatology. *Expert Opin. Drug Discov.* 1, 539–548 (2006).

66. Nair, N. *et al.* Mass cytometry as a platform for the discovery of cellular biomarkers to guide effective rheumatic disease therapy. *Arthritis Res. Ther.* 17, 127 (2015).

67. Tasaki, S. *et al.* Multi-omics monitoring of drug response in rheumatoid arthritis in pursuit of molecular remission. *Nat. Commun.* 9, (2018).

68. Ritchlin, C. Biomarker development in psoriatic arthritis. *J. Rheumatol. Suppl.* 89, 57–60 (2012).

69. Miyagawa, I. *et al.* Precision medicine using different biological DMARDs based on characteristic phenotypes of peripheral T helper cells in psoriatic arthritis. *Rheumatology* 58, 336–344 (2019).

70. Miyagawa, I., Nakayamada, S. & Tanaka, Y. Optimal Biologic Selection for Treatment of Psoriatic Arthritis: the Approach to Precision Medicine. *Curr. Rheumatol. Rep.* 21, 21 (2019).

71. Ceccarelli F., *et al.* T regulatory cells as biomarker of disease activity and response in psoriatic Arthritis patients: Results from apremilast-treated cohort. *Ann. Rheum. Dis.* 78, 864 (2019).

72. David, T., Jani, M., Bowes, J., Chinoy, H.. Is HLA-B27 a predictor of treatment response to biologics in psoriatic arthritis? *Rheumatol. (United Kingdom)* 58, iii128 (2019).

73. Mascia, E., Orru, S., Mathieu, A., Cauli, A. Genetic variants in the TNF-alpha region: A novel biomarker of clinical response to anti TNF-alpha drugs in psoriatic arthritis patients. *J. Psoriasis Psoriatic Arthritis* 4, 99 (2019).

74. Ridker, P. M. High-Sensitivity C-Reactive Protein. *Circulation* 103, 1813–1818 (2001).

75. Musunuru, K. *et al.* The use of high sensitivity C-reactive protein in clinical practice. *Nat. Clin. Pract. Cardiovasc. Med.* 5, 621 (2008).

76. Jani, M., Chinoy, H. & Barton, A. The Journal of Rheumatology Treatment in Psoriatic Arthritis Study Syndicate of long-term disability in patients with psoriatic arthritis: results from the Outcomes Association of pharmacological biomarkers with treatment response and. *J. Rheumatol. Rheumatol. J. Oct.* 10, (2019).

77. Orbai, A.-M. *et al.* International patient and physician consensus on a psoriatic arthritis core outcome set for clinical trials. *Ann. Rheum. Dis.* 76, 673–680 (2017).

78. Gossec, L. *et al.* EULAR points to consider for the use of big data in rheumatic and musculoskeletal diseases. *Ann. Rheum. Dis.* 79, 69-76 (2020).

79. Häupl, T. *et al.* Biomarker und Bildgebung zur Diagnose und Stratifizierung der rheumatoiden Arthritis und Spondylarthritis im BMBF-Verbund ArthroMark. *Z. Rheumatol.* 77, 16–23 (2018).

80. Orange, D. E. *et al.* Identification of Three Rheumatoid Arthritis Disease Subtypes by Machine Learning Integration of Synovial Histologic Features and RNA Sequencing Data. *Arthritis Rheumatol. (Hoboken, N.J.)* 70, 690–701 (2018).

81. Zhang, F. *et al.* Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* 20, 928–942 (2019).

82. Duyx, B., Swaen, G. M. H., Urlings, M. J. E., Bouter, L. M. & Zeegers, M. P. The strong focus on positive results in abstracts may cause bias in systematic reviews: a case study on abstract reporting bias. *Syst. Rev.* 8, 174 (2019).

Supplemental Information

Category	Biomarker	Psoriatic arthritis
MeSH term	Biomarkers	Arthritis, Psoriatic
Emtree term	Biological marker	Psoriatic arthritis
Synonyms	Biologic marker* Biological marker* Biomarker* Laboratory marker* Serum marker*	Arthritic psoriasis Arthropathic psoriasis Psoriasis arthropathica Psoriatic arthritis Psoriatic arthropathies Psoriatic arthropathy

Supplemental Table A. Literature search. Abbreviations: Emtree: Embase subject headings; MeSH: medical subject headings.

Search term	Syntax	Results [a)]
1. Biomarker	(((((Biomarkers[MeSH Major Topic]) OR Biologic Marker*[Title/Abstract]) OR Biological Marker*[Title/Abstract]) OR Biomarker*[Title/Abstract]) OR Laboratory Marker*[Title/Abstract]) OR Serum Marker*[Title/Abstract]	449.854
2. Psoriatic arthritis	((((((Arthritis, Psoriatic[MeSH Major Topic]) OR Arthritic psoriasis[Title/Abstract]) OR Arthropathic psoriasis[Title/Abstract]) OR Psoriasis arthropathica[Title/Abstract]) OR Psoriatic arthritis[Title/Abstract]) OR Psoriatic arthropathies[Title/Abstract]) OR Psoriatic arthropathy[Title/Abstract]	9.163
1 AND 2	(((((((Biomarkers[MeSH Major Topic]) OR Biologic Marker*[Title/Abstract]) OR Biological Marker*[Title/Abstract]) OR Biomarker*[Title/Abstract]) OR Laboratory Marker*[Title/Abstract]) OR Serum Marker*[Title/Abstract])) AND (((((((Arthritis, Psoriatic[MeSH Major Topic]) OR Arthritic psoriasis[Title/Abstract]) OR Arthropathic psoriasis[Title/Abstract]) OR Psoriasis arthropathica[Title/Abstract]) OR Psoriatic arthritis[Title/Abstract]) OR Psoriatic arthropathies[Title/Abstract]) OR Psoriatic arthropathy[Title/Abstract])	314

Supplemental Table B. Search syntax PubMed. [a)] Search conducted on the 3rd of September 2019.

Search term	Syntax	Results [a)]
1. Biomarker	'biological marker'/de OR 'biologic marker*':ab,ti OR 'biological marker*':ab,ti OR biomarker*:ab,ti OR 'laboratory marker*':ab,ti OR 'serum marker*':ab,ti	464.737
2. Psoriatic arthritis	'psoriatic arthritis'/de OR 'arthritic psoriasis':ab,ti OR 'psoriasis arthropathica':ab,ti OR 'psoriatic arthritis':ab,ti OR 'psoriatic arthropathies':ab,ti OR 'psoriatic arthropathy':ab,ti	23.233
1 AND 2	('biological marker'/exp OR 'biological marker' OR 'biologic marker*':ab,ti OR 'biological marker*':ab,ti OR biomarker*:ab,ti OR 'laboratory marker*':ab,ti OR 'serum marker*':ab,ti) AND ('psoriatic arthritis'/exp OR 'psoriatic arthritis' OR 'arthritic psoriasis':ab,ti OR 'psoriasis arthropathica':ab,ti OR 'psoriatic arthritis':ab,ti OR 'psoriatic arthropathies':ab,ti OR 'psoriatic arthropathy':ab,ti)	805

Supplemental Table C. Search syntax Embase. [a)] Search conducted on the 4th of September 2019.

Inclusion	- Adult study participants - Humans - Original study - Full text available - Molecular biomarkers predicting DMARD response
Exclusion	- Meta-analysis - Review - Case-report - Expert opinion - Authors reply - Language other than English - Only other diseases than PsA

Supplemental Table D. Eligibility criteria. Abbreviations: DMARD: disease modifying anti-rheumatic drug; PsA: psoriatic arthritis.

Chapter 9

Summary and general discussion

Medicine is a science of uncertainty and an art of probability.

– prof. William Osler (1849 -1919)

This phrase – although it dates back more than 100 years - has stood the test of time. Despite increasing experience, knowledge and therapies, in modern medicine clinicians still handle great uncertainties while treating patients. It is evident that this applies to patients with psoriatic arthritis (PsA), as underlined by **Chapter 1**. Currently, there is incomplete understanding of pathophysiology, uncertainty about the critical factors that contribute to PsA transition, and lack of robust methods to select treatment. With new treatment options becoming available, it is increasingly difficult for rheumatologists to know which therapy is best for which patient.(1) Postponing efficacious therapy - even for six months - can result in progression of joint erosions, decreased long-term physical function and reduced risk of medication-free remission.(2–5) Hence, there is an urgent clinical need to tailor medical treatment to the individual patient: precision medicine. Over recent years, basis scientists and academic researchers have studied various strategies for patient stratification to select therapies for individual patients. Moreover, genetics and immune dysregulation were investigated to discover patient-specific drivers for inflammation. Identification of these mechanisms is deemed essential for personalized healthcare, as they provide insights for therapeutic targets, risk stratification and prediction of treatment response.(6) In **Chapter 9** we discuss advances – including our contributions – that were made to unravel the complexity of PsA, on a journey towards precision medicine.

Stratification by …

… Disease

The fact that PsA is different from rheumatoid arthritis (RA) was not common knowledge at the time that PsA was discovered.(7) It took decades to conclude that these types of inflammatory arthritis affect different components of the immune system, that lead to divergent clinical features.(8) Meanwhile, treatment regimens were copied from RA to PsA without evaluation of their efficacy in clinical trials, resulting in the pragmatic prescription of conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs): methotrexate, leflunomide and sulfasalazine.(9) After it became clear that RA and PsA are different disease entities, only a handful of controlled trials was performed to study csDMARD efficacy in PsA.(10–15) Results were not always consistent,

but most placebo-controlled studies observed small effects on peripheral arthritis. In addition, four studies - including our retrospective cohort study presented in **Chapter 7** – provide indirect evidence for csDMARD efficacy by investigating their retention rate.(16–18) Despite these efforts, evidence supporting the recommendation of csDMARD use in PsA remains limited.(19–21) Moreover, uncertainty remains for their efficacy concerning radiographic progression and disease domains other than arthritis, including dactylitis, enthesitis and axial spondyloarthritis.(9) Another (more general) difficulty concerning the use of csDMARDs – especially methotrexate – are their safety and tolerability.(22) A recent prospective study again confirmed that the magnitude and frequency of nausea and fatigue experienced by PsA patients that use MTX is substantial.(23) In support, in **Chapter 7** we observed a significant proportion of patients (28%) that failed csDMARD monotherapy, because of insurmountable side-effects including gastro-intestinal complaints, malaise and hepatotoxicity.

Despite their side effect profile and lack of high-level evidence for efficacy, csDMARDs are still recommended as first-line treatment of peripheral arthritis in PsA by international guidelines.(24–26) Moreover, health insurance companies in The Netherlands do not reimburse costs of biologic treatments for csDMARD naive patients. I find this surprising, given the fact that current medical practice is based on evidence based medicine. The question arises why csDMARDs are still frequently prescribed, and why randomized controlled trials investigating csDMARD efficacy in PsA are so scarce. Might it be explained by the fact that rheumatologists considered csDMARD efficacy sufficiently proven based on expert opinions? Or that placebo-controlled trials were considered unethical with presumably effective therapies available? Or could pharmaceutical companies have focused only on new (more profitable) therapies? Regardless of the reasons, the increasing number of biologicals (bDMARDs) and targeted synthetics (tsDMARDs) becoming available underline the necessity for head-to-head trials to directly compare efficacy in all domains of PsA.(22) The ongoing TOFA-PREDICT clinical trial aims to fill the void by comparing treatment response of methotrexate, etanercept and tofacitinib (EudraCT Number: 2017-003900-28, further discussed under 'Novel approaches'). With regards to treatment recommendations in light of precision medicine, taking into consideration all available evidence, I find that the American guideline – that recommends starting tumor necrosis factor alpha inhibitors (TNFi) over csDMARDs in therapy naive PsA – is better attuned to our patients' needs.(27)

… Patient preferences

An obvious note, but clinicians have to consider personal preferences of patients while selecting one of many available therapies. A patient may strongly prefer topical over systemic treatment, oral over subcutaneous administration, or weekly over daily dosing. Moreover, the available DMARD therapies for PsA are associated with a wide range of side effects, that may prompt patients to decline or discontinue their treatment. These side effects and alarm symptoms of serious side effects are to be discussed with our patients, before they agree with initiating treatment. Hence, to achieve patient's personal treatment goals and to improve drug adherence, shared decision making is fundamental for precision medicine.(24)

… Clinical characteristics

In former times, clinicians could only rely on clinical characteristics for clinical decision making in PsA. Some of these characteristics – including disease domain, severity, comorbidity and response to previous treatments – associate with differential response to nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular glucocorticoids and DMARDs. For example, patients with predominantly enthesial or axial disease respond to biologics and not to csDMARDs.(24,28) For cutaneous psoriasis, interleukin (IL)-17 or IL-12/23 inhibitors appear the most efficacious.(29–31) Data from clinical trials revealed that patients with concomitant inflammatory bowel disease or uveitis are best treated with TNFi.(32) Further, clinical factors were used to develop a prognostic model for leflunomide toxicity.(33) Moreover, PsA patients with poor prognostic factors (polyarthritis, radiographic damage, structural damage, elevated acute phase reactants, extra-articular manifestations) benefit from early initiation of DMARD therapy, as opposed to starting treatment with NSAIDs or glucocorticoid injections.(24)

Advantages of using clinical characteristics for personalizing treatment are that these are readily accessible and can – to some extent – predict prognosis and treatment response. That explains why international guidelines implemented these criteria in their treatment algorithms. The resulting treatment recommendations have been summarized in complex flow diagrams by the European Alliance of Associations for Rheumatology (EULAR), Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) and American College of Rheumatology (ACR).(24–27,34) However, despite using these algorithms, clinicians and patients are still confronted with unsatisfactory and differential treatment responses in clinical practice. This is the case even in patients with similar clinical phenotypes, which suggests that there is more than meets the eye.

... Genotype

For decades researchers were unable to explain the heterogeneous manifestations of psoriatic disease. However, recent evidence supports the observation that differential expression of human leukocyte antigen (HLA) susceptibility alleles could be part of the answer. Five HLA alleles linked to PsA - B*08:01, B*27:05, B*38, B*39 and C*06:02 – have been associated with diverse psoriatic disease phenotypes, disease courses, therapeutic responses and differential expression of cytokines at joints, skin and entheses.(21,35,36) For example, C*06 associates with early onset of severe psoriasis and low prevalence of PsA, B*27 favors concomitant onset of skin and severe musculoskeletal disease, and B*08 is related to a phenotype with asymmetrical spinal involvement.(35,37) According to 'the peptide binding hypothesis', differential phenotypes are explained by the specific binding preference of anchoring amino acids of 9-amino acid peptides by these HLA allotypes. Presentation of different self-peptides may drive a distinct adaptive immune responses through preferred stimulation of a specific autoreactive CD8+ T cell receptor repertoire, that migrates towards local sites of inflammation and targets different autoantigens. In that way, PsA phenotypes are under control of specific HLA susceptibility alleles, that orchestrate patterns of inflammation characterized by different pro-inflammatory mediators and tissues. To extend these findings, another fascinating observation was published: although HLA-B and -C susceptibility alleles differ in peptide binding preferences, they have a shared negative charge of amino acids in the 'B' pocket of the peptide binding groove, preferentially binding peptides with arginine at position two or three.(35) This finding is highly relevant for the field, as this may aid future researchers in their quest to identify the driving self-peptides and autoantigens in PsA.

The aforementioned findings may pave the way for individualized treatment in PsA, since genetic stratification of patients could enhance personalized therapies.(35) In theory, in addition to clinical phenotype, heterogeneous treatment responses might be explained by (HLA) genotype. A handful of studies have tested the hypothesis of HLA class I alleles being predictors of response to biologics, but only in cohorts of psoriasis patients and yielding conflicting results.(38–45) In addition to HLA, in theory, patients with Single-Nucleotide Polymorphisms (SNPs) in genes of the IL-23/IL-17 axis could respond better to biologicals targeting these cytokines, including secukinumab, ixekizumab and ustekinumab.(46) Three studies found evidence for associations of TNF genes with DMARD response in PsA. One study identified polymorphisms in TNFAIP3 associated with better quality of life after six months of TNFi therapy, but did not observe an association with skin disease severity (as measured by Psoriasis Area and Severity Index (PASI) 75) and did not include PsA

composite indices for disease activity.(47) Another study observed that spondyloarthritis patients with a TNF-308A allele had better survival of their first TNFi therapy.(48) A third study showed that PsA patients with TNF+489A alleles responded better to etanercept.(49) Despite the currently limited available evidence and some contradictions, these strategies could be promising for the use of pharmacogenetic markers to tailor treatment in PsA. The field looks forward to studies on genetic stratification strategies in large cohorts of patients, while including additional DMARD therapies and genetic variants, including SNPs, mutations, insertions, deletions and substitutions. At this moment, the field is evolving and studies are being published that answer to the increasing need for appropriate methods to select an optimal number of features from large genetic datasets.(50) Nevertheless, as demonstrated by a heritability of less than 100%, genetics alone do not explain the full story of psoriatic disease.(51)

... Immune phenotype

Emerging analytical techniques have enabled us to study dysregulated immune pathways on a molecular level, which has significantly improved our understanding of psoriatic disease pathogenesis. These developments made it possible to focus on finding the key immune drivers for inflammation in individual patients. Discovery of these main mechanisms facilitated identification of new therapeutic targets and biomarkers for clinical outcomes – including diagnosis, prognosis and therapy response – thus continuing on our journey towards precision medicine.

Localization matters

To study the main drivers of PsA in detail, many studies compared psoriasis and PsA, hypothesizing that differences between patients with and without musculoskeletal signs and symptoms are important in PsA pathogenesis. We too have compared psoriasis and PsA patients in our attempt to identify PsA-specific serum proteins, that could direct us towards the driving factors for arthritis in psoriasis patients (**Chapter 6**). In contrast to what we expected, we found few differences between psoriasis and PsA proteomic signatures and were unable to develop a model to discriminate between patients with skin versus musculoskeletal phenotypes based on serum proteins. To put this in perspective: over recent years multiple studies investigated proteomic differences in circulation between psoriasis and PsA using varying techniques: Olink, mass-spectrometry and Luminex. Although all studies identified some differentially expressed proteins, these have not been replicated in large studies and results were contradicting.(52–55) The

contradictions might be explained by small sample sizes or by differences in analytical techniques, demographic characteristics and methodological approaches. In addition, the lack of robust, replicable proteomics results suggest that we have not studied the most discriminative type of biosample.

Because of its accessibility, peripheral blood has been the most studied compartment in the body. However, as psoriatic disease manifests at various tissue sites with diverse stromal cell populations – including the skin, entheses, joints, spine and nails – signals found in circulation are perhaps not the most representative to better understand disease pathogenesis.(56) This notion is supported by **Chapter 6** and our work presented in **Chapter 3**, in which we found very few differences between psoriasis and PsA with regards to circulatory regulatory T cell (Treg) characteristics, but we observed major differences in comparison to Tregs derived from inflamed joints. The same holds true for **Chapter 4**: we found no differences in expression levels of CD155, DNAX accessory molecule 1 (DNAM1) or T cell immunoreceptor with Ig and ITIM domains (TIGIT) by antigen presenting cells (APCs) and T cells in peripheral blood. The importance of tissue sites is underlined by emerging insights on site-specific differences in stromal cells, interactions between stromal and immune cells and inflammatory responses.(57) Heterogeneity of stromal cells across different tissues, locations within tissues and different (immune) cells in tissues could in part explain the distribution of inflammation in various tissues in PsA.(57) Moreover, studies in psoriatic skin identified oligoclonal, pro-inflammatory, tissue resident memory T cell (Trm) populations, that do not recirculate in peripheral blood or lymphoid organs.(58–61) These Trm cells may establish a site specific disease memory. Recent studies support a pathogenic role for Trm in synovial inflammation in PsA. These synovial Trm are characterized by pro-inflammatory cytokine production (TNF, IL-17, interferon gamma (IFNγ)) and resemble a T helper cell (Th)1 and Th17 effector phenotype (expression of Granzyme A/N, RORC, CD161).(62–65) Furthermore, gene signatures in skin and synovium are different: IL-17 signatures are dominant in psoriatic skin as opposed to TNF and IFNγ in synovial tissue.(66) These findings help explain the tissue-specific divergence of treatment response in PsA, as discussed under 'Clinical characteristics'. Hence, the selection of specific tissue sites is highly relevant for translational research of psoriatic disease.

Studying skin, synovial compartment or entheses is complicated by the fact that obtaining these tissue from living human beings is not practical. As an alternative, use of murine models have provided us with revolutionary insights, including proposing the enthesial site as initial localization of inflammation in PsA by the group of McGonagle

and colleagues (**Chapter 1**: Disease models). Another major step forward has been the increasing use of synovial biopsies for clinical translational research.(67,68) Synovial biopsies not only enable assessment of stromal and immune cells characteristics directly *ex vivo*, but as shown by studies in RA, make it possible to gain deeper insights by studying patient-derived primary synovial organoids.(69,70) To further unravel the complexity of psoriatic disease, I deem it essential that future researchers keep expanding their focus from peripheral blood to additional tissue sites and to integrate the diverse molecular, cellular and tissue layers.

Autoinflammation and -immunity

As discussed in detail in **Chapter 2**, PsA was long considered an autoinflammatory disease, characterized by self-directed inflammation from activated innate immune cells and absence of autoantibodies.(71) However, after recent discovery of multiple PsA-specific autoantibodies and increasing evidence for loss of peripheral immune tolerance, it is now generally accepted that autoimmune mechanisms also contribute to pathogenesis.(72) This knowledge is of utter importance for precision medicine, because it means that therapies primarily directed at components of both the innate and the adaptive immune system – including autoreactive effector T cells and autoantibodies – could be effective.

In line with the autoimmune hypothesis, we discovered three new aspects about the adaptive immune system in psoriatic disease. First, we found evidence for a potentially pathogenic subset of regulatory T cells (Tregs) at inflamed joints, as characterized by intermediate forkhead box p3 (Foxp3) expression, IL-17A production and expression of CD161, RORγt and ICOS (**Figure 1**). If future functional experiments and suppression assays would indeed confirm a pathogenic role of this Treg plasticity, it could be worthwhile to investigate therapies that target pathogenic Tregs or to study cellular therapies with functional Tregs. Second, our results suggest a role for TIGIT expression by CD4+ and CD8+ T cells in the preservation of immune homeostasis. We confirm that TIGIT expression inhibits T cell proliferation and that reduced TIGIT expression associates with systemic inflammation as measured by acute phase reactants (**Figure 2**). Third, in **Chapter 3**, we confirm the presence of autoantibodies against A Disintegrin-Like and Metalloprotease Domain Containing Thrombospondin Type 1 Motif-like 5 (ADAMTSL5), an autoantigen discovered in psoriasis patients in 2015 and recently suggested to be implicated in PsA pathophysiology.(73) Autoantibodies are an area of interest, because they have been implicated in the pathogenesis and may function as biomarkers for clinical outcomes.(73,74) With regards to ADAMTSL5, we show that serum anti-ADAMTSL5

Immunoglobulin G (IgG) is significantly higher in PsA as compared to psoriasis patients, but we did not find an association with clinical outcomes nor a relevant increase in synovial fluid. We speculate that our results argue against an role of ADAMTSL5 as an important autoantigen in PsA.

It remains to be elucidated whether autoantibodies play an important role in PsA pathophysiology (either pathogenic or protective), or whether they are mere consequence of loss of immune tolerance.(72,75) The clinical relevance of identified autoantibodies in PsA is currently under debate. Some argue against an important role of autoantibodies in the disease course, because results were not replicated in other studies, correlations with clinical outcomes were often weak, clinically irrelevant or absent, and differences between PsA patients and controls were relatively small. However, evidence for an important role of one autoantigen is fairly convincing: cathelicidin LL37.(76) To date, this is the only autoantigen to which autoantibodies have been found that are significantly higher in PsA versus controls, and increased in synovial fluid, and correlate with a PsA composite disease activity score.

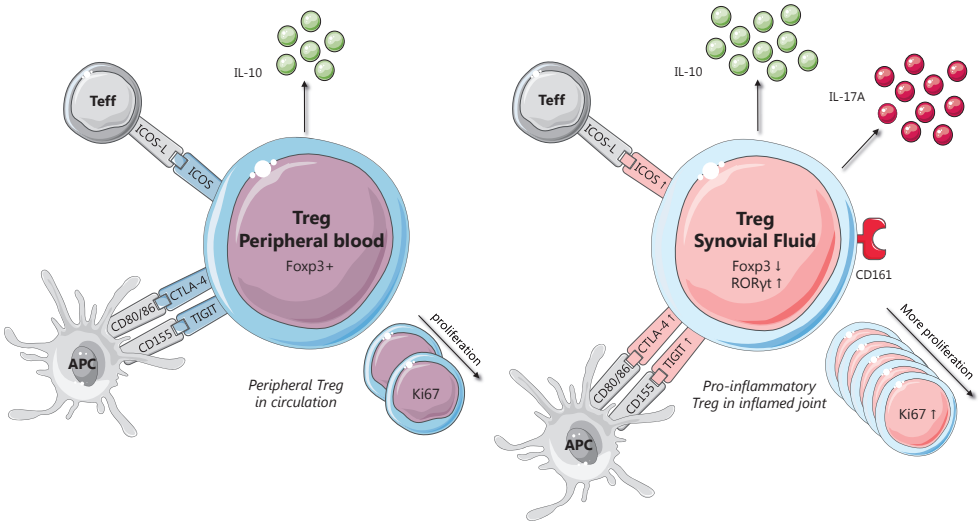

Figure 1. Plasticity of Tregs at inflamed joints in Psoriatic Arthritis. In comparison with their circulating counterparts, Tregs from inflamed joints express increased levels of ICOS, CTLA-4 and TIGIT. Furthermore, synovial fluid-derived Tregs have a pro-inflammatory phenotype, characterized by IL-17A production and upregulation of CD161 and RORyt. We identified a subset of Tregs with intermediate Foxp3 expression as the major cytokine producer. Abbreviations: APC: antigen-presenting cell; CTLA-4: cytotoxic T-lymphocyte-associated protein 4 (CD152); Foxp3: forkhead box p3; ICOS: inducible T-cell costimulator (CD278); ICOS-L: ligand for inducible T-cell costimulator; IL: interleukin; Ki67: Antigen KI-67; RORyt: retinoic acid receptor-related orphan receptor gamma; Teff: effector T cell; TIGIT: T cell immunoreceptor with Ig and ITIM domains; Treg: regulatory T cell.

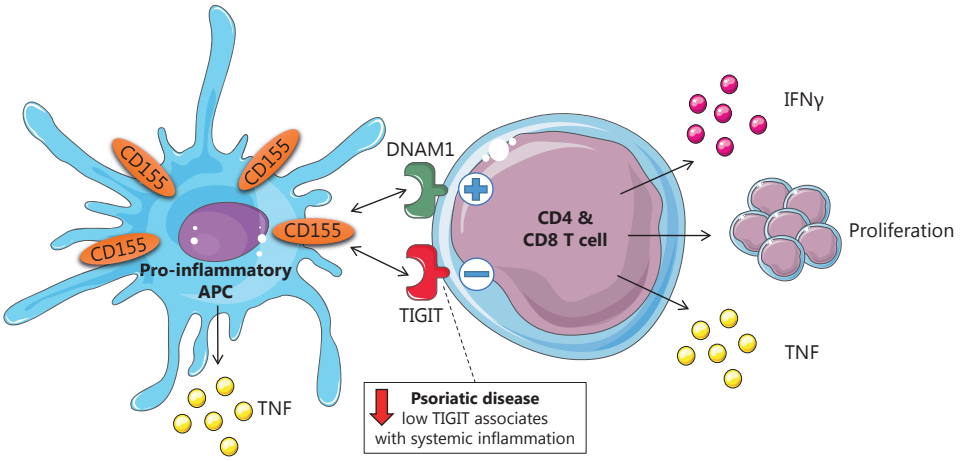

Figure 2. TIGIT downregulation by peripheral T cells associates with systemic inflammation in psoriatic disease. CD155 is increased on pro-inflammatory APCs, while the receptors DNAM1 and TIGIT expressed on T cells balance the inflammatory response by T cells. In psoriatic disease, low TIGIT expression on T cells is associated with systemic inflammation. Abbreviations: APC: antigen-presenting cell, DNAM1: DNAX-accessory molecule-1, IFNγ: interferon gamma, TIGIT: T cell immunoreceptor with Ig and ITIM domains, TNF: tumor necrosis factor.

Clinical applications

Studying immune phenotype in psoriatic disease patients has not only improved our understanding of pathophysiology, but also paved the way for direct clinical application of individualized therapy. One study demonstrated that it is conceptually possible to stratify patients for therapy by peripheral Th cell phenotype.(77) Using flow cytometry analyses of peripheral blood mononuclear cells (PBMCs) from 26 PsA patients, four groups of Th cell subsets were identified: predominant Th1, predominant Th17, combined Th1 and Th17, and nor Th1 neither Th17. In comparison with six months biologic therapy using standard selection (n=38), differential allocation of secukinumab, ustekinumab and TNFi resulted in significantly higher responses (P values <0.05 of simplified disease activity index (SDAI) – low disease activity (LDA), disease activity score 28 (ESR)-LDA, and ACR20 response criterion). The concept of immune phenotype for treatment selection has also been studied in RA. *Tao et al.* followed 80 patients prospectively and demonstrated good prediction of response (≥79%) to adalimumab and etanercept based on multicellular multi-omics data, using machine learning models to analyze epigenetic and transcriptomic features of PBMC, monocytes and CD4+ T cells.(78) Although these were relatively small pilot studies, these results could pave the way for future precision medicine in inflammatory arthritis.

… Molecular biomarkers

Prompted by the promising data from genetic and immune stratification studies in PsA, we queried what is known about robust molecular biomarkers that predict treatment outcomes. The review of literature presented in **Chapter 8** provides experimental support for the predictive value of molecular biomarkers for therapy response. However, despite the promising data presented in these studies, none of the predictors has been implemented in clinical care.(56) We may speculate about the underlying reasons: could the biomarkers not be replicated or validated in large cohorts (only one study (79) had validated their results)? Were the experimental methods and analytical techniques too labor-intensive, complicated or costly? Are some of the studied tissue sites – including synovial tissue – too difficult to access? Or did the markers not reach sufficiently robust sensitivity and specificity for use in the clinic? All these factors could have contributed to the lack of biomarker validation, clinical implementation and evaluation.

Novel approaches

Despite promising research reviewed in this discussion, treatment algorithms for PsA still yield unsatisfactory therapy responses.(56) It has not been elucidated why trials have failed to consistently achieve a satisfactory DMARD responses in more than 60% of patients, nor why drug responses are strongly divergent.(9) These shortcomings leave clinicians to prescribing DMARDs on a 'trial-and-error' basis: a relevant problem in clinical care, because delayed initiation of effective treatment negatively impacts clinical outcomes.(20,80) Hence, scientists may need to embrace new strategies to accurately predict response and progress towards precision medicine. With advancing experimental methods, analytical techniques and computational modeling approaches, there is now access to increasing range of clinical, (epi)genetic, immune, and other biomarkers for patient stratification. In the course of my studies, I have come to realize that the complexity of psoriatic disease might warrant a broader approach to predict response, one that integrates the combined predictive capacity of divergent patient characteristics (**Figure 3**). Combining different data platforms is considered challenging, but pipelines for standardized analyses to integrate these data are indeed emerging.(56) For example, in cancer research integrated data sets – comprising epigenetics, genetics and proteomics – demonstrated that the 'precision oncology' approach is feasible.(81–83)

In PsA, literature on such approaches for therapy prediction is scarce. One study integrated CD4+ T cell transcriptomics with protein data and identified a signaling pathway that strongly associates with IL-17 inhibitor (IL-17i) response.(84) In addition, a

recent proof-of-concept study applied machine learning techniques to clinical data and identified patient clusters that follow variable IL-17i response trajectories.(85) This study demonstrates that precision medicine does not necessarily imply using multi-omics, but that application of novel techniques to analyze less complex data can provide relevant insights. Further, important data are expected from OUTPASS, a multi-center prospective observational study in the UK. They investigate serological, clinical, pharmacological, genetic and psychological factors that influence response to TNFi in PsA, by using high-throughput techniques and applying machine learning and statistical modeling. In addition, I look forward to results of the PredictORPsA study, that aspires to identify and validate predictive biomarkers by studying clinical and protein biomarkers with PAPRICA assays, mass spectrometry and multivariate analyses in cohorts provided by the GRAPPA collaborative research network.(86)

Figure 3. Proposed integrated approach to precision medicine in Psoriatic Arthritis. DMARD: disease modifying anti-rheumatic drugs; HLA: human leukocyte antigen; PsA: psoriatic arthritis; RNA: ribonucleic acid.

Additionally, besides a broad approach that includes integration of diverse predictors, a reductionist approach is necessary to develop clinically applicable models. To answer to this need, the TOFA-PREDICT clinical trial is ongoing (EudraCT Number: 2017-003900-28). Our objective is to integrate clinical, molecular, and imaging parameters to discover patient profiles that predict treatment response. A relevant and pioneering detail is the aim to both discover and to validate the prediction model by using common methods such as enzyme-linked immunoassay, quantitative polymerase chain reaction and flow cytometry. Through replication and translation to conventional experimental methods we endeavour to bridge the gap between research and clinical practice, taking one step further on our journey towards precision medicine. However, it needs to be taken into account that the designs of discussed ongoing studies are exploratory and relatively small sample sizes may hinder the discovery and validation of strong signals from the omics data. Therefore, research initiatives such as the HIPPOCRATES consortium are highly relevant.(87) HIPPOCRATES is a new research program that aims to improve precision treatment strategies by combining the expertise of clinical and scientific researchers, patient representatives and the pharmaceutical industry across Europe. The project plans to combine molecular analysis, artificial intelligence and machine learning to analyse large shared cohorts and datasets, in order to progress in biomarker development. I consider such international research efforts to be the future of research on PsA.

Future perspective

With all this exciting ongoing research, the field is evolving rapidly and I am curious as to what the future may hold. Nevertheless, we have to consider the option that – despite emerging techniques and analyses methods – it might not be possible to accurately predict therapy response. Moreover, certain patients may never consistently respond to one of the currently available therapies. Hence, in light of precision medicine, patient stratification for existing therapies is not the only goal to strive for. Research should continue to broaden the perspective and focus on identification of new therapeutic targets, development of innovative therapies and risk stratification – as discussed below.(6)

Window of opportunity

As highlighted in **Chapter 1**, cutaneous symptoms precede musculoskeletal manifestations on average by ten years in psoriatic disease patients. This time window offers the opportunity to study the critical factors that drive musculoskeletal disease, to facilitate early diagnosis and to investigate prevention of PsA.(88,89) PsA prevention has gained interest over recent years, since multiple studies found evidence that DMARD initiation in psoriasis

patients reduces the risk of transition to PsA.(90–94) Although large prospective studies are necessary to confirm the results, these data suggest that it is possible to delay, attenuate or even prevent PsA by initiating biologic treatment. As high costs and potentially serious side-effects of biologics impede preventive prescription of these drugs, clinicians need methods to identify patients at risk for PsA to initiate early (and potentially preventive) therapy.

Predictors for PsA

Various predictors for PsA transition have been studied over the years. In the meta-analysis presented in **Chapter 5** we investigated a phenotypical characteristic that is quick and easily assessable in the outpatient clinic: psoriasis severity. Insufficient data impeded us to conclude its predictive value for development of PsA. However, other clinical risk factors were identified including disease duration, nail dystrophy, arthralgia, imaging findings and certain psoriasis localizations.(95–98) Moreover, environmental factors (including trauma), comorbidities (obesity, hypercholesterolemia), genetic variants (HLA class I alleles, variants implicated in IFN and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling) and serum biomarkers (C-X-C motif chemokine 10 (CXCL10)) associate with PsA transition.(21,52,95) As literature on soluble predictors was scarce, we aimed to find serum protein signatures (**Chapter 6**) and studied ADAMTSL5 autoantibodies as predictors for PsA transition (**Chapter 3**). Unfortunately, we were unable to draw definitive conclusions, as only few patients progressed to PsA during follow-up (*our unpublished data*). Although multiple features were shown to associate with the development of PsA, individual predictors alone are unable to accurately predict the onset of musculoskeletal disease.(95) The first steps to include multiple predictors were taken by cross-sectional studies that identified models with good discriminative ability between psoriasis and PsA patients by including either genetic markers, or a combination of variables (medical history, phenotype, laboratory parameters).(99–101) These models could help stratification of patients at risk, but their predictive value remains to be confirmed in prospective studies.

PsA prevention?

Although the field is evolving, no prediction models that accurately predict PsA progression at the level of an individual patient have been implemented.(21) To develop robust prediction models in the future, high-throughput and machine-learning analyses are necessary to combine divergent variables into predictive algorithms.(95) Models that integrate different types of biomarkers could provide the cornerstone for design

of future clinical trials aimed at PsA prevention. These efforts may sound challenging, as they require integrated approaches and close collaboration between caregivers and researchers of different backgrounds, including dermatologists, rheumatologists, mathematicians, statisticians and basis scientists. Nevertheless, the research field is currently undergoing fast and successful developments, and large longitudinal cohort studies are ongoing to address the important matter of prediction models.(87,102,103) Prevention trials are already underway for lupus and RA (SMILE, NCT03030118; Stop-RA, NCT02603146). Meanwhile, awaiting robust prediction models, awareness of clinical predictors is important in the outpatient clinic to assist early identification of PsA.

Concluding remarks

At present, the quest for predictive biomarkers and personalized therapy in PsA is ongoing. Despite many efforts on the journey towards precision medicine, robust methods to select optimal treatment are lacking and it is not possible to predict drivers of musculoskeletal disease in individual patients. Hence, PsA patients and health care providers still need to deal with great uncertainties in clinical care. One way to handle these medical uncertainties is by trying to reduce them, through performing high-quality, non-biased and objective clinical, translational and fundamental research. However, another matter – of equivalent significance – needs to be addressed here. It is of vital importance that caregivers concentrate on mastering the tolerance of uncertainty, and that they become comfortable to address medical uncertainties. Only through recognition of its presence and discussing it with their patients, clinical academics are able to practice good and effective clinical care. How should it be explained to patients that there is incomplete understanding why they developed PsA? That it is not possible to predict how severe their joint inflammation will become? Or that it could take years to find a drug that provides the desired effect? Answers to these types of questions are essential for patients, that need to develop coping strategies to deal with uncertainty in their everyday lives. In my opinion, care givers are not only responsible for implementation of best research evidence, but also for a contribution to patients wellbeing by conveying skills to handle and tolerate uncertainty in medical practice. Mastering the art of probabilities in modern medicine may appear even more difficult than straight-forward implementation of evidence-based research. These are skills one does not develop from reading papers or books, but from gaining clinical experience, from considering expert opinion and mostly, from carefully listening to our individual patient's needs. The subjective nature of handling probability in medicine explains why Osler defined it as an art, which after more than 100 years still is a well-fitting term for our everyday work.

References

1. Haroon M, FitzGerald O. Psoriatic arthritis: complexities, comorbidities and implications for the clinic. Expert Rev Clin Immunol. 2016;12:405–16.
2. Haroon M, Gallagher P, FitzGerald O. Diagnostic delay of more than 6 months contributes to poor radiographic and functional outcome in psoriatic arthritis. Ann Rheum Dis. 2015;74:1045–50.
3. Tillett W, Jadon D, Shaddick G, Cavill C, Korendowych E, Vries CS de, et al. Smoking and delay to diagnosis are associated with poorer functional outcome in psoriatic arthritis. Ann Rheum Dis. 2013;72:1358–61.
4. Theander E, Husmark T, Alenius G-M, Larsson PT, Teleman A, Geijer M, et al. Early psoriatic arthritis: short symptom duration, male gender and preserved physical functioning at presentation predict favourable outcome at 5-year follow-up. Results from the Swedish Early Psoriatic Arthritis Register (SwePsA). Ann Rheum Dis. 2014;73:407–13.
5. Gladman DD, Thavaneswaran A, Chandran V, Cook RJ. Do patients with psoriatic arthritis who present early fare better than those presenting later in the disease? Ann Rheum Dis. 2011;70:2152–4.
6. Castillo R, Scher JU. Not your average joint: Towards precision medicine in psoriatic arthritis. Clin Immunol. 2020;217:108470.
7. Wright V. Psoriasis and arthritis. Ann Rheum Dis. 1956;15:348–56.
8. Coates LC, FitzGerald O, Helliwell PS, Paul C. Psoriasis, psoriatic arthritis, and rheumatoid arthritis: Is all inflammation the same? Semin Arthritis Rheum. 2016;46:291–304.
9. Van Den Bosch F, Coates L. Psoriatic arthritis 2 Clinical management of psoriatic arthritis. Lancet Rheumatol. 2018;391:2285–94.
10. Kingsley GH, Kowalczyk A, Taylor H, Ibrahim F, Packham JC, McHugh NJ, et al. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. Rheumatol (United Kingdom). 2012;51:1368–77.
11. Mease PJ, Gladman DD, Collier DH, Ritchlin CT, Helliwell PS, Liu L, et al. Etanercept and Methotrexate as Monotherapy or in Combination for Psoriatic Arthritis: Primary Results From a Randomized, Controlled Phase III Trial. Arthritis Rheumatol. 2019;71:1112–24.
12. Clegg DO, Reda DJ, Mejias E, Cannon GW, Weisman MH, Taylor T, et al. Comparison of sulfasalazine and placebo in the treatment of psoriatic arthritis. Arthritis Rheum. 1996;39:2013–20.
13. Kaltwasser JP, Nash P, Gladman D, Rosen CF, Behrens F, Jones P, et al. Efficacy and safety of leflunomide in the treatment of psoriatic arthritis and psoriasis: A multinational, double-blind, randomized, placebo-controlled clinical trial. Arthritis Rheum. 2004;50:1939–50.
14. Baranauskaite A, Raffayová H, Kungurov N V., Kubanova A, Venalis A, Helmle L, et al. Infliximab plus methotrexate is superior to methotrexate alone in the treatment of psoriatic arthritis in methotrexate-naive patients: The RESPOND study. Ann Rheum Dis. 2012;71:541–8.
15. Coates LC, Helliwell PS. Methotrexate efficacy in the tight control in psoriatic arthritis study. J Rheumatol. 2016;43:356–61.
16. Lie E, Van Heijde D Der, Uhlig T, Heiberg MS, Koldingsnes W, Rødevand E, et al. Effectiveness and retention rates of methotrexate in psoriatic arthritis in comparison with methotrexate-treated patients with rheumatoid arthritis. Ann Rheum Dis. 2010;69:671–6.
17. Landi M, Zaffarana C, Cerda O, Gallino Y, Schneeberger E, Carrillo I, et al. Methotrexate and leflunomide survival in patients with psoriatic arthritis. Int J Clin Rheumtol. 2018;13:102–7.

18. Ricci M, De Marco G, Desiati F, Mazzocchi D, Rotunno L, Battafarano N, et al. [Long-term survival of methotrexate in psoriatic arthritis]. Reumatismo. 2009;61:125–31.
19. Vivekanantham A, McGagh D, Coates LC. Current treatments and recommendations for Psoriatic Arthritis. Best Pract Res Clin Rheumatol. 2021;35:101680.
20. Hawkes J, Yan B, Chan T, Krueger J. Discovery of the IL-23/IL-17 Signaling Pathway and the Treatment of Psoriasis. Br Med J. 2018;6:1605–13.
21. FitzGerald O, Ogdie A, Chandran V, Coates LC, Kavanaugh A, Tillett W, et al. Psoriatic arthritis. Nat Rev Dis Prim. 2021;7.
22. Merola JF, Ogdie A. SEAM-PsA: Seems Like Methotrexate Works in Psoriatic Arthritis? Arthritis Rheumatol. 2019;71:1027–9.
23. Nowell WB, Karis E, Gavigan K, Stradford L, Zhao H, Chen L, et al. Patient-Reported Nausea and Fatigue Related to Methotrexate: A Prospective, Self-Controlled Study in the ArthritisPower® Registry. Rheumatol Ther. 2021;[In press].
24. Gossec L, Baraliakos X, Kerschbaumer A, De Wit M, McInnes I, Dougados M, et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. Ann Rheum Dis. 2020;79:S700–12.
25. Coates LC, Kavanaugh A, Mease PJ, Soriano ER, Laura Acosta-Felquer M, Armstrong AW, et al. Group for Research and Assessment of Psoriasis and Psoriatic Arthritis 2015 Treatment Recommendations for Psoriatic Arthritis. Arthritis Rheumatol. 2016;68:1060–71.
26. Coates L, Soriano E, Corp N, Bertheussen H, Callis-Duffin K, Barbosa Campanholo C. The group for research and assessment of psoriasis and psoriatic arthritis (GRAPPA) treatment recommendations 2021. Ann Rheum Dis. 2021;80:139–40.
27. Singh JA, Guyatt G, Ogdie A, Gladman DD, Deal C, Deodhar A, et al. Special Article: 2018 American College of Rheumatology/National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis. Arthritis Care Res. 2019;71:2–29.
28. Kerschbaumer A, Smolen JS, Dougados M, De Wit M, Primdahl J, McInnes I, et al. Pharmacological treatment of psoriatic arthritis: A systematic literature research for the 2019 update of the EULAR recommendations for the management of psoriatic arthritis. Ann Rheum Dis. 2020;79:S778–86.
29. Reich K, Armstrong AW, Foley P, Song M, Wasfi Y, Randazzo B, et al. Efficacy and safety of guselkumab, an anti-interleukin-23 monoclonal antibody, compared with adalimumab for the treatment of patients with moderate to severe psoriasis with randomized withdrawal and retreatment: Results from the phase III, double-blind, p. J Am Acad Dermatol. 2017;76:418–31.
30. Blauvelt A, Reich K, Tsai TF, Tyring S, Vanaclocha F, Kingo K, et al. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate-to-severe plaque psoriasis up to 1 year: Results from the CLEAR study. J Am Acad Dermatol. 2017;76:60–9.
31. Papp KA, Blauvelt A, Bukhalo M, Gooderham M, Krueger JG, Lacour J-P, et al. Risankizumab versus Ustekinumab for Moderate-to-Severe Plaque Psoriasis. N Engl J Med. 2017;376:1551–60.
32. Mease PJ, Kavanaugh A, Reimold A, Tahir H, Rech J, Hall S, et al. Secukinumab Provides Sustained Improvements in the Signs and Symptoms of Psoriatic Arthritis: Final 5-year Results from the Phase 3 FUTURE 1 Study. ACR Open Rheumatol. 2020;2:18–25.
33. Nakafero G, Grainge MJ, Card T, Taal MW, Aithal GP, Zhang W, et al. Development and validation of a prognostic model for leflunomide discontinuation with abnormal blood-tests during long-term treatment: cohort study using data from Clinical Practice Research Datalink Gold and Aurum. Rheumatology. 2021;keab790:[In press].

34. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, et al. Psoriasis. Nat Rev Dis Prim. 2016;2.
35. Winchester R, Fitzgerald O. MHC class i associations beyond HLA-B27: The peptide binding hypothesis of psoriatic arthritis and its implications for disease pathogenesis. Curr Opin Rheumatol. 2020;32:330–6.
36. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. Autoimmun Rev. 2017;16:10–5.
37. Winchester R, Minevich G, Steshenko V, Kirby B, Kane D, Greenberg DA, et al. HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype. Arthritis Rheum. 2012;64:1134–44.
38. Burlando M, Russo R, Clapasson A, Carmisciano L, Stecca A, Cozzani E, et al. The HLA-Cw6 dilemma: Is it really an outcome predictor in psoriasis patients under biologic therapy? A monocentric retrospective analysis. J Clin Med. 2020;9:3140.
39. Gulliver W. HLA-Cw6 polymorphism predict response to the biologic therapy efalizumab in patients with chronic plaque psoriasis. J Am Acad Dermatol. 2009;60(Suppl 1):AB162 [Abstract].
40. Talamonti M, Galluzzo M, van den Reek JM, de Jong EM, Lambert JLW, Malagoli P, et al. Role of the HLA-C*06 allele in clinical response to ustekinumab: evidence from real life in a large cohort of European patients. Br J Dermatol. 2017;177:489–96.
41. Gallo E, Cabaleiro T, Roman M, Solano-Lopez G, Abad-Santon F, Garcia-Diez A, et al. The relationship between tumour necrosis factor (TNF)-a promoter and IL12B/IL-23R genes polymorphisms and the efficacy of anti-TNF-a therapy in psoriasis: a case–control study. Br J Dermatol. 2013;169:819–29.
42. Chiu H, Wang T, Chan C, Cheng Y, Lin S, Tsai T. Human leucocyte antigen-Cw6 as a predictor for clinical response to ustekinumab, an interleukin-12/23 blocker, in Chinese patients with psoriasis: a retrospective analysis. Br J Dermatol. 2014;171:1181–8.
43. Li K, Huang CC, Randazzo B, Li S, Szapary P, Curran M, et al. HLA-C*06:02 Allele and Response to IL-12/23 Inhibition: Results from the Ustekinumab Phase 3 Psoriasis Program. J Invest Dermatol. 2016;136:2364–71.
44. Prieto-Pérez R, Llamas-Velasco M, Cabaleiro T, Solano-López G, Márquez B, Román M, et al. Pharmacogenetics of ustekinumab in patients with moderate-to-severe plaque psoriasis. Pharmacogenomics. 2016;18:157–64.
45. Costanzo A, Spallone G, Talamonti M, Botti E, Chimenti S. New Developments in the Pharmacogenetics of Psoriasis: HLA-Cw6 as a Marker for the Treatment of Psoriasis with Biological Therapies. Adv Psoriasis Inflamm Ski Dis. 2010;1:125–30.
46. Vecellio M, Hake VX, Davidson C, Carena MC, Wordsworth BP, Selmi C. The IL-17/IL-23 Axis and Its Genetic Contribution to Psoriatic Arthritis. Front Immunol. 2021;11:596086.
47. Ovejero-Benito MC, Muñoz-Aceituno E, Reolid A, Fisas LH, Llamas-Velasco M, Prieto-Pérez R, et al. Polymorphisms associated with anti-TNF drugs response in patients with psoriasis and psoriatic arthritis. J Eur Acad Dermatology Venereol. 2019;33:e175–7.
48. Fabris M, Quartuccio L, Fabro C, Sacco S, Lombardi S, Ramonda R, et al. The-308 TNFα and the -174 IL-6 promoter polymorphisms associate with effective anti-TNFα treatment in seronegative spondyloarthritis. Pharmacogenomics J. 2016;16:238–42.
49. Murdaca G, Gulli R, Spanò F, Lantieri F, Burlando M, Parodi A, et al. TNF-α gene polymorphisms: Association with disease susceptibility and response to anti-TNF-α treatment in psoriatic arthritis. J Invest Dermatol. 2014;134:2503–9.

50. Jalali F, Stadler M, Dand N, Jadon D, Soomro M, Ho P, et al. Application of information theoretic feature selection and machine learning methods for the development of genetic risk prediction models. Sci Rep. 2021;11:1–14.
51. Pedersen OB, Svendsen AJ, Ejstrup L, Skytthe A, Junker P. On the heritability of Psoriatic arthritis. Disease concordance among monozygotic and dizygotic twins. Ann Rheum Dis. 2008;67:1417–21.
52. Abji F, Pollock RA, Liang K, Chandran V, Gladman DD. Brief Report: CXCL10 Is a Possible Biomarker for the Development of Psoriatic Arthritis Among Patients With Psoriasis. Arthritis Rheumatol. 2016;68:2911–6.
53. Reindl J, Pesek J, Kruger T, Wendler S, Nemitz S, Muckova P, et al. Proteomic biomarkers for psoriasis and psoriasis arthritis. J Proteomics. 2016;140:55–61.
54. Matsuura T, Sato M, Nagai K, Sato T, Arito M, Omoteyama K, et al. Serum peptides as putative modulators of inflammation in psoriasis. J Dermatol Sci. 2017;87:36–49.
55. Zhu J, Han L, Liu R, Zhang Z, Huang Q, Fang X, et al. Identification of proteins associated with development of psoriatic arthritis in peripheral blood mononuclear cells: a quantitative iTRAQ-based proteomics study. J Transl Med. 2021;19:331.
56. Jadon DR, Stober C, Pennington SR, FitzGerald O. Applying precision medicine to unmet clinical needs in psoriatic disease. Nat Rev Rheumatol. 2020;16:609–27.
57. Noack M, Miossec P. Importance of lymphocyte–stromal cell interactions in autoimmune and inflammatory rheumatic diseases. Nat Rev Rheumatol. 2021;17:550–64.
58. von Adrian U, Mackay C. T-Cell function and migration; two sided of the same coin. N Engl J Med. 2000;343:1020–34.
59. Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K, Dowgiert RK, et al. The Vast Majority of CLA + T Cells Are Resident in Normal Skin. J Immunol. 2006;176:4431–9.
60. Cheuk S, Wikén M, Blomqvist L, Nylén S, Talme T, Ståhle M, et al. Epidermal Th22 and Tc17 Cells Form a Localized Disease Memory in Clinically Healed Psoriasis. J Immunol. 2014;192:3111–20.
61. Matos TR, O'Malley JT, Lowry EL, Hamm D, Kirsch IR, Robins HS, et al. Clinically resolved psoriatic lesions contain psoriasis-specific IL-17-producing αβ T cell clones. J Clin Invest. 2017;127:4031–41.
62. Menon B, Gullick N, Walter G, Rajasekhar M, Garrood T, Evans H, et al. Interleukin-17+CD8+ T Cells Are Enriched in the Joints of Patients With Psoriatic Arthritis and Correlate With Disease Activity and Joint Damage Progression. Arthritis Rheumatol. 2014;66:1272–81.
63. Wade SM, Canavan M, McGarry T, Low C, Wade SC, Mullan RH, et al. Association of synovial tissue polyfunctional T-cells with DAPSA in psoriatic arthritis. Ann Rheum Dis. 2018;4:350–4.
64. Raychaudhuri SK, Abria C, Raychaudhuri SP. Polyfunctional TEM cells in psoriatic arthritis synovium skewed towards Th17 cells. Ann Rheum Dis. 2019;0:1-2 [Correspondence].
65. Steel K, Srenathan U, Ridley M, Durham L, Wu S-Y, Ryan S, et al. Polyfunctional, Proinflammatory, Tissue- Resident Memory Phenotype and Function of Synovial Interleukin- 17A+CD8+ T Cells in Psoriatic Arthritis. Arthritis Rheumatol. 2020;72:435–47.
66. Belasco J, Louie JS, Gulati N, Wei N, Nograles K, Fuentes-Duculan J, et al. Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis. Arthritis Rheumatol. 2015;67:934–44.
67. Orr C, Sousa E, Boyle DL, Buch MH, Buckley CD, Cañete JD, et al. Synovial tissue research: A state-of-the-art review. Nat Rev Rheumatol. 2017;13:463–75.

68. Smits M, van de Groes S, Thurlings RM. Synovial Tissue Biopsy Collection by Rheumatologists: Ready for Clinical Implementation? Front Med. 2019;6:10–2.

69. Calvo IO, Byrne RA, Karonitsch T, Niederreiter B, Kartnig F, Alasti F, et al. 04.19 3D synovial organoid culture reveals cellular mechanisms of tissue formation and inflammatory remodelling. Ann Rheum Dis. 2017;76(Suppl 1):A49-A50 [Abstract].

70. Rothbauer M, Höll G, Eilenberger C, Kratz SRA, Farooq B, Schuller P, et al. Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing. Lab Chip. 2020;20:1461–71.

71. McGonagle D, McDermott MF. A Proposed Classification of the Immunological Diseases. PLoS Med. 2006;3:e297.

72. Schön MP. Adaptive and Innate Immunity in Psoriasis and Other Inflammatory Disorders. Front Immunol. 2019;10:1764.

73. Yuan Y, Qiu J, Lin Z, Li W, Haley C, Mui UN, et al. Identification of Novel Autoantibodies Associated With Psoriatic Arthritis. Arthritis Rheumatol. 2019;71:941–51.

74. Conigliaro P, Chimenti MS, Triggianese P, Sunzini F, Novelli L, Perricone C, et al. Autoantibodies in inflammatory arthritis. Autoimmun Rev. 2016;15:673–83.

75. Zhu J, Shi XF, Chu CQ. Autoantibodies in psoriatic arthritis: Are they of pathogenic relevance? Chin Med J (Engl). 2020;133:2899–901.

76. Frasca L, Palazzo R, Chimenti MS, Alivernini S, Tolusso B, Bui L, et al. Anti-LL37 Antibodies Are Present in Psoriatic Arthritis (PsA) Patients: New Biomarkers in PsA. Front Immunol. 2018;9:1936.

77. Miyagawa I, Nakayamada S, Nakano K, Kubo S, Iwata S, Miyazaki Y, et al. Precision medicine using different biological DMARDs based on characteristic phenotypes of peripheral T helper cells in psoriatic arthritis. Rheumatol (United Kingdom). 2019;58:336–44.

78. Tao W, Concepcion AN, Vianen M, Marijnissen ACA, Lafeber FPGJ, Radstake TRDJ, et al. Multiomics and Machine Learning Accurately Predict Clinical Response to Adalimumab and Etanercept Therapy in Patients With Rheumatoid Arthritis. Arthritis Rheumatol. 2021;73:212–22.

79. Ademowo OS, Hernandez B, Collins E, Rooney C, Fearon U, Van Kuijk AW, et al. Discovery and confirmation of a protein biomarker panel with potential to predict response to biological therapy in psoriatic arthritis. Ann Rheum Dis. 2016;75:234–41.

80. Pitzalis C, Choy EHS, Buch MH. Transforming clinical trials in rheumatology: towards patient-centric precision medicine. Nat Rev Rheumatol. 2020;16:590–9.

81. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, et al. Proteogenomic characterization of human colon and rectal cancer. Nature. 2014;513:382–7.

82. Mertins P, Mani DR, Ruggles K V., Gillette MA, Clauser KR, Wang P, et al. Proteogenomics connects somatic mutations to signalling in breast cancer. Nature. 2016;534:55–62.

83. Rodriguez H, Pennington SR. Revolutionizing Precision Oncology through Collaborative Proteogenomics and Data Sharing. Cell. 2018;173:535–9.

84. Rahmati S, O'Rielly DD, Li Q, Codner D, Dohey A, Jenkins K, et al. Rho-GTPase pathways may differentiate treatment response to TNF-alpha and IL-17A inhibitors in psoriatic arthritis. Sci Rep. 2020;10:21703.

85. Pournara E, Kormaksson M, Nash P, Ritchlin CT, Kirkham BW, Ligozio G, et al. Clinically relevant patient clusters identified by machine learning from the clinical development programme of secukinumab in psoriatic arthritis. RMD Open. 2021;7:e001845.

86. Stober C, Jadon DR, Armstrong AW, Chandran V, de Wit M, Helliwell PS, et al. Proceedings of the 2020 GRAPPA Collaborative Research Network (CRN) Meeting. J Rheumatol. 2021;97(Suppl):1–6.

87. Fitzgerald O, Pennington SR. HIPPOCRATES : improving diagnosis and outcomes in psoriatic arthritis. Nat Rev Rheumatol. 2022;[In press].
88. Ritchlin C, Colbert R, Gladman D. Psoriatic Arthritis. NEJM. 2017;10:957–70.
89. Raychaudhuri SP, Wilken R, Sukhov AC, Raychaudhuri SK, Maverakis E. Management of psoriatic arthritis: Early diagnosis, monitoring of disease severity and cutting edge therapies. J Autoimmun. 2017;76:21–37.
90. Solmaz D, Ehlebracht A, Karsh J, Bakirci S, McGonagle D, Aydin SZ. Evidence that systemic therapies for psoriasis may reduce psoriatic arthritis occurrence. Clin Exp Rheumatol. 2020;38:257–61.
91. Gisondi P, Bellinato F, Targher G, Idolazzi L, Girolomoni G. Biological disease-modifying antirheumatic drugs may mitigate the risk of psoriatic arthritis in patients with chronic plaque psoriasis. Ann Rheum Dis. 2021;[In press].
92. Acosta Felquer ML, Logiudice L, Galimberti ML, Rosa J, Mazzuoccolo L, Soriano ER. Treating the skin with biologics in patients with psoriasis decreases the incidence of psoriatic arthritis. Ann Rheum Dis. 2021;[In press].
93. Shalev Rosenthal Y, Schwartz N, Sagy I, Pavlovsky L. Psoriatic arthritis incidence among patients receiving biologic medications for psoriasis: A nested case control study. Arthritis Rheumatol. 2021;[In press].
94. Zabotti A, Giovannini I, McGonagle D, De Vita S, Stinco G, Errichetti E. Arthritis Interception in Patients with Psoriasis Treated with Guselkumab. Dermatol Ther (Heidelb). 2021;[In press].
95. Scher JU, Ogdie A, Merola JF, Ritchlin C. Preventing psoriatic arthritis: focusing on patients with psoriasis at increased risk of transition. Nat Rev Rheumatol. 2019;15:153–66.
96. Rouzaud M, Sevrain M, Villani AP, Barnetche T, Paul C, Richard MA, et al. Is there a psoriasis skin phenotype associated with psoriatic arthritis? Systematic literature review. J Eur Acad Dermatology Venereol. 2014;28:17–26.
97. Zabotti A, De Lucia O, Sakellariou G, Batticciotto A, Cincinelli G, Giovannini I, et al. Predictors, Risk Factors, and Incidence Rates of Psoriatic Arthritis Development in Psoriasis Patients: A Systematic Literature Review and Meta-Analysis. Rheumatol Ther. 2021;8:1519–34.
98. Zabotti A, McGonagle DG, Giovannini I, Errichetti E, Zuliani F, Zanetti A, et al. Transition phase towards psoriatic arthritis: Clinical and ultrasonographic characterisation of psoriatic arthralgia. RMD Open. 2019;5:e001067.
99. Yan D, Ahn R, Leslie S, Liao W. Clinical and Genetic Risk Factors Associated with Psoriatic Arthritis among Patients with Psoriasis. Dermatol Ther (Heidelb). 2018;8:593–604.
100. Chen Z, Wang Y, Lan X, Yang M, Ding L, Li G, et al. Nomogram for accurate and quantitative prediction of the risk of psoriatic arthritis in Chinese adult patients with moderate and severe plaque psoriasis. Eur J Dermatol. 2021;[In press].
101. Patrick MT, Stuart PE, Raja K, Gudjonsson JE, Tejasvi T, Yang J, et al. Genetic signature to provide robust risk assessment of psoriatic arthritis development in psoriasis patients. Nat Commun. 2018;9:1–10.
102. Eder L, Haddad A, Rosen CF, Lee KA, Chandran V, Cook R, et al. The Incidence and Risk Factors for Psoriatic Arthritis in Patients with Psoriasis: A Prospective Cohort Study. Arthritis Rheumatol. 2016;68(4):915–23.
103. Simon D, Watad A, Rodrigues-manica S, Perricone C. Editorial : Early Origins of Psoriatic Arthritis. 2021;8(November):1–2.

Appendices

A. Abstract

English

Psoriatic Arthritis (PsA) is a complex, musculoskeletal disease characterized by a heterogeneous clinical phenotype and variable disease course. With new treatment options emerging, it has become difficult for rheumatologists to know which therapy is best for which patient. Postponing efficacious therapy - even for six months - can result in progression of joint erosions, decreased long-term physical function and reduced risk of medication-free remission. Hence, there is an urgent clinical need to tailor medical treatment to individual patients. This thesis discusses recent advances to unravel the complexity of PsA, on a journey towards precision medicine. The clinical work presented in this thesis provides indirect evidence for the efficacy of conventional synthetic disease modifying anti-rheumatic drugs in PsA. The results of a literature review and experimental laboratory research underline the importance of the adaptive immune system in disease pathogenesis. Two new dysregulated T cell mechanisms are discovered, which could be investigated as therapeutic targets. Furthermore, by clinical research and reviewing literature the need for robust predictors is confirmed, both for the development of PsA in psoriasis patients and for the response to therapy. In recent years, advancing experimental methods, analytical techniques and computational modeling approaches have enabled researchers to study an increasing range of clinical, (epi)genetic, immune, and other biomarkers. Future research is warranted to discover and validate robust prediction models to facilitate personalized treatment and prevention of PsA.

Nederlands

Artritis Psoriatica (PsA) is een complexe, musculoskeletale aandoening die wordt gekenmerkt door een heterogeen fenotype en een variabel ziektebeloop. Een groeiend aantal behandelopties maakt het voor reumatologen steeds moeilijker te weten welke behandeling het meest geschikt is voor welke patiënt. Uitstellen van effectieve therapie - zelfs voor zes maanden - kan resulteren in progressie van gewrichtserosies, verminderde functionaliteit en een verlaagde kans op medicatie-vrije remissie. Daarom is het noodzakelijk om therapie op maat aan individuele patiënten aan te bieden. In dit proefschrift worden recente ontwikkelingen beschreven die meer inzicht in het ziektebeeld geven, op weg naar precisiegeneeskunde. Het klinische werk gepresenteerd in dit proefschrift verstrekt indirect bewijs voor de effectiviteit van conventionele synthetische ziekte-modificerende reuma-remmende medicatie bij PsA. De resultaten van een literatuurstudie en experimenteel laboratoriumonderzoek benadrukken het belang van het adaptieve immuunsysteem in de pathogenese van deze ziekte. Bovendien worden twee nieuwe ontregelde T cel mechanismen ontdekt, welke interessant kunnen zijn als therapeutisch aangrijpingspunt. Verder bevestigt dit proefschrift, door klinisch onderzoek en beoordeling van literatuur, de behoefte aan robuuste voorspellers voor zowel het ontwikkelen van PsA in psoriasis patiënten, alsmede de respons op therapie. De laatste jaren hebben vernieuwende analytische technieken en rekenmodellen ervoor gezorgd dat onderzoekers nu in toenemende mate klinische, (epi)genetische, immunologische en andere biomarkers kunnen bestuderen. Toekomstig onderzoek is nodig om robuuste voorspelmodellen te ontdekken en valideren, om gepersonaliseerde behandeling en preventie van PsA mogelijk te maken.

B. Wetenschappelijke samenvatting

Achtergrond

Artritis Psoriatica (PsA) is een musculoskeletale aandoening die wordt gekenmerkt door een complexe pathofysiologie. PsA is de op een na meest voorkomende vorm van inflammatoire artritis met een wereldwijde prevalentie van 0.2%. Tot ver na de eerste beschrijving van het ziektebeeld in 1956 werd PsA gezien als een relatief milde vorm van reumatoïde artritis. Gedurende de laatste decennia is echter duidelijk geworden dat PsA een potentieel destructieve ziekte is, die gepaard gaat met significante morbiditeit, systemische inflammatie en sociale stigmatisatie met grote impact op kwaliteit van leven en levensverwachting.

Tot wel 30% van de psoriasis patiënten ontwikkelt PsA gedurende het leven, gemiddeld tien jaar na het ontstaan van cutane symptomatologie. Psoriasis en PsA worden – door grote overlap in pathogenese, co-morbiditeit en behandeling - ook wel beschouwd als twee uitingsvormen van één ziektebeeld: het spectrum van psoriatische ziekte. PsA kan zich manifesteren als ontsteking in huid (psoriasis), nagels (dystrofie), gewrichten (artritis), bekken en wervelkolom (axiale spondyloartritis), weke delen in vingers of tenen (dactylitis), pezen (enthesitis) en extra-articulair (uveïtis, inflammatoire darmziekte). Bovendien gaat PsA gepaard met uitgebreide invaliderende co-morbiditeit, waaronder cardiovasculaire ziekte, vermoeidheid, depressie en angststoornissen.

Het therapeutisch repertoire voor PsA omvat momenteel meer dan 15 ziekte-modificerende reuma-remmende medicijnen (DMARDs), die op basis van het werkingsmechanisme worden ingedeeld in drie groepen: conventioneel synthetisch (csDMARD, remming van het immuunsysteem in bredere zin), biologicals (bDMARD, gericht op een specifieke immunologische route) en doelgericht synthetisch (tsDMARD, gericht op specifieke intracellulaire signaaltransductie-enzymen). Het tijdig starten van effectieve therapie bij PsA is van essentieel belang, omdat uitstel van behandeling – zelfs gedurende zes maanden – kan leiden tot progressie van gewrichtserosies, verminderd fysiek functioneren op lange termijn en verminderde kans op medicatie-vrije remissie.

Doelstelling proefschrift

Ondanks het toenemende aantal therapeutische keuzemogelijkheden zijn behandelresultaten van patiënten met PsA met DMARDs niet bevredigend geweest. Zo zijn tot op heden klinische trials er niet in geslaagd om met een DMARD bij meer dan 60% van de patiënten een klinisch relevante respons te genereren. Bovendien is niet opgehelderd waarom de behandelrespons sterk uiteenloopt tussen patiënten. Momenteel ontbreken er robuuste methodes om de meest optimale behandeling voor de individuele patiënt te selecteren. Door deze tekortkomingen zijn clinici overgeleverd aan het voorschrijven van DMARDs met een 'trial-and-error' methode, waardoor patiënten maanden tot soms jaren op zoek zijn naar een effectief medicijn. Gezien de kans op ernstige gewrichtsschade vroeg in het ziektebeloop, is het noodzakelijk dat therapie wordt toegespitst op de individuele PsA patiënt.

Op zoek naar precisie geneeskunde hebben basale wetenschappers en klinische onderzoekers verschillende methodes onderzocht voor gepersonaliseerde behandeling. Onder andere heeft de wetenschap zich gericht op het stratificeren van patiënten voor specifieke behandelingen op basis van klinische, genetische en immunologische factoren. Eveneens hebben onderzoekers patiënt-specifieke ontregelde immunologische werkingsroutes getracht te identificeren. Desalniettemin bestaan er tot op heden een aantal uitdagingen in de klinische zorg voor PsA patiënten, die het personaliseren van behandeling verhinderen. Drie van deze uitdagingen worden beschreven in gelijknamige delen van dit proefschrift, waarmee wordt gestreefd de complexiteit van PsA te ontrafelen op weg naar precisiegeneeskunde.

Onderzoeken in dit proefschrift

Deel 1 – Pathofysiologische mechanismen onderliggend aan Artritis Psoriatica

Allereerst worden op dit moment de pathofysiologische mechanismen die ten grondslag liggen aan PsA en haar fenotypische diversiteit onvoldoende begrepen. Jarenlang werd – onder andere door ontbreken van de 'klassieke' auto-antistoffen – PsA gezien als een seronegatieve auto-inflammatoire reumatische ziekte. Nieuwe inzichten hebben er echter toe geleid dat deze classificatie is herzien. Om het wetenschappelijke dogma van PsA als seronegatieve artritis te doorbreken, richt **Hoofdstuk 2** zich middels een verhalende review op het belang van B cel tolerantie en auto-antilichamen in de pathogenese van spondylartropathieën - de groep inflammatoire ontstekingsziekten waar PsA onder valt. Ook bij PsA patiënten worden namelijk aanwijzingen gezien voor

verminderde immuuntolerantie resulterend in ziekte-specifieke auto-antilichamen in serum en synoviale vloeistof, welke correleren met ziekteactiviteit. Onderzoek naar de pathogeniciteit van deze auto-antilichamen en gebruik als biomarker is momenteel gaande.

Bovendien spelen ontregelingen van het adaptieve immuunsysteem (auto-immuniteit) een belangrijke rol in de pathogenese van PsA. Dit blijkt ook uit het translationeel laboratoriumonderzoek gepresenteerd in **Hoofdstuk 3**, waarin dieper wordt ingegaan op de mechanismen die leiden tot verlies van perifere immuuntolerantie. In dit hoofdstuk wordt een potentieel pathogenetische rol van regulatoire T cellen (Tregs) in een inflammatoir micro-milieu geïdentificeerd, gekenmerkt door interleukine (IL)-17A productie, downregulatie van de Treg-specifieke transcriptiefactor Forkhead box P3 (Foxp3), en het verkrijgen van een effector T cel fenotype (CD161 en RORγt expressie). Daarnaast wordt een verband gevonden tussen verminderde Foxp3 expressie met een hogere concentratie van anti-A Disintegrin-Like and Metalloprotease Domain Containing Thrombospondin Type 1 Motif-like 5 (ADAMTSL5) immunoglobuline G (IgG), suggestief voor een rol van Foxp3 instabiliteit bij de onderdrukking van autoantistof productie.

Vervolgens wordt door middel van het laboratorium onderzoek beschreven in **Hoofdstuk 4** de organisatie van de adaptieve immuunrespons door antigen-presenterende cellen (APCs) bestudeerd in psoriatische ziekte, en in het bijzonder CD155/DNAM1/TIGIT signalering. Hier wordt een associatie gevonden van hoge CD155 expressie door pro-inflammatoire APCs, welke interacteren met T cellen via de immuunreceptoren DNAM1 (stimulerend) en TIGIT (remmend). Bovendien wordt een verband gevonden tussen verminderde T cel TIGIT expressie met systemische inflammatie – gemeten middels C-reactief proteine en bezinking bij patiënten met psoriatische ziekte.

Deel 2 – Transitie van psoriasis naar Artritis Psoriatica

Ten tweede, klinische, genetische en immunologische factoren die bijdragen aan de overgang van psoriasis naar PsA zijn momenteel onvoldoende bekend. Bovendien is het niet goed mogelijk om het ontwikkelen van PsA in patiënten met psoriasis adequaat te voorspellen. Predictie van PsA ontwikkeling is essentieel voor vroege initiatie van behandeling en mogelijk zelfs het voorkomen van PsA. Daarom wordt middels een meta-analyse in **Hoofdstuk 5** de ernst van cutane psoriasis – een relatief snelle en non-invasieve klinische uitkomstmaat – onderzocht als voorpeller voor PsA in psoriasis patiënten. Hieruit blijkt dat méér activiteit van de huid (gemeten middels Psoriasis Area

and Severity Index (PASI) of Body Surface Area (BSA)) associeert met de aanwezigheid van PsA, al betreft het hier kleine verschillen. Door onvoldoende data kan geen eenduidige conclusie worden getrokken over de associatie van de ernst van psoriasis met ontwikkelen van PsA in de toekomst.

Vervolgens is er met een relatief nieuwe onderzoeksmethode (*proximity extension assay*) in **Hoofdstuk 6** in het serum proteoom gezocht naar drijvende factoren voor het ontwikkelen van artritis in psoriasis patiënten. In tegenstelling tot wat werd verwacht, blijken er grote overeenkomsten te bestaan in het eiwitprofiel van perifeer bloed van patiënten met psoriasis en PsA. Deze resultaten leveren aanvullend bewijs voor het beschouwen van psoriasis en PsA als twee uitingsvormen van één ziekte. Bovendien werden enkele nieuwe associaties gevonden van serum eiwitten met ziekteactiviteit, suggestief voor een rol in de pathofysiologie.

Deel 3 – Therapie respons en voorspelling

Ten derde ontbreken er robuuste methoden om de optimale behandeling van individuele PsA patiënten te selecteren. Een relevante omissie hieromtrent betreft het geringe bewijs voor effectiviteit van csDMARDs, ondanks dat deze medicijnen in meerdere behandelrichtlijnen worden aanbevolen als eerstelijnstherapie. Derhalve wordt in **Hoofdstuk 7** door middel van retrospectief statusonderzoek bewijs onderzocht voor de werkzaamheid van csDMARDs bij PsA. Voor deze effectiviteit worden inderdaad indirect aanwijzingen gevonden, gezien de lange duur van het gebruik van sulfasalazine en methotrexaat (mediaan bijna drie jaar). Aanvullend worden belangrijke redenen geobserveerd voor staken van therapie, namelijk matig-ernstige bijwerkingen zoals malaise, gastro-intestinale klachten en levertoxiciteit.

Op zoek naar voorspellers voor behandelrespons wordt in **Hoofdstuk 8** een overzicht van in de vakliteratuur beschreven moleculaire biomarkers gepresenteerd naar aanleiding van een systematische literatuur zoekopdracht. Meerdere genetische, circulerende en weefsel biomarkers zijn in de afgelopen decennia ontdekt, maar toch is er op dit moment in de dagelijkse klinische praktijk geen voorspelmodel geïmplementeerd om adequaat behandelrespons te voorspellen. Verschillende verklaringen voor deze omissie worden beschouwd in dit hoofdstuk, waaronder de hoge kosten van biomarker bepalingen, complexiteit van gebruikte technieken en het gebrek aan validatie studies.

Discussie

In **Hoofdstuk 9** worden de belangrijkste bevindingen van dit proefschrift in breder perspectief geplaatst door de resultaten te bespreken in de context van recente literatuur. Verschillende stratificatie methoden voor selectie van therapie worden beschouwd: stratificatie van patiënten op basis van ziekte, patiënt voorkeuren, klinische factoren, genotype, immunologisch fenotype en moleculaire biomarkers. Bovendien worden vernieuwende methoden besproken, welke gericht zijn op integratie van verschillende dataplatforms. Tot slot wordt het belang aangestipt van identificatie van psoriasis patiënten die een hoog risico hebben op het ontwikkelen van PsA, nu meerdere studies suggereren dat het mogelijk is om met vroege initiatie van biologicals PsA te voorkomen.

Op dit moment is de zoektocht naar precisiegeneeskunde voor PsA nog steeds gaande. Ondanks de toegenomen kennis en ervaringen beschreven in dit proefschrift, is het nog niet mogelijk om optimale gepersonaliseerde therapie te bieden aan patiënten met PsA. Wel hebben de laatste jaren vernieuwende experimentele methoden, analytische technieken en rekenmodellen ervoor gezorgd dat onderzoekers nu in toenemende mate klinische, (epi)genetische, immunologische en andere biomarkers kunnen bestuderen. Op het moment van het schrijven dit proefschrift (begin 2022) zijn er reeds grote studies onderweg, die door integratie van verschillende data platformen trachten te voorspellen welke behandeling het meest effectief zal zijn voor individuele PsA patiënten en te voorspellen welke psoriasis patiënten een hoog risico hebben om PsA te ontwikkelen.

Conclusie

Al met al beschrijft dit proefschrift recente ontwikkelingen ten aanzien van het ontrafelen van de complexiteit van PsA op het gebied van pathofysiologie, transitie van psoriasis naar PsA, en voorspelling van behandelrespons door patiënt-stratificatie. Om in de toekomst precisiegeneeskunde toe te kunnen passen, worden de resultaten van grote longitudinale onderzoeken met geïntegreerde benaderingswijzen noodzakelijk geacht, zodat robuuste voorspelmodellen kunnen worden geïdentificeerd en gevalideerd. In afwachting van deze toepassingen is het belangrijk dat clinici en patiënten effectieve copingstrategieën gebruiken bij het omgaan met de onzekerheden in het behandeltraject van deze complexe ziekte.

C. List of Publications

Thesis

1. **Pouw JN**, Leijten E, Radstake T, Boes M. Emerging molecular biomarkers for predicting therapy response in psoriatic arthritis: A review of literature. *Clin Immunol*. 2020;211:108318.

2. **Pouw JN**, Leijten EFA, van Laar JM, Boes M. Revisiting B cell tolerance and autoantibodies in seropositive and seronegative autoimmune rheumatic disease (AIRD). *Clin Exp Immunol*. 2021;203:160-173.

3. Leijten E*, Tao W*, **Pouw JN**, van Kempen T, Olde Nordkamp M, Balak D, Tekstra J, Muñoz-Elías E, DePrimo S, Drylewicz J, Pandit A, Boes M, Radstake T. Broad proteomic screen reveals shared serum proteomic signature in patients with psoriatic arthritis and psoriasis without arthritis. *Rheumatology (Oxford)*. 2021;60:751-761.

4. Jacobs ME, **Pouw JN**, Welsing P, Radstake TRDJ, Leijten EFA. First-line csDMARD monotherapy drug retention in psoriatic arthritis: methotrexate outperforms sulfasalazine. *Rheumatology (Oxford)*. 2021;60:780-784.

5. **Pouw JN***, Jacobs ME*, Olde Nordkamp MA, Radstake TRDJ, Leijten EFA, Boes M. DNAM1 and TIGIT balance the T cell response, with low T cell TIGIT expression corresponding to inflammation in psoriatic disease. *Immunotherapy Advances (Oxford)*. 2020: Itaa004.

6. **Pouw JN***, Jacobs ME*, Balak DMW, van Laar JM, Welsing PMJ, Leijten EFA. Do patients with psoriatic arthritis have more severe skin disease than patients with psoriasis only? A systematic review and meta-analysis. *Dermatology*. [In press]

7. **Pouw JN**, Olde Nordkamp M, van Kempen T, Concepcion AN, van Laar JM, van Wijk F, Spierings J, Leijten EFA, Boes M. Plasticity of regulatory T cells in psoriatic arthritis: an IL-17A-producing, Foxp3^{int}CD161+RORγt+ICOS+ pro-inflammatory phenotype, that associates with the presence of ADAMTSL5 autoantibodies. [Submitted]

** Equal contribution*

Not in Thesis

1. van Dijkhuizen EH, **Pouw JN**, Scheuern A, Hügle B, Hardt S, Ganser G, Kümmerle-Deschner JB, Horneff G, Holzinger D, Bulatović Ćalasan M, Wulffraat NM. Methotrexate intolerance in oral and subcutaneous administration in patients with juvenile idiopathic arthritis: a cross-sectional, observational study. *Clin Exp Rheumatol*. 2016;34:148-54.

2. **Pouw JN**, de Winter - de Groot KM, Berkers G, van Mourik P, van de Graaf EA, Vries R, Beekman JM, Clevers JC, van der Ent CK. Lumacaftor/Ivacaftor in CF patients with homozygous P.PHE508DEL mutations: improving personalized medicine utilizing intestinal organoids. *Pediatr Pulmonol*. 2016;51: 291.

3. **Pouw JN**, Klooster K, Slebos DJ, van der Zeijden H. Spontane klinische verbetering bij een COPD-patiënt [Spontaneous clinical improvement in a COPD patient]. *Ned Tijdschr Geneeskd*. 2018;162:D2336.

4. Scheltema NM, Nibbelke EE, **Pouw JN**, Blanken MO, Rovers MM, Naaktgeboren CA, Mazur NI, Wildenbeest JG, van der Ent CK, Bont LJ. Respiratory syncytial virus prevention and asthma in healthy preterm infants: a randomised controlled trial. *Lancet Respir Med*. 2018;6:257-64.

5. Scheltema NM, Nibbelke EE, **Pouw JN**, Blanken MO, Rovers MM, Naaktgeboren CA, Mazur NI, Wildenbeest JG, van der Ent CK, Bont LJ. RSV prevention in infancy and asthma in later life - Authors' reply. *Lancet Respir Med*. 2018;6:e33.

6. Leijten EF, van Kempen TS, Olde Nordkamp MA, **Pouw JN**, Kleinrensink NJ, Vincken NL, Mertens J, Balak DMW, Verhagen FH, Hartgring SA, Lubberts E, Tekstra J, Pandit A, Radstake TR, Boes M. Tissue-resident memory CD8+ T cells from skin differentiate psoriatic arthritis from psoriasis. *Arthritis Rheumatol*. 2021;73:1220-32.

7. **Pouw JN**, Leijten EFA, Tekstra J, Balak DMW, Radstake TRDJ. Spectrum van psoriatische aandoeningen [Spectrum of psoriatic conditions]. *Ned Tijdschr Geneeskd*. 2019;163:D3936.

8. Kleinrensink NJ, **Pouw JN**, Leijten EFA, Takx RAP, Welsing PMJ, de Keizer B, de Jong PA, Foppen W. Increased vascular inflammation on PET/CT in psoriasis and the effects of biologic treatment: systematic review and meta-analyses. *Clin Transl Imaging*. [In press]

9. Deng J, Leijten E, Olde Nordkamp M, Hartgring S, Tao W, **Pouw JN**, Balak D, Rijken R, Huang R, Radstake T, Lu C, Pandit A. Multi-omics Integration Reveals a Core Network Involved in Host Defense and Hyper-keratinization in Psoriasis. [Submitted]

10. **Pouw JN**, Olde Nordkamp M, O'Toole T, Radstake TRDJ, Leijten EFA, Boes M. Activation induced colocalization of SCAMP5 with IFNα in human plasmacytoid dendritic cells. *Lupus Sci Med.* [In press]

11. Deng J, Leijten E, Olde Nordkamp M, Hartgring S, Tao W, **Pouw JN**, Balak D, Meyaard L, Radstake T, Lu C, Pandit A. PI3: A Potential Biomarker for Disease Severity and Hyper-keratinization in Psoriasis. [Submitted]

12. **Pouw JN***, Kleinrensink NJ*, Perton FT*, Vincken NLA*, Hartgring S, Jansen MP, Arbabi S, TOFA-PREDICT author group, Foppen W, de Jong PA, Tekstra J, Leijten EFA, Spierings J, Lafeber FPJG, Welsing PMJ, Heijstek MW. The TOFA-PREDICT study protocol: a phase III, multi-center, randomized controlled trial to predict response to methotrexate, TNFi (etanercept) and JAKi (tofacitinib) in patients with psoriatic arthritis using a systems medicine approach. [In preparation]

* *Equal contribution*

A

D. About the author

Juliëtte Pouw was born on 8 July 1991 in the city of Leiden, the Netherlands. She is the second child to Roland Pouw and Trudi Smit, and sister of Mathijs Pouw. She graduated cum laude from the Stedelijk Gymnasium Leiden in 2009. She commenced her bachelor and master in medicine at Utrecht University in 2009 and 2012, respectively. Her fascination for rheumatology was sparked in 2014, being part of a research project on MTX intolerance in juvenile idiopathic arthritis under supervision of prof. Nico Wulffraat. Enthusiastic about immunologic diseases, Juliëtte wrote her master's thesis under supervision of by prof. Cornelis van der Ent, prof. Louis Bont and dr. Nienke Scheltema at the Department of Pediatric Infectious Diseases and Immunology at the Wilhelmina Children's Hospital in 2015. Here she studied the association of respiratory syncytial virus prevention and asthma. After graduation in 2016, Juliëtte worked as a resident at the internal medicine department in the St. Antonius Hospital. She started her PhD at the Department of Rheumatology & Clinical Immunology at the University Medical Center Utrecht in 2018, with a main focus on psoriatic arthritis. The composition of her supervisory team changed over time and included prof. Timothy Radstake, dr. Marianne Boes, dr. Emmerik Leijten, prof. Jacob van Laar and dr. Julia Spierings. During her PhD Juliëtte performed laboratory experiments in the Center for Translational Immunology, executed retrospective cohort studies, worked on literature reviews and meta-analyses, was junior board member of the European Association of Systems Medicine, participated in teaching of medical bachelor and master students, and operated as clinical investigator for the multi-centre TOFA-PREDICT clinical trial. After finishing her PhD programme and travelling the world with Remi Stevelink in 2022, she will return to the St. Antonius Hospital to start her specialty training to become a rheumatologist.

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Afdeling Reumatologie & Klinische Immunologie

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Research Groups

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