Biomarker Need in Paediatric Rheumatology:

from Discovery to Clinical Translation

Faekah Gohar

572961-L-bw-PS4U
Processed on: 25-1-2022

Cover: Peter Top
Lay-out and design: Faekah Gohar
Printing: PrintSupport4u

Printing of this thesis was financially supported by the Immunity and Infection graduate school (Paediatric Rheumatology) Utrecht

Biomarker Need in Paediatric Rheumatology:
from Discovery to Clinical Translation

Noodzaak van biomarkers in pediatrische reumatologie:
Van ontdekking tot klinische translatie

met een samenvatting in het Nederlands

Proefschrift

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

vrijdag 18 maart 2022 des middags te 12.15 uur

door
Faekah Gohar
geboren op 9 juni 1984
te Huddersfield, Verenigd Koninkrijk

572961-L-bw-PS4U
Processed on: 25-1-2022

Promotoren:

Prof. dr. A.B.J. Prakken
Prof. dr. D. Foell

Table of contents

Chapter 1

Introduction

572961-L-bw-PS4U
Processed on: 25-1-2022

1 Arthritis in children: a spectrum of disease

Introduction

The speciality of paediatric rheumatology covers a diverse spectrum of clinical conditions ranging from the most common juvenile idiopathic arthritis (JIA), to the much rarer systemic autoinflammatory diseases (SAIDs). All forms of arthritis involve by definition joint inflammation, swelling with or without limited movement and joint pain, all of which can lead to both short and long-term complications such as joint contractures, bone destruction, limb length discrepancies and chronic pain. Many patients with paediatric rheumatological disorders still face delays in their diagnosis and treatment, which can arise from a variety of factors, including a lack of recognition of inflammatory disease as the cause. A diagnosis therefore remains heavy reliant on the time to presentation to a rheumatologist. Barriers to an early diagnosis include the lack of disease-specific diagnostic markers. The role for biomarkers in the diagnosis and management of JIA and the commonest SAID, Familial Mediterranean Fever (FMF) will be introduced below.

Juvenile idiopathic arthritis

JIA refers to a heterogeneous group of chronic inflammatory arthritis subtypes that begin before the age of 16 and persist for a minimum of six weeks. The criteria for diagnosis and subclassification of JIA is defined by the International League of Associations for Rheumatology (ILAR) criteria (Figure 1).(1) A small group of markers are referenced in the classification criteria: rheumatoid factor (RF), anti-nuclear antibody (ANA) titres, and the cell surface antigen Human Leukocyte Antigen B27 (HLA-B27), which help define a shared pattern of disease progression or increased risk of disease-specific complications.(2) ANA- and HLA-B27-positivity confer a higher risk of ocular involvement with uveitis and associated complications.(3) HLA-B27 allele positivity is found in up to 10 % of Europeans and in JIA is associated with enthesitis, the inflammation of tendon and ligament insertion points, as well as with reactive arthritis and inflammatory bowel disease. More importantly, the presence of HLA-B27 is associated with the later development of sacroiliitis and spondylitis, particularly in boys older than 6 years. This explains the classification of such patients into the ILAR Enthesitis Related Arthritis

572961-L-bw-PS4U
Processed on: 25-1-2022

category, as HLA-B27 positivity can predate the development of the usual clinical signs.(4) The measurement of RF predicts the development of erosive arthritis, whilst anti-cyclic citrullinated peptide (anti-CCP) antibodies are early occurring positive predictive factors for the development of rheumatoid arthritis (RA) in adults.(2) In children, anti-CCP antibodies are associated with the RF-positive JIA subgroup, and in polyarthritis, positive RF and/or anti-CCP indicate a poorer prognosis.(5–7) In summary, whilst the diagnosis of JIA remains clinical, the measurement of a number of autoantibodies can help in identifying a pattern of disease and risk of complications in patients with JIA.

Systemic-onset juvenile idiopathic arthritis

Systemic-onset juvenile idiopathic arthritis (SJIA) is also classified under the umbrella of JIA. However, SJIA is a rarer subgroup, with an average incidence of 6.6-15 cases per 100,000 children and is recognised to be a distinct form of JIA due to its systemic features. The diagnosis requires the presence of arthritis in at least one joint for a minimum of six weeks and fever over 2 weeks that is quotidian for at least 3 days, occurring with either a transient evanescent erythematous rash, hepato- or splenomegaly.(8) Up to 40 % of patients will not have any clinical signs of arthritis at presentation, meaning that in the early stages ILAR classification criteria are often not fulfilled.(9,10) A major differential diagnosis of SJIA in the early phase is infection, as both cause raised inflammatory markers and fever as the main symptoms, further complicating the diagnostic process.(10) Also, the clinical course of SJIA is heterogeneous, with around half of patients having a monocyclic course with fever associated relapses, while other predominant phenotypes include a polycyclic or persistent course.(11,12) A rare but significant associated complication occuring in up to 10 % of patients with SJIA is macrophage activation syndrome (MAS). Classification criteria for MAS were published in 2016.(13) Persistent fever is characteristic as are laboratory findings of cytopenias, liver abnormalities and coagulopathy. Bone marrow aspirates are diagnostic and show macrophages well-differentiated into haemophagocytes. MAS represents an acquired form of the inherited disease hemophagocytic lymphohistiocytosis (HLH).(14)

572961-L-bw-PS4U
Processed on: 25-1-2022

Familial mediterranean fever

Autoinflammatory disorders have common immunological and clinical findings, such as fever and organomegaly. Dysregulation of the innate immune system is characteristic, with self-perpetuating inflammation cascades resulting in a proinflammatory environment that damages the host tissues. Both genetic and non-genetic autoinflammatory disorders fall under the spectrum of autoinflammatory paediatric rheumatic diseases.(15) FMF is an inherited periodic fever autoinflammation syndrome, defined by self-limiting inflammatory episodes or "attacks" of variable duration (hours to 3-5 days) with symptom-free interval periods. Arthritis and the periodic fever are the defining symptoms of FMF, while skin involvement (erysipelas) is a rare but classic feature. Serositis, inflammation involving any serosal surface including pleuritis or peritonitis, are important complications and features of FMF severity.(16) A specific late complication is amyloidosis, which can lead to renal failure.(17,18) Inheritance is autosomal recessive and mutation carrier frequencies are linked to geography and highest in families originating from the Mediterranean basin. The MEFV gene was identified on chromosome 16p13.3 in 1997 and encodes the protein pyrin, which is encoded by ten exons or 781 amino acids. To date, 368 potentially disease-causing mutations in the MEFV gene are recognised (see Infevers website, http://fmf.igh.cnrs.fr/ISSAID/infevers/).(19) The four exon 10 mutations M680I, M694V, M694I, and V726A account for the vast majority of identified disease-causing chromosomes.(20) Whilst homozygous M694V mutations are recognised to be the most pathogenic, increasingly heterozygous or compound heterozygous mutations are identified which may be associated with typical FMF symptoms or signs, whilst others have undetermined clinical relevance.(21,22) Complicating the clinical interpretation further, is the varying prevalence and pathogenicity of mutations in different ethnicity groups. A diagnosis of FMF should therefore be clinically likely, e.g. due to ethnicity, a positive family history, presence of parental consanguinity and a pathogenic disease mutation and confirmed by genetic analysis.(23) Additionally, a positive response to a treatment trial with colchicine should be confirmed.(16)

In summary, significant diagnostic challenges remain for both JIA and FMF. Both diseases continue to rely on a primarily clinical diagnosis. In JIA, further elucidation of the pathogenesis is vital to improving both diagnosis and management. Also in FMF, though the underlying genetic cause is known, fu-

572961-L-bw-PS4U
Processed on: 25-1-2022

rther understanding of the pathogenicity and relevance of detected mutations with symptoms is still required. The detection and relevance of subclinical disease is also relevant to the management of JIA and FMF. Additionally, both diseases are associated with significant acute and/or chronic complications, which reflects the urgency and importance of identifying the correct diagnosis and instituting appropriate early management to reduce the burden of disease.

Biomarkers and mechanisms of disease

Routine laboratory markers

There remains a lack of sensitive and specific diagnostic markers for paediatric rheumatic diseases. The routinely measured inflammatory markers erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) are characteristically elevated during acute episodes in patients with SJIA and FMF, and can be elevated in JIA, but are also raised in infection. A normal ESR or CRP value can also not be used to exclude the presence of arthritis or inflammation. These markers are also unreliable for defining remission and making therapy decisions e.g. escalation or de-escalation of therapy. ANA, HLA-B27, anti-CCP and RF are more specific markers for rheumatological disease, but are also not diagnostic. An additional useful routinely measurable marker for FMF is serum amyloid A (SAA). The release of SAA from hepatocytes is induced by inflammation, and persistently elevated SAA is strongly associated with the development of amyloidosis and is a marker of disease activity or treatment response.(24,25)

Biomarker Discovery

Biomarkers are biochemical molecules, which when quantified can provide information for diagnosis, monitoring or management of a biological process or disease. Ideally these molecules should be stable and reliably measurable in easily accessible samples e.g. serum, in a cost-effective manner which provides clinically useful information. Various methods have been used for the discovery and validation of biomarkers, including cytokine assays and proteomics.(26–28) Biomarker quantification should be performed on a statistically valid number of samples, in multiple populations/cohorts where possible.(29) The process of identifying biomarker need and potential markers (discovery studies) to testing in a research setting and validation for clinical use is often

referred to as the biomarker pipeline. A translational strategy is frequently employed, which refers to the process of clinical need and knowledge informing laboratory strategies and vice versa, commonly referred to as 'bench to bedside'.(30) Interdisciplinary working is uniquely helpful in achieving the objectives of bench to bedside research and as a result the methodology is increasingly a focus of research.(31) One example of a successful pathway in autoinflammation is the elucidation of the role of interleukins 1 and 6 (IL-1 and IL-6) and consequent development of effective therapies which themselves have provided light on the role of cytokines, which will be further discussed below.(15,32)

Role of the innate immune system

Over-activation of the innate immune system and resulting systemic inflammation is seen in both SJIA and FMF. The innate immune system is responsible for the non-specific but rapid first-line response to infection that is predominantly carried out by neutrophil, macrophage and monocyte cells. In contrast, the acquired immune response requires antigen presentation and the development of antibodies. Autoimmunity is the process by which the acquired immune system recognises self-antigens as being foreign. An innate immune response is triggered by the recognition of pathogen associated molecular patterns (PAMPs), e.g. peptidoglycan or lipopolysaccharide (LPS) in the cell wall of bacteria and cellular components, or damage-associated molecular patterns (DAMPs). PAMP and DAMP molecules activate toll-like receptors (TLRs) and NACHT-Leucine Rich (NLR) proteins on cells of the innate immune system. This activates intracellular cascades resulting in the release of intracellular molecules which may trigger further inflammatory cascades. If the normal inhibitory response to this process is defective, autoinflammation can result. Predominant autoinflammatory mechanisms active in JIA and SAIDs involve the TNF-alpha and IL-1 family of cytokines, which has resulted in the development of targeted biological therapies to these antibodies and receptors.(33,34) These cytokines and their associated receptors have upregulated gene expression and elevated serum concentrations in FMF and JIA. IL-1 blockade has proven to be an effective therapy for FMF. IL-18 has also shown usefulness as a biomarker of disease activity in both FMF (35) and SJIA (36) and a marker of treatment response in SJIA.(37) Higher serum IL-18 concentrations in SJIA are associated with higher risk of MAS.(38) The activity of IL-18 is mediated through its effect of neutrophil activation, cau-

sing neutrophil migration and degranulation with consequent cytokine and chemokine release.

The S100 proteins

Two intracellular molecules released in high concentrations from monocytes and neutrophils in part as a result of TLR activation are the S100 proteins.(39) S100A8/A9, also referred to as myeloid-related protein 8/14 (MRP8/14), and S100A12 are endogenous intracellular stored proinflammatory molecules. Once the aforementioned inflammatory cascades are activated and the S100 proteins released extracellularly, the serum levels of these molecules can be quantified. Cell death is a normal process of release of these S100 proteins, which means low levels may always be measurable in the serum of healthy controls. However, in active autoinflammatory disease, serum concentrations of these proteins are significantly elevated. The release of such proinflammatory intracellular molecules can itself initiate further cytokine release, meaning innate immune system dysregulation can lead to a self-perpetuating release of cytokines and inflammation.(39,40) Phagocyte activation is a finding in both FMF and SJIA, indicated by high serum ferritin and particularly high S100A8/A9 and S100A12 concentrations, the latter are significantly higher than the concentrations seen in other SAIDs, JIA-subtypes and infection, making these S100 proteins useful as diagnostic markers.(41,42) Both S100 proteins have also been evaluated as predictors of relapse risk and renewed disease activity in patients with JIA.(43–45)

Autoinflammatory mechanisms and genetic influence

In FMF autoinflammation is triggered by mutations in the MEFV gene which result in changes to pyrin referred to as gain-of-function mutations. As pyrin is predominantly expressed in innate cells and fibroblasts in serosal and synovial tissues, it has a pivotal role in the regulation of inflammation in these tissues.(46) Pyrin is composed of separate domains which interact with other proteins containing a pyrin-domain, for example the adaptor protein ASC (apoptosis-associated speck-like protein with a CARD) which activates the NALP3 (nucleotide-binding oligomerisation domain-like receptors) inflammasome. Inflammasome activation mediates the maturation of pro-inflammatory cytokines, including the cleavage of pro-caspase to its active enzyme form caspase-1 which can cleave the interleukin-1b (IL-1ß) precursor into the biologically active IL-1ß cytokine. The role of pyrin in inflammasome

572961-L-bw-PS4U
Processed on: 25-1-2022

regulation was elucidated from mouse models by Chae and colleagues who provided the first evidence for the gain of function occurring as a result of MEFV mutations and the consequent auto-inflammatory features of FMF.(46–48) Unlike FMF, JIA does not have a clear genetic cause. However, an increased risk of SJIA has been associated with genetic variants within the MHC (major histocompatibility complex) class II gene cluster, most strongly the presence of the HLA-DRB1*11 antigen gene complex.(49) As MHC molecules are usually found on antigen-presenting cells, this finding indicates a significant role for the adaptive immune response in the pathogenesis of SJIA and a complex interplay of both autoinflammation and autoimmunity mechanisms.(50,51)

Management strategies and remaining questions

Therapeutic options

The goal of treatment is to achieve and maintain a state of disease remission without flares, and to limit short-term complications and prevent persisting disability or pain. Rheumatic diseases also have significant impact on school attendance, sport participation and chronic pain.(52) The mainstay of arthritis treatment remains non-steroidal anti-inflammatory drugs (NSAIDs) plus disease-modifying antirheumatic drugs (DMARDs), specifically methotrexate which is efficacious for several categories of JIA.(53) Glucocorticoids are still important therapy options, being used in various forms including as intra-articular injections or as intravenous or oral therapies e.g. to induce remission at disease onset or as bridging therapy. At the start of the 20th century the availability of the biological DMARDs (bDMARDs) further revolutionised treatment possibilities providing efficacious alternatives to the previously available armamentarium.

JIA treatment strategies and their effectiveness

The use of biologics allowed the development of treat to target (T2T) strategies - first in adult rheumatology, and then in JIA. T2T strategies promote use of the most effective treatment, ideally at an early stage of disease referred to as the ‘window of opportunity’ in order to achieve pre-defined targets defined individually for the patient.(54,55) Regular monitoring of response is required, a concept referred to as ‘tight control’, which may necessitate ‘step-up’ or

'step-down' therapy. Non-response to therapy however remains an important clinical issue in both FMF and all JIA subtypes, with up to 40 % of patients with JIA not responding to treatment with bDMARDs.(56) Whilst a number of measures of therapy response exist, no universal measure has been identified for use in T2T strategies, and the biopsychosocial aspects of the disease remain poorly addressed.(57) For example, chronic pain is an important and frequent associated complication of JIA, even in the absence of active disease, but its cause is multifactorial und not well defined as the clinical course varies greatly between patients.(58) If left unmanaged, chronic pain can have significant consequences on the daily lives of affected children. The roles that existing and new biomarkers can play in tailoring management of both SAIDs and JIA include prediction of flares or early identification of disease activity. Addressing clinical dilemmas such as how best to manage clinically varying phenotypes of the same disease for example may first require a more thorough biomolecular characterisation of the phenotypes, to identify targets for individualised management.

Therapy options in FMF

The effectiveness of oral colchicine therapy for preventing the development and progression of the significant disease-related complications like amyloidosis in FMF has already been touched on above. Colchicine, is an anti-microtubule agent, which is thought to institute its disease modifying effects in FMF by disrupting microtubule function and consequently suppressing inflammatory processes. For example, intracellular vesicular motility is inhibited which interferes with signalling pathways preventing the secretion of endogenous cytokines and chemokines. However, up to 10 % of children are recognised to be colchicine-resistant while some of these and more may be incompliant.(59,60) Clinical unresponsiveness may be defined as incomplete disease remission with persistent FMF attacks despite the maximum colchicine dose possible, or persistently high inflammatory markers, particularly SAA, or the occurrence of renal, hepatic or amyloidosis-related complications. For such cases, biological therapies, specifically IL-1 targeting therapies such as Anakinra and Canakinumab can be effective therapies.(61)

Managing subclinical inflammation

The significance of subclinical inflammation, ongoing inflammation without the presence of clinical symptoms or signs, remains an issue of debate. Iden-

tifiable in up to 30 % of patients and potentially over prolonged periods, subclinical inflammation may or may not lead to overt clinical flares.(62) Additionally, persisting subclinical inflammation can lead to the development of disease related complications such as organomegaly and anaemia, in the same way as overt clinical disease.(63) However, how much of a risk persisting subclinical inflammation is, remains unknown. Whilst aggressive treatment of subclinical inflammation may induce immunological remission, it is unclear if this has clinical relevance.

572961-L-bw-PS4U
Processed on: 25-1-2022

Figure 1 Summary of the ILAR classification of juvenile idiopathic arthritis
Adapted from the International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: 2nd revision, Edmonton, 2001. J Rheumatol 2004;31;390-392

JIA is arthritis of unknown aetiology that begins before the 16th birthday and persists for at least 6 weeks; other known conditions excluded. All categories are mutually exclusive, represented by this list of exclusions:

a) Psoriasis or history of psoriasis in the patient or first-degree relative
b) Arthritis in an HLA-B27 positive male beginning after the 6th birthday
c) Ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative
d) The presence of IgM rheumatoid factor on at least 2 occasions at least 3 months apart
e) *The application of exclusions is indicated under each category, and may change as new data become available.*

Systemic Arthritis *Exclusions:* a, b, c, d.
Arthritis in ≥ 1 joint with or preceded by fever of at least 2 weeks duration that is documented to be daily ("quotidian"), for at least 3 days, and accompanied by one or more of the following:

1. Evanescent (nonfixed) erythematous rash
2. Generalized lymph node enlargement
3. Hepatomegaly and/or splenomegaly
4. Serositis

Oligoarthritis *Exclusions:* a, b, c, d, e.
Arthritis affecting one to 4 joints during the first 6 months of disease. Two subcategories are recognised:

1. *Persistent* Maximum 4 joints affected throughout the disease
2. *Extended* More than 4 joints after the first 6 months

Polyarthritis (Rheumatoid Factor Negative) *Exclusions:* a, b, c, d, e.
Arthritis affecting 5 or more joints during the first 6 months of disease, a test for RF is negative.

Polyarthritis (Rheumatoid Factor Positive) *Exclusions:* a, b, c, e.
Arthritis affecting 5 or more joints during the first 6 months of disease, 2 or more tests for RF at least 3 months apart during the first 6 months of disease are positive.

Psoriatic Arthritis *Exclusions:* b, c, d, e.
Arthritis and psoriasis, or arthritis and at least 2 of the following:

1. Dactylitis
2. Nail pitting or onycholysis
3. Psoriasis in a first-degree relative

Enthesitis Related Arthritis *Exclusions:* a, d, e.
Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of the following:

1. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain
2. The presence of HLA-B27 antigen
3. Onset of arthritis in a male over 6 years of age
4. Acute (symptomatic) anterior uveitis
5. History of ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative

Undifferentiated Arthritis
Arthritis that fulfils criteria in no category or in 2 or more of the above categories

1 Thesis Outline

Part I of this thesis focuses on the need for biomarkers for juvenile idiopathic arthritis (JIA) and existing and newly identified biomarkers are discussed. In Chapter 2, the management of JIA, including SJIA, is discussed in detail. Current treatment protocols and guidelines from around the world are summarised and concepts of management, such as the "window of opportunity" and treatment targets are also covered along with the role that biomarkers could and currently play in the diagnosis and management of JIA. In Chapter 3 an in-depth systematic literature review of biomarkers in SJIA is presented. Papers were evaluated for their identification of potential biomarkers pertaining to the diagnosis and management of SJIA. This paper highlighted the great lack of validation studies for SJIA biomarkers, but also more positively indicates the huge variety of potential biomarkers that are being explored by various research groups around the world in this field. Continuing to study such potential biomarkers could further our understanding of the different mechanisms at play in this systemic autoinflammatory disease.

Part II focuses on two projects aimed at discovering new biomarkers for the AIDs Familial Mediterranean Fever (FMF) and Systemic onset JIA (SJIA). Chapter 4 outlines the search to identify if sub-phenotypes of SJIA can be identified from a biomarker profile and to identify a potential biomarker panel to discriminate SJIA from patients presenting with infection. Secondary objectives were to evaluate the use of multiple platforms to identify biomarkers of various molecular sizes and to use proteomic techniques which have been so far under-used in SJIA for both discovery and validation studies. The focus of Chapter 5 was to identify patterns of S100A12 and IL-18 neutrophil secretion of patients with FMF, which was measured in the serum of patients and in vitro secreted from patient and healthy control neutrophils. Results were correlated with genotype and disease activity.

Part III changes tact by focusing on the translation of biomarkers into clinical practice. The most important aim of biomarker studies is the development of clinically relevant and applicable tests which will positively impact patient care and experience. A study of the biomarker S100A12 and response to therapy is included as well as two commentary papers which focus on the importance of innovation and the translational process of biomarker develop-

ment. Chapter 6 investigated the association of S100A12 and treatment response in patients with JIA treated with methotrexate or anti-TNF biological therapy. Patients were categorised as treatment responders or non-responders based on various clinical evaluation parameters including ACRpedi50 score, JADAS-10 score and achievement of inactive disease and baseline and follow-up S100A12 measured. Concluding Part III, the two commentary papers are strongly influenced by personal experience in translational research settings (Chapter 7 and Chapter 8) and illustrate the practical role of the clinical scientist in translational medicine and the directly related concept of interdisciplinarity as a way of driving innovation to reach the overriding goals as discussed above.

In summary, this thesis focuses specifically on the clinical translation of biomarkers from discovery to clinical practice in two paediatric autoinflammatory diseases SJIA and FMF. The thesis is organised using the well-known "bench-to-bedside" translational pathway, starting with the background research, critical appraisal of studies and need for the research outlined in Part I, followed by basic science studies ("bench") utilising a variety of techniques in Part II. Part III concludes with a clinical study ("bedside") of one of the investigated biomarkers outlined in the previous chapters, S100A12, as well as a discussion of the requirement for translational clinical researchers and medical innovation to complete the pathway. The above mentioned work is summarised and discussed in Chapters 9 and 10.

1

References

1. Petty, R. E. et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J. Rheumatol. 31, 390–2 (2004).
2. Mahmud, S. A. & Binstadt, B. A. Autoantibodies in the pathogenesis, diagnosis, and prognosis of juvenile idiopathic arthritis. Frontiers in Immunology 10, 3168 (2019).
3. Ravelli, A. et al. Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. Arthritis Rheum. 52, 826–32 (2005).
4. Berntson, L. et al. HLA-B27 predicts a more extended disease with increasing age at onset in boys with Juvenile idiopathic arthritis. J. Rheumatol. 35, 2055–2061 (2008).
5. Brunner, J. & Sitzmann, F. C. The diagnostic value of anti-cyclic citrullinated peptide (CCP) antibodies in children with Juvenile Idiopathic Arthritis. Clin. Exp. Rheumatol. 24, 449–51
6. Gilliam, B. E., Chauhan, A. K., Low, J. M. & Moore, T. L. Pediatric rheumatology. Measurement of biomarkers in juvenile idiopathic arthritis patients and their significant association with disease severity : a comparative study. Clin. Exp. Rheumatol. 26, 492–497 (2008).
7. Beukelman, T. et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. Arthritis Care Res. (Hoboken). 63, 465–82 (2011).
8. Petty, R. E. et al. International League of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis: Second Revision. 31, (2001).
9. Behrens, E. M. et al. Evaluation of the presentation of systemic onset juvenile rheumatoid arthritis: Data from the Pennsylvania Systemic Onset Juvenile Arthritis Registry (PASOJAR). J. Rheumatol. 35, 343–348 (2008).
10. Hinze, C. H. et al. Practice and consensus-based strategies in diagnosing and managing systemic juvenile idiopathic arthritis in Germany. Pediatr. Rheumatol. 16, 7 (2018).
11. Singh-Grewal, D., Schneider, R., Bayer, N. & Feldman, B. M. Predictors of disease course and remission in systemic juvenile idiopathic arthritis: Significance of early clinical and laboratory features. Arthritis Rheum. 54, 1595–1601 (2006).
12. Martini, A. et al. Toward new classification criteria for juvenile idiopathic arthritis: First steps, pediatric rheumatology international trials organization international consensus. J. Rheumatol. 46, 190–197 (2019).
13. Ravelli, A. et al. 2016 Classification Criteria for Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis: A European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborat. Arthritis Rheumatol. 68, 566–576 (2016).
14. Sawhney, S., Woo, P. & Murray, K. Macrophage activation syndrome: a potentially fatal complication of rheumatic disorders. Arch. Dis. Child. 85, 421–426 (2001).
15. Holzinger, D., Kessel, C., Omenetti, A. & Gattorno, M. From bench to bedside and back again: translational research in autoinflammation. Nat. Rev. Rheumatol. advance on, (2015).
16. Livneh, A. et al. Criteria for the diag-

nosis of familial Mediterranean fever. Arthritis Rheum. 40, 1879–85 (1997).
17. Medlej-Hashim, M. et al. Amyloidosis in familial Mediterranean fever patients: correlation with MEFV genotype and SAA1 and MICA polymorphisms effects. BMC Med. Genet. 5, 4 (2004).
18. Ben-Chetrit, E. & Levy, M. Colchicine prophylaxis in familial Mediterranean fever: Reappraisal after 15 years. Semin. Arthritis Rheum. 20, 241–246 (1991).
19. Milhavet, F. et al. The infevers autoinflammatory mutation online registry: update with new genes and functions. Hum. Mutat. 29, 803–8 (2008).
20. Touitou, I. Standardized Testing for Mutations in Familial Mediterranean Fever. Clin. Chem. 49, 1781–1782 (2003).
21. Marek-Yagel, D. et al. Clinical disease among patients heterozygous for familial Mediterranean fever. Arthritis Rheum. 60, 1862–6 (2009).
22. Booty, M. G. et al. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? Arthritis Rheum. 60, 1851–61 (2009).
23. Livneh, A. et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum. 40, 1879–1885 (1997).
24. Merlini, G. & Bellotti, V. Molecular mechanisms of amyloidosis. N. Engl. J. Med. 349, 583–596 (2003).
25. Urieli-Shoval, S., Linke, R. P. & Matzner, Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. Curr. Opin. Hematol. 7, 64–69 (2000).
26. Ling, X. B. et al. Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. Proteomics 10, 4415–30 (2010).
27. Miyamae, T. et al. Serum protein profile in systemic-onset juvenile idiopathic arthritis differentiates response versus nonresponse to therapy. Arthritis Res. Ther. 7, R746-55 (2005).
28. Gibson, D. S. et al. Validation of Protein Biomarkers to Advance the Management of Autoimmune Disorders.
29. McArdle, A. et al. Developing Clinically Relevant Biomarkers in Inflammatory Arthritis: A Multi-Platform Approach for Serum Candidate Protein Discovery. PROTEOMICS - Clin. Appl. n/a-n/a (2015). doi:10.1002/prca.201500046
30. Cohrs, R. J. et al. Translational Medicine definition by the European Society for Translational Medicine. New Horizons Transl. Med. 2, 86–88 (2015).
31. Waldman, S. A. & Terzic, A. Clinical and translational science: from bench-bedside to global village. Clin. Transl. Sci. 3, 254–7 (2010).
32. De Benedetti, F. et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. N. Engl. J. Med. 367, 2385–2395 (2012).
33. Vastert, S. J. et al. Effectiveness of First-Line Treatment With Recombinant Interleukin-1 Receptor Antagonist in Steroid-Naive Patients With New-Onset Systemic Juvenile Idiopathic Arthritis: Results of a Prospective Cohort Study. Arthritis Rheumatol. 66, 1034–1043 (2014).
34. Quartier, P. et al. Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type. Arthritis Rheum. 48, 1093–101 (2003).
35. Gohar, F. et al. Correlation of Secretory Activity of Neutrophils With Genotype in Patients With Familial Mediterranean Fever. Arthritis Rheumatol. 68, 3010–3022 (2016).
36. Maeno, N. et al. Highly elevated serum levels of interleukin-18 in systemic juvenile idiopathic arthritis but not in other juvenile idiopathic arthritis subty-

pes or in Kawasaki disease: comment on the article by Kawashima et al. Arthritis Rheum. 46, 2539–41; author-reply 2541-2 (2002).
37. Quartier, P. et al. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). Ann. Rheum. Dis. 70, 747–754 (2011).
38. Shimizu, M. et al. Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis. Rheumatology (Oxford). 49, 1645–53 (2010).
39. Frosch, M. et al. The myeloid-related proteins 8 and 14 complex, a novel ligand of toll-like receptor 4, and interleukin-1beta form a positive feedback mechanism in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 60, 883–91 (2009).
40. Foell, D. et al. Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4. Am. J. Respir. Crit. Care Med. 187, 1324–34 (2013).
41. Wittkowski, H. et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum. 58, 3924–31 (2008).
42. Kessel, C., Holzinger, D. & Foell, D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. Clin. Immunol. 147, 229–41 (2013).
43. Holzinger, D. et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann. Rheum. Dis. 71, 974–80 (2012).
44. Rothmund, F. et al. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care Res. (Hoboken). 66, 949–955 (2013).
45. Foell, D. Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? Ann. Rheum. Dis. 63, 206–208 (2004).
46. Chae, J. J., Aksentijevich, I. & Kastner, D. L. Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. Br. J. Haematol. 146, 467–78 (2009).
47. Chae, J. J. et al. The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. Proc. Natl. Acad. Sci. 103, 9982–9987 (2006).
48. Chae, J. J. et al. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. Immunity 34, 755–68 (2011).
49. Ombrello, M. J. et al. HLA-DRB1*11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis. Proc. Natl. Acad. Sci. 112, 15970–5 (2015).
50. Nigrovic, P. A. Autoinflammation and autoimmunity in systemic juvenile idiopathic arthritis. Proc. Natl. Acad. Sci. 112, 15785–15786 (2015).
51. Kessel, C., Hedrich, C. M. & Foell, D. Innately Adaptive or Truly Autoimmune: Is There Something Unique About Systemic Juvenile Idiopathic Arthritis? Arthritis Rheumatol. 72, 210–219 (2020).

52. van Dijkhuizen, E. H. P. et al. Patient's experiences with the care for juvenile idiopathic arthritis across Europe. Pediatr. Rheumatol. 16, 10 (2018).
53. Giannini, E. H. et al. Methotrexate in Resistant Juvenile Rheumatoid Arthritis. N. Engl. J. Med. 326, 1043–1049 (1992).
54. Hinze, C., Gohar, F. & Foell, D. Management of juvenile idiopathic arthritis: hitting the target. Nat. Rev. Rheumatol. 11, 290–300 (2015).
55. Nigrovic, P. A. Review: is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis? Arthritis Rheumatol. 66, 1405–13 (2014).
56. Gohar, F. et al. S100A12 Is Associated with Response to Therapy in Juvenile Idiopathic Arthritis. J Rheumatol. 45, 547–554 (2019).
57. Chaplin, H. et al. O25 Exploring a biopsychosocial approach to pain in adolescents and young adults with juvenile idiopathic arthritis (young investigator award winner 2018). Rheumatology 57, (2018).
58. Lomholt, J. J., Thastum, M. & Herlin, T. Pain experience in children with juvenile idiopathic arthritis treated with anti-TNF agents compared to non-biologic standard treatment. Pediatr. Rheumatol. 11, (2013).
59. Ben-Zvi, I. & Livneh, A. Colchicine failure in familial mediterranean fever and potential alternatives: Embarking on the Anakinra trial. Isr. Med. Assoc. J. 16, 271–273 (2014).
60. Özen, S., Batu, E. D. & Demir, S. Familial mediterranean fever: Recent developments in pathogenesis and new recommendations for management. Front. Immunol. 8, (2017).
61. De Benedetti, F. et al. Canakinumab for the treatment of autoinflammatory recurrent fever syndromes. N. Engl. J. Med. 378, 1908–1919 (2018).
62. Lachmann, H. J. et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford). 45, 746–50 (2006).
63. Ben-Zvi, I. & Livneh, A. Chronic inflammation in FMF: markers, risk factors, outcomes and therapy. Nature reviews. Rheumatology 105–12 (2011).

Chapter 2

Management of juvenile idiopathic arthritis: Hitting the target

Claas Hinze, Faekah Gohar and Dirk Foell

Nat Rev Rheumatol. 2015 May;11(5):290-300

Abstract

The treatment of juvenile idiopathic arthritis (JIA) is evolving. The growing number of effective drugs has led to successful treatment and prevention of long-term sequelae in most patients. Although patients with JIA frequently achieve lasting clinical remission, sustained remission off medication is still elusive for most. Treatment approaches vary substantially among paediatric rheumatologists owing to the inherent heterogeneity of JIA and, until recently, to the lack of accepted and well-evidenced guidelines. Furthermore, many pertinent questions related to patient management remain unanswered, in particular regarding treatment targets, and selection, intensity and sequence of initiation or withdrawal of therapy. Existing JIA guidelines and recommendations do not specify treat-to-target or tight control strategies, in contrast to adult rheumatology in which these approaches have been successful. The concepts of window of opportunity (early treatment to improve long-term outcomes) and immunological remission (abrogation of subclinical disease activity) are also fundamental when defining treatment methodologies.

This review explores the application of these concepts to JIA and their possible contribution to the development of future clinical guidelines or consensus treatment protocols. The article also discusses how diverse forms of standardized, guideline-led care and personalized treatment can be combined into a targeted, patient-centred approach to optimize management strategies for patients with JIA.

2

Key points

- The development of biologic DMARDs has revolutionized the therapeutic management of juvenile idiopathic arthritis (JIA)
- A treat-to-target approach combined with tight control, in which patients are frequently reassessed, can help improve disease outcomes and achieve disease remission off medication
- Initiating intensive therapy early in the course of JIA (during the window of opportunity) is likely to improve long-term disease outcomes, and is a focus of ongoing clinical trials
- Guidelines and consensus protocols have been pivotal in improving the management of children with JIA, but further development and investigator-led clinical trials are required, including by multinational collaborations
- Optimal management strategies for patients with JIA can be achieved by combining guideline-led care and personalized treatment approaches, and by focusing efforts on targeted treatment

572961-L-bw-PS4U
Processed on: 25-1-2022

Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of disorders characterized by childhood-onset chronic arthritis of unknown cause.(1) This phenotypic variability reflects fundamental underlying biological diversity, as evidenced, for example, by differences in gene expression patterns in peripheral blood mononuclear cells and serum cytokine profiles.(2,3) The greatest improvements in care have occurred with the development of biological DMARDs which, together with existing therapies, have improved the efficacy and tolerability of treatments for JIA (Figure 1). Owing to knowledge gaps related to the optimal use of this growing armamentarium of effective therapies, the pharmacological treatment options for patients with JIA are more complex than ever. Inception cohorts developed to address treatment strategies inevitably vary in terms of patient selection, availability of therapies and treatment schedules, reflecting variations in clinical practice. Additionally, with few exceptions, most strategies used in the management of JIA have been informed by data from adult studies, with resulting criticisms over the lack of innovation in paediatric rheumatology.(4)

Whereas appropriate disease control is achievable for most patients, a substantial proportion do not achieve long-term drug-free remission or cure. In a study of 1,500 patients with JIA treated with a step-up approach, which included NSAIDs, glucocorticoids and conventional as well as biologic DMARDs,(5) overall 70% of patients achieved inactive disease within 2 years, but the proportion was markedly lower in some categories (for example, 48% in rheumatoid factor [RF]-positive polyarthritis), and only around 50% of patients were able to discontinue antirheumatic medications within 5 years, reflecting data from retrospective studies.(6–9)

Review criteria

We searched PubMed for original and review articles published from January 2000 to May 2014, published in English. Related articles were included from reference lists of identified articles. Additionally, searches of specific websites were also performed to identify unpublished pathways, guidelines and protocols, such as The Institute of Health (USA) and the British Society for Paediatric and Adolescent Rheumatology. Specific search terms were: „treatment recommendations“, „juvenile arthritis“, „personalized treatment“, „rheumatology“, „standardized treatment“, „guideline“, „biomarker“, „treatment algorithm“, „pharmacogenetics“ and „gene expression profiling“.

Important questions about treatment efficacy remain, in particular regarding initiation and discontinuation of therapy to achieve lasting clinical remission. Therefore, in this Review we provide an overview of historical and current treatment strategies, with special consideration of approaches targeting the window of opportunity. We describe existing guidelines and discuss their impact and limitations in improving and standardizing care, as well as the role of personalized treatment. Finally, we bring together the seemingly contradictory concepts of standardized and personalized treatment, to direct the development of patient-centred, optimized management strategies.

Treatment strategies

Step-up (pyramid) therapy

Historically, JIA treatment was escalated in steps (referred to as pyramid therapy) typically guided by expert opinion. In general, treatment began with NSAIDs, which, if ineffective, would be replaced by glucocorticoids and longer-acting drugs such as penicillamine, gold or antimalarial medication.(10) This approach was based on several assumptions now proven to be false: that chronic arthritis is benign; that NSAIDs are similarly benign; that immunosuppressive drugs are toxic; and that highly effective drugs are not available.(11)

Expanding armamentarium

Methotrexate was the first conventional DMARD with marked efficacy in several categories of JIA,(12) signalling the expansion of the armamentarium of antirheumatic drugs. Subsequently, changes in paediatric regulations on conducting trials in children (as a prerequisit for the approval of drugs for paediatric use) of the European Medicines Agency (EMA) in 2007 and the FDA led to industry-sponsored, placebo-controlled trials of conventional and biologic DMARDs being successfully performed in children, leading to approval of therapies specifically for paediatric indications (Figure 1).(13–15)

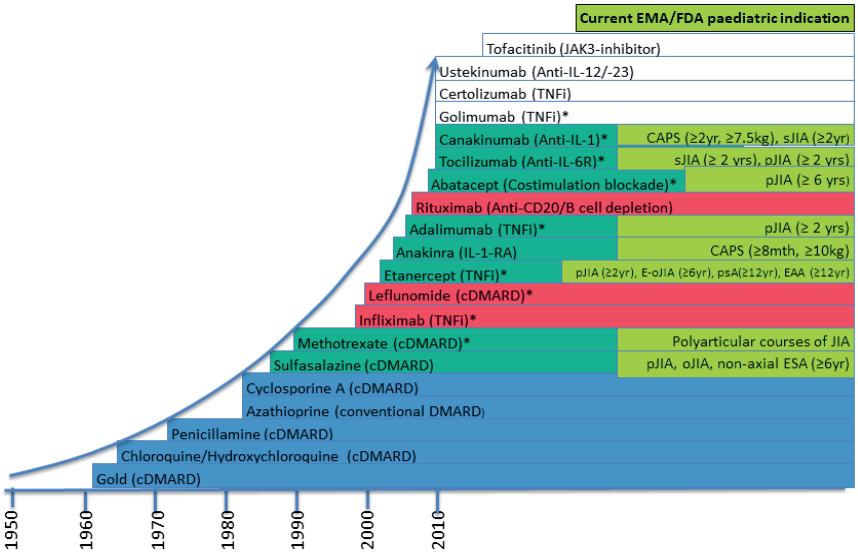
Top-down therapy („Hit hard and early")

The step-up or pyramid-therapy approach has been questioned since the early 1990s by investigators calling for early introduction of aggressive treatment.16 Improved long-term benefit to risk ratios were demonstrated for conventional and biologic DMARD therapies when used early in management,(17) which

Figure 1: The armamentarium of antirheumatic drugs available for the treatment of JIA.

The evolution of biologic DMARDs in past decades revolutionized the therapeutic management of arthritis. However, although the number and variety of therapies available for use in paediatric rheumatology (excluding glucocorticoids and NSAIDs) is greater than ever, deciding on a treatment strategy has correspondingly increased in complexity. Whereas some therapies have been tested in high-quality paediatric studies, only some have received specific approval for use in children with rheumatological diseases, and such approval may also be limited to certain JIA categories. Additionally, some drugs approved in adult rheumatology have been recommended for use in JIA without EMA or FDA approval. *Therapies tested in high-quality paediatric studies.

Abbreviations: CAPS, cryopyrin-associated autoinflammatory syndromes; cDMARD, conventional disease-modifying drug; EMA, European Medicines Agency; E-oJIA, extended oligoatricular juvenile idiopathic arthritis; ERA, enthesitis-related arthritis; IL-1-RA, IL-1-receptor antagonist; JIA, juvenile idiopathic arthritis; oJIA, oligoarticular juvenile idiopathic arthritis; pJIA, polyarticular juvenile idiopathic arthritis; psA, psoriatic arthritis; sJIA, systemic juvenile idiopathic arthritis; TNFi, tumour necrosis factor inhibitor; yr, years old.



led to the initiation of several trials investigating early aggressive therapy. The ACUTE–JIA and TREAT studies, which combined methotrexate, glucocorticoid and anti-TNF therapies, were encouraging examples of how aggressive combination strategies enable patients to achieve clinically inactive disease (CID) status earlier than those receiving monotherapeutic management. (18,19)

„Treat-to-target" and tight control

Treat-to-target (T2T) was first advocated for the treatment of adult rheumatoid arthritis (RA) in 2010, based on its successful use in chronic conditions such as hypertension and diabetes mellitus.(20) Key components of T2T include explicitly defining a target (such as remission or low disease activity) for each patient, combined with tightly controlled, regular visits for monitoring their progress towards achieving these treatment targets (Box 1).(21-22) In adult rheumatology, RA was seen as a chronic condition, with patients being seen infrequently and irregularly; the implementation of these strategies was effective and achieved good response rates, which were not simply due to the use of biologic DMARDs.(22-24) The 2010 EULAR recommendations for the management of RA, based on evidence from systematic reviews, were founded on a T2T-directed strategy, as were the later joint ACR/EULAR recommendations.(25-26) A 2013 update of the EULAR recommendations went even further in highlighting T2T as a fundamental foundation of treatment strategies, and readjusting the individual recommendations to keep T2T at its core.(27)

Defining treatment targets for JIA

Importance of treatment targets

Categories of JIA have been defined for some time, but the identification of risk factors associated with different subphenotypes and the development of parameters to score disease activity has allowed further definition of treatment targets. The ultimate treatment target is disease remission, but acceptable disease control can be an alternative valid target in some patients; nevertheless, treatment should always aim to prevent long-term damage and impairment of quality of life. The definition of categories of disease activity in JIA, including remission off medication, remission on medication, CID,

Box 1: Defining treatment targets on JIA

Clinically inactive disease (Wallace criteria)
- No joints with active arthritis
- No fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA
- No active uveitis according to standardization of uveitis nomenclature criteria
- ESR or CRP level within normal limits (high levels only acceptable if cause is not JIA)
- Best possible score on PGA of disease activity (e.g. 0 on a scale of 0–10)
- Duration of morning stiffness <15 min

Wallace criteria:
Clinical remission on medication: CID for ≥6 months on therapy
Clinical remission off medication: CID for ≥12 months off therapy

JADAS criteria:
Clinical remission: JADAS cut-off = 1
Minimal disease activity: Oligoarthritis: JADAS cut-off = 2, Polyarthritis: cut-off = 3.8
Acceptable symptom state: Oligoarthritis: JADAS cut-off = 3, Polyarthritis: cut-off = 4.3

ACR criteria:
Low disease activity: One or no active joints, ESR or CRP level normal, PGA of overall disease activity score <3 on a scale of 0–10. Patient or parent global assessment of overall well-being score <2 on a scale of 0–10

Abbreviations: CRP, C reactive protein; CID, clinically inactive disease, ESR, erythrocyte sedimentation rate (mm/h); JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; PGA, physician global assessment.

minimal disease activity, acceptable symptom state and active disease, has been a major progressive step (Table 1).(28–30) However, and in contrast to RA, treatment targets required for the implementation of a T2T approach in JIA have not been explicitly defined in any existing paediatric rheumatology recommendations or consensus pathways (Table 2), a gap partly owed to limitations in measuring JIA disease activity. Even though not explicitly stated, T2T components have since been integrated into the ACR treatment recommendations for JIA, in which therapies are stratified according to low, moderate or high disease activity categories.

In contrast to adults with RA, children with JIA are more likely to achieve remission off medication; this potential outcome means that, in addition to reaching an initial target of remission with therapy, the long-term target of

remission without the need for further therapy is reachable for many patients (Figure 2).(6–9) Therefore, protocols and recommendations that include strategies to taper or safely withdraw therapy are urgently needed in paediatric rheumatology.

CID and immunological remission

Up to 40% of patients with JIA in clinical trials reach CID after 6 months of therapy.(19) CID is defined according to the Wallace criteria as the absence of symptoms associated with JIA (Box 1).(30) Approximately 40% of patients who fulfil the criteria for CID and receive methotrexate or anti-TNF therapy have evidence of ongoing subclinical inflammation, with elevated circulating levels of the S100A8/A9 protein complex (also known as the MRP8/14 complex) or S100A12, which function as phagocyte activation markers.(31-32) Patients with evidence of persisting subclinical inflammation (inferred by high S100 protein levels) have a substantially increased risk of disease flare after discontinuation of methotrexate therapy versus patients with normal levels, who are considered to be in immunological remission. However, the same has not been demonstrated for anti-TNF therapy.(31-33) Therefore, whether the concept of subclinical disease activity or immunological remission is applicable to all patients with JIA, or whether continuing with therapy during subclinical disease activity could prevent disease flares, is currently unclear. The PREVENT–JIA prospective clinical trial was designed to answer some of these questions and is currently ongoing, enrolling patients with polyarticular disease within any JIA category.

Measuring disease activity and improvement

The Disease Activity Score in 28 joints (DAS28) used in adult RA is a global and continuous disease activity score that is sensitive to change and used in both clinical practice and trials. This composite score incorporates multiple, easily obtainable measurements, including the erythrocyte sedimentation rate (ESR), a global health parameter, and the number of tender and swollen joints.(34) By contrast, clinical trials in JIA have commonly used change in a core set of criteria as end points, such as the ACR Pediatric (ACRp) 30, 50, 70 or 90 responses, reflecting an improvement in at least three of the six core set variables by at least 30, 50, 70 or 90%, with no more than one of the remaining variables worsening by more than 30, 50, 70 or 90%, respectively (Table 1). Nevertheless, these core sets have not been adopted into clinical

practice, because the extent of improvement or worsening in responses is not equivalent to absolute current disease activity, and these measurements can differ from defined clinical targets.

The Juvenile Arthritis Disease Activity Score (JADAS 71, 27 or 10) is used in clinical practice by simply adding the points scored in four domains: physician global (0–10), patient or parent global (0–10), active joint count (in 71, 27 or 10 joints, respectively) and normalized ESR.(35) For the JADAS 27 score, the levels of disease activity, how responsive the score is to change and how well it detects clinically important differences have been documented.(36) In 2012, validated cut-offs were published for a three-variable version of the JADAS (cJADAS), that excluded ESR, which simplifies the application of this score in clinical settings.(37)

Table 1 Measuring disease activity in JIA

Score variables	ACRp core set	JADAS	DAS28
Components	Patient or parent global assessment Physician global assessment CHAQ Active joint count LOM joint count ESR	Physician global assessment (0-10) Patient or parent global assessment (0-10) Active joint count (0-10, 0-27, or 0-71) ESR (normalized to 20mm/h) (0-10)	Global health score (0-100) Tender joint count (0-28) Swollen joint count (0-28) ESR
Absolute measure of disease activity?	No	Yes	Yes
Calculation	Not applicable	Simple addition	Calculated
Sensitive to change	Yes	Yes	Yes
Derived disease states	No	Yes	Yes

Abbreviations: ACRp, ACR paediatric; CHAQ, Childhood Assessment Score; CRP, C-reactive protein; DAS28, Disease Activity Score in 28 joints; ESR, erythrocyte sedimentation rate (mm/h); JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; LOM, limitation of motion.

Biomarkers and imaging

Biomarkers can be broadly classified as biochemical (identified in serum, plasma or synovial fluid), immunological (such as cytokines), molecular

(proteomics, transcriptomics or metabolomics) or genetic. Biomarkers can be used as markers for diagnosis,(38) relapse or remission,(39) response to treatment or treatment withdrawal,(31) or as surrogate markers. In the clinically heterogeneous group of patients with JIA, the use of biomarkers could help define the window of opportunity for early treatment (40) and further aid the stratification of patients with JIA for specific therapies.(41) The heterogeneity of patients with JIA also dictates that multiple or a combination of biomarkers are likely to be required.(40) Measurement of the most recently discovered biomarkers S100A8/A9 (MRP8/14) and S100A12 proteins is now possible using commercial ELISAs, for which assay-specific cut-offs for the prediction of relapse have been validated.(42,43) Levels of these protein complexes are hugely elevated in active systemic JIA (sJIA) and during disease flares. (42) Despite these advances, clinically useful biomarkers are still lacking - even though several biomarker discovery or investigative studies exist, the vast majority still requires validation.

A substantial proportion of patients with CID JIA have evidence of joint inflammation on MRI or ultrasonography, but whether nonradiographic imaging techniques can provide a clinically relevant measurement of disease activity is still unknown.(44,45) In addition, these modalities might not be widely available and image interpretation is operator-dependent, which currently limits their general use.

Legislation and research networks

The introduction of legislation regarding the development of paediatric medicines in the USA and the European Union in the late 1990s and early 2000s led to the funding and completion of multiple clinical trials in paediatric rheumatology.(15) This legislation requires pharmaceutical companies to submit a paediatric investigation plan for novel drugs if equivalent diseases exist in children. Owing to the lower prevalence of paediatric rheumatic diseases than adult rheumatic diseases, the establishment of collaborative and international research networks has been of huge importance to enable clinical trials to be conducted. The Pediatric Rheumatology Collaborative Study Group (PRCSG) in North America and the Paediatric Rheumatology International Trials Organisation (PRINTO, which covers 47 countries in Africa, Asia, Europe and South America) are of particular relevance, and have collaborated on multiple clinical trials.

How to optimize treatment strategies?

Window of opportunity

Early intensive therapy in JIA during the window of opportunity can alter the biology of the disease and improve long-term disease outcomes, including preven¬tion of cumulative joint damage. In sJIA, early anti-IL 1 therapy quickly leads to inactive disease and improves long-term outcomes.(46,47) For example, in a cohort of steroid-naive patients with new-onset sJIA treated with IL 1 receptor antagonist (IL-1RA) therapy, 85% of patients had achieved inactive disease or an ACRp90 response by 3 months of treatment.(46) The benefits of early treatment with other agents and in other JIA categories are less clear, but have been increasingly suggested for polyarticular JIA (pJIA). In the ACUTE–JIA trial,(18) initiation of anti-TNF therapy plus methotrexate in DMARD-naive patients with pJIA was more effective in achieving minimally active or inactive disease than methotrexate alone, or methotrexate plus hydroxychloroquine and sulfasalazine. The TREAT trial also showed a benefit with this early strategy, even though it failed to reach its primary endpoint;(19) a second trial of early initial treatment with sulfasalazine demonstrated long-term benefit and improved outcome versus the same treatment initiated later on.(19,48-49) Other trials investigating early treatment strategies for JIA are currently ongoing, including the BeST for kids study in the Netherlands.(50)

Standardized treatment

To standardize JIA treatment, various protocols, pathways and clinical practice guidelines (as well as recommendations and evidence-based statements contributing to these guidelines) have been developed.(51-52) Clinical practice guidelines and standardized strategies are aimed at harmonizing and improving treatments in particular, because not all children with JIA are treated by paediatric rheumatologists. Thus, these guidelines are extraordinarily important to help improve the treatment of children by non-paediatric rheumatologists.

Treatment recommendations and guidelines

Although other consensus and evidence-based treatment recommendations had been created earlier, for example in Germany and the UK (Table 2), publication of the 2011 ACR evidence-based treatment recommendations for patients with JIA was a major milestone, as it gave practitioners a practical

framework.(53) JIA is a heterogeneous disease, and seven distinct categories as defined according to the International League of Associations for Rheumatology (ILAR) criteria were used in the German guidelines;(54) however, the validity and usefulness of this classification has been questioned.(55) By contrast, the ACR recommendations distinguish between arthritis affecting ≤4 joints, arthritis affecting ≥5 joints, degree of disease activity (low, moderate or high) and the presence or absence of poor prognostic factors; additionally, sJIA is considered separately.

Despite being included in international treatment recommendations, several treatments have not received regulatory approval for use in children and are used off-label in most countries (Figure 1), leading to refusal of reimbursement by insurance companies and to lack of availability in resource-poor countries. Some treatments are controversial: for example, the use of glucocorticoids for the treatment of JIA has been strongly debated. Whereas systemic glucocorticoids are not recommended for treatment of non-systemic arthritis in the 2011 ACR recommendations, owing to lack of evidence from randomized clinical trials on the optimal use and potential adverse effects of these agents, their use as 'bridging' therapy a short-term approach prior to longer-acting agents becoming effective has received support.(53,56) By contrast, the use of intra-articular glucocorticoid injections is supported in most guidelines (Table 2).

Table 2 Comparison of published JIA treatment guidelines

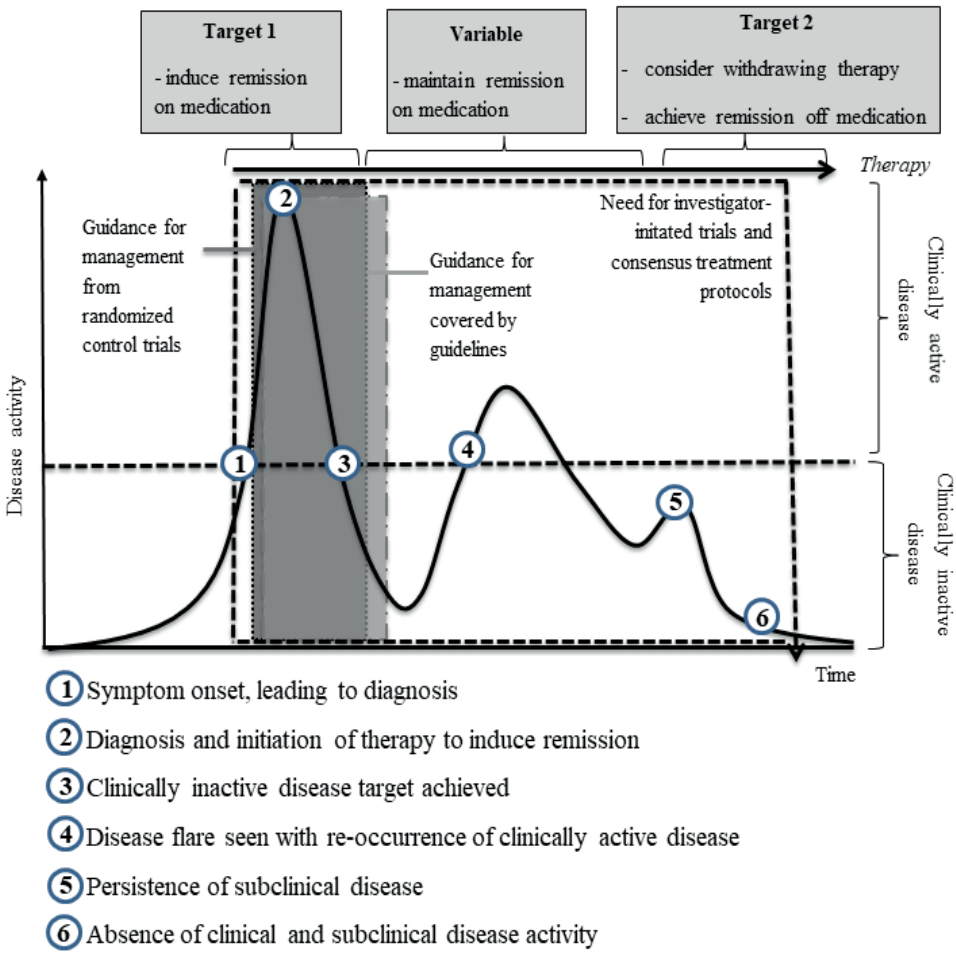
	Australia	Germany	UK/Ireland	USA	USA
Type	Guideline, 2009	Guideline, 2011	Guideline, 2001	Recommendation, 2011	Recommendation, 2013
Years literature searched	2000-2007	Up to 2010	Unclear	1966-2009	Up to 2013
Authors or members	Multidisciplinary expert working group	Multidisciplinary expert working group	British paediatric research group	Core expert panel, task force panel	
Methodology	Literature review, group meetings, email circulation, feedback	Systematic review of the literature, formal consensus building, NGT, Delphi method	Consensus and informal	Systematic review of the literature, RAND/UCLA appropriate method	
Target audience	Primary care physicians	Paediatric rheumatologists	General	Paediatric rheumatologists	Paediatric rheumatologists
Categories applicable	NA	Oligoarthritis, polyarthritis, systemic	Oligoarthritis, polyarthritis, systemic	Distinct treatment groups	Systemic
NSAIDs addressed?	Yes	Yes	Yes	Yes	Yes
Intra-articular glucocorticoids addressed?	No	Yes	Yes	Yes	Yes
DMARDS incl?	No	Yes	Yes	Yes	Yes
Biologics addressed?	No	Yes	No	Yes	Yes
Physiotherapy addressed?	Yes	Yes	No	No	No
Pathways addressed?	No	Limited	Yes	Yes	Yes
Explicit treatment target included?	Yes (minimizing pain, preventing complications)	No	Yes (remission)	No	No
Implicit treatment target included?	NA	No	NA	Low disease activity	Inactive disease

Abbreviations: NA, not available; NGT, nominal group technique; RAND/UCLA, Research and Development/ University of California at Los Angeles

2

Figure 2: Matching evidence-based guidance to individual disease courses in clinical practice

A typical disease sequence experienced by a patient with JIA begins with disease onset and diagnosis, after which disease activity (manifesting as periods of disease flare or disease remission) typically varies with time; scenarios of clinically inactive disease, in which subclinical disease persists, can also occur. Clinical practice guidelines and recommendations are founded on evidence-based statements, data from randomised controlled trials or consensus-derived definitions, but these are focused on particular aspects of the management of JIA such as initiation of therapy or achieving remission on medication. The disease activity patterns seen over time indicate other important long-term targets, including managing subclinical active disease, preventing disease flares and timing withdrawal of medication to achieve the ultimate goal of remission off medication. These targets are not yet adequately addressed by existing evidence or guidelines; thus, continued efforts to establish consensus-derived treatment strategies and investigator-initiated trials are required.



Consensus treatment plans

Evolving from the ACR recommendations, the North American Childhood Arthritis and Rheumatology Research Alliance (CARRA) developed consensus-based treatment plans for the initial treatment of patients with pJIA and sJIA.(57) The CARRA consensus-based treatment plans are primarily aimed at treatment standardization, to improve the power of comparative effectiveness research, and are not intended as guidelines for the care of patients in the way that the ACR recommendations are. For example, three treatment strategies for pJIA are defined, which represent the typical clinical spectrum: a step-up plan (conventional DMARD therapy, followed by a biologic DMARD in case of non-response) similar to the ACR recommendations; early combined treatment (conventional and biologic DMARD given at onset), similar to the most intensive treatment arm in the TREAT study;(19) and a biologic-DMARD-only plan, which will enable long-term comparisons of the effectiveness of these strategies. The British Society for Paediatric and Adolescent Rheumatology and the Arthritis Musculoskeletal Alliance also published consensus-derived standards of care in 2009, which focused on access to treatment and standards for care provision, rather than strategies for individual therapies.(58-59)

Impact and limitations of standard treatments

One limitation of clinical practice guidelines or recommendations is that they can often be outdated even before their publication.(54,60) For example, the IL 6 receptor antibody tocilizumab was not included in the 2011 ACR recommendations, but was approved by the FDA before these recommendations were published; the ACR recommendations were updated afterwards.(61) The Institute of Medicine's definition of clinical practice guidelines requires the inclusion of evidence from systematic reviews;(51) however, this evidence is often not available in JIA, leading to an inclusion bias towards quantifiable outcomes. For example, non-drug-based therapies are omitted from the ACR recommendations, but are included in the German recommendations.(53-54) A search of the Cochrane library in May 2014 using the keywords "juvenile arthritis" only retrieved six systematic reviews covering methotrexate and intra-articular steroid treatments, exercise and psychological therapies or nonsurgical interventions.(62–67) By contrast, the keywords "rheumatoid arthritis" retrieved 97 reviews, highlighting the reasons why treatments in paediatric rheumatology have been informed by adult studies. Even when

randomized controlled trials (RCTs) are available, these vary in both their inclusion criteria and in the presence or absence of an active comparator in the trial, limiting their validity and generalizability for use in individual patients. Current guidelines are also limited by the scanty reporting of medication-related adverse effects, comorbidities and non-drug-based therapies, and therefore cannot be considered comprehensive. Owing to these limitations and the often narrow scope of guidelines and RCTs for the management of JIA, consensus-derived treatment strategies and investigator-initiated trials are still needed (Figure 2).

Discontinuation of treatment

For patients who maintain CID during treatment, knowing when to withdraw therapy (to avoid unnecessary medication) without compromising disease control is a challenging question influenced by multiple variables (Figures 2 and 3). The risk of disease flare after achieving CID and withdrawal of methotrexate is independent of the duration of CID, and can be as high as 50% within 2 years; patients who achieve sustained drug-free remission are rare. (31) Returning to remission after a flare is possible for many patients, but can be difficult for some.

Personalized treatment

Individualized or stratified therapies

A core value of modern health services is the provision of patient-centred care, with clinical decisions guided by the needs and preferences of each individual patient while maintaining standard best practice.(68) Possible strategies include treatment adjustments based on disease duration, treatment preferences and perceptions, previous response and tolerance to treatments, as well as measurement of quantifiable, defining characteristics such as genomic or pharmacokinetic parameters.(69) Furthermore, as discussed previously in this review, the development of accurate biomarkers to define and quantify disease activity might assist further in assessing an individual's need for additional therapies. Personalizing treatment of JIA could lead to the selection of the most efficacious therapies, with optimal durations and possibly avoiding adverse reactions.(70-71) Strategies that maximize treatment efficacy should be balanced with efforts to minimize manifest or potential adverse effects. Discrete choice studies involving parents of children with JIA demonstrate that the majority of parents place most emphasis on efficacy and reducing

pain, even if that decision involves accepting adverse effects from medication.(72) Fully personalized treatment is as yet unattainable, mainly owing to persisting large gaps of knowledge, in particular regarding the pathogenesis of JIA; however, certain elements are or might soon be achievable.

Patient stratification to optimize treatment

Given the heterogeneity of JIA and the variability in responses to therapies, personalized treatment of this disease requires comprehensive stratification strategies that include the consideration of genetic, clinical and biochemical (or biomarker) factors. The relative rarity of JIA and its largely unknown aetiology result in much of the knowledge in this field being extrapolated from studies of genotype–phenotype correlations in adult patients.(73) Environmental and genetic risk factors are likely to play a part in disease development. Several paediatric trials, including the Research in Arthritis in Canadian Children emphasizing Outcomes (ReACCh-Out) and Childhood Arthritis Risk Factor Identification Study (CLARITY) studies, are currently underway to investigate these factors.(74-75) Genome-wide association studies in patients with JIA have confirmed an association with non-HLA gene regions, as well as the previously established HLA gene regions.(76) The identification of other relevant genetic and epigenetic variations (for example in microRNAs, DNA methylation or histone acetylation) might also pave the way for a stratification strategy to personalize and optimize treatment for patients with JIA.(77)

Gene expression signatures and genetic profiles, including single nucleotide polymorphisms, could be used to stratify patients further according to their response to treatment as evaluated by either individual profiling (pharmacogenetics) or profiling of subgroups of individuals (pharmacogenomics).(78) The diversity in genetic signatures, particularly those of drug-metabolizing enzymes, contributes to differences in drug response.(79) Several differences in clinical responses to treatment have already been associated with a number of gene variants, but further evidence is required before incorporating this type of screening into routine clinical care. Nevertheless, the quantification of mRNA expression of thousands of genes (transcriptomics) is already helping to rationalize therapies in other diseases.(80)

Figure 3: Target definition and achievement in JIA management.

Various protocols, management pathways, treatment guidelines and recommendations exist for the management of JIA, each with the aim of standardizing treatment and optimization of outcomes. A standardized approach based on the ACR 2011 treatment recommendations can incorporate the "treat-to-target" and "tight control" concepts.(52) Prognostic factors and disease activity measurements should also include biomarkers that can help guide therapeutic decision-making and monitor the patient's responses.

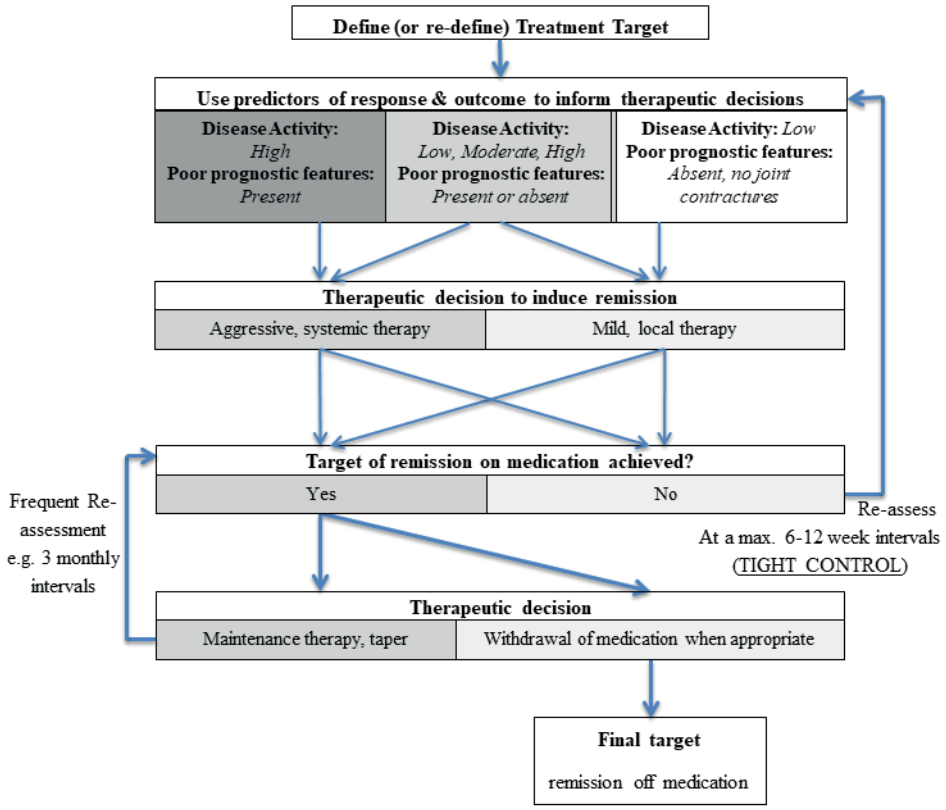
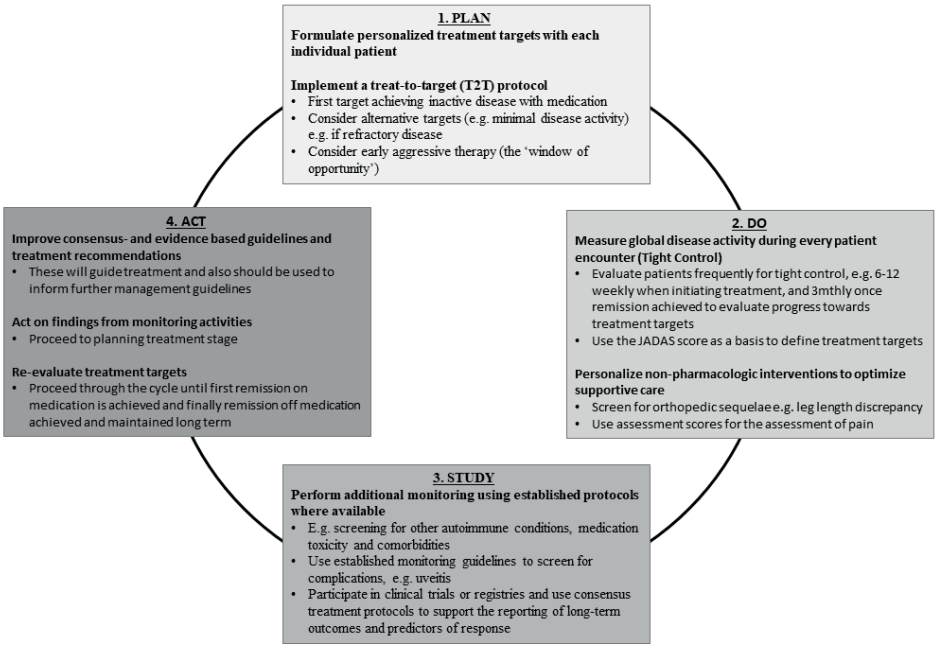


Figure 4 Optimization of JIA management in clinical practice

Where possible, effective management strategies in JIA should incorporate both personalized (for example patient-specific treatment targets) and standardized treatment strategies (based on published guidelines and data from randomized controlled trials) to achieve optimal treatment outcomes. Any treatment strategy requires a continuous process of reassessment, which can be illustrated in the form of a 'Plan, Do, Study, Act' cycle.(90) First, treatment planning (Plan) should define treatment targets, taking into account the specific needs of the patient as well as current treatment recommendations; next, the treatment plan is implemented (Do), supported by quantitative disease activity measurement while incorporating a tight control strategy by frequently reviewing parameters—personalized treatment is achieved by assessing each individual's need for supportive therapies; the response to treatment should then be assessed (Study), for example by performing recommended screening for medication toxicity or uveitis screening; finally, clinicians should act on the outcomes of the previous steps (Act), and return to the 'Plan' step to address results of screening, ongoing treatment and readdress treatment targets. In parallel, data sharing and reporting in consensus protocols should aid optimization of future treatment guidelines and recommendations.
Abbreviations: JADAS, Juvenile Arthritis Disease Activity Score; T2T, treat-to-target.



Can personalized care be standardized?

Guidelines based on relevant research take a population-centred approach to providing standardized clinical care. By contrast, personalized care is patient-centred and, to date, has not been addressed satisfactorily by standardized guidelines. Standardized guidelines based on data from RCTs assume that such data are generalizable to all individuals, and that an individual patient treated according to the guideline would have fulfilled the inclusion criteria for that trial; however, this assumption is rarely correct. Subgroup analyses within RCTs could improve this limitation, but even when such analyses are performed, they are often inappropriate.(69) Therefore, the concept of standardized care seems to be in stark contrast to the concept of personalized care. Experts have argued that giving less attention to personalized medicine and more attention to improving adherence to treatment guidelines (as different biologic DMARDs are similarly efficacious) would improve remission rates in patients with RA.(81) Adherence to a clinical practice guideline can be explicit (direct statements in guidelines) or inferred (encouraged by audits of adherence to guidelines). However, comprehensive RCTs or systematic reviews to support or disprove this claim are lacking.82 The definition of clinical practice guidelines argues against imposing standardization, as these guidelines should aid clinical practice, not strictly define it.(51) Overgeneralization can have potentially negative consequences if, for example, a subgroup of patients with JIA would benefit from a different approach but clinicians are encouraged to treat all patients in the same way.(69)

Progress in using biomarkers to direct personalized treatment can be incorporated into clinical study design and treatment algorithms.(40,70,83,84) In a study of patients with RA, pharmacokinetics and drug level measurements from each patient were considered for the evaluation of cost-effectiveness and clinical efficacy of adalimumab therapy.(70) Actively determining levels of therapeutic drugs and neutralizing anti-drug antibodies, referred to as ‘theranostics’ (therapeutics–diagnostics), could represent another aspect of personalized treatment.(85) Effective guidelines should include or retain scope for patient-centred management; examples of integrated management strategy for use in clinical practice (Figure 3) can begin with a personalized treatment plan and defined targets, and incorporate fundamental elements of standardized care, such as established monitoring practices.

Non-drug-based therapies

Many paediatric rheumatologists consider holistic care, with close cooperation with physiotherapists and occupational therapists, a vital adjunct to achieving optimal pain management and prevention of long-term morbidity.(54,86) Controlled studies are lacking for most non-drug-based therapies, but consensus statements for these therapies have been developed. Non-drug approaches should be used as indicated by individual need, previous proven benefit and expert opinion.(54) Important areas of focus include exercise therapy, optimization of bone health and management of pain and psychological issues.(54,58) Exercise, comprising active and passive movements, strengthening, weight-bearing and cardiovascular activities, should be performed even during periods of flare to help maintain muscle strength and function, and can be supported with hydrotherapy.(58) Bone health requires special attention since both persistent disease activity (for example, for systemic and local inflammation in pJIA and sJIA), and corticosteroid treatment are associated with an increased risk of osteoporosis.(24,58,87) Despite improvements in therapies, pain is still an important symptom for children with JIA and can persist even with use of biologic DMARDs and in the absence of disease activity.(88,89) Apart from the use of analgesics, pain management can include the use of splinting or other assistive devices, orthotics or even surgical care.(58) Psychological therapists, similarly to pain specialists, can also be integral to a multidisciplinary team, helping to deal with any behavioural or psychological issues that might arise from the underlying pain or physiological abnormalities associated with JIA.(54)

Conclusions

Treatment of JIA remains difficult, despite huge progress in available trial-tested pharmacological treatments and established evidence-based treatment recommendations. Achieving optimal and defined treatment targets is more probable when a combination of standardized and personalized treatment approaches is employed, particularly when implemented as early as possible in the disease course. Clinical trials incorporating personalized drug withdrawal regimens and non-pharmacological measures are required to facilitate comparisons of individualized therapeutic plans. Whereas clinical assessment is the foundation of current disease activity scores, improving future definitions and cut-offs for disease activity, CID and subclinical disease activity will probably involve the use of imaging modalities and novel biomarkers. The

development and application of treatment plans that implement both standardized and personalized approaches contributes to continuous efforts to improve the quality of management of JIA (Figure 4). Therefore, continued efforts are required to achieve a consensus regarding definitions of disease activity, treatment targets, recommendations and protocols, as well as trial design and definition of outcome measures; agreement over all these aspects can contribute to the standardization of personalized treatments, potentially improving and optimizing the management of JIA.

Acknowledgements
F.G. is supported by the European Union Seventh Framework Programme for Research and Technological Development (FP7) grant EUTRAIN (European Translational Training for Autoimmunity & Immune Manipulation Network, ref. 289903). D.F. is supported by the FP7 grant MIAMI (Monitoring Innate Immunity in Arthritis and Mucosal Inflammation project, ref. 305266) and by a Clinical Research Award (CRA 04) from the Interdisciplinary Centre of Clinical Research (IZKF), Münster, Germany.

References

1. Prakken, B., Albani, S. & Martini, A. Juvenile idiopathic arthritis. Lancet 377, 2138-49 (2011).
2. Barnes, M.G. et al. Subtype-specific peripheral blood gene expression profiles in recent-onset juvenile idiopathic arthritis. Arthritis Rheum 60, 2102-12 (2009).
3. van den Ham, H.J., de Jager, W., Bijlsma, J.W., Prakken, B.J. & de Boer, R.J. Differential cytokine profiles in juvenile idiopathic arthritis subtypes revealed by cluster analysis. Rheumatology (Oxford) 48, 899-905 (2009).
4. Lehman, T.J. The future of pediatric rheumatology: many questions remain. Arthritis Rheum 56, 2815-6 (2007).
5. Guzman, J. et al. The outcomes of juvenile idiopathic arthritis in children managed with contemporary treatments: results from the ReACCh-Out cohort. Ann Rheum Dis (2014).
6. Nordal, E. et al. Ongoing disease activity and changing categories in a long-term nordic cohort study of juvenile idiopathic arthritis. Arthritis Rheum 63, 2809-18 (2011).
7. Oen, K. et al. Disease course and outcome of juvenile rheumatoid arthritis in a multicenter cohort. J Rheumatol 29, 1989-99 (2002).
8. Selvaag, A.M. et al. Early disease course and predictors of disability in juvenile rheumatoid arthritis and juvenile spondyloarthropathy: a 3 year prospective study. J Rheumatol 32, 1122-30 (2005).
9. Wallace, C.A., Huang, B., Bandeira, M., Ravelli, A. & Giannini, E.H. Patterns of clinical remission in select categories of juvenile idiopathic arthritis. Arthritis Rheum 52, 3554-62 (2005).
10. Ansell, B.M. & Swann, M. The management of chronic arthritis of children. J Bone Joint Surg Br 65, 536-43 (1983).
11. Fries, J.F. Current treatment paradigms in rheumatoid arthritis. Rheumatology (Oxford) 39 Suppl 1, 30-5 (2000).
12. Giannini, E.H. et al. Methotrexate in resistant juvenile rheumatoid arthritis. Results of the U.S.A.-U.S.S.R. double-blind, placebo-controlled trial. The Pediatric Rheumatology Collaborative Study Group and The Cooperative Children's Study Group. N Engl J Med 326, 1043-9 (1992).
13. Evaluation, H.M.D.a. in EMA/250577/2013 (ed. Agency, E.M.) (2013).
14. Stoll, M.L. & Cron, R.Q. Treatment of juvenile idiopathic arthritis: a revolution in care. Pediatr Rheumatol Online J 12, 13 (2014).
15. Ruperto, N., Vesely, R., Saint-Raymond, A., Martini, A. & Paediatric Rheumatology International Trials, O. Impact of the European paediatric legislation in paediatric rheumatology: past, present and future. Ann Rheum Dis 72, 1893-6 (2013).
16. Levinson, J.E. & Wallace, C.A. Dismantling the pyramid. J Rheumatol Suppl 33, 6-10 (1992).
17. Recommendations of the German Society of Rheumatology for therapy with tumor necrosis factor inhibitors. Pharmacotherapy Committee of the German Society of Rheumatology]. Z Rheumatol 59, 291-2 (2000).
18. Tynjala, P. et al. Aggressive combination drug therapy in very early polyarticular juvenile idiopathic arthritis (ACUTE-JIA): a multicentre randomised open-label clinical trial. Ann Rheum Dis 70, 1605-12 (2011).
19. Wallace, C.A. et al. Trial of early aggressive therapy in polyarticular juvenile idiopathic arthritis. Arthritis Rheum 64, 2012-21 (2012).
20. Smolen, J.S. et al. Treating rheumatoid

arthritis to target: recommendations of an international task force. Ann Rheum Dis 69, 631-7 (2010).
21. Consolaro, A. et al. Toward a treat-to-target approach in the management of juvenile idiopathic arthritis. Clin Exp Rheumatol 30, S157-62 (2012).
22. Smolen, J.S. Treat-to-target: rationale and strategies. Clin Exp Rheumatol 30, S2-6 (2012).
23. Henderson, C.J., Specker, B.L., Sierra, R.I., Campaigne, B.N. & Lovell, D.J. Total-body bone mineral content in non-corticosteroid-treated postpubertal females with juvenile rheumatoid arthritis: frequency of osteopenia and contributing factors. Arthritis Rheum 43, 531-40 (2000).
24. Grigor, C. et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomsied controlled trial. Lancet 364, 263-269 (2004).
25. Felson, D.T. et al. American College of Rheumatology/European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. Ann Rheum Dis 70, 404-13 (2011).
26. Smolen, J.S. et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis 69, 964-75 (2010).
27. Smolen, J.S. et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis 73, 492-509 (2014).
28. Consolaro, A. et al. Remission, minimal disease activity, and acceptable symptom state in juvenile idiopathic arthritis: defining criteria based on the juvenile arthritis disease activity score. Arthritis Rheum 64, 2366-74 (2012).
29. Wallace, C.A. et al. American College of Rheumatology provisional criteria for defining clinical inactive disease in select categories of juvenile idiopathic arthritis. Arthritis Care Res (Hoboken) 63, 929-36 (2011).
30. Wallace, C.A., Ruperto, N. & Giannini, E. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. J Rheumatol 31, 2290-4 (2004).
31. Foell, D. et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. JAMA 303, 1266-73 (2010).
32. Hinze, C. et al. A68: The Role of Serum S100A12 Protein Levels in Maintaining Inactive Disease on Anti-tumor Necrosis Factor Therapy in Polyarticular Forms of Juvenile Idiopathic Arthritis. Arthritis Rheumatol 66 Suppl 11, S99-S100 (2014).
33. Hinze, C. et al. A12: The Role of Serum S100A12 Protein Levels in Disease Flare After Withdrawal of Anti-tumor Necrosis Factor Therapy in Polyarticular Forms of Juvenile Idiopathic Arthritis. Arthritis Rheumatol 66 Suppl 11, S19-20 (2014).
34. Prevoo, M.L. et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38, 44-8 (1995).
35. Consolaro, A. et al. Development and validation of a composite disease activity score for juvenile idiopathic arthritis. Arthritis Rheum 61, 658-66 (2009).
36. Bulatovic Calasan, M., de Vries, L.D., Vastert, S.J., Heijstek, M.W. & Wulffraat, N.M. Interpretation of the Juvenile Arthritis Disease Activity Score:

responsiveness, clinically important differences and levels of disease activity in prospective cohorts of patients with juvenile idiopathic arthritis. Rheumatology (Oxford) 53, 307-12 (2014).
37. McErlane, F. et al. Validity of a three-variable Juvenile Arthritis Disease Activity Score in children with new-onset juveile idiopathic arthritis. Ann. Rheum Dis. 72, 1983-1988 (2013).
38. Wittkowski, H. et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum 58, 3924-31 (2008).
39. Gerss, J. et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. Ann Rheum Dis 71, 1991-7 (2012).
40. Miossec, P. et al. Biomarkers and personalised medicine in rheumatoid arthritis: a proposal for interactions between academia, industry and regulatory bodies. Ann Rheum Dis 70, 1713-8 (2011).
41. Hunter, P.J. & Wedderburn, L.R. Pediatric rheumatic disease: can molecular profiling predict the future in JIA? Nat Rev Rheumatol 5, 593-4 (2009).
42. Kessel, C., Holzinger, D. & Foell, D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. Clin Immunol 147, 229-41 (2013).
43. Rothmund, F. et al. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care Res (Hoboken) 66, 949-55 (2014).
44. Collado, P. et al. Detection of synovitis by ultrasonography in clinically inactive juvenile idiopathic arthritis on and off medication. Clin Exp Rheumatol (2014).
45. Javadi, S., Kan, J.H., Orth, R.C. & DeGuzman, M. Wrist and ankle MRI of patients with juvenile idiopathic arthritis: identification of unsuspected multicompartmental tenosynovitis and arthritis. AJR Am J Roentgenol 202, 413-7 (2014).
46. Vastert, S.J. et al. Effectiveness of first line use of recombinant IL-1RA treatment in steroid naive systemic juvenile idiopathic arthritis: Results of a prospective cohort study. Arthritis Rheum (2013).
47. Nigrovic, P.A. Review: is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis? Arthritis Rheumatol 66, 1405-13 (2014).
48. van Rossum, M.A. et al. Long-term outcome of juvenile idiopathic arthritis following a placebo-controlled trial: sustained benefits of early sulfasalazine treatment. Ann Rheum Dis 66, 1518-24 (2007).
49. Ting, T.V. & Lovell, D.J. Does early sulfasalazine treatment provide long-term benefits to patients with juvenile idiopathic arthritis? Nat Clin Pract Rheumatol 4, 344-5 (2008).
50. Hissink Muller, P.C.E et al. A comparison of three treatment strategies in recent onset DMARD naïve juvenile idiopathic arthritis: 3-months results of the BeSt for Kids-Study. Late breaking abstracts presented at the ACR Annual Meeting 2014 [online], http://acrabstracts.org/abstracts/a-comparison-of-three-treatment-strategies-in-recent-onset-dmard-naïve-juvenile-idiopathic-arthritis-3-months-results-of-the-best-for-kids-study (2014).
51. Institute of Medicine (U.S.). Committee on Standards for Developing Trust-

worthy Clinical Practice Guidelines. & Graham, R. Clinical practice guidelines we can trust (National Academies Press, Washington, DC, 2011).
52. McMaster, P., Rogers, D., Kerr, M. & Spencer, A. Getting guidelines to work in practice. Arch Dis Child 92, 104-6 (2007).
53. Beukelman, T. et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. Arthritis Care Res (Hoboken) 63, 465-82 (2011).
54. Dueckers, G. et al. Evidence and consensus based GKJR guidelines for the treatment of juvenile idiopathic arthritis. Clin Immunol 142, 176-93 (2012).
55. Martini, A. JIA in 2011: New takes on categorization and treatment. Nat Rev Rheumatol 8, 67-8 (2012).
56. Huppertz, H.I. Recommendations for juvenile idiopathic arthritis by the American College of Rheumatology: comment on the article by Beukelman et al. Arthritis Care Res (Hoboken) 63, 1354-5; author reply 1355-6 (2011).
57. Ringold, S. et al. Childhood arthritis and rheumatology research alliance consensus treatment plans for new onset polyarticular juvenile idiopathic arthritis. Arthritis Care Res (Hoboken) (2013).
58. Davies, K. et al. BSPAR Standards of Care for children and young people with juvenile idiopathic arthritis. Rheumatology (Oxford) 49, 1406-8 (2010).
59. Wallace, C.A. Developing standards of care for patients with juvenile idiopathic arthritis. Rheumatology (Oxford) 49, 1213-4 (2010).
60. Hull, R.G. & British Paediatric Rheumatology, G. Guidelines for management of childhood arthritis. Rheumatology (Oxford) 40, 1309-12 (2001).
61. Ringold, S. et al. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. Arthritis Rheum 65, 2499-512 (2013).
62. Eccleston, C. et al. Psychological therapies for the management of chronic and recurrent pain in children and adolescents. Cochrane Database Syst Rev 12, CD003968 (2012).
63. Hawke, F., Burns, J., Radford, J.A. & du Toit, V. Custom-made foot orthoses for the treatment of foot pain. Cochrane Database Syst Rev, CD006801 (2008).
64. Rome, K., Ashford, R.L. & Evans, A. Non-surgical interventions for paediatric pes planus. Cochrane Database Syst Rev, CD006311 (2010).
65. Takken, T. et al. Exercise therapy in juvenile idiopathic arthritis. Cochrane Database Syst Rev, CD005954 (2008).
66. akken, T., Van Der Net, J. & Helders, P.J. Methotrexate for treating juvenile idiopathic arthritis. Cochrane Database Syst Rev, CD003129 (2001).
67. Wallen, M. & Gillies, D. Intra-articular steroids and splints/rest for children with juvenile idiopathic arthritis and adults with rheumatoid arthritis. Cochrane Database Syst Rev, CD002824 (2006).
68. Institute of Medicine (U.S.). Committee on Quality of Health Care in America. Crossing the quality chasm : a new health system for the 21st century (National Academy Press, Washington, D.C., 2001).
69. Goldberger, J.J. & Buxton, A.E. Personalized medicine vs guideline-based medicine. JAMA 309, 2559-60 (2013).

70. Krieckaert, C.L. et al. Personalised treatment using serum drug levels of adalimumab in patients with rheumatoid arthritis: an evaluation of costs and effects. Ann Rheum Dis (2013).
71. Malik, N.N. Biologics: Personalized drugs should cut care costs. Nature 485, 582 (2012).
72. Burnett, H.F., Regier, D.A., Feldman, B.M., Miller, F.A. & Ungar, W.J. Parents' preferences for drug treatments in juvenile idiopathic arthritis: a discrete choice experiment. Arthritis Care Res (Hoboken) 64, 1382-91 (2012).
73. Becker, M.L. Pharmacogenomics in pediatric rheumatology. Curr Opin Rheumatol 24, 541-7 (2012).
74. Ellis, J.A. et al. CLARITY - ChiLdhood Arthritis Risk factor Identification sTudY. Pediatr Rheumatol Online J 10, 37 (2012).
75. Oen, K. et al. Predictors of early inactive disease in a juvenile idiopathic arthritis cohort: results of a Canadian multicenter, prospective inception cohort study. Arthritis Rheum 61, 1077-86 (2009).
76. Cobb, J.E., Hinks, A. & Thomson, W. The genetics of juvenile idiopathic arthritis: current understanding and future prospects. Rheumatology (Oxford) 53, 592-9 (2014).
77. Hawtree, S., Muthana, M. & Wilson, A.G. The role of histone deacetylases in rheumatoid arthritis fibroblast-like synoviocytes. Biochem Soc Trans 41, 783-8 (2013).
78. Wellcome Trust Sanger Institute. yourgenome.org [online], http://www.yourgenome.org. (2014).
79. Szekanecz, Z. et al. Pharmacogenetics and pharmacogenomics in rheumatology. Immunol Res 56, 325-33 (2013).
80. Smith, S.L., Plant, D., Eyre, S. & Barton, A. The potential use of expression profiling: implications for predicting treatment response in rheumatoid arthritis. Ann Rheum Dis 72, 1118-24 (2013).
81. Smolen, J.S. & Aletaha, D. Forget personalised medicine and focus on abating disease activity. Ann Rheum Dis 72, 3-6 (2013).
82. Wilke, W.S. Reply to: forget personalized medicine and focus on abating disease activity. Ann Rheum Dis 72, e8 (2013).
83. Dunn, G., Emsley, R., Liu, H. & Landau, S. Integrating biomarker information within trials to evaluate treatment mechanisms and efficacy for personalised medicine. Clin Trials 10, 709-19 (2013).
84. Tak, P.P. A personalized medicine approach to biologic treatment of rheumatoid arthritis: a preliminary treatment algorithm. Rheumatology (Oxford) 51, 600-9 (2012).
85. Bendtzen, K. Personalized medicine: theranostics (therapeutics diagnostics) essential for rational use of tumor necrosis factor-alpha antagonists. Discov Med 15, 201-11 (2013).
86. Spamer, M. et al. [Physiotherapy for juvenile idiopathic arthritis]. Z Rheumatol 71, 387-95 (2012).
87. Lien, G. et al. Frequency of osteopenia in adolescents with early-onset juvenile idiopathic arthritis: a long-term outcome study of one hundred five patients. Arthritis Rheum 48, 2214-23 (2003).
88. Consolaro, A. & Ravelli, A. Paediatric rheumatology: Juvenile idiopathic arthritis--are biologic agents effective for pain? Nat Rev Rheumatol 9, 447-8 (2013).
89. La Hausse de Lalouviere, L., Ioannou, Y. & Fitzgerald, M. Neural mechanisms underlying the pain of juvenile idiopathic arthritis. Nat Rev Rheumatol 10, 205-11 (2014).
90. Taylor, M. J. et al. Systematic review of

the application of the plan-do-study-act method to improve quality in healthcare. BMJ Qual. Saf. 23, 290-298 (2014).

Chapter 3

Review of biomarkers in systemic juvenile idiopathic arthritis: Helpful tools or just playing tricks?

Faekah Gohar, Christoph Kessel, Miha Lavric,
Dirk Holzinger, Dirk Foell

Arthritis Research & Therapy, 2016: 18:163

Abstract

Background

Diagnosing systemic juvenile idiopathic arthritis (SJIA) can be extremely challenging if typical arthritis is lacking. A variety of biomarkers have been described for the diagnosis and management of SJIA. However, very few markers have been well validated. In addition, increasing numbers of biomarkers are identified by high throughput or multi-marker panels.

Method

We identified diagnostic or prognostic biomarkers by systematic literature review, evaluating each according to a predefined level of verification, validation or clinical utility. Diagnostic biomarkers were those identifying SJIA versus (1) non-SJIA conditions or healthy controls (HC) or (2) other non-systemic JIA subtypes. Prognostic biomarkers were those specifically tested for the prediction of (1) disease flare, (2) increased disease activity +/- discrimination of active versus inactive disease, or (3) macrophage activation syndrome (MAS).

Results

Fifty-five studies fulfilled the inclusion criteria identifying 68 unique biomarkers, of which 50 (74 %) were investigated by only a single research group. Candidate marker verification and clinical utility was evaluated according to whether markers were readily and reliably measurable, investigated by independent study groups, discovered by more than one method (i.e. verified markers) and validated in independent cohorts. This evaluation revealed diagnostic biomarkers of high interest for further evaluation in the diagnostic approach to SJIA that included heme oxygenase-1, interleukin-6 (IL-6), IL-12, IL-18, osteoprotegerin, S100 calcium-binding protein A12 (S100A12) and S100A8/A9.

Conclusion

In summary, a number of biomarkers were identified, though most had limited evidence for their use. However, our findings combined with the identified studies could inform validation studies, whether in single or multi-marker assays, which are urgently needed.

Background

Systemic juvenile idiopathic arthritis (SJIA), or Still's disease/syndrome, is a childhood rheumatic condition that is typically characterized by spiking fever in a quotidian pattern, transient rash and arthritis. Patients may alternate between periods of disease activity (flare) and inactive disease. SJIA accounts for around 10–20 % of juvenile idiopathic arthritis (JIA), which has an incidence of around 6.6–15 per 100,000 children.(1) Although defined as a subtype of JIA, patients often present with rather unspecific signs and symptoms initially, with the hallmark fever of unknown origin, but without chronic arthritis. Diagnosing SJIA is challenging in these cases as the disease is recognized as an autoinflammatory syndrome rather than classical autoimmune arthritis.(2, 3) Accordingly, most clinical symptoms can be attributed to dysregulated innate immune mechanisms with only minor involvement of adaptive immunity. Gene expression studies of circulating cells show increased levels of transcripts, reflecting monocyte/macrophage-associated activation in SJIA.(4-6) The innate immune cells such as monocytes and macrophages are thought to be drivers of SJIA, producing several mediators implicated in the pathogenesis of SJIA, including interleukin-1 (IL-1), IL-6 and IL-18 and phagocyte-specific S100 proteins.(7) IL-1 in particular seems to have a prominent role in SJIA. Serum from patients with SJIA induces the transcription of genes of the innate immune system including IL-1 in peripheral blood mononuclear cells (PBMC). Furthermore, activated monocytes from patients with SJIA secrete significantly more IL-1β in comparison with monocytes from healthy controls.(6)

Significant challenges to improving the clinical care of patients with SJIA include the discrimination of SJIA from other causes of fever, evidence-based evaluation of response to treatment, detection and limitation of subclinical inflammation and discrimination of SJIA without macrophage activation syndrome (MAS) from SJIA with MAS.(8) MAS is a serious complication of SJIA with a 10 % mortality risk, defined as an acute episode of overwhelming inflammation and characterized by activation and expansion of T lymphocytes and hemophagocytic macrophages. In the early stages, development of MAS is difficult to predict and diagnostic and prognostic biomarkers might enable early intervention.

These challenges could be addressed by the identification and validation of clinically relevant biomarkers, of which those circulating in serum and plasma are useful and easily obtainable from peripheral blood.(9-13) Mechanistic markers are those that are elevated or decreased in response to underlying pathological processes, whereas proxy markers, such as C-reactive protein (CRP), do not have a definite role in the pathology of the disease, and are non-specific markers of inflammation.(14) Therefore, measurement of a mechanistic biomarker can quantify a pathologic process. With such quantification, a level of severity can be defined and cut-offs determined, allowing the use of such biomarkers as treatment targets (Figure 1).(8, 15) Diagnostic biomarkers, proxy or mechanistic, can aid detection of a disease or confirm it in uncertain cases e.g., evolving SJIA versus sepsis.(15,16)

Although a number of publications describe potential biomarkers, none have been recently validated or used in clinical studies aside from the IL-1 family cytokines and the S100-proteins, S100A12 and S100A8/A9.(17) To date, discovery studies vastly outnumber validation studies, which are more challenging to perform given their requirement for independent cohorts and statistically valid sample sizes. Additionally, the number of identified candidates is usually large and the cost of validation high, leading to a need for unbiased prioritization of candidates for validation.(18)

In conclusion, a combination of sensitive biomarkers could allow targeted and personalized treatment and improve treatment outcomes.(8) We therefore identified current candidate diagnostic and prognostic biomarkers from the literature, additionally evaluating their potential for validation/clinical use, function and association with other identified biomarkers. We also discuss the current and future potential of biomarkers for SJIA.

Method

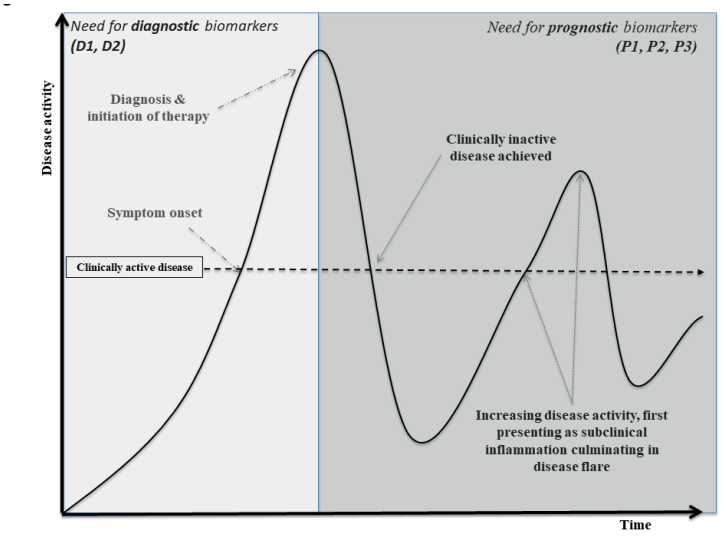
Search criteria

A PubMed search was performed using the search terms as follows: “Arthritis, Juvenile“[Mesh] AND ((”2000/11/ 01”[PDAT]: “2015/11/01”[PDAT]) AND “humans”[MeSH Terms] AND English [lang]) along with the additional keywords: 1) cytokine (“cytokines”[MeSH Terms] OR “cytokines”[All Fields] OR “cytokine”[All Fields]) (n = 544 individual studies identified), OR 2) biomarker (“biological markers”[MeSH Terms] OR (“biological”[All

Fields] AND "markers"[All Fields]) OR "biological markers"[All Fields] OR "biomarker"[All Fields]) (n = 307), OR 3) validation (n = 114). Abstracts of identified studies were reviewed and any fulfilling exclusion criteria at the outset were excluded and the full text scrutinized for those remaining.

Figure 1 Biomarker need in clinical context

Typical clinical sequence in systemic juvenile idiopathic arthritis (SJIA) from disease onset, diagnosis to clinical resolution and flare. Specific time points where there is a need for diagnostic and prognostic biomarkers are indicated. Diagnostic markers are indicated as follows: D1 SJIA versus other non-JIA conditions, D2 SJIA versus other JIA subtypes. Prognostic markers are indicated as follows: P1 prognostic for flare, P2 prognostic for increased disease activity, P3 prognostic for macrophage activation syndrome (MAS) or differentiating MAS from SJIA flare.Adapted from Hinze et al. 2015 [8]



Inclusion and exclusion criteria

Inclusion criteria were as follows: studies in which serum or plasma markers were analysed; original research studies; studies that specifically addressed the biomarkers with the diagnostic or prognostic functions as indicated in Table 1 and studies that also included SJIA-specific analyses. Exclusion criteria were: case studies or review articles; studies that included fewer than three patients with SJIA; studies with only negative findings reported (i.e. no statistically significant finding for the candidate marker for the use evaluated) and studies describing functional/cell-based assays or enzyme activity assays. We also excluded studies on adult-onset Still's disease (AOSD) (19) and genetic array/genotype or phenotype studies that described individual patients rather than disease signatures, without evaluation of individual biomarkers, even if performed as unbiased discovery studies. Genetic markers and gene expression profiles in SJIA have been previously discussed in a review by Nirmala et al.(20)

Data analysis and categorisation of biomarkers

Details recorded from identified studies included the aims, numbers of included patients and methods of biomarker assessment (Supplemental Table 1). Biomarkers from each study were categorised as diagnostic (discriminating SJIA from non-JIA disorders or healthy controls (HC) termed "D1 biomarkers" or differentiating SJIA from other JIA subtypes, "D2 biomarkers") or prognostic (for flare, "P1 biomarkers", increased disease activity or discriminating active versus inactive disease, "P2 biomarkers", or prognostic for MAS or differentiating SJIA with and without MAS, "P3 biomarkers"), as defined in Table 1, according to the study objectives, and indicated in Fig. 1.

Evaluation of identified markers

Identified candidate biomarkers were scored and ranked by their potential to reach validation or clinical use, with potentially spurious or unreproducible candidate findings ranked the lowest. The biomarker scoring system (BMS) used (Table 2) was developed to identify whether identified candidates (1) were readily measurable, i.e. in standard collected biological samples and without special equipment, (2) had been measured by independent study groups, as confirmation that the biomarker is detectable, (3) had been discovered by more than one method, e.g. proteomic and enzyme-linked immunosorbent assay (ELISA) methods, (4) had been measured by an established assay, i.e. an assay that is well described, with normal cut-off values availa-

ble, as this would allow easier translation to clinical practice and finally (5) had been validated for the stated clinical question. Each evaluation question making up the BMS (Table 2) was answered using only the information collected during the review process, and each of the included five questions was scored 0 or 1.

Table 1: Diagnostic and prognostic criteria for inclusion

Biomarker function	Description	Biomarkers identified (n)	No. of studies in which biomarkers were identified (n)
Diagnostic	D1: SJIA versus other non-arthritis conditions or HC	36	48
	D2: SJIA versus other JIA subtypes	25	25
Prognostic	P1: for flare (or relapse)	14	16
	P2: for increased disease activity and/or the discrimination of active and inactive disease	15	21
	P3: for MAS or discriminating MAS from SJIA flare	7	12

Table 2: Scoring system used to perform an unbiased evaluation of identified biomarkers

Q1	Readily measurable (e.g. in serum)	Yes=1	No=0
Q2	Measured by more than one independent study group	Yes=1	No=0
Q3	Discovered by more than one single method	Yes=1	No=0
Q4	Measured by a reproducible assay	Yes=1	No=0
Q5	Validated in a validation cohort	Yes=1	No=0
	Maximum score = 5, minimum score = 0		

Results and discussion

Identified candidate biomarkers

A total of 57 studies describing 68 unique biomarkers were identified (Table 3). All reported biomarkers were identified in serum unless otherwise indicated (Additional file 1: Table S1). The mean number of patients with SJIA included in studies was 21 (range 4–60). There were 50 biomarkers (74 %) investigated in studies performed by a single research group and 29/57 studies evaluated a single biomarker. Biomarkers included cytokines, soluble receptors, antibodies, alarmins and other functional molecules (Table 3). The most studied biomarkers were IL-18 (n = 7 individual studies), IL-6 (n = 5), S100A8/A9 (n = 5), S100A12 (n = 4) and soluble CD25 (IL-2 receptor) (n = 4) (Table 3). Only two identified biomarkers, namely S100A8/A9 and S100A12, were described in JIA (but not SJIA) validation studies.(21) Hepcidin, also included as a diagnostic marker, was validated for differentiating SJIA-associated anaemia from anaemia of other causes, but not specifically for SJIA diagnosis.(22)

Current clinical uses of identified biomarkers

This study identified some well-established markers of inflammation and/or SJIA, such as the S100-proteins (S100A12 and S100A8/A9 complex), IL-18 and IL-6, autoantibodies, non-specific inflammatory markers and some markers not classically associated with SJIA, such as B cell markers. S100A8/A9 is a predictive biomarker for subclinical disease activity and a predictor of JIA relapse after stopping medication.(17, 21, 23) IL-18 concentration is a known marker of disease activity in SJIA, while IL-18 and IL-6 can define subsets of SJIA.(24-26) While IL-6 and IL-1 are targets of the biological therapies tocilizumab, canakinumab, rilonacept and anakinra, respectively, neither cytokine is routinely measured in patients.(27-30) IL-1b, as already discussed, is usually undetectable in serum and IL-18 is not regularly measured due to technical limitations in performing bioassays (31); however the reason for IL-6 not being used in routine care is unclear.(32, 33)

A number of autoantibodies were identified as candidate biomarkers, including rheumatoid factor (RF), anti-nuclear antibodies (ANA) and anti-citrullinated protein antibodies (ACPA). RF has long been recognised as distinguishing RF-positive and RF-negative forms of polyarthritis (JIA subtypes).(34) ANA are routinely evaluated in JIA as a screening factor for JIA-associated

572961-L-bw-PS4U
Processed on: 25-1-2022

uveitis.(35) However, Shin et al. showed that ANA levels can change over time in patients with SJIA, which is a finding replicated by Huegle et al.(36, 37) ACPA are associated with joint damage, and are included in the classification criteria for rheumatoid arthritis, though they do not have an established use in SJIA.(38-40)

CRP and ferritin, which are routinely measured, non-specific, acute phase reactants used as surrogate markers, were described as baseline parameters in most of the identified studies, but were not the subject of investigation in the studies, so were therefore excluded from Tables 1 and 3 and from further analyses. Other non-specific identified biomarkers of inflammation were serum amyloid (SAA), fibrin D-dimer and complement 4 (C4). While previously important in detecting long-term complications of inflammation such as amyloidosis, SAA measurement has become less important since the introduction of biological treatments, which have reduced complications in SJIA.

*Candidate biomarkers categorised as diagnostic or prognos*tic

Some biomarkers were identified in more than one study as described above and evaluated for more than one clinical question (Tables 1 and 3). There were 51 markers characterised as diagnostic, 33 as prognostic and 16 were both diagnostic and prognostic (Table 3): these were ACPA, A proliferation-inducing ligand (APRIL), B-cell activating factor (BAFF), cartilage oligomeric matrix protein (COMP), follistatin-like protein 1 (FSTL-1), heme oxygenase-1 (HO-1), interferon gamma (IFNg), IL-10, IL-18, IL-18 binding protein (IL-18BP), IL-6, S100A12, S100A8/A9, SAA, soluble ST2/IL-1 receptor-like 1 (sST2) and transthyretin (TTr).

Table 3 Identified serum and plasma biomarkers

Biomarker		Detection method	Intended Use (P/D) + Ref		BMS score* Q1+Q2+Q3+Q4+Q5 = Total
Abbreviation/ gene name	Full/alternative name		D	P	
A2M	Alpha-2-macroglobulin	Commercial ELISA		(45)	1+0+0+1+0=2
AB-oxLDL	Antibodies to oxidized low-density lipoprotein	Commercial ELISA	(68)		1+0+0+1+0=2
ACAN	Aggrecan core protein, cartilage-specific core protein	Immunoassay	(69)		1+0+0+1+0=2
ACPA	Anti-citrullinated protein antibodies	Commercial ELISA	(39)	(39)	1+0+0+1+0=2
ACT	Alpha-1-antichymotrypsin	Commercial ELISA		(45)	1+0+0+1+0=2
AECA	Anti-endothelial cell antibodies	In-house ELISA	(70)		1+0+0+1+0=2
AGP1	Alpha-1-acid-glycoprotein	Commercial ELISA		(45)	1+0+0+1+0=2
ANA	Antinuclear antibody	Fluorescence assay Commercial ELISA		(36) (37)	1+1+0+1+0=3
Anti-BiP	Anti-immunoglobulin binding protein / glucose regulated protein 78 (GRP78)	In-house ELISA	(71)		1+0+0+1+0=2
Anti-CCP	Anti cyclic citrullinated peptide	Commercial ELISA	(72)		1+0+0+1+0=2
APO A1	Apolioprotein A1	Commercial ELISA		(45)	1+0+0+1+0=2
APO VI	Apolipoprotein VI	Commercial ELISA		(45)	1+0+0+1+0=2
APRIL	A proliferation-inducing ligand	Commercial ELISA	(73)	(73)	1+0+0+1+0=2
B2M	Beta -2-microglobulin	Not indicated		(74)	1+0+0+1+0=2
BAFF	B-cell activating factor	Commercial ELISA	(73)	(73)	1+0+0+1+0=2
C4	Complement C4	Commercial ELISA		(45)	1+0+0+1+0=2
CCL3	Chemokine (C-C motif) ligand 3	Luminex assay	(57)		1+0+0+1+0=2
CD10	Cluster of differentiation antigen 10, also called Neprilysin	Fluorimetric assay	(75)		1+0+0+1+0=2
CFH	Complement Factor H	Commercial ELISA		(45)	1+0+0+1+0=2
COMP	Cartilage oligomeric matrix protein	Commercial ELISA	(39) (76)	(39) (77) (78)	1+1+0+1+0=3
CXCL9	Chemokine (C-X-C Motif) Ligand 9	Luminex assay	(57)		1+0+0+1+0=2
Fibrin D-dimer		Commercial assay		(79)	1+0+0+1+0=2
FSTL-1	Follistatin-like protein 1	Commercial ELISA	(80)	(80) (81)	1+1+0+1+0=3
GHRL	Ghrelin, appetite regulating hormone	Commercial ELISA	(82)		1+0+0+1+0=2
GSN	Gelsolin	Commercial ELISA		(45)	1+0+0+1+0=2
Hepcidin	Peptide hormone, released by hepatocytes	Commercial assay	(22)		1+0+0+1+0=2
HMGB1	High mobility group box protein 1	Commercial assay	(83)		1+0+0+1+0=2
HO-1	Heme oxygenase-1	Commercial ELISA	(84)	(85)	1+1+0+1+1=4
HP	Haptoglobin	Commercial ELISA		(45)	1+0+0+1+0=2

IFNg	Interferon gamma	Commercial ELISA	(86)	(86)	1+0+0+1+0=2
IgA RF	Ig A rheumatoid factor isotype	In-house ELISA	(87)		1+0+0+1+0=2
IgM RF	Ig M rheumatoid factor isotype	In-house ELISA	(87)		1+0+0+1+0=2
IL-10	Interleukin-10	Commercial ELISA	(88)	(85)	1+0+0+1+0=2
IL-12	Interleukin-12	Luminex assay Commercial ELISA	(57) (89)		1+1+1+1+0=4
IL-18	Interleukin-18	Commercial assay Luminex assay	(25) (57) (86) (90) (91)	(24) (90) (92)	1+1+1+1+0=4
IL-18BP	Interleukin-18 binding protein	Commercial assay	(86) (90)	(90)	1+1+0+1+0=3
IL-1b	Interleukin-1beta	Commercial ELISA	(89)		1+0+0+1+0=2
IL-6	Interleukin-6	Luminex assay Commercial ELISA	(57) (86) (89) (76)	(24)	1+1+1+1+0=4
IP-10/CXCL10	IFNg-induced protein 10, or C-X-C motif chemokine 10.	Commercial ELISA Luminex assay	(57) (86) (93)		1+1+0+1+0=3
LGALS3	Galectin-3	Commercial ELISA	(94)		1+0+0+1+0=2
MIF	Macrophage migration inhibitory factor	Luminex assay	(57)		1+0+0+1+0=2
MMP-3	Matrix metalloprotinease-3 / Stromelysin-1 (SL-1)	Commercial ELISA	(72)		1+0+0+1+0=2
Neopterin		Commercial ELISA		(85)	1+0+0+1+0=2
NO	Nitric oxide	Spectrophotometry		(95)	1+0+0+1+0=2
OPG	Osteoprotegerin / TNF 11B	Luminex assay Commercial ELISA	(57) (96)		1+1+1+1+0=4
OPN	Osteopontin, phosphoglycoprotein	Commercial ELISA	(97)		1+0+0+1+0=2
RA33	Anti-heterogeneous nuclear ribonucleoprotein A2 antibodies	Commercial ELISA	(98)		1+0+0+1+0=2
RANKL	TNF ligand superfamily member 11 / Receptor activator of nuclear factor kappa B ligand	Commercial ELISA	(96)		1+0+0+1+0=2
Resistin	Protein adipokine	Commercial ELISA	(99)		1+0+0+1+0=2
S100A12	S100 calcium-binding protein A12	In-house ELISA Commercial ELISA	(45) (100) (101)	(45) (100) (102)	1+1+1+1+0=4
S100A8/A9	MRP8/14 (myeloid regulatory protein 8/14) complex, complex of S100A8 (Calgranulin A) and S100A9 (Calgranulin B)	In-house ELISA Commercial ELISA	(23) (45) (101) (103)	(23) (17) (45)	1+1+1+1+0=4
SAA	Serum amyloid A	Commercial ELISA	(45) (76)	(45)	1+1+0+1+0=3
SAP	Serum amyloid P	Commercial ELISA		(45)	1+0+0+1+0=2
sCD163	Soluble cluster of differentiation 163 / Haemoglobin scavenging receptor	Commercial ELISA		(85) (104)	1+1+0+1+0=3
sCD21	Soluble cluster of differentiation 21	Commercial ELISA	(105)		1+0+0+1+0=2
sCD23	Soluble cluster of differentiation 23 / soluble low affinity immunoglobulin epsilon Fc receptor)	Commercial ELISA	(105)		1+0+0+1+0=2
sCD25	Soluble cluster of differentiation 25 / soluble interleukin-2	Commercial ELISA		(74) (104)	1+1+0+1+0=3

	receptor alpha			(106) (107)	
sE-selectin	Soluble E-selectin adhesion molecule	Commercial ELISA	(108) (109) (110)		1+1+0+1+0=3
sICAM-1	Soluble intracellular adhesion molecule-1	Commercial ELISA	(108) (109) (110)		1+1+0+1+0=3
sRAGE	Soluble receptor for advanced glycation end products	Commercial assay	(83)		1+0+0+1+0=2
sST2	Soluble ST2, also called Interleukin 1 receptor-like 1 (IL-1RL1)	Commercial ELISA	(111)	(111)	1+0+0+1+0=2
sTM	Soluble thrombomodulin / CD141	Commercial ELISA	(112)		1+0+0+1+0=2
sTNFR55	Soluble tumour necrosis factor receptor 55	Commercial ELISA	(113)		1+0+0+1+0=2
sTNFR75	Soluble tumour necrosis factor receptor 75	Commercial ELISA	(113)		1+0+0+1+0=2
Survivin		Commercial ELISA	(76)		1+0+0+1+0=2
TIMP	Tissue inhibitors of metalloproteinases	Commercial ELISA	(96)		1+0+0+1+0=2
TNF-alpha	Tumor necrosis factor-alpha	Commercial ELISA	(88)		1+0+0+1+0=2
TTR	Transthyretin	Commercial ELISA	(45)	(45)	1+0+0+1+0=2

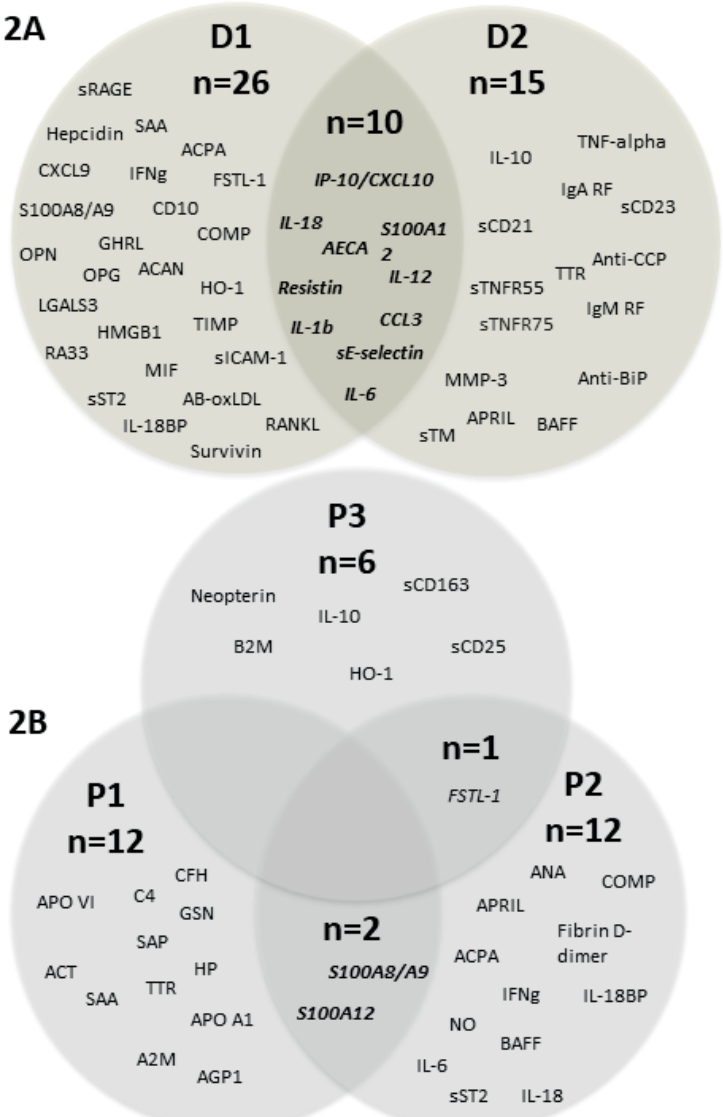
***BMS** biomarker score, each answer is scored as follows: yes = 1, no=0.
Q1 Readily measurable (e.g. in serum), **Q2** Measured by more than one independent study group, **Q3** Discovered by more than one single method, **Q4** Measured by a reproducible assay, **Q5** Validated in a validation cohort.

Figure 2 Identified biomarkers grouped by clinical question

A Diagnostic biomarkers are shown that differentiated systemic juvenile idiopathic arthritis (SJIA) from healthy controls (HC) or other non-JIA disease (D1), SJIA vs other JIA subtypes (D2) or both (D1 and D2).
B Prognostic biomarkers for flare (P1), increased disease activity or discriminating active disease from inactive (P2), for macrophage activation syndrome (MAS) or discriminating MAS from SJIA flare (P3), or a combination of these are shown. The specific clinical question is very important in interpreting the results of biomarker studies. Little overlap between different diagnostic questions suggests a predominance of different pathways during different stages of disease and therefore a specific hypothesis and clinical question is more useful in studies to understand mechanisms. Biomarkers that are broad enough to cover more than one diagnostic or prognostic category may be more likely to have a specific role in the underlying immunological pathology, and as broad markers will be more useful for wider clinical care. By performing this analysis we can create a shortlist of biomarkers on which to focus. Indeed, only a few markers fall into this group, but perhaps they should receive most attention for future validation in preference to other markers.

Abbreviations: *ACAN aggrecan core protein cartilage-specific core protein, ACCP anti-cyclic citrul-*

Figure 2 Identified biomarkers grouped by clinical question



linated peptide, ACPA anti-citrullinated protein antibodies, ACT alpha-1-antichymotrypsin, AECA anti-endothelial cell antibodies, ANA antinuclear antibodies, Anti-BiP anti-immunoglobulin binding protein/glucose regulated protein 78 (GRP78), APO apolioprotein, APRIL A proliferation-inducing ligand, B2M Beta -2-microglobulin, BAFF B-cell activating factor, COMP cartilage oligomeric matrix protein, CRP C-reactive protein, FSTL-1 follistatin-like protein 1, GSN Gelsolin, HO-1 heme oxygenase-1, IFN interferon, IL-18BP IL-18 binding protein, LGAL galectin, MMP matrix metalloproteinase, ONP osteopontin, SAA serum amyloid A, SAP serum amyloid P, sICAM-1 soluble intracellular adhesion molecule-1, sST2 soluble ST2/IL-1 receptor-like 1, TIMP tissue inhibitors of metalloproteinase, TTr transthyretin

Evaluation of identified markers by clinical question

There were 36 biomarkers that differentiated SJIA from HC or other non-JIA disease (D1 biomarkers) and 25 markers differentiated SJIA from other JIA subtypes (D2 biomarkers). Of the prognostic markers, 14 P1 (flare), 15 P2 (disease activity) and seven P3 (MAS) biomarkers were identified (Table 1). Ten biomarkers were common to D1 and D2 (Figure 2a); however, few markers overlapped between the prognostic groups (Figure 2b). This analysis suggests that some biomarkers could have broad use as diagnostic or prognostics markers, rather than being useful only for specific questions. These markers might therefore be more useful than others in a clinical setting, and might therefore be prioritised for validation.

Evaluation of candidate markers

For unbiased and valid results, biomarker evaluation should be performed according to a predefined hypothesis (41) in order to identify candidates more likely to be specific, rather than a high number of unspecific candidates. High throughput methods are increasingly sensitive and producing ever larger numbers of candidate biomarkers; however, they can still be impeded by methodological limitations, such as in LC-MS/MS, by the presence of high abundant proteins.(42, 43) Therefore, careful and evidence-based hypothesis-driven evaluation and prioritisation of candidates for validation studies is vital. While discovery studies are usually unbiased, the prioritisation of identified markers for further evaluation is much more variable, and might be reported as being based on reproducibility, availability of antibodies or levels of protein expression.(44) However, too often these data are omitted, leading to bias in the selection procedure. Ling et al. detected 26 proteins in plasma from patients with SJIA, which differentiated flare from quiescence plasma, of which 18 proteins were significant, and from these the top 15 were selected for unsupervised analysis and shown to remain significant.(45) However, only a limited panel of 9/15 were further tested, chosen according to the availability of antibodies and ELISA. As there is no quantitative and unbiased approach for prioritising candidate markers, we created the novel but unvalidated BMS (Table 2) for this study.

We evaluated each identified biomarker (Table 3) using the BMS (Table 2). No biomarker had the maximum score (5/5). The highest-scoring markers (score 4/5) were HO-1, IL-6, IL-12, IL-18, osteoprotogerin (OPG), S100A12 and S100A8/A9 (n = 7). There were 10 and 51 biomarkers with scores of 3/5

and 2/5, respectively. A score of 3/5 or greater, therefore, identified 17 (25 %) of the total biomarkers. The highest scoring markers grouped according to diagnostic or prognostic subgroup are indicated in Figure 1. Next, the 36 identified D1 biomarkers, the largest group of identified biomarkers for any of the clinical questions asked, were scored and ranked as an example to show how the BMS could prioritise candidates for further evaluation (shown in Figure 2, scores in Table 3). Seven biomarkers scored 4/5 (as listed above) and seven others scored 3/5, while the remaining 22 markers scored 2/5. This resulted in a panel of 14 markers when the cutoff was applied at a score of 3/5 or above (or n = 15 when S100A8 and S100A9 were analysed as separate proteins). Further ranking of markers within these broad groups was not performed. The online Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) platform was used to identify if any of these 15 proteins had known functions in common.(46) To differing extents STRING identified direct or indirect functional links or interactions between all proteins except S100A12, FSTL-1 and COMP (when tested at the "medium" confidence level). All proteins were identified to be extracellular, consistent with their measurement in peripheral blood, and had an identified immune function role. A summary of the functions of this protein set is shown in Supplemental Table 2.

The biomarker panel approach

Multiplex cytokine analysis can (1) differentiate SJIA from differential diagnoses and (2) identify distinct profiles in individual patients. Identification of cytokine patterns in individual patients could lead to the identification of subphenotypes within SJIA and also provide insight into the underlying biological basis for the clinical heterogeneity seen in SJIA.(47, 48) This clinical variation and the variety in identified biomarkers supports a prevailing view that a biomarker panel is required.(8) A "multimarker approach" is already used to predict risk of cardiovascular events and the multibiomarker assessment of disease activity (MBDA) is validated for rheumatoid arthritis (RA).(49-55) The MBDA outperforms clinical assessment alone, imaging and single biomarker measurement, and is also cost-effective, measuring 12 biomarkers in just 0.2 ml of serum. A potential panel of biomarkers has recently been identified for paediatric systemic lupus erythematosus, which had good predictive value for detecting the complication lupus nephritis.(56) Jager et al. identified cytokine profiles in paired plasma and synovial fluid samples in 20 patients with SJIA using a bead array based multiplex immunoassay which

measured 30 soluble inflammatory mediators in only 50 µl of sample and showed the blind measurement of IL-18 predicted patients with active SJIA with 93 % accuracy.(57, 58) While the identified studies in our analysis often evaluated more than one candidate marker, combinations of markers were not tested and did not feature in study hypotheses/design and/or sample numbers.

Prerequisites for clinical biomarkers

Sample-specific and method-specific factors should be considered before performing either discovery or validation studies.(59) Sample requirements differ according to the planned methodology and platform to be used.(60, 61) Some cytokines, such as IL-1β, are extremely sensitive to degradation by freeze-thawing, whereas IL- 18 is comparatively more stable.(61) A clinical bio- marker should also fulfil an unmet need and improve existing tests, while also being cost-effective, criteria which will also help define candidates for validation.(10, 59, 62) We did not investigate the cost-effectiveness of markers. However, the validation and clinical use of many of the biomarkers, as described, is limited by the cost and/or local availability of diagnostic tests.

Validation of biomarkers

Most candidate markers (86 %) were identified in a single study and/or by a single group, respectively. While this indicates that multiple groups are working on SJIA biomarkers, each with different strategies, it also reflects a lack of current understanding of the pathology of SJIA. Methods of biomarker verification, as intermediary steps towards validation, become increasingly important as new and improved biomarker discovery techniques result in large numbers of candidates.(35, 63) Identification of the same biomarker by multiple research groups could be seen as a verification step, suggesting a false positive finding to be less likely. Other verification factors might include confirmation that a candidate biomarker can be robustly measurable in peripheral blood, or the use of specific verification methods such as proteomic mass-spectrometry-based selected reaction monitoring (SRM) analysis.(18) SRM measures multiple target proteins, identified from discovery studies or existing literature simultaneously, without requiring specific antibodies as with antibody-based validation techniques, but it does not replace validation. Biomarker validation, most frequently performed using antibody-based assays, is a difficult, costly and time-consuming process.(35) An example of validation is the included study by Rothmund et al. which compared different

assays for measuring S100-proteins in JIA .(21, 64) Biomarker validation, also termed "qualification", can be separated from clinical validation as a process referring more specifically to the process of linking biomarkers to a clinical endpoint based on evidence and statistical analysis.(65) Validation is widely acknowledged to be a more difficult process than identification, due to the requirement of large numbers of samples of well-defined patients from populations not used in the discovery step. An example use of a validated diagnostic biomarker or panel could allow earlier diagnosis of SJIA, allowing treatment to be started during the "window of opportunity", the time point early enough in disease that intensive targeted treatment could be used to achieve early disease remission.(8, 29, 66) We therefore focused on identifying potentially clinically relevant diagnostic or prognostic biomarkers for SJIA from studies addressing specific clinical questions.

Conclusions

There remains a need for the simultaneous evaluation of multiple biomarkers and an unbiased method of selecting candidate biomarkers for further evaluation. The parallel use of different methodological platforms such as microbead arrays (e.g. Luminex xMAP), aptamer-based assay or label-free liquid mass spectrometry (LC- MS/MS) could improve the spectrum of detected proteins,(67) while the BMS used here is an example of how candidate markers could be prioritised. Markers that exclude SJIA would also be useful in the clinical setting. In particular, markers diagnostic for the main differential diagnoses of SJIA, such as the causes of fever of unknown origin, which might include infection or malignancy, would help exclude SJIA as a diagnosis. While this review was not designed to explore markers of differential diagnoses of SJIA, including them in a potential multi-marker panel would likely improve such a diagnostic assay.

Sixty-eight unique candidate markers evaluated for the management of SJIA were identified by this literature review. Only one identified study was a validation study and very few identified biomarkers were evaluated by more than one study group. Therefore, there is a clear and urgent need to confirm and consolidate findings from discovery studies and validate findings. The use of emerging technologies, with collaborative efforts, may ultimately help achieve the goal of validating new diagnostic or prognostic biomarkers, or panels of biomarkers, for improving the management of SJIA.

Supplemental Table 1: Full summary of included studies

Author and year of study	Study aims	SJIA + other included patients/ controls/ comparison groups	Biomarkers included	Method of biomarker measurement	Summary of results	Diagnostic or Prognostic biomarker described
Aggarwal 2007	To investigate chemokine and their receptors in ERA.	9 SJIA, 12 PA, 18 HC	CCL5/RANTES and IP-10/ CXCL10	Commercial ELISA	Lower serum IP10/ CXCL10 levels were found in SJIA vs ERA. SF concentrations were less than serum for JIA subtypes.	Diagnostic
Bica 2007	To evaluate the distribution and correlation of NO with disease severity in JIA.	34 SJIA, 34 OA, 29 PA	NO	In-house spectrophotometric assay	NO was greater when disease activity was greater.	Prognostic
Bleesing 2007	To assess if sIL-2Ralpha (sCD25) and sCD163 can diagnose acute MAS complicating SJIA.	16 SJIA +/- MAS	sCD163 and sIL-2R alpha (sCD25)	Commercial ELISA	sCD163 and aIL-2R alpha were elevated in SJIA-MAS compared with SJIA without MAS.	Diagnostic
Bloom 2005	To determine whether soluble forms of ICAM-1 and E-selectin correlate with clinical measures or other markers of endothelial activation.	8 SJIA, 10 PA, 10 OA, 30 HC	sICAM-1 and sE-selectin	Commercial ELISA	sICAM-1 was elevated in all JIA subtypes compared with HCs, and was elevated in active vs non-active SJIA.	Prognostic
Bloom 2007	To determine the prevalence of AECA in JIA vs HC	8 SJIA, 10 PA, 10 OA	AECA	In-house ELISA	AECA was elevated in SJIA compared with HC, and elevated in SJIA compared with OA JIA.	Diagnostic
Bloom 2009	To test if the persistence of D-dimer elevation over long follow-up would signal poor outcome.	31 SJIA	Fibrin D Dimer	Quantitative commercial assay using latex particles	When SJIA patients with persistent elevation of D-dimer were more likely to have a severe outcome.	Prognostic
Bobek 2014	To determine the presence of HMGB1 and sRAGE in different subtypes of JIA and compared to SLE and HC.	27 SJIA, 34 OA, 35 PA, 19 SLE, 28 HC	HMGB1, sRAGE	Commercial ELISA	Serum HMGB1 was significantly increased in SJIA vs HC and serum sRAGE was significantly reduced in SJIA vs HC.	Diagnostic
Bodman-Smith 2004	Aimed to determine presence of BiP antibodies according to JIA subtype.	37 SJIA, 41 OA, 33 persistent OA, 43 PA, 16 HC	Anti-BiP antibody	In-house ELISA	No difference in anti-BIP levels between SJIA and HC , but was elevated in RF-positive PA JIA.	Diagnostic

Cangemi 2013	To clinically validate a commercial kit for the measurement of serum hepcidin in healthy children and pathological conditions including children with SJIA related anaemia.	19 SJIA, 86 HC, 49 patients (including thalassaemia and 16 iron replacement therapy).	Hepcidin	Commercial ELISA	Serum hepcidin was significantly higher in children with anaemia with SJIA compared to the controls. Hepicidin measurement may discriminate anaemia of inflammatory cause in SJIA from iron deficiency anaemia.	Diagnostic
Chen 2002	To measure sE-selectin and sICAM-1 levels among JIA subtypes and controls, and correlate with disease activity	12 SJIA, 13 PA, 15 OA, 16 HC	sICAM and sE-Selectin	Commercial ELISA	Both sICAM-1 and sE-selectin were elevated in JSIA compared with HC. sE-selectin was elevated in SJIA compared to OA.	Both
Chen 2013	To further investigate IL-18 in the pathogenesis of SJIA.	45 active SJIA, 23 inactive SJIA, 20 HC	IL-18 and IL-18BP and IL-18:IL-18BP ratio	Commercial ELISA	Patients with active SJIA had higher IL-18 plasma levels and higher IL-18:IL-18BP ratio compared to inactive SJIA patients and HC.	Both
De Benedetti 2000	To measure circulating levels of sE-selectin, sP-selectin and sICAM-1 in patients with JIA and correlate results with disease activity and cytokine levels.	42 SJIA, 15 HC, 42 total JIA	sE-selectin and sICAM-1	Commercial immunno-assays.	Still need full paper Both sICAM-1 and sE-selectin were elevated in active SJIA compared with HC.	Diagnostic
de Jager 2007	To determine plasma cytokine levels using a 30-panel multiplex assay in different subtypes of JIA and controls and correlate with disease activity.	20 SJIA, 30 OA, 15 PA, 9 Diabetes, 20 HC	Panel of 30 significant diagnostic cytokines discussed in detail in this review are: IL-6, IL-12, IL-18, CCL3, CXCL9, XCXL10, OPG and MIF.	Particle based mulitplex immunoassay (LUMINEX)	IL-6, CCL3, CXCL9, CXCL10, OPG and MIF were significantly raised in plasma of SJIA patients vs controls. CCL3, IL-18 and IL-12 were elevated in SJIA plasma vs other JIA subtypes.	Diagnostic
El-Gamal 2004	sTM is elevated in RA and therefore hypothesized to be raised in SJIA.	6 SJIA, 17 PA, 30 HC	sTM	Commercial ELISA	sTM was elevated in SJIA patients compared to other JIA subtypes.	Both
El-Sayed 2001	Evaluated serum and SF aggrecan as a metabolic marker and predictor of cartilage destruction in JIA.	6 SJIA, 20 PA, 5 OSA, 10 arthritis related to CVD	ACAN	Commercial enzyme based immunoassay	Aggrecan (ACAN) was elevated in SJIA patients compared with HC and elevated in SJIA compared with other CVD.	Diagnostic
Ezzat 2011	Increased serum Galectin-3 is noted in RA and implicated as a inflammatory response regulator and was tested in SJIA	10 SJIA, 20 PA, 12 extended OA, 8 persistent OA	LGALS3	Commercial ELISA	Elevated galectin-3 distinguished SJIA from HC but not from PA.	Diagnostic
Ferreira 2007	Aimed to determine levels of IgM and IgA RF by ELISA in JIA and correlated with clinical and laboratory markers.	38 SJIA, 28 OA, 25 PA, 45 HC	IgA RF and IgM RF	In-house ELISA	IgM+/IgA+ RF is lower in SJIA patients compared with PA. IgM-/IgA+ is elevated in SJIA patients compared to PA.	Diagnostic
Foell 2004	To determine neutrophil activation in JIA by analysing serum S100A12	33 SJIA, 53 OA, 38 PA, 74 HCs, 16 bacterial infection	S100A12	In-house ELISA	S100A12 correlated with disease activity and was significantly elevated in SJIA compared to JIA.	Both

Foell 2010	To test whether patients at risk of flare after methotrexate withdrawal can be identified by S100A8/A9 analysis.	35 SJIA, 96 persistent OA, 56 extended OA, 152 PA, 10 ERA, 15 PsA	S100A8/A9	In-house ELISA	S100A8/A9 was prognostic for risk of flaring, relapse rate and time to relapse.	Both
Frosch 2009	To test MRP8/14 as a diagnostic tool for the differentiation of SJIA and systemic infections.	60 SJIA, 50 HC	S100A8/A9	In-house ELISA	S100A8/A9 distinguished SJIA from infection.	Diagnostic
Galeotti 2008	To measure survivin as a marker of JIA in plasma	11 SJIA, 12 OA, 23 PA, 46 HC	SAA, Survivin, COMP and IL-6	Commercial ELISA	SAA, survivin and IL-6 are elevated in SJIA versus HC.	Diagnostic
Gheita 2012	To assess the level of BAFF and APRIL in different JIA subtypes in relation to disease activity	20 SJIA, 34 HC, 31 OA, 23 OA	BAFF and APRIL	Commercial ELISA	APRIL and BAFF were higher (but not statistically) in SJIA versus JIA subtypes. BAFF and APRIL correlated with increase in CHAQ and JADAS27 in all JIA patients.	Both
Gheita 2013	Resistin levels were tested in patients with JIA.	19 SJIA, 33 HC, 28 OA, 21 PA	Resistin	Commercial ELISA	Resistin was elevated in SJIA compared with HC and other JIA subtypes.	Diagnostic
Gilliam 2008	A panel of biomarkers in JIA including COMP were tested for association with disease severity.	9 SJIA, 17 OA, 18 HC	COMP and ACPA	Commercial ELISA	ACPA is elevated in SJIA compared with HC. COMP and ACPA correlated with disease severity.	Both
Gorelik 2013	To correlate FSTL-1 levels with gene expression, known biomarkers and measures of disease activity in SJIA +/- MAS.	28 SJIA, 30 HC	FSTL-1	Commercial ELISA	FSTL-1 was elevated in SJIA patients before treatment.	Prognostic
Holzinger 2012	To test the ability of S100A8/A9 serum concentrations to monitor disease activity in patients with SJIA and stratify patients at risk of relapse.	52 Active and inactive SJIA	S100A8/A9	In-house ELISA	Predicted relapse in SJIA and was an indicator of disease activity.	Prognostic
Huegle 2014	To determine whether ANA and rheumatoid factor will develop in patients with SJIA over disease course.	32 SJIA	ANA	Fluorescence assay	Positive ANA titers developed in patients over time: 8/32 patients were positive at diagnosis, 22/32 at follow up.	Prognostic
Ishikawa 2013 the receptor for IL-33, in the pathogenesis of systemic juvenile idiopathic arthritis (s-JIA	To assess the role of IL-33 and ST2 in the pathogenesis of SJIA and correlation with disease activity and severity.	24 SJIA, 20 HC, 5 PA, 4 SJIA-MAS	sST2	Commercial ELISA	sST2 was elevated with increased disease activity in SJIA, and increased in SJIA patients compared with HC. Serum IL-33 concentrations did not statistically significantly differ between HC and SJIA.	Both
Jelusic 2007	To describe serum IL-18 levels in active and inactive JIA.	17 SJIA, 31 OA, 33 PA, 18 HC	IL-18	Commercial ELISA	Elevated in SJIA compared with other JIA subtypes. Elevated in active SJIA compared with inactive SJIA.	Diagnostic

Kounami 2005	To assess the clinical features in 9 MAS events, 5 in SJIA.	5 SJIA-MAS	B2M and soluble IL-2 receptor (sCD25)	Not indicated.	B2M was elevated in SJIA-MAS patients compared to SJIA without MAS.	Prognostic
Karagiozoglou-Lampoudi 2011	Serum ghrelin in JIA and association with anti-TNF therapy, disease activity and nutritional status was evaluated.	8 SJIA, 19 PA, 23 OA, 2 ERA, 50 HC	Ghrelin	Commercial ELISA	Serum Ghrelin in SJIA was significantly lower compared to HC.	Diagnostic
Lehmberg 2013	To identify measures distinguishing MAS in SJIA from FHL and VA-HLH and to define cut off levels. To evaluate suggested dynamic measures differentiating MAS in SJIA from SJIA flares.	27 SJIA, 90 FHL, 42 VA-HLH, each patient with SJIA had MAS	Ferritin and sCD25	Commercial ELISA	Serum sCD25, ferritin and CRP were significantly lower in MAS-SJIA patients compared to FHL or VA-HLH.	Prognostic
Ling 2010	To identify a protein signature that differentiates patients with SJIA flare from those with quiescent disease.	17 SJIA, 10 SJIA patients in flare and quiescence, 5 PA	TTR, CFH, APO A1, A2M, GSN, C4, AGP1, ACT, APO VI, SAP, HP, CRP, S100A8/A9, S100A12, SAA	Commercial ELISA	26 plasma proteins were associated with SJIA flare: 15 were highly significant. Nine biomarkers were further evaluated in ELISA and shown to significantly differentiate flare from quiescent disease in SJIA.	Prognostic
Lotito 2007	To verify the importance of interleukin 18 (IL-18) in the pathogenesis of JIA.	13 SJIA, 13 PA, 24 OA, 25 HCs	IL-6 and IL-18	Commercial ELISA	IL-18 was increased in active SJIA vs inactive disease.	Prognostic
Maeno 2002	To further the understanding of IL-18 in the pathogenesis of SJIA.	29 active SJIA, 29 PA, 18 OA, 10 KD, 33 HC	IL-18 and IL-18BP	Commercial ELISA	Serum IL-18 was significantly higher in SJIA vs other JIA and HC.	Diagnostic
Masi 2009	To assess if OPN is a marker of response to methotrexate at disease onset.	5 SJIA, 30 PA, 23 OA, 2 PsA	OPN	Commercial ELISA	OPN was elevated in SJIA patients compared with HC.	Diagnostic
Muzaffer 2002	To find a biological basis for clinically distinct JIA subtypes, levels of soluble TNF receptors were measured as an indirect measure of cytokine activity.	11 SJIA, 13 PA, 10 OA	sTNFR55 and sTNFR75	Commercial ELISA	Levels of aTNFR55 and sTNFR75 differ by JIA subtype and therefore maybe prognostic for subtypes. IL-1RA was igher in SJIA vs pauciarticular JIA, but not statistically.	Diagnostic
Nakajima 2009	To determine COMP levels in SJIA patients during active and inactive disease.	11 SJIA, 201 HC	COMP	Commercial ELISA	Decreased levels of COMP were in active compared to remission in SJIA, and was decreased in active SJIA compared with HCs.	Prognostic
Ozawa 2012	To detect the differences between SJIA and PA by measuring serum proteins and radiological findings.	20 SJIA, 16 PA	Anti-CCP antibody and MMP-3	Not indicated	MMP-3 was higher in SJIA vs PA. Anti-CCP antibody was lower in SJIA vs PA.	Diagnostic

Put 2015	To study the role of IFNg and IFNg associated cytokines in SJIA, SJIA associated MAS and haemophagocytic lymphohistiocytosis (HLH).	20 SJIA (10 active, 10 inactive), 2 HLH, 3 SJIA-MAS, 16 HC	IP-10, IL-18BP, indoleamine 2,3-dioxygenase, IL-18, IFNg, IL-6	Commercial ELISA	IFNg was approximately five times higher in active SJIA vs inactive sJIA or HC. IFNg was higher in HLH vs SJIA. Plasma IL-6 was higher in SJIA (active or inactive) vs HC. Plasma IL-18 was higher in active SJIA vs inactive SJIA, and higher in SJIA vs HC. Plasma IP-10 was higher in HLH patients vs patients with inactive SJIA or HC. IL-18BP was elevated more than 5-fold in HLH vs active SJIA, and was higher in SJIA vs HC.	Diagnostic
Reddy 2014	To investigate the prevalence of clinical and traditional laboratory markers of MAS as well as soluble CD163 and soluble interleukin (IL)-2Ra (CD25) in active SJIA patients.	33 SJIA, 2 SJIA + MAS, 11 PA	sCD25 and sCD163	Commercial ELISA	2 patients with MAS had elevated sCD25 vs PA. However, sCD25 was also raised in almost half patients with SJIA without MAS.	Prognostic
Sarma 2008	To determine RANKL, TIMP and OPG levels in SJIA versus controls.	13 SJIA, 24 ERA, 22 PA, 8 OA, 3 others.	TIMP, RANKL and OPG	Commercial ELISA	RANKL, TIMP and OPG were elevated in SJIA patients compared to HC.	Diagnostic
Shahin 2002	Circulating IL-6, sIL-2R (sCD25), TNF-alpha and IL-10 was measured in SJIA and PA and correlated with Doppler sonography.	10 SJIA, 9 PA	IL-6, TNF-alpha, IL-10	Commercial ELISA	TNF-alpha serum levels were higher in SJIA vs PA. IL-10 and IL-6 were higher in patients with PA vs SJIA.	Diagnostic
Shenoi 2015	Shenoi et al. investigated diagnostic markers for SJIA in children with fever who had SJIA compared with children who did not.	10 active SJIA, 10 febrile non-SJIA controls	S100A12, S100A8, S100A9, S100A8/A9	Commercial ELISA	S100 proteins were significantly elevated in SJIA vs control, while PD-1 expression was significantly lower. Procalcitonin, CRP and ESR) were not specific for SJIA.	Diagnostic
Shimizu 2010	To compare the cytokine profiles and kinetics in patients with MAS due to SJIA in both active and inactive disease SJIA without MAS, compared to EBV-induced HLH and KD, and to investigate the significance of IL-18 in the pathogenesis of SJIA.	5 SJIA-MAS, 10 EBV-HLH, 22 KD, 28 HCs	IL-18	Commercial ELISA	Serum IL-18 was significantly higher in SJIA-MAS patients than in other HLH groups or HC.	Prognostic

Shimizu 2012	To investigate the role of the CD163/ HO-1 axis in SJIA, and pro-inflammatory cytokines (IL-10, IL-18, IL-6, neopterin, soluble TNF-α receptor types I and II) in patients with SJIA complicated by MAS.	4 patients with SJIA-MAS, 10 HC, 10 KD	Neopterin, HO-1, sCD163 and IL-10	Commercial ELISA	HO-1, sCD163 and IL-10 were elevated in SJIA-MAS, active and inactive SJIA without MAS in comparison to levels of other pro-inflammatory cytokines, and significantly higher than KD and EBV-HLH.	Prognostic
Shin 2008	Screened children with different subtypes of JIA at presentation for ANA. ANA is prognostic for uveitis in patients with OA, but its prognostic use in other subtypes is not known.	17 SJIA, 16 OA, 10 PA	ANA	Commercial ELISA	Serum ANA patterns and levels change during follow-up in SJIA and other JIA subtypes.	Prognostic
Simonini 2001	Two endproducts of lipid peroxidation and the formation of antibodies against oxidized low density lipoproteins (Ab oxLDL) in different JIA subsets was investigated.	14 SJIA, 28 OA, 15 PA	AB-oxLDL	Commercial ELISA	AB-oxLDL are higher in SJIA compared with HC, but similarly elevated in all subtypes of JIA.	Diagnostic
Simonini 2005	Neprilysin, a cell surface enzyme particularly found on neurons, is thought to play a role in inflammation via its action of degrading neuropeptides involved in neurogenic inflammation which terminates their inflammatory effects.	8 SJIA, 52 HC, 34 OA, 16 PA	CD10	Commercially available coumarin used in a fluorimetric assay.	Patients with SJIA had lower plasma Neprilysin (CD10) levels compared to HC.	Diagnostic
Singh 2012	The authors previously demonstrated reduced sCD21 in various autoimmune disorders, and here investigated sCD21 and sCD23 in subtypes of JIA.	20 SJIA, 20 OA, 20 PA	sCD21 and sCD23	Commercial ELISA	sCD21 was significantly decreased in all JIA subtypes. sCD23 was significantly decreased in PA and SJIA but not OA.	Diagnostic
Takahashi 2009	To determine the serum levels of HO-1 in patients with SJIA compared to HC and other rheumatic diseases-	56 SJIA, 15 PA, 13 SLE, 25 MTCD, 17 KD, 6 Takayasu aortitis, SJIA-MAS, HC	HO-1	Commercial ELISA	HO-1 was elevated in SJIA compared with HC and the other disease conditions tested.	Diagnostic
Tomoum 2009	To investigate the value of anti-RA33 for diagnosis of JIA, and its relation to disease activity markers and bone resorption.	7 SJIA, 44 HC, 18 PA, 9 OA	RA33	Commercial ELISA	RA33 was elevated in in all SJIA subtypes vs HC, but not elevated in SJIA vs HC.	Diagnostic
Urakami 2006	To investigate COMP as a marker of arthritis and/or growth impairment	8 SJIA, 6 OA, 10 PA, 82 HCs	COMP	Commercial ELISA	Decreased with greater disease activity in SJIA.	Prognostic

Wilson 2010	To examine the source of FSTL-1, factors inducing its expression in arthritis and whether it's over-expressed in JIA.	15 SJIA, 54 OA, 26 PA, 15 HC	FSTL-1	Commercial ELISA	FSTL-1 was elevated in SJIA patients vs HC, and elevated with increased disease activity in SJIA.	Both
Wittkowski 2008	To investigate whether serum concentrations of S100A12 help in deciding whether to treat patients with FUO with antibiotics or immunosuppressive agents	60 SJIA, 45 HC, 17 FMF, 18 NOMID, 17 MWS, 40 ALL, 5 AML, 83 systemic infection	S100A12	In-house ELISA	S100A12 is highly overexpressed in SJIA compared to the other disease groups tested (except FMF).	Prognostic
Yilmaz 2001	To investigate serum levels of cytokines in SJIA during inactive and active disease, and compared to HC and other JIA subtypes.	8 SJIA, 21 HC, 15 PA, 11 OA.	IL-1b, IL-6, IL-12, IL-8, TNF-alpha	Commercial ELISA	IL-1b was significantly higher in SJIA vs PA, and in SJIA vs OA during inactive and active SJIA disease. IL-1beta was significantly higher in inactive SJIA vs HC. IL-12 was significantly higher in SJIA vs OA during inactive disease. IL-6 was significantly higher in SJIA vs OA and SJIA vs PA during active disease.	Diagnostic

Abbreviations: disease / control groups: HC: healthy controls, OA: oligoarthritis, PA: polyarthritis, SJIA: systemic-onset juvenile ideopathic arthritis, JIA: juvenile idiopathic arthritis, OSA: osteoarthritis, SLE: systemic lupus erythematosus, ERA: enthesitis-related arthritis, PsA: psoriatic arthritis, RA: Rheumatoid Arthritis, KD: Kawasaki Disease, MAS: macrophage activation syndrome, SJIA-MAS: MAS in patients with SJIA, FHL: familial hemophagocytic lymphohistiocytosis, VA-HLH: virus-associated haemophagocytic lymphohistiocytosis, EBV-HLH: Epstein-Barr virus haemophagocytic lymphohistiocytosis, MTCD: mixed connective tissue disease CVD: collagen vascular disorders (SLE/scleroderma), ELISA: enzyme-linked immunoassay, vs: versus.
Abbreviations: biomarkers: A2M: alpha-2-macroglobulin, AB-oxLDL: antibodies to low-density lipoprotein, ACAN: aggrecan core protein, ACPA: anti-citrullinated protein antibodies, ACT: alpha-1-antichymotrypsin, AECA: anti-endothelial cell antibodies, AGP1: alpha-1-acid-glycoprotein, ANA: antinuclear antibody, anti-BIP: anti-immunoglobulin binding protein, anti-CCP: anti-cyclic citrullinated peptide, APO A1: apolipoprotein A1, APO VI: apolipoprotein A VI, APRIL: a proliferation inducing ligand, B2M: beta-2-microglobulin, BAFF: B-cell activating factor, C4: complement C4, CCL3: chemokine (C-C motif) ligand 3, CD10: cluster of differentiation antigen 10, CFH: complement factor H, COMP: cartilage oligomeric matrix protein, CXCL9: chemokine (C-X-C motif) ligand 9, FSTL-1: follistatin-like protein 1, GHRL: ghrelin, appetite regulating hormone, GSN: gelsolin, HMGB1: high mobility group box protein 1, HO-1: heme-oxygenase-1, HP: haptoglobin, IFNG: interferon gamma, IgA RF: immunoglobulin A rheumatoid factor, IgM RF: immunoglobulin M rheumatoid factor, IL-10: interleukin-10, IL-12: interleukin-12, IL-18: interleukin 18, IL-18BP: IL-18 binding protein, IL-1b: interleukin 1b, IL-6: interleukin 6, IP-10/CXCL10: ifng-induced protein 10, or c-x-c motif chemokine 10, LGALS3: galectin-3, MIF: macrophage migration inhibitory factor, MMP-3: matrix metalloproteinase-3, NO: nitric oxide, OPG: osteoprotogerin, OPN: osteopontin, RA33: anti-heterogeneous nuclear ribonucleoprotein A2 antibodies, RANKL: tumour necrosis factor (TNF) ligand superfamily member 11, S100A12: s100 calcium-binding protein A12, S100A8/A9 or MRP8/14: myeloid regulatory protein 8/14 complex, SAA: serum amyloid A, SAP: serum amyloid P, sCD163: soluble cluster of differentiation 163, sCD21: soluble cluster of differentiation 21, sCD23: soluble cluster of differentiation 23, sCD25: soluble cluster of differentiation 25, sE-selectin: soluble E-selectin adhesion molecule, sICAM-1: soluble intracellular adhesion molecule-1, sRAGE: soluble recetor for advanced glycation end products, sST2: soluble ST2, sTM: soluble thrombomodulin, sTNFR55: soluble tumour necrosis factor receptor 55, sTNFR75: soluble tumour necrosis factor receptor 75, TIMP: tissue inhbitors of metalloproteinases, TNF-alpha, tumour necrosis factor-alpha, TTR: transthyretin.

Supplemental Table 2

Functional enrichments in the top scoring 15 proteins identified as differentiating SJIA from non-arthritis conditions or healthy controls. The false discovery rate was < 0.05 for all pathways listed. Adapted from www.string-db.org analysis.

Biological Processes n=172 GO terms (gene count > 10 shown only)		
pathway ID	pathway description	count in gene set
GO:0050794	regulation of cellular process	14
GO:0051716	cellular response to stimulus	12
GO:0070887	cellular response to chemical stimulus	11
GO:0048583	regulation of response to stimulus	11
GO:0048522	positive regulation of cellular process	11
GO:0044707	single-multicellular organism process	11
Molecular Function n=8 GO terms		
pathway ID	pathway description	count in gene set
GO:0005102	receptor binding	10
GO:0050786	RAGE receptor binding	3
GO:0005515	protein binding	13
GO:0035662	Toll-like receptor 4 binding	2
GO:0005125	cytokine activity	4
GO:0008201	heparin binding	4
GO:0050544	arachidonic acid binding	2
GO:0005509	calcium ion binding	5
Cellular component n=5 GO terms		
pathway ID	pathway description	count in gene set
GO:0005615	extracellular space	13
GO:0005576	extracellular region	13
GO:0044421	extracellular region part	12
GO:0009897	external side of plasma membrane	4
GO:0070062	extracellular exosome	8
KEGG Pathways, n=11		
pathway ID	pathway description	count in gene set
05144	Malaria	5
05143	African trypanosomiasis	4
04060	Cytokine-cytokine receptor interaction	5
04668	TNF signaling pathway	4
05164	Influenza A	4
04623	Cytosolic DNA-sensing pathway	3
05321	Inflammatory bowel disease (IBD)	3
05323	Rheumatoid arthritis	3
05134	Legionellosis	2
04621	NOD-like receptor signaling pathway	2
05132	Salmonella infection	2

3

References

1. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, He X, Maldonado- Cocco J, Orozco-Alcala J, Prieur A, Suarez-Almazor ME, Woo P; International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004;31;390–92.
2. Martini A. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. Ann Rheum Dis. 2012;71(9):1437–9.
3. Gattorno M, Piccini A, Lasigliè D, Tassi S, Brisca G, Carta S, et al. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2008;58(5):1505–15.
4. Fall N, Barnes M, Thornton S, Luyrink L, Olson J, Ilowite NT, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. Arthritis Rheum. 2007;56(11):3793–804.
5. Macaubas C, Nguyen KD, Peck A, Buckingham J, Deshpande C, Wong E, et al. Alternative activation in systemic juvenile idiopathic arthritis monocytes. Clin Immunol. 2012;142(3):362–72.
6. Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med. 2005;201(9):1479–86.
7. Mellins ED, Macaubas C, Grom AA. Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions. Nat Rev Rheumatol. 2011;7(7):416–26.
8. Hinze C, Gohar F, Foell D. Management of juvenile idiopathic arthritis: hitting the target. Nat Rev Rheumatol. 2015;11(5):290–300.
9. Smolen JS, Aletaha D, Grisar J, Redlich K, Steiner G, Wagner O. The need for prognosticators in rheumatoid arthritis. Biological and clinical markers: where are we now? Arthritis Res Ther. 2008;10(3):208.
10. Tektonidou MG, Ward MM. Validation of new biomarkers in systemic autoimmune diseases. Nat Rev Rheumatol. 2011;12:708–17.
11. Kessel C, Holzinger D, Foell D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. Clin Immunol. 2013;147(3):229–41.
12. Duurland CL, Wedderburn LR. Current developments in the use of biomarkers for juvenile idiopathic arthritis. Curr Rheumatol Rep. 2014;16(3):406.
13. Consolaro A, Varnier GC, Martini A, Ravelli A. Advances in biomarkers for paediatric rheumatic diseases. Nat Rev Rheumatol. 2015;11(5):265–75.
14. Robinson WH, Lindstrom TM, Cheung RK, Sokolove J. Mechanistic biomarkers for clinical decision making in rheumatic diseases. Nat Rev Rheumatol. 2013;9(5):267–76.
15. Oldenhuis CNAM, Oosting SF, Gietema JA, de Vries EGE. Prognostic versus predictive value of biomarkers in oncology. Eur J Cancer. 2008;44(7):946–53.
16. Søreide K. Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research. J Clin Pathol. 2009;62(1):1–5. 17.
17. Holzinger D, Frosch M, Kastrup A, Prince FHM, Otten MH, Van Suijle-

kom-Smit LWA, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis. 2012;71(6):974–80.
18. Lau TYK, Power KA, Dijon S, Gardelle I De, Mcdonnell S, Duffy MJ, et al. Prioritization of candidate protein biomarkers from an in vitro model system of breast tumor progression toward clinical verification research articles. J Proteome Res. 2010;9(3):1450–9.
19. Bae CB, Suh CH, An JM, Jung JY, Jeon JY, Nam JY, et al. Serum S100A12 may be a useful biomarker of disease activity in adult-onset still's disease. J Rheumatol.2014;41(12):2403–8.
20. Nirmala N, Grom A, Gram H. Biomarkers in systemic juvenile idiopathic arthritis: a comparison with biomarkers in cryopyrin-associated periodic syndromes. Curr Opin Rheumatol. 2014;26(5):543–52.
21. Rothmund F, Gerss J, Ruperto N, Däbritz J, Wittkowski H, Frosch M, et al. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care Res. 2013;66(6):949–55.
22. Cangemi G, Pistorio A, Miano M, Gattorno M, Acquila M, Bicocchi MP, et al. Diagnostic potential of hepcidin testing in pediatrics. Eur J Haematol. 2013;90(4):323–30.
23. Foell D, Wulffraat N, Wedderburn LR, Wittkowski H, Frosch M, Gerss J, et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. JAMA Am Med Assoc. 2010;303(13):1266–73.
24. Lotito APN, Campa A, Silva CAA, Kiss MHB, Mello SBV. Interleukin 18 as a marker of disease activity and severity in patients with juvenile idiopathic arthritis. J Rheumatol. 2007;34(4):823–30.
25. Jelusić M, Lukić IK, Tambić-Bukovac L, Dubravcić K, Malcić I, Rudan I, et al. Interleukin-18 as a mediator of systemic juvenile idiopathic arthritis. Clin Rheumatol. 2007;26(8):1332–4.
26. Shimizu M, Nakagishi Y, Yachie A. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine. 2013;61(2):345–8.
27. De Benedetti F, Brunner HI, Ruperto N, Kenwright A, Wright S, Calvo I, et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. N Engl J Med. 2012;367(25):2385–95.
28. Pascual V, Allantaz F, Patel P, Palucka AK, Chaussabel D, Banchereau J. How the study of children with rheumatic diseases identified interferon-alpha and interleukin-1 as novel therapeutic targets. Immunol Rev. 2008;223:39–59.
29. Vastert SJ, de Jager W, Noordman BJ, Holzinger D, Kuis W, Prakken BJ, et al. Effectiveness of first-line treatment with recombinant interleukin-1 receptor antagonist in steroid-naive patients with new-onset systemic juvenile idiopathic arthritis: results of a prospective cohort study. Arthritis Rheumatol (Hoboken, NJ). 2014;66(4):1034–43.
30. Ruperto N, Brunner HI, Quartier P, Constantin T, Wulffraat N, Horneff G, et al. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. N Engl J Med. 2012;367(25):2396–406.
31. Butterfield L, Whiteside T. Cytokine Assays. Chichester: John Wiley & Sons, Ltd; 2016. p. 1–11. eLS.
32. Gaines Das RE, Poole S. The international standard for interleukin-6. J Immunol Methods. 1993;160(2):147–53.
33. Ledur A, Fitting C, David B, Hamberger

C, Cavaillon J-M. Variable estimates of cytokine levels produced by commercial ELISA kits: results using international cytokine standards. J Immunol Methods. 1995;186(2):171–9.
34. Taylor P, Gartemann J, Hsieh J, Creeden J. A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Dis. 2011;2011:815038.
35. Gibson DS, Rooney ME, Finnegan S, Qiu J, Thompson DC, Labaer J, et al. Biomarkers in rheumatology, now and in the future. Rheumatology (Oxford). 2012;51(3):423–33.
36. Hügle B, Hinze C, Lainka E, Fischer N, Haas J-P. Development of positive antinuclear antibodies and rheumatoid factor in systemic juvenile idiopathic arthritis points toward an autoimmune phenotype later in the disease course. Pediatr Rheumatol Online J. 2014;12(1):28.
37. Shin JI, Kim KH, Chun JK, Lee TJ, Kim KJ, Kim HS, et al. Prevalence and patterns of anti-nuclear antibodies in Korean children with juvenile idiopathic arthritis according to ILAR criteria. Scand J Rheumatol. 2008;37(5):348–51.
38. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569–81.
39. Gilliam BE, Chauhan AK, Low JM, Moore TL. Pediatric rheumatology. Measurement of biomarkers in juvenile idiopathic arthritis patients and their significant association with disease severity: a comparative study. Clin Exp Rheumatol. 2008;26(3):492–7.
40. Berntson L, Nordal E, Fasth A, Aalto K, Herlin T, Nielsen S, et al. Anti-type II collagen antibodies, anti-CCP, IgA RF and IgM RF are associated with joint damage, assessed eight years after onset of juvenile idiopathic arthritis (JIA). Pediatr Rheumatol Online J. 2014;12:22.
41. Cummings J, Raynaud F, Jones L, Sugar R, Dive C. Fit-for-purpose biomarker method validation for application in clinical trials of anticancer drugs. Br J Cancer. 2010;103(9):1313–7.
42. Swan AL, Stekel DJ, Hodgman C, Allaway D, Alqahtani MH, Mobasheri A, et al. A machine learning heuristic to identify biologically relevant and minimal biomarker panels from omics data. BMC Genomics. 2015;16 Suppl 1:S2.
43. Anderson NGL, Anderson NGL. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics. 2002;1(11):845–67.
44. Solé X, Crous-Bou M, Cordero D, Olivares D, Guinó E, Sanz-Pamplona R, et al. Discovery and validation of new potential biomarkers for early detection of colon cancer. PLoS One. 2014;9(9):e106748.
45. Ling XB, Park JL, Carroll T, Nguyen KD, Lau K, Macaubas C, et al. Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. Proteomics. 2010;10(24):4415–30.
46. STRING: functional protein association networks [Internet]. [cited 2016 Jan 7]. Available from: http://string-db.org/. Accessed 27 July 2016.
47. van den Ham H-J, de Jager W, Bijlsma JWJ, Prakken BJ, de Boer RJ, Van Den Ham H, et al. Differential cytokine profiles in juvenile idiopathic arthritis subtypes revealed by cluster analysis. Rheumatology (Oxford). 2009;48(8):899–905.

48. Eng SWM, Duong TT, Rosenberg AM, Morris Q, Yeung RSM. The biologic basis of clinical heterogeneity in juvenile idiopathic arthritis. Arthritis Rheumatol (Hoboken, NJ). 2014;66(12):3463–75.
49. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh Ch, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Eng J Med. 2006;355(25):2631–9.
50. Eastman PS, Manning WC, Qureshi F, Haney D, Cavet G, Alexander C, et al. Characterization of a multiplex, 12-biomarker test for rheumatoid arthritis. J Pharm Biomed Anal. 2012;70:415–24.
51. Curtis JR, van der Helm-van Mil AH, Knevel R, Huizinga TW, Haney DJ, Shen Y, et al. Validation of a novel multibiomarker test to assess rheumatoid arthritis disease activity. Arthritis Care Res (Hoboken). 2012;64(12):1794–803.
52. Centola M, Cavet G, Shen Y, Ramanujan S, Knowlton N, Swan KA, et al. Development of a multi-biomarker disease activity test for rheumatoid arthritis. PLoS One. 2013;8(4):e60635.
53. Bakker MF, Cavet G, Jacobs JW, Bijlsma JWJ, Haney DJ, Shen Y, et al. Performance of a multi-biomarker score measuring rheumatoid arthritis disease activity in the CAMERA tight control study. Ann Rheum Dis. 2012;71(10):1692–7.
54. Michaud K, Strand V, Shadick NA, Degtiar I, Ford K, Michalopoulos SN, et al. Outcomes and costs of incorporating a multibiomarker disease activity test in the management of patients with rheumatoid arthritis. Rheumatology. 2015;54(9):1640–9.
55. Segurado OG, Sasso EH. Vectra DA for the objective measurement of disease activity in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2014;32(Suppl. 85):S29–S34.
56. Smith E, Oni L, Midgley A, Ekdawy D, Corkhill R, Jones C, et al. Demonstration of an "excellent" biomarker panel for identifying active lupus nephritis in children. Ann Rheum Dis. 2015;331.
57. de Jager W, Hoppenreijs EPAH, Wulffraat NM, Wedderburn LR, Kuis W, Prakken BJ. Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross-sectional study. Ann Rheum Dis. 2007;66(5):589–98.
58. De Jager W, Velthuis H, Prakken BJ, Rijkers GT, Kuis W. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol. 2003;10(1):133–9.
59. Sturgeon C, Hill R, Hortin GL, Thompson D. Taking a new biomarker into routine use–a perspective from the routine clinical biochemistry laboratory. Proteomics Clin Appl. 2010;4(12):892–903.
60. Gillio-Meina C, Cepinskas G, Cecchini EL, Fraser DD. Translational research in pediatrics II: blood collection, processing, shipping, and storage. Pediatrics. 2013;131(4):754–66.
61. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunol. 2009;10(1):52.
62. Van den Bruel A, Cleemput I, Aertgeerts B, Ramaekers D, Buntinx F. The evaluation of diagnostic tests: evidence on technical and diagnostic accuracy, impact on patient outcome and cost-effectiveness is needed. J Clin Epidemiol. 2007;60(11):1116–22.
63. Gibson DS, Finnegan S, Pennington S,

Qiu J, LaBaer J, Rooney ME, Duncan MW. Chapter 8: Validation of Protein Biomarkers to Advance the Management of Autoimmune Disorders. In: Huang F-P, editor. Autoimmune Disorders - Current Concepts and Advances from Bedside to Mechanistic Insights. InTech; 2011. p. 614. ISBN 978-953-307-653-9.

64. Hunter DJ, Losina E, Guermazi A, Burstein D, Lassere MN, Kraus V. A pathway and approach to biomarker validation and qualification for osteoarthritis clinical trials. Curr Drug Targets. 2010;11(5):536–45.
65. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006;23(2):312–28.
66. Nigrovic PA. Review: is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis? Arthritis Rheumatol (Hoboken, NJ). 2014;66(6):1405–13.
67. McArdle A, Butt AQ, Szentpetery A, Jager W De, Roock S de, FitzGerald O, et al. Technical brief. Developing clinically relevant biomarkers in inflammatory arthritis: a multi-platform approach for serum candidate protein discovery. Proteomics Clin Appl. 2015;1–20.
68. Simonini G, Matucci Cerinic M, Cimaz R, Anichini M, Cesaretti S, Zoppi M, et al. Evidence for immune activation against oxidized lipoproteins in inactive phases of juvenile chronic arthritis. J Rheumatol. 2001;28(1):198–203.
69. El-Sayed ZA, Saleh MT, Al-Wakkad AS, Sherief LS, AM N e-D. Cartilage proteoglycan aggrecan as a predictor of joint damage in juvenile rheumatoid arthritis. East Mediterr Health J. 2001;7(6):992–1003.
70. Bloom BJ, Toyoda M, Petrosian A, Jordan S. Anti-endothelial cell antibodies are prevalent in juvenile idiopathic arthritis: implications for clinical disease course and pathogenesis. Rheumatol Int. 2007;27(7):655–60.
71. Bodman-Smith MD, Fife MF, Wythe H, Corrigal VM, Panayi GS, Wedderburn LR, et al. Anti-BiP antibody levels in juvenile idiopathic arthritis (JIA). Rheumatology (Oxford). 2004;43(10):1305–6.
72. Ozawa R, Inaba Y, Mori M, Hara R, Kikuchi M, Higuchi R, et al. Definitive differences in laboratory and radiological characteristics between two subtypes of juvenile idiopathic arthritis: systemic arthritis and polyarthritis. Mod Rheumatol. 2012;22(4):558–64.
73. Gheita TA, Bassyouni IH, Emad Y, el-Din AMN, Abdel-Rasheed E, Hussein H. Elevated BAFF (BLyS) and APRIL in juvenile idiopathic arthritis patients: relation to clinical manifestations and disease activity. Joint Bone Spine. 2012;79(3):285–90.
74. Kounami S, Yoshiyama M, Nakayama K, Okuda M, Okuda S, Aoyagi N, et al. Macrophage activation syndrome in children with systemic-onset juvenile chronic arthritis. Acta Haematol. 2005;113(2):124–9.
75. Simonini G, Azzari C, Gelli AMG, Giani T, Calabri GB, Leoncini G, et al. Neprilysin levels in plasma and synovial fluid of juvenile idiopathic arthritis patients. Rheumatol Int. 2005;25(5):336–40.
76. Galeotti L, Adrian K, Berg S, Tarkowski A, Bokarewa M, Silvia Q. Pediatric rheumatology. Circulating survivin indicates severe course of juvenile idiopathic arthritis. Clin Exp Rheumatol. 2008;26(2):373–8.
77. Nakajima S, Naruto T, Miyamae T,

Imagawa T, Mori M, Nishimaki S, et al. Improvement of reduced serum cartilage oligomeric matrix protein levels in systemic juvenile idiopathic arthritis patients treated with the anti-interleukin-6 receptor monoclonal antibody tocilizumab. Mod Rheumatol. 2009;19(1):42–6.
78. Urakami T, Manki A, Inoue T, Oda M, Tanaka H, Morishima T. Clinical significance of decreased serum concentration of cartilage oligomeric matrix protein in systemic juvenile idiopathic arthritis. J Rheumatol. 2006;33(5):996–1000.
79. Bloom BJ, Alario AJ, Miller LC. Persistent elevation of fibrin D-dimer predicts long term outcome in systemic juvenile idiopathic arthritis. J Rheumatol. 2009;36(2):422–6.
80. Wilson DC, Marinov AD, Blair HC, Bushnell DS, Thompson SD, Chaly Y, et al. Follistatin-like protein 1 is a mesenchyme-derived inflammatory protein and may represent a biomarker for systemic-onset juvenile rheumatoid arthritis. Arthritis Rheum. 2010;62(8):2510–6.
81. Gorelik M, Fall N, Altaye M, Barnes MG, Thompson SD, Grom AA, et al. Follistatin-like protein 1 and the ferritin/erythrocyte sedimentation rate ratio are potential biomarkers for dysregulated gene expression and macrophage activation syndrome in systemic juvenile idiopathic arthritis. J Rheumatol. 2013;40(7):1191–9.
82. Karagiozoglou-Lampoudi T, Trachana M, Agakidis C, Pratsidou-Gertsi P, Taparkou A, Lampoudi S, et al. Ghrelin levels in patients with juvenile idiopathic arthritis: relation to anti-tumor necrosis factor treatment and disease activity. Metabolism. 2011;60(10):1359–62.
83. Bobek D, Grčević D, Kovačić N, Lukić IK, Jelušić M. The presence of high mobility group box-1 and soluble receptor for advanced glycation end- products in juvenile idiopathic arthritis and juvenile systemic lupus erythematosus. Pediatr Rheumatol Online J. 2014;12(1):50.
84. Takahashi A, Mori M, Naruto T, Nakajima S, Miyamae T, Imagawa T, et al. The role of heme oxygenase-1 in systemic-onset juvenile idiopathic arthritis. Mod Rheumatol. 2009;19(3):302–8.
85. Shimizu M, Yachie A. Compensated inflammation in systemic juvenile idiopathic arthritis: role of alternatively activated macrophages. Cytokine. 2012;60(1):226–32.
86. Put K, Avau A, Brisse E, Mitera T, Put S, Proost P, et al. Cytokines in systemic juvenile idiopathic arthritis and haemophagocytic lymphohistiocytosis: tipping the balance between interleukin-18 and interferon-γ. Rheumatology. 2015;54(8):1507–17.
87. Ferreira RA, Silva CHM, Silva DAO, Sopelete MC, Kiss MHB, Mineo JR, et al. Is measurement of IgM and IgA rheumatoid factors (RF) in juvenile rheumatoid arthritis clinically useful? Rheumatol Int. 2007;27(4):345–9.
88. Shahin AA, Shaker OG, Kamal N, Hafez HA, Gaber W, Shahin HA. Circulating interleukin-6, soluble interleukin-2 receptors, tumor necrosis factor alpha, and interleukin-10 levels in juvenile chronic arthritis: correlations with soft tissue vascularity assessed by power Doppler sonography. Rheumatol Int. 2002;22(2):84–8.
89. Yilmaz M, Kendirli SG, Altintas D, Bingöl G, Antmen B. Cytokine levels in serum of patients with juvenile rheumatoid arthritis. Clin Rheumatol. 2001;20(1):30–5.
90. Chen O, Shan N, Zhu X, Wang Y, Ren P, Wei D, et al. The imbalance of IL-18/IL-18BP in patients with systemic juvenile idiopathic arthritis. Acta Biochim Bio-

phys Sin Shanghai. 2013;45(4):339–41.
91. Shimizu M, Yokoyama T, Yamada K, Kaneda H, Wada H, Wada T, et al. Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis. Rheumatology (Oxford). 2010;49(9):1645–53.
92. Maeno N, Takei S, Nomura Y, Imanaka H, Hokonohara M, Miyata K. Highly elevated serum levels of interleukin-18 in systemic juvenile idiopathic arthritis but not in other juvenile idiopathic arthritis subtypes or in Kawasaki disease: comment on the article by Kawashima et al. Arthritis Rheum. 2002;46(9):2539–41. author reply 2541–2.
93. Aggarwal A, Agarwal S, Misra R. Chemokine and chemokine receptor analysis reveals elevated interferon-inducible protein-10 (IP)-10/CXCL10 levels and increased number of CCR5+ and CXCR3+ CD4 T cells in synovial fluid of patients with enthesitis-related arthritis (ERA). Clin Exp Immunol. 2007;148(3):515–9.
94. Ezzat MHM, El-Gammasy TMA, Shaheen KYA, Osman AOY. Elevated production of galectin-3 is correlated with juvenile idiopathic arthritis disease activity, severity, and progression. Int J Rheum Dis. 2011;14(4):345–52.
95. Bica BERG, Gomes NM, Fernandes PD, Luiz RR, Koatz VLG. Nitric oxide levels and the severity of juvenile idiopathic arthritis. Rheumatol Int. 2007;27(9):819–25.
96. Sarma PK, Misra R, Aggarwal A. Elevated serum receptor activator of NFkappaB ligand (RANKL), osteoprotegerin (OPG), matrix metalloproteinase (MMP)3, and ProMMP1 in patients with juvenile idiopathic arthritis. Clin Rheumatol. 2008;27(3):289–94.
97. Masi L, Ricci L, Zulian F, Del Monte F, Simonini G, Capannini S, et al. Serum osteopontin as a predictive marker of responsiveness to methotrexate in juvenile idiopathic arthritis. J Rheumatol. 2009;36(10):2308–13.
98. Tomoum HY, Mostafa GA, El-Shahat EMF. Autoantibody to heterogeneous nuclear ribonucleoprotein-A2 (RA33) in juvenile idiopathic arthritis: clinical significance. Pediatr Int. 2009;51(2):188–92.
99. Gheita TA, El-Gazzar II, El Shazly RI, El-Din AMN, Abdel-Rasheed E, Bassyouni RH. Elevated serum resistin in juvenile idiopathic arthritis: relation to categories and disease activity. J Clin Immunol. 2013;33(1):297–301.
100. Foell D, Wittkowski H, Hammerschmidt I, Wulffraat N, Schmeling H, Frosch M, et al. Monitoring neutrophil activation in juvenile rheumatoid arthritis by S100A12 serum concentrations. Arthritis Rheum. 2004;50(4):1286–95.
101. Shenoi S, Ou J-N, Ni C, Macaubas C, Gersuk VH, Wallace CA, et al. Comparison of biomarkers for systemic juvenile idiopathic arthritis. Pediatr Res International Pediatric Research Foundation, Inc. 2015;78(5):554–9.
102. Wittkowski H, Frosch M, Wulffraat N, Goldbach-Mansky R, Kallinich T, Kuemmerle-Deschner J, et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum. 2008;58(12):3924–31.
103. Frosch M, Ahlmann M, Vogl T, Wittkowski H, Wulffraat N, Foell D, et al. The myeloid-related proteins 8 and 14 complex, a novel ligand of toll-like receptor 4, and interleukin-1beta form a positive feedback mechanism in systemic-onset juvenile idiopathic arthritis.

Arthritis Rheum. 2009;60(3):883–91.
104. Bleesing J, Prada A, Siegel DM, Villanueva J, Olson J, Ilowite NT, et al. The diagnostic significance of soluble CD163 and soluble interleukin-2 receptor alpha-chain in macrophage activation syndrome and untreated new-onset systemic juvenile idiopathic arthritis. Arthritis Rheum. 2007;56(3):965–71.
105. Singh A, Vastert SJ, Prakken BJ, Illges H. Decreased levels of sCD21 and sCD23 in blood of patients with systemic-juvenile arthritis, polyarticular-juvenile arthritis, and pauciarticular-juvenile arthritis. Rheumatol Int. 2012;32(6):1581–7.
106. Lehmberg K, Pink I, Eulenburg C, Beutel K, Maul-Pavicic A, Janka G. Differentiating macrophage activation syndrome in systemic juvenile idiopathic arthritis from other forms of hemophagocytic lymphohistiocytosis. J Pediatr. 2013;162(6):1245–51.
107. Reddy VV, Myles A, Cheekatla SS, Singh S, Aggarwal A. Soluble CD25 in serum: a potential marker for subclinical macrophage activation syndrome in patients with active systemic onset juvenile idiopathic arthritis. Int J Rheum Dis. 2014;17(3):261–7.
108. Chen C, Tsao C, Ou L, Yang M, Kuo M, Huang J. Comparison of soluble adhesion molecules in juvenile idopathic arthritis between the active and remission stages. Ann Rheum Dis. 2002;61:167–70.
109. De Benedetti F, Vivarelli M, Pignatti P, Oliveri M, Massa M, Pistorio A, et al. Circulating levels of soluble E-selectin, P-selectin and intercellular adhesion molecule-1 in patients with juvenile idiopathic arthritis. J Rheumatol. 2000;27(9):2246–50.
110. Bloom BJ, Nelson SM, Eisenberg D, Alario AJ. Soluble intercellular adhesion molecule-1 and E-selectin as markers of disease activity and endothelial activation in juvenile idiopathic arthritis. J Rheumatol. 2005;32(2):366–72.
111. Ishikawa S, Shimizu M, Ueno K, Sugimoto N, Yachie A. Soluble ST2 as a marker of disease activity in systemic juvenile idiopathic arthritis. Cytokine. 2013;62(2):272–7.
112. El-Gamal YM, Heshmat NM, El-Kerdany TH, Fawzy AF. Serum thrombomodulin in systemic lupus erythematosus and juvenile idiopathic arthritis. Pediatr Allergy Immunol. 2004;15(3):270–7.
113. Muzaffer MA, Dayer J-M, Feldman BM, Pruzanski W, Roux-Lombard P, Schneider R, et al. Differences in the profiles of circulating levels of soluble tumor necrosis factor receptors and interleukin 1 receptor antagonist reflect the heterogeneity of the subgroups of juvenile rheumatoid arthritis. J Rheumatol. 2002;29(5):1071–8.

Chapter 4

Molecular signature characterization of different inflammatory phenotypes of systemic juvenile idiopathic arthritis

Faekah Gohar, Angela McArdle, Melissa Jones, Niamh Callan, Belinda Hernandez, Christoph Kessel, Maria Miranda-Garcia, Miha Lavric, Dirk Holzinger, Carolin Pretzer, Elke Lainka, Sebastiaan J Vastert, Sytze de Roock, Oliver FitzGerald, Stephen R Pennington, Dirk Foell

Ann Rheum Dis 2019;0:1-7.

Abstract

Objectives
The International League of Associations for Rheumatology classification criteria define systemic juvenile idiopathic arthritis (SJIA) by the presence of fever, rash and chronic arthritis. Recent initiatives to revise current criteria recognise that a lack of arthritis complicates making the diagnosis early, while later a subgroup of patients develops aggressive joint disease. The proposed biphasic model of SJIA also implies a 'window of opportunity' to abrogate the development of chronic arthritis. We aimed to identify novel SJIA biomarkers during different disease phases.

Methods
Children with active SJIA were subgrouped clinically as systemic autoinflammatory disease with fever (SJIAsyst) or polyarticular disease (SJIApoly). A discovery cohort of n=10 patients per SJIA group, plus n=10 with infection, was subjected to unbiased label-free liquid chromatography mass spectrometry (LC-MS/MS) and immunoassay screens. In a separate verification cohort (SJIAsyst, n=45; SJIApoly, n=29; infection, n=32), candidate biomarkers were measured by multiple reaction monitoring MS (MRM-MS) and targeted immunoassays.

Results
Signatures differentiating the two phenotypes of SJIA could be identified. LC-MS/MS in the discovery cohort differentiated SJIAsyst from SJIApoly well, but less effectively from infection. Targeted MRM verified the discovery data and, combined with targeted immunoassays, correctly identified 91% (SJIAsyst vs SJIApoly) and 77% (SJIAsyst vs infection) of all cases.

Conclusions
Molecular signatures differentiating two phenotypes of SJIA were identified suggesting shifts in underlying immunological processes in this biphasic disease. Biomarker signatures separating SJIA in its initial autoinflammatory phase from the main differential diagnosis (i.e. infection) could aid early-stage diagnostic decisions, while markers of a phenotype switch could inform treat-to-target strategies.

Key Messages

What is already known about this subject?
► Insights into the biological basis of systemic juvenile idiopathic arthritis (SJIA) increasingly support the existence of an initial autoinflammatory phase of the disease offering a therapeutic 'window of opportunity'.
► Early initiation of effective treat-to-target strategies may change the course of SJIA (Still's disease) and prevent the development of a chronic polyarticular disease phenotype.

What does this study add?
► We identified molecular signatures discriminating SJIA phenotypes and separating SJIA from infection.
► Diagnostic biomarkers could aid early-stage therapeutic decisions in initial SJIA phases, while markers of a phenotype switch could inform treat-to-target strategies.

How might this impact on clinical practice or future developments?
► These findings may have future implications for improving SJIA classification criteria and for the development of precision medicine.

Introduction

Systemic juvenile idiopathic arthritis (SJIA, or Still's disease) is an autoinflammatory disorder of unknown pathogenesis that accounts for 10%–15% cases of juvenile idiopathic arthritis (JIA).(1) The International League of Associations for Rheumatology (ILAR) criteria for JIA classification define SJIA by the presence of quotidian fever at onset for a minimum of 2 weeks, a transient rash and chronic arthritis.(2) Early in disease, arthritis is minimal or absent, which complicates establishing the diagnosis when features also fitting differential diagnoses such as infections dominate. However, early diagnosis is key to initiate effective treat-to-target approaches.(3,4)
In a subgroup of patients, stable remission status can be reached rapidly (monophasic disease),(5) though in most cases SJIA progresses to become recurrent or persistent. Recent findings on pathophysiology suggest a biphasic model of SJIA.(6,7) Innate immune dysregulation is present early with systemic features of an autoinflammatory disorder, while adaptive immunity is thought to dominate later phases with destructive joint disease. Therefore, initial immune dysregulation induced by unknown triggers may then drive

autoimmune arthritis. A genetic association of SJIA with major histocompatibility complex class II specific allele HLA-DRB1*11 also suggests an autoimmune component.(8)

Early diagnosis and recognition of phenotypes could therefore be key to initiate effective treat-to-target–based management strategies during a window of opportunity and also to prevent the progression of SJIA into a more aggressive chronic arthritis phenotype. A revision of the ILAR criteria to facilitate the implementation of targeted and personalised management strategies is a current initiative.(3, 9,10)

There is an unmet need for laboratory tests to diagnose and monitor SJIA disease activity.(11) Differential gene expression profiles in peripheral blood mononuclear cells and cytokine signatures in serum have been investigated in new-onset and active versus inactive SJIA.(12) Notably, several immune mediators including interleukin-1 (IL-1), IL-18 as well as the S100 proteins S100A12 and S100A8/A9 (MRP8/14, myeloid related protein 8/14) might be important players in the pathogenesis of SJIA.(13–15) S100A12 and MRP8/14 are highly elevated in SJIA compared with infection or other causes of fever of unknown origin (16–18) and are predictive for subclinical inflammatory activity and disease flares.(19–21)

We aimed to identify molecular markers of systemic-autoinflammatory and chronic-polyarticular SJIA phenotypes by applying unbiased label-free proteomics and multiplex immunoassays in a discovery cohort and targeted candidate biomarker analyses in a verification cohort.

Patients and methods

Patients

Serum samples were collected between 2009 and 2012 from 136 paediatric patients with SJIA or infections (clinically diagnosed and/or confirmed by serology/microbiology; see online supplementary Table S1) attending various hospitals in Germany. In 2015 to 2016, treating clinicians retrospectively reported whether patients with SJIA developed a predominantly systemic or (poly)arthritic phenotype and a monophasic or chronic disease course. Patients were subgrouped as classical autoinflammatory (SJIAsyst) or chronic articular-dominant (SJIApoly) based on the overall clinical disease course. Patients with SJIApoly had a polyarthritic course (≥5 joints affected) and active arthritis at sampling, but no fever, rash, serositis, splenomegaly or generalised lymphadenopathy. In contrast, patients with SJIAsyst had a systemic course with fever, rash, serositis, splenomegaly, lymphadenopathy, elevated acute phase reactants or white cell count and <5 joints involved at time of sampling. No patient fulfilled the criteria for a diagnosis of macrophage activation syndrome (MAS).(22)

Ten patients each with either SJIAsyst, SJIApoly or infection (total n=30) formed the 'discovery cohort'. Another 106 patients (45 SJIAsyst, 29 SJIApoly and 32 infections; Table 1) formed the 'verification cohort'. Patients were not included in both cohorts. At sampling, all patients had clinically active disease (eg, active joints or fever) and laboratory signs of inflammation (eg, elevated acute phase reactants). In the verification cohort, disease duration was significantly shorter in patients with SJIAsyst (median 0.1 years; range, 0.1–8.3) compared with SJIApoly (median 4.7 years; range, 0.1–10; p=0.001). Patients using biological treatments at time of sampling were excluded. The study overview is shown in online supplementary figure S2.

Serum collection

Samples were collected in routine clinical settings. A 20 min centrifugation of the blood sample (collected in a serum gel tube) was performed within 2 hours of collection and the serum directly separated from the cell pellet. Serum was aliquoted into 1.5 mL or 2 mL Eppendorf tubes and posted at room temperature, to be stored at −80°C on arrival after the measurement of serum S100 proteins. Discovery cohort samples were subjected to unbiased label-free proteomics using liquid chromatography mass spectrometry (LC-MS/MS) and a broad multiplexed immunoassay screen (Luminex). Multi-

ple reaction monitoring MS (MRM-MS) and targeted Luminex assays were performed on verification cohort samples. All samples were analysed for S100A12 and MRP8/14 by ELISAs (Figures 1 and 2).

Proteomic analysis

Serum for LC-MS/MS analysis was prepared by depletion of the 14 most highly abundant proteins using a MARS Hu-14 affinity depletion column (Agilent Technologies, P/N 5188-6557) and visually confirmed using SDS chromatography as previously described.(23) Depleted samples were further concentrated by centrifugation and then subjected to in-solution tryptic digestion followed by desalting using C18 zip tips (online supplementary methods S3). Technical reference samples were included. Samples were stored at −80°C before analyses using a Q-Exactive mass spectrometer.(24) LC-MS/MS data were analysed by MaxQuant (V.1.4.1.3) with protein identifications generated using Andromeda.(25) Qualitative analysis was performed using PEAKS Studio (BSI) and Spectrum Mill (Agilent). Statistical analyses of the LC-MS/MS data were performed using Perseus (V.1.4.1.3).

MRM analysis of the verification cohort

To evaluate the candidate biomarkers identified during discovery, a unique MRM assay was generated, incorporating as many candidate proteins as possible (online supplementary methods S3).

Bead array assays and ELISA

In the discovery cohort, a total of 150 immune-related proteins (online supplementary table S4) were screened by a bead-based (Luminex) multiplex panel.(26) Proteins with little or no variation between samples (less than 75% unique values) or proteins with low expression levels were excluded. In the verification cohort, a customised panel of 17 cytokines was designed using commercially available Luminex analytes (eBioscience): interleukin (IL) 1 alpha (IL1α), IL1 receptor antagonist (IL1RA), IL2 receptor (IL-2R), IL6, IL8, IL10, IL18, IL20, IL21, IL23, macrophage colony stimulating factor, osteoprotegerin, thymic stromal lymphopoietin, monocyte chemotactic protein 2, eotaxin, TNF-like weak inducer of apoptosis (TWEAK) and monokine induced by gamma interferon.(24,27,28) S100A12 and MRP8/14 concentrations were measured in all serum samples using standardised in-house ELISAs as previously reported.(29,30) Both assays included reference internal control

Table 1: Clinical and laboratory characteristics of sampled patients

	Discovery Cohort, n=30			***Verification Cohort, n=106***		
	SJIA^{syst}	SJIA^{poly}	Infection	SJIA^{syst}	SJIA^{poly}	Infection
Patients, n	10	10	10	45	29	32
Gender (M:F)	4:6	3:7	4:6	30:15	11:18	11:21
Age at sample, year	7 (5)	15 (6)	6 (7)	9 (8)	11 (5)	6 (9)
Phenotype at sampling, n						
Fever	10	0	10	40	0	27
Arthralgia	7	5	4	25	9	7
Arthritis	4	10	n/a	14	29	n/a
Active joints (mean,	1.3 (0-3)	3.6 (3-	n/a	2 (0-5)	6 (5-23)	n/a
Exanthema	6	0	3	18	0	6
Serositis	1	0	1	9	0	1
Hepatosplenomegaly	4	0	4	9	0	2
Phenotype after sampling						
Monophasic:chronic	7:3	0:10	n/a	26:19	5:24	n/a
Medication at sampling						
Antibiotics	1	0	5	0	2	16
NSAID	4	2	2	13	7	6
Methotrexate	2	5	0	6	16	0
Biologics	0	0	0	0	0	0
Steroid	3	2	0	15	12	2
Markers of inflammation at sampling						
ESR (mm/h)	95 (55)	5 (11)	60 (56)	80 (67)	11 (17)	68 (39)
Missing ESR, n	1	1	3	7	3	16
CRP (mg/dL)	11 (7.3)	0.2 (1.4)	13 (5.6)	12.8	0.4 (1.8)	9.7 (8.0)
Missing CRP, n	0	0	0	1	0	4
WCC (x10^3/ml)	21 (12)	7 (5.3)	11.8	19 (10)	8 (4)	12 (14)
Missing WCC, n	0	0	0	1	0	4

SJIA^{syst}: SJIA with fever/systemic disease, SJIA^{poly}: SJIA with polyarthritis but systemic disease. Values are Median (IQR) unless otherwise specified

sera with established cut-off values and were performed blinded to the patient characteristics.

Data analysis
Random Forest models were used to discriminate between the clinical groups (Random Forest package in R V.4.3.2). Reported performance measures were cross-validated in all models using leave-one-out cross-validation. Measurements of accuracy included the classification rate (percentage of total cases correctly classified by the model) and the area under the receiver operating characteristic (ROC) curve (AUC), calculated using the pROC package in R V.3.4.4. The Random Forest model provides a measure of importance of each variable contributing towards the overall performance, calculated using the Gini decrease in impurity (higher decrease in impurity means the variable is more predictive). In each model, the overall importance of variables was taken as the average decrease in the Gini decrease in impurity over each of the n random forest models run (n=number of samples in a given cohort). Kruskal-Wallis test, corrected for multiple comparisons, was applied to compare biomarkers between the groups. Data are shown as median (IQR), unless otherwise specified. Network analyses were performed using the GeneMANIA platform by applying Gene Ontology network weighting based on biological processes.(31)

Results
Clinical and laboratory characteristics of the cohorts
Clinical characteristics and laboratory markers of participants are detailed in Table 1. Patients with infection had similarly high levels of C-reactive protein (CRP), WCC and erythrocyte sedimentation rate as patients with SJIAsyst. Frequencies of fever and exanthema were highest in SJIAsyst followed by infection and lowest in SJIApoly. Patients with infection and SJIApoly had similar frequencies of joint pain and hepatosplenomegaly, which were lower than in SJIAsyst.

LC-MS/MS proteomic analysis in the discovery cohort
Univariate analysis revealed 41 differentially expressed proteins between SJIAsyst and SJIApoly and 31 non-overlapping proteins differing between SJIAsyst and infections (total 72 candidate markers). Heat maps showing upregulated and downregulated proteins indicated good separation of the groups

(Figure 1). Some markers that discriminated groups in the immunoassay panel (eg, SAA1, S100A12 and sCD163) were also discovered by unbiased proteomics and thus acted as independent confirmation.

Proteomic analysis in the verification cohort

Of the 72 differentially expressed proteins from the LC-MS/MS discovery phase, 48 could be included in the MRM assay. Univariate and multivariate analysis of the data confirmed the presence of protein signatures capable of differentiating SJIAsyst from SJIApoly and separately SJIAsyst from infection (Figure 2A). The top 30 performing peptides and their recognised proteins for each analysis are shown in the variable importance plot in Figure 3A–D. Interestingly, a CRP peptide was the top-ranked peptide for SJIAsyst versus SJIApoly and also featured in the top 30 for SJIAsyst versus infection. CRP featured more than once in the list due to the inclusion of several different peptides in the assay panel. Analyses using the Random Forest model distinguished the SJIAsyst phenotype from SJIApoly with 91% accuracy (sensitivity 86%, specificity 93%; Table 2). Patients with SJIAsyst were less well distinguished from those with infection (accuracy, 62%; sensitivity, 38%; specificity, 80%). ROC curves showed a good differentiation of the two SJIA phenotypes with an AUC of 0.91 (95% CI 0.83 to 0.99), which outperformed SJIAsyst versus infection (AUC 0.66, 95% CI 0.54 to 0.79).

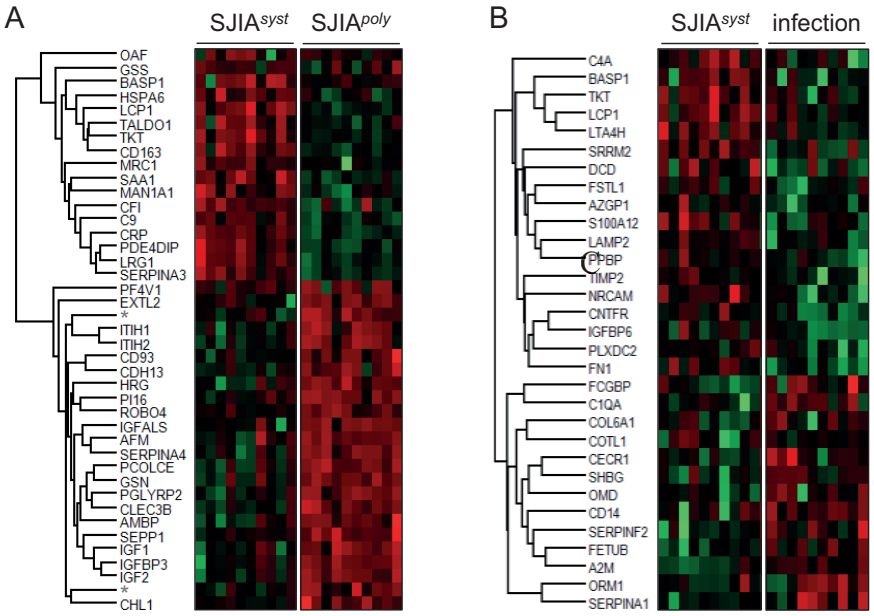
Table 2: Accuracy of the proteomic marker panels

	SJIA^{syst} vs **SJIA^{poly}**			**SJIA^{syst}** vs **Infection**		
	MRM	Immune	Combined	MRM	Immune	Combined
Sensitivity	0.86	0.76	0.86	0.80	0.69	0.69
Specificity	0.93	0.91	0.93	0.38	0.82	0.82
Accuracy	0.91	0.85	0.91	0.65	0.77	0.77
PPV	0.89	0.85	0.89	0.80	0.73	0.73
NPV	0.91	0.85	0.91	0.57	0.79	0.79
LR+	14.33	8.44	14.33	1.28	4.06	4.06
LR-	0.15	0.26	0.15	0.53	0.39	0.39
AUC	0.909	0.895	0.918	0.661	0.840	0.803

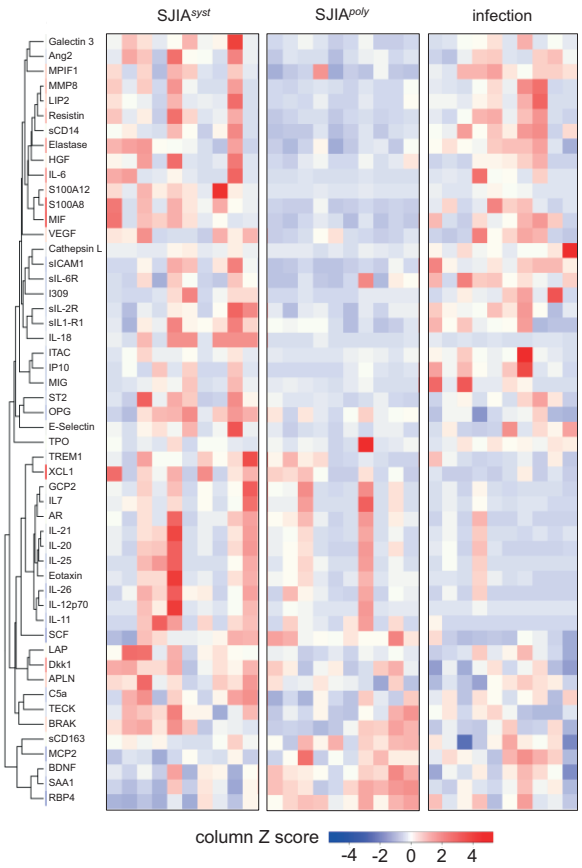
SJIA^{syst} = SJIA with fever/systemic disease; SJIA^{poly} = SJIA with polyarthritis but systemic disease;
PPV = Positive Predictive Value; NPV = Negative Predictive Value; LR+ = Positive Likelihood Ratio; LR- = Negative Likelihood Ratio; AUC = Area Under Curve

Figure 1 Proteomic and immune assay analyses in the discovery cohort

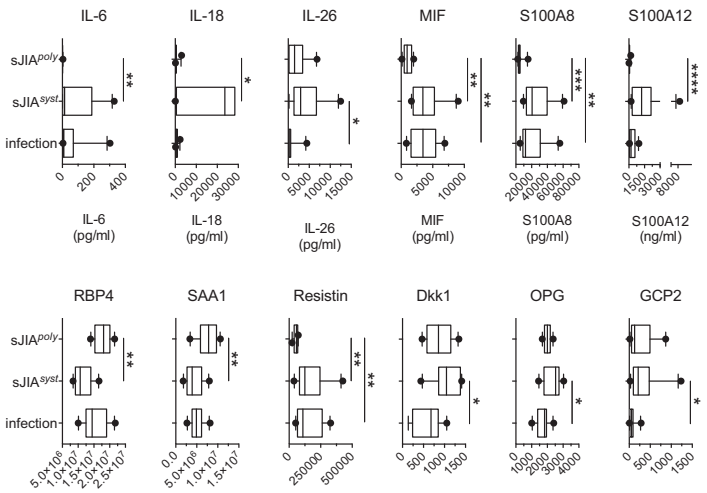
Serum was analysed by LC-MS/MS in a discovery cohort with the differences between the phenotypes shown as follows: (A) SJIA with systemic disease (SJIAsyst, n=10) vs SJIA with polyarticular disease (SJIApoly, n=10) and (B) SJIA with systemic disease (SJIAsyst,n=10) vs infections (n=10). Heatmaps are shown with red squares representing overexpressed proteins and green representing lower expression (*indicates peptides without protein assignment). (C) Sera of identical patients as in (A) and (B) were analysed by 150-plex bead array Luminex assay. Analytes differentiating between groups with a p value <0.08 (see online supplementary table S4) are shown as heatmap based on Spearman rank correlation and average linkage clustering. S100A12 levels in respective sera were determined by ELISA and data are included in the heatmap. (D) Top performing serum markers based on significant separation of SJIAsyst from SJIApoly or infections are shown as box-and-whisker plots (10th–90th percentile). Black full circles indicate outliers. Data were analysed by Kruskal-Wallis test and corrected for multiple comparisons, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



C


SJIA^syst
SJIA^poly
infection
Galectin 3
Ang2
MPIF1
MMP8
LIP2
Resistin
sCD14
Elastase
HGF
IL-6
S100A12
S100A8
MIF
VEGF
Cathepsin L
sICAM1
sIL-6R
I309
sIL-2R
sIL1-R1
IL-18
ITAC
IP10
MIG
ST2
OPG
E-Selectin
TPO
TREM1
XCL1
GCP2
IL7
AR
IL-21
IL-20
IL-25
Eotaxin
IL-26
IL-12p70
IL-11
SCF
LAP
Dkk1
APLN
C5a
TECK
BRAK
sCD163
MCP2
BDNF
SAA1
RBP4
column Z score
-4 -2 0 2 4

D


IL-6
IL-18
IL-26
MIF
S100A8
S100A12
sJIA^poly
sJIA^syst
infection
IL-6 (pg/ml)
IL-18 (pg/ml)
IL-26 (pg/ml)
MIF (pg/ml)
S100A8 (pg/ml)
S100A12 (ng/ml)
RBP4
SAA1
Resistin
Dkk1
OPG
GCP2

Inflammatory markers analysed by immune assays

In the discovery cohort, Luminex and ELISA analyses showed differences between the groups (Figure 1). However, sample size limited statistical analysis. Therefore, a verification panel including commercially available analytes that were discriminative in the discovery cohort or in published literature, allowing a semitargeted reproducible approach, was designed. Univariate analyses of the best-performing individual markers in differentiating SJIAsyst and SJIApoly measured by ELISA and Luminex are shown in Figure 2B and levels of most important markers per group are plotted in Figure 2C. Variable importance plots ranking each of the markers tested within the combined analysis revealed that MRP8/14, IL18 and S100A12 were the most important variables for differentiating SJIAsyst from infection, whereas MRP8/14, S100A12 and IL-1Ra were the top variables differentiating SJIAsyst from SJIApoly (online supplementary figure 5). The ROC curve showed a good differentiation of the two SJIA phenotypes (AUC 0.90, 95% CI 0.81 to 0.98), similar to the discrimination of SJIAsyst and infection (AUC 0.84, 95% CI 0.75 to 0.93).

Lastly, to compare the performance of known biomarkers, ROC analyses of the single markers MRP8/14, S100A12, IL18 and ferritin were performed in the verification cohort (summarised in online supplementary figure S6 and online supplementary table S7). For the distinction between SJIAsyst versus infection, the single markers S100A12 (AUC 0.81) and MRP8/14 (AUC 0.82) performed almost as well as the immune assay panel (AUC 0.84). The best discriminator between SJIAsyst and SJIApoly was MRP8/14 (AUC 0.93), which was also ranked high in the variable importance plots of the multiplex analyses.

Figure 2 Proteomic and immune assay analyses in the verification cohort

(A) Heatmap of MRM peak area data for peptides derived from the indicated proteins as detected in sera of patients with systemic juvenile idiopathic arthritis with systemic (SJIAsyst, n=46) or polyarticular disease (SJIApoly, n=47) or infections (n=32) based on Spearman rank correlation and average linkage clustering. (B) Heatmap of serum marker concentrations by bead array assay in cohorts as described in (A) based on Spearman rank correlation and average linkage clustering. (C) Box-and-whisker plots (10th–90th percentile) of top performing serum markers. Black full circles indicate outliers. Data were analysed by Kruskal-Wallis test and corrected for multiple comparisons, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

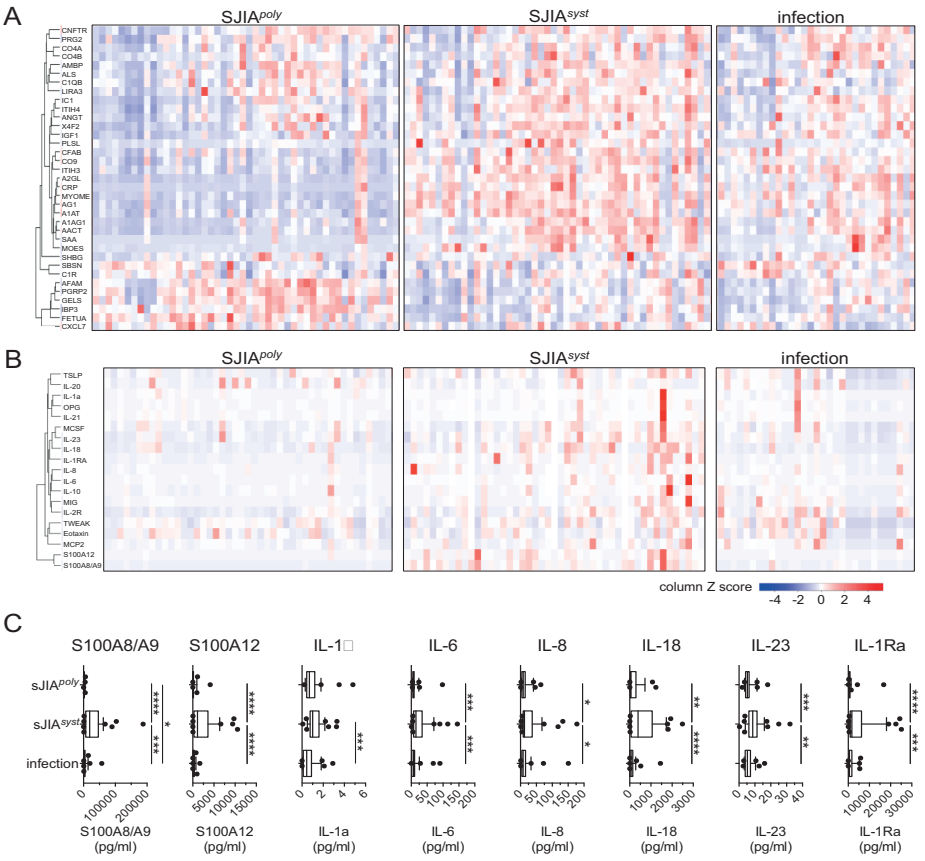
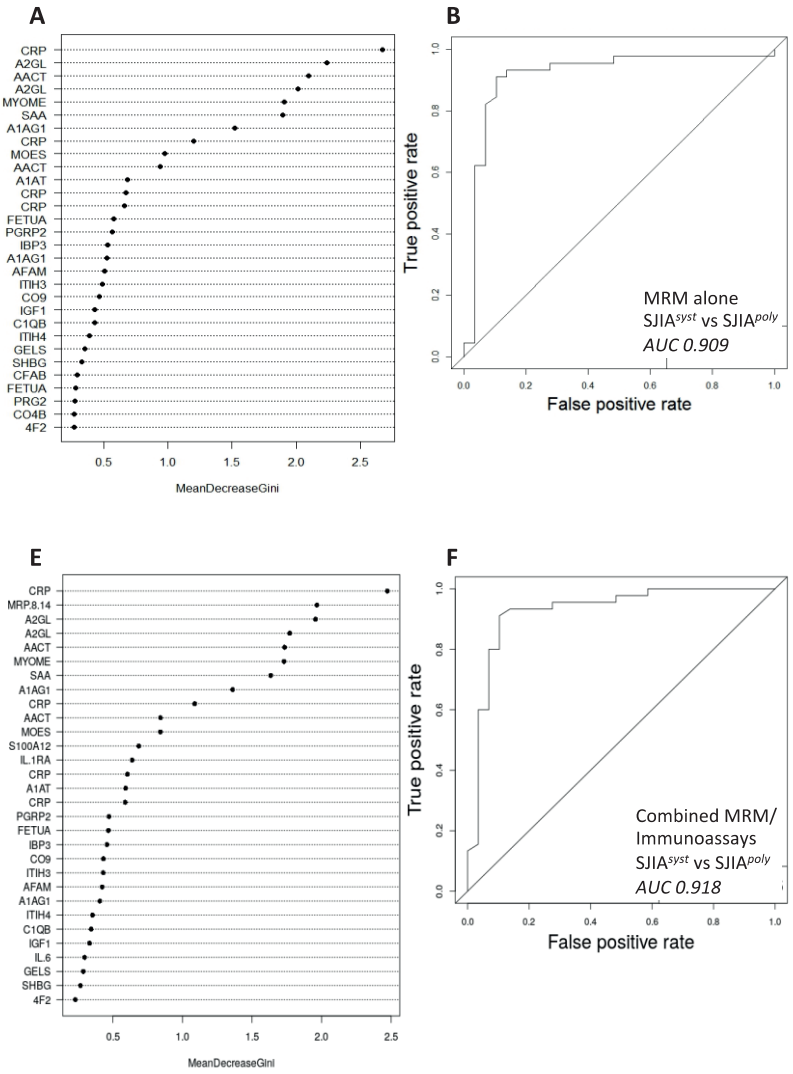
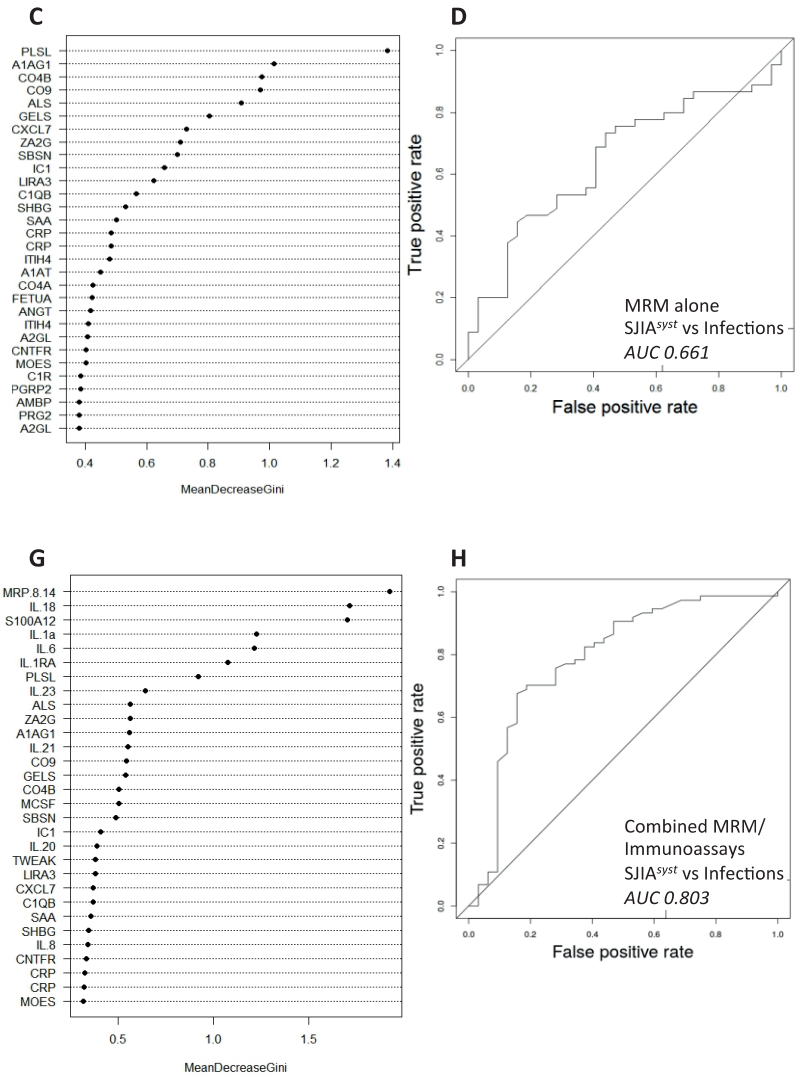


Figure 3 Predictive power of biomarker signatures

The accuracy of either the proteomic MRM panel alone (upper panels A–D) or a combination of MRM and immunoassays Luminex/ELISA (lower panels E–H) was analysed using a Random Forest model with 'leave-one-out cross-validation' statistical method. In the top rank list of the Random Forest models, the same protein could be identified by multiple peptides. Based on the ranked markers, receiver operating characteristic (ROC) curves were plotted for different comparisons. The analyses were performed for comparison groups SJIAsyst vs SJIApoly (A, B or E, F, respectively) and for the differentiation of SJIAsyst vs infections (C, D or G, H, respectively). The ROC curve and area under the curve (AUC) are shown.





Accuracy of combining proteomic and immune assays

The top 30 variables in the combined multimarker panels comprising MRM, ELISA and Luminex were ranked (Figure 3E–H). The top five discriminating biomarkers for SJIAsyst versus SJIApoly were CRP, leucine-rich alpha-2-glycoprotein (A2GL), MRP8/14, alpha-1-antichymotrypsin (AACT) and myomegalin (myome); for SJIAsyst versus infection, MRP8/14, IL18, S100A12, IL1α and IL6. MRP8/14 was the only marker featuring in the top five of both panels. Eleven biomarkers were common to both panels: CRP, MRP8/14, AACT, serum amyloid A (SAA), alpha-1-acid glycoprotein 1 (A1AG1), moesin (MOES), S100A12, IL1RA, gelsolin (GELS), IL6 and sex hormone– binding globulin. In the combined model, distinction between SJIAsyst versus SJIApoly was possible with an overall accuracy of 90.5% with 67/74 cases correctly identified (AUC 0.93). Accuracy of the SJIAsyst versus infection model was 76.6% and 59/77 cases were correctly classified (Table 2). The combined multimodal panel (MRM and immunoassay) improved the distinction between SJIAsyst and SJIApoly, but not SJIAsyst from infections, with immunoassays outperforming MRM.

To better understand potential relationships between the diverse identified markers, network analyses based on Gene Ontology biological process annotations using the GeneMANIA platform (figure 4 and online supplementary figures S8, S9) were performed. Serum marker panels identified by the combined Random Forest models to discriminate SJIAsyst from SJIApoly (Figure 3E) or SJIAsyst from infections (Figure 3G) revealed multiple associations between each other as well as additional proteins that were not in the analysed panels (Figure 4A,B). The top performing markers (mean Gini decrease >0.5) of both Random Forest model panels associated best with members of the IL1 signalling pathway, namely IL1R2 (Figure 4). The association with IL1 signalling appeared more pronounced with markers in the panel differentiating SJIAsyst from SJIApoly, with IL1R2 further linking to IL1β and IL1R1 (Figure 4B), which was not pronounced for the separation of SJIAsyst from infections.

Discussion

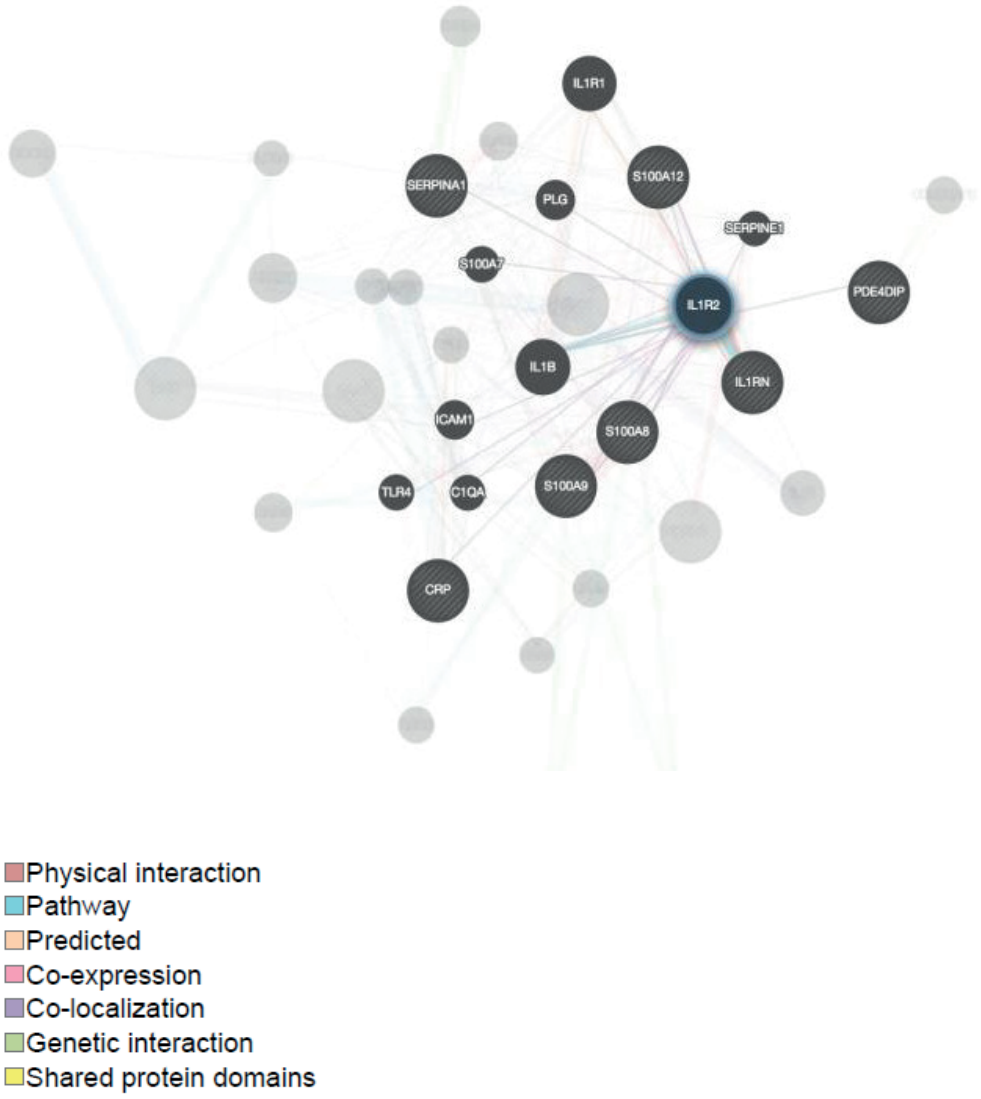
Using proteomic analyses and immunoassays, signatures of serum proteins that distinguish two clinical phenotypes of SJIA and help differentiate autoinflammatory SJIA from infections were discovered. Proteomics has been relatively underused in paediatric rheumatology with most studies focusing on synovial fluid protein expression in JIA.(32,33) Proteomic analyses have, however, identified serum protein profiles of SJIA and revealed biomarkers for monitoring response to therapy in SJIA.(28,34) Clinical heterogeneity is a well-recognised feature of JIA and assumed to have a biological basis,(35,36) with differential PBMC gene expression profiles found in patients with SJIA versus non-systemic JIA.(12) Serum cytokine profiles have so far predominantly focused on discriminating active and inactive SJIA, or predicting response to treatment. With that regard, specifically RNA expression studies have been performed.(37–39) However, recent analyses failed to show distinct transcriptional profiles that could be attributed to diverse subphenotypes of SJIA.(39,40)

This study is the first aiming to systematically discriminate SJIA phenotypes with proteomic biomarkers. Correct discrimination of SJIAsyst from SJIApoly was achieved in over 90% of cases using any of the three identified biomarker panels (MRM alone, ELISA/Luminex alone and combined). Our study included a number of markers with reported potential value for the diagnosis of SJIA.(41–43) Of these, IL18 is not regularly measured due to technical limitations in performing bioassays, while the routine use of IL6 is still limited for various reasons.(44,45) In the MRM assay, peptides of S100A12, MRP8/14 and the bead assay–measured cytokines were below the limit of detection for MRM and were therefore excluded from panels. Lack of available analytes and/ or low sensitivity are limitations of proteomic analyses and may explain the variation in results from the different approaches. The use of the combined approach could overcome this to some extent. However, our analyses of MRP8/14 and S100A12 by immunoassays show that these single analytes, partially already in routine care, perform very well as surrogate markers.

Interestingly, a number of markers in our combined panel that discriminated SJIAsyst from SJIApoly were also identified in a published panel of markers that differentiated flare from quiescent SJIA.(28) The common markers

Figure 4 Association of identified discriminating serum markers
Plots show GeneMANIA-generated networks seeded with the proteins identified by Random Forest analysis discriminating SJIAsyst from SJIApoly (A) and SJIAsyst from infections (B) above a mean Gini decrease cutoff of 0.5. Seeded markers are depicted as hatched circles of uniform size, while those that were added as relevant based on gene ontology biological process annotations are depicted as solid circles. Circle size is proportional to the number of interactions. The most relevant identified associations are highlighted.

A: SJIA^{syst} vs SJIA^{poly}



B: SJIA^{syst} vs Infections

572961-L-bw-PS4U
Processed on: 25-1-2022

were alpha-2-macroglobulin (A2M or A2GL), alpha-1-acid glycoprotein 1 (A1AG1 or AGP1), serpin a3/alpha-1-antichymotrypsin (AACT), GELS, SAA, MRP8/14 and CRP, which as part of the full panel published by Ling et al also differentiated SJIA from acute febrile illnesses. Of these biomarkers, A1AG1, GELS, SAA, MRP8/14 and CRP featured in both the SJIAsyst versus SJIApoly and the SJIAsyst versus infection comparisons. Kininogenin (KLKB1), a high molecular weight protein which plays a role in the pathogenesis of inflammatory reactions, was previously identified by MS and also featured among the top gene ontology associated markers in the SJIAsyst from SJIApoly network analysis performed here.(28)

A differentiation of phenotypes is not currently included in SJIA classification criteria. As knowledge of underlying immunological processes increases, leading to new treatment strategies,(46) it is important that treat-to-target approaches are supported by reliable biomarkers. The primary target is disease remission, and the available data support the hypothesis of a therapeutic window of opportunity in the autoinflammatory phase of the disease.(4) Our patients with clinically discriminated SJIA phenotypes had significantly different disease durations, which itself supported the biphasic model of SJIA. It is therefore important to start therapy early, which requires timely diagnosis before chronic arthritis develops. Recent data show that about half of the patients treated as SJIA do not have arthritis and therefore do not fulfil the ILAR classification criteria,(47) resulting in SJIA often being a diagnosis of exclusion. Here, the tested biomarker panel can help the earlier differentiation of SJIA versus infections. Another important aspect of treat-to-target protocols is the monitoring of the therapeutic response to check for necessary treatment adaptation. Phenotype switches occurring during the clinical course may require a corresponding therapy adjustment. The identification of underlying immunological imbalances could be facilitated by biomarker panels as described here.

Our study has a number of limitations. The sample size was relatively small. The quality of some samples may have been suboptimal for unbiased proteomic profiling, although clinically useful diagnostic biomarkers should be robust and stable.(48) Multiple preanalytical factors are thought to affect results, including the handling, shipping and storage of samples.(49) However, internal evaluation of the impact of freeze–thawing samples for MRM ana-

lysis and tryptic digestion of proteins before proteomic analysis showed that any preanalytical proteolysis had no effect on the final measurements (data not shown).

Conclusion

In summary, differing biomarker profiles between two phenotypes of SJIA were identified, strengthening the biological basis for subphenotypes in SJIA. Moreover, separate panels discriminating patients with SJIAsyst from those with infections were established. Biomarker panels were measurable using MRM, ELISA and Luminex assays or a combination of these, which improved the accuracy of the discrimination of SJIAsyst from infection, but not the discrimination of SJIAsyst from SJIApoly, which already performed very well with single-platform panels. The identified protein signature of SJIA versus infections can help to establish an early diagnosis. The discrimination of SJIA subphenotypes may improve the understanding of the pathophysiology underlying different disease phases and courses, which may inform future treat-to-target strategies. Future work could include biomarker measurements at specific time points including at diagnosis and flare as well as in established phenotype switches in a larger cohort.

Acknowledgements

The authors would like to thank the patients and colleagues who contributed their time and samples to the bio-bank collections. We are also thankful to Melanie Saers, Susanne Schleifenbaum, Sabrina Fuehner (Muenster) and Kieran Wynne (Dublin) for their excellent technical assistance in performing the experiments and assays.

Collaborators

Antje Hellige; Andreas Urban; Aleš Janda; Annette Jansson; Bernd-Ulrich Keck; Boris Hügle; Eggert Lilienthal; Elisabeth Weißbarth-Riedel; Frank Weller-Heinemann; Gerd Ganser; Gerd Horneff; Gert Reutter-Simon; Gertrud Wannenmacher; Gregor Dückers; Gundula Böschow; Hans-Iko Huppertz; Hartwig Lehmann; Jeannine Rettschlag; Jens Berrang; Johannes-Peter Haas; Jürgen Brunner; Markus Knuf; Kirsten Mönkemöller; Klaus Tenbrock; Markus Hufnagel; Prasad Thomas Oommen; Rainer Berendes; Sandra Hansmann; Thomas Berger; Toni Hospach.

Contributors

DF, OF and SRP designed and planned the study. FG, DH, CP, CK, SdR, AM, MJ, NC and SRP performed experiments. FG, AM, MJ, BH, CP, CK, EL, SJV, SdR, DF and SRP analysed data. FG, AM, MJ, BH, NC, MM-G, CK, ML, EL, SJV, SdR, OF, DH, SRP and DF participated in data interpretation and discussion. All authors were involved in writing the manuscript and all made substantial contributions to the content and approved the final manuscript.

Collaborators provided clinical data and samples (full details listed in online supplementary material).

Funding
FG was supported by the EU FP7 EUTRAIN (European Translational Training for Autoimmunity and Immune manipulation Network) project grant (ref. 289903) until December 2016. FG, AM, NC, ML, SdR, SRP and DF received funding from the EU FP7 MIAMI (Monitoring innate Immunity in Arthritis and Mucosal Inflammation) project grant (ref. 305266). BH is funded by the HRB (Health Research Board) ref. LP-HSR-2017-021. The AID-Net was funded by the German Ministry of Research and Education (BMBF 01GM1512A/D). The UCD Conway Institute and Proteome Research Centre is funded by the Programme for Research in Third Level Institutions, as administered by Higher Education Authority of Ireland.

Competing interests
OF declares the receipt of research grants and personal fees from Pfizer, Abbott and personal fees from Roche, Amgen, UCB, Celgene and Lilly.
DF declares the receipt of research grant support and honoraria from Pfizer, Novartis, Sobi and Chugai-Roche. SRP declares honoraria from Celgene and is founder of Atturos, a UCD spin-out company developing advanced diagnostic tests. The other authors declare no conflicts of interest.

Ethics approval: University of Münster (ref 2009-031-f-S and 2014-637-f-S).

Provenance and peer review. Not commissioned; externally peer reviewed.

Data sharing statement All data relevant to the study are included in the article or uploaded as supplementary information.

References

1. Thierry S, Fautrel B, Lemelle I, et al. Prevalence and incidence of juvenile idiopathic arthritis: a systematic review. Joint Bone Spine 2014;81:112–7.
2. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision,Edmonton, 2001. J Rheumatol 2004;31:390–2.
3. Ravelli A, Consolaro A, Horneff G, et al. Treating juvenile idiopathic arthritis to target: recommendations of an international task force. Ann Rheum Dis 2018;77:annrheumdis-2018-213030–28.
4. Vastert SJ, de Jager W, Noordman BJ, et al. Effectiveness of first-line treatment with recombinant interleukin-1 receptor antagonist in steroid-naive patients with new-onset systemic juvenile idiopathic arthritis: results of a prospective cohort study. Arthritis Rheumatol 2014;66:1034–43.
5. Singh-Grewal D, Schneider R, Bayer N, et al. Predictors of disease course and remission in systemic juvenile idiopathic arthritis: significance of early clinical and laboratory features. Arthritis Rheum 2006;54:1595–601.
6. Nigrovic PA. Review: is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis? Arthritis Rheumatol 2014;66:1405–13.
7. Nigrovic PA. Autoinflammation and autoimmunity in systemic juvenile idiopathic arthritis. Proc Natl Acad Sci USA 2015;112:15785–6.
8. Ombrello MJ, Remmers EF, Tachmazidou I, et al. HLA-DRB1*11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis. Proc Natl Acad Sci USA 2015;112:15970–5.
9. Martini A, Ravelli A, Avcin T, et al. Toward new classification criteria for juvenile idiopathic arthritis: first steps, Pediatric Rheumatology International Trials Organization international consensus. J Rheumatol 2019;46:190–7.
10. Martini A. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. ARD 2012;71:1437–9.
11. Gohar F, Kessel C, Lavric M, et al. Review of biomarkers in systemic juvenile idiopathic arthritis: helpful tools or just playing tricks? Arthritis Res Ther 2016;18.
12. Ogilvie EM, Khan A, Hubank M, et al. Specific gene expression profiles in systemic juvenile idiopathic arthritis. Arthritis Rheum 2007;56:1954–65.
13. Kessel C, Lippitz K, Weinhage T, et al. Proinflammatory cytokine environments can drive interleukin-17 overexpression by γ/δ T cells in systemic juvenile idiopathic arthritis. Arthritis Rheumatol 2017;69:1480–94.
14. Pascual V, Allantaz F, Arce E, et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201:1479–86.
15. De Benedetti F, Brunner HI, Ruperto N, et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. NEJM 2012;367:2385–95.
16. Wittkowski H, Frosch M, Wulffraat N, et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum 2008;58:3924–31.
17. Frosch M, Ahlmann M, Vogl T, et al. The myeloid-related proteins 8 and 14 complex, a novel ligand of Toll-like receptor 4, and interleukin-1beta form a positive feedback mechanism in syste-

mic-onset juvenile idiopathic arthritis. Arthritis Rheum 2009;60:883–91.
18. Shenoi S, Ou J-N, Ni C, et al. Comparison of biomarkers for systemic juvenile idiopathic arthritis. Pediatr Res 2015;78:554–9.
19. Holzinger D, Frosch M, Kastrup A, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. ARD 2012;71:974–80.
20. Rothmund F, Gerss J, Ruperto N, et al. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care Res 2014;66:949–55.
21. Foell D, Wulffraat N, Wedderburn LR, et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. JAMA 2010;303:1266–73.
22. Ravelli A, Minoia F, Davì S, et al. 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: a European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation collaborative initiative. Arthritis Rheumatol 2016;68:566–76.
23. Morrissey B, O'Shea C, Armstrong J, et al. Development of a label-free LC-MS/MS strategy to approach the identification of candidate protein biomarkers of disease recurrence in prostate cancer patients in a clinical trial of combined hormone and radiation therapy. Prot. Clin. Appl. 2013;7:316–26.
24. McArdle A, Qasim Butt A, Szentpetery A, et al. Developing clinically relevant-biomarkers in inflammatory arthritis: a multiplatform approach for serum candidate protein discovery. Proteomics Clin Appl 2016;10:691–8.
25. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol 2008;26:1367–72.
26. Scholman RC, Giovannone B, Hiddingh S, et al. Effect of anticoagulants on 162 circulating immune related proteins in healthy subjects. Cytokine 2018;106:114–24.
27. de Jager W, Hoppenreijs EPAH, Wulffraat NM, et al. Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross-sectional study. ARD 2007;66:589–98.
28. Ling XB, Park JL, Carroll T, et al. Plasma profiles in active systemic juvenile idiopathic arthritis: biomarkers and biological implications. Proteomics 2010;10:4415–30.
29. Gerss J, Roth J, Holzinger D, et al. Phagocyte-specific S100 proteins and highsensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. ARD 2012;71:1991–7.
30. Frosch M, Strey A, Vogl T, et al. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum 2000;43:628–37.
31. Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010;38:W214–W220.
32. Rosenkranz ME, Wilson DC, Marinov AD, et al. Synovial fluid proteins diffe-

rentiate between the subtypes of juvenile idiopathic arthritis. Arthritis Rheum 2010;62:1813–23.
33. Finnegan S, Robson J, Scaife C, et al. Synovial membrane protein expression differs between juvenile idiopathic arthritis subtypes in early disease. Arthritis Res Ther 2014;16.
34. Miyamae T, Malehorn DE, Lemster B, et al. Serum protein profile in systemic-onset juvenile idiopathic arthritis differentiates response versus nonresponse to therapy. Arthritis Res Ther 2005;7:R746–55.
35. van den Ham H-J, de Jager W, Bijlsma JWJ, et al. Differential cytokine profiles in juvenile idiopathic arthritis subtypes revealed by cluster analysis. Rheumatology 2009;48:899–905.
36. E ng SWM, Duong TT, Rosenberg AM, et al. The biologic basis of clinical heterogeneity in juvenile idiopathic arthritis. Arthritis Rheumatol 2014;66:3463–75.
37. Allantaz F, Chaussabel D, Stichweh D, et al. Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade. J Exp Med 2007;204:2131–44.
38. Barnes MG, Grom AA, Thompson SD, et al. Subtype-specific peripheral blood gene expression profiles in recent-onset juvenile idiopathic arthritis. Arthritis Rheum 2009;60:2102–12.
39. Brachat AH, Grom AA, Wulffraat N, et al. Early changes in gene expression and inflammatory proteins in systemic juvenile idiopathic arthritis patients on canakinumab therapy. Arthritis Res Ther 2017;19:1–10.
40. Hügle B, Schippers A, Fischer N, et al. Transcription factor motif enrichment in whole transcriptome analysis identifies Stat4 and BCL6 as the most prominent binding motif in systemic juvenile idiopathic arthritis. Arthritis Res Ther 2018;20.
41. Lotito APN, Campa A, Silva CAA, et al. Interleukin 18 as a marker of disease activity and severity in patients with juvenile idiopathic arthritis. J Rheumatol 2007;34:823–30.
42. Jelusić M, Lukić IK, Tambić-Bukovac L, et al. Interleukin-18 as a mediator of systemic juvenile idiopathic arthritis. Clin Rheumatol 2007;26:1332–4.
43. Shimizu M, Nakagishi Y, Yachie A. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine 2013;61:345–8.
44. Gaines Das RE, Poole S. The international standard for interleukin-6. J Immunol Methods 1993;160:147–53.
45. Ledur A, Fitting C, David B, et al. Variable estimates of cytokine levels produced by commercial ELISA kits: results using international cytokine standards. J Immunol Methods 1995;186:171–9.
46. Hunter PJ, Wedderburn LR. Pediatric rheumatic disease: can molecular profiling predict the future in JIA? Nat Rev Rheumatol 2009;5:593–4.
47. Hinze CH, Holzinger D, Lainka E, et al. Practice and consensus-based strategies in diagnosing and managing systemic juvenile idiopathic arthritis in Germany. Pediatr Rheumatol 2018;16.
48. Sturgeon C, Hill R, Hortin GL, et al. Taking a new biomarker into routine use—a perspective from the routine clinical biochemistry laboratory. Prot. Clin. Appl. 2010;4:892–903.
49. Gillio-Meina C, Cepinskas G, Cecchini EL, et al. Translational research in pediatrics II: blood collection, processing, shipping, and storage. Pediatrics 2013;131:754–66.

4

572961-L-bw-PS4U
Processed on: 25-1-2022

Chapter 5

Secretory activity of neutrophils correlates with genotype in Familial Mediterranean Fever

Faekah Gohar, Banu Orak, Tilmann Kallinich, Marion Jeske, Mareike Lieber, Horst von Bernuth, Arnd Giese, Elisabeth Weissbarth-Riedel, Johannes-Peter Haas, Frank Dressler, Dirk Holzinger, Peter Lohse, Ulrich Neudorf, Elke Lainka, Claas Hinze, Katja Masjosthusmann, Christoph Kessel, Toni Weinhage, Dirk Foell, Helmut Wittkowski.

Arthritis Rheumatol. 2016 Dec;68(12):3010-3022

Abstract

Objectives
Familial Mediterranean Fever (FMF) is an autoinflammatory disorder caused by pyrin-encoding MEFV gene mutations. Patients present with recurrent but self-limiting episodes of acute inflammation and often have persistent subclinical inflammation. The pathophysiology is only partially understood, but neutrophil overactivation is a hallmark of the disease. S100A12 is a neutrophil-derived pro-inflammatory danger signal that is strongly elevated in active FMF. We characterised neutrophilic secretory activity in vitro and investigated the association of S100A12 with disease activity and genotype in FMF patients.

Methods
Neutrophils from one compound heterozygous and five homozygous p.M694V mutation carriers and four healthy controls (HC) were purified and stimulated in vitro. Secretion of S100A12, IL-18, IL-1beta and caspase-1 was determined. Based on these in vitro analyses, serum concentrations of S100A12, IL-18 and IL-1beta were also analysed in 128 clinically and genetically characterized FMF patients.

Results
In vitro, unstimulated neutrophils from p.M694V-positive patients spontaneously secreted more S100A12, IL-18 and caspase-1 compared to HC. S100A12 serum concentrations correlated with disease activity and genotype, being highest in homozygotes > compound heterozygotes > heterozygotes. Heterozygous, compound heterozygous or homozygous p.M694V-positive patients had higher serum levels of S100A12 and IL-18 during inactive and subclinical disease than p.M694V-negative individuals.

Conclusion
FMF phenotype is known to be more severe in patients carrying the p.M694V mutation. We describe two molecules secreted by unconventional secretory pathways, S100A12 and IL-18, which correlate with clinical disease activity and FMF genotype. In this clinically and genetically heterogeneous disease, these surrogate markers might help to improve patient care and outcome.

Introduction

Familial Mediterranean Fever (FMF), the most frequently diagnosed inherited auto-inflammatory disease, is caused by pyrin-encoding MEFV (Familial Mediterranean Fever gene) gene mutations. Recurrent self-limiting acute flares of inflammatory disease involving abdominal, chest or joint pain and fever are characteristic.(1) Wide clinical and genetic heterogeneity exists within FMF.(2,3) The most frequently detected mutation is p.Met694Val (p.M694V) and p.M694V homozygotes are regarded to have a more severe clinical phenotype compared with other genotypes.(4–6) Homozygous p.M694V patients present with more frequent joint and skin involvement, higher acute phase reactants during clinically inactive disease, a higher rate of secondary amyloidosis and higher colchicine dose requirement.(3,5,7)

Currently, 297 sequence variants (ref: Infevers online database: http://fmf.igh.cnrs.fr/ISSAID/infevers) of the MEFV gene are recognized.(8) Many of these disease-causing mutations are missense, vary in penetrance, differ in prevalence and carrier frequencies between populations and may involve gene regions less commonly associated with FMF, complicating its diagnosis.(9–11) A spectrum of disease activity is recognised ranging from clinically and biologically active disease to clinically and biologically inactive disease. Subclinical disease, meaning clinically inactive disease with ongoing biological disease activity, is currently best detected, though not sensitively, by elevated conventional inflammatory markers (c-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and serum amyloid-A (SAA)).(10,12,13) Persisting subclinical disease probably increases the likelihood of long-term complications such as amyloidosis and therefore it's detection could improve management and long-term outcomes for patients with FMF.(14)

S100A12 is a pro-inflammatory damage associated molecular pattern (DAMP) molecule and an endogenous neutrophil-derived TLR4 ligand. S100A12 serum concentrations are raised during active FMF to levels significantly higher than in other fever syndromes and decrease with colchicine treatment.(15) Consequently, S100A12 is suggested to have a greater sensitivity and specificity for identifying FMF-related inflammation than conventional inflammatory markers.(16,17) Additionally, elevated S100A12 is found in asymptomatic carriers of two MEFV mutations when conventional markers are within normal ranges, suggesting S100A12 as a marker for subclinical disease. Homozygous p.M694V mutation has also been associated with higher S100A12 concentrations compared to other mutations.(16) Inter-

leukin-18 (IL-18), a pro-inflammatory IL-1 family is another postulated FMF biomarker. Serum IL-18 concentrations can discriminate patients with FMF and healthy controls (HC). However, IL-18 has not been evaluated for its potential to discriminate genotype, or disease activity and genotype in FMF.(18) Here, we characterize neutrophilic secretory activity in FMF patients in vitro and correlate S100A12 and IL-18 concentrations with FMF disease activity and genotype.

Patients and Methods

In vitro analyses

Purified neutrophils from six patients fulfilling Tel Hashomer FMF diagnostic criteria and four HC were tested in vitro.(1) At inclusion, patients had clinically inactive FMF, without signs of infection or flare as evaluated using a standardized questionnaire and were receiving recommended doses of colchicine therapy.(19) HC were also healthy, without infection, fever or medication use. Patient and HC characteristics are shown in Supplementary Table 1.

Cells were isolated from whole blood within 30-60 min of collection in both patient and control samples, using Percoll (GE Healthcare, Freiburg, Germany) two-density centrifugation as previously described. (20) Isolated neutrophils were >95% pure (measured using Sysmex (Norderstedt, Germany) cell counting flow cytometry) and viability was 97-99% (measured using Trypan Blue). Neutrophils ($5x10^6$cells/mL) were untreated or stimulated for five hours with PMA (10 nM, Sigma Aldrich, USA) or LPS (10 ng/mL, LPS-RS Ultrapure, InvivoGen, San Diego, USA) with or without colchicine (5ug/mL, Sigma, Munich, Germany) added at time zero and ATP (Adenosine 5'-triphosphate disodium salt, Sigma) added at 3.5h (Figure 1). Cell supernatants were analysed by double sandwich enzyme-linked immune-absorbent assays (ELISA) following standard protocols for S100A12 (in–house assay, described below), IL-18 (Human IL-18 ELISA kit, MBL, concentration range in healthy controls: 36.1 – 257.8 pg/mL according to the manufacturer), IL-1beta (OptEIA Human IL-1ß, BD, Heidelburg, Germany) and caspase-1 (Human Caspase-1/ICE Immunoassay, R&D, Wiesbaden-Nordenstadt, Germany). Lactate dehydrogenase (LDH) activity was measured in cell supernatants and compared to the kit-supplied positive control according to the manufacturer's instructions (Pierce LDH Cytotoxicity Assay Kit, Thermoscientific, Rockford, USA).

Patient cohort: inclusion and categorisation

Patients were retrospectively identified from the German Auto-Inflammatory Disease Network (AID-Net) online registry in May 2014. The registry maintains a pseudonymous record including clinical and laboratory details for patients with autoinflammatory disorders (available online: http://www.aid-register.de).(21) A total of 481 patients with FMF have been included in the AID-Net registry since its inception in 2009. Patients with a documented genotype, zygosity and an FMF diagnosis according to the Tel Hashomer criteria treated with colchicine were identified.(1) For inclusion, at least one clinical visit was required where both S100A12 and CRP were measured and clinical disease activity was documented allowing categorisation as inactive, subclinical or active disease. Patients fulfilling the strict inclusion criteria were included from the following AID-Net centres: Berlin, Essen, Münster, Hamburg, Garmisch-Partenkirchen, Hannover, Herne, Lüneburg, Landshut and Aachen.

Patients with active disease had FMF specific clinical activity, regardless of biomarker or inflammatory marker levels. Inactive disease patients were clinically inactive or asymptomatic along with normal range biochemical markers (CRP < 5mg/L +/- ESR, and if ESR measured, must be < 20 mm/h). Patient visits with clinically inactive disease (without signs of acute inflammatory attack) but elevated inflammatory markers (CRP ≥ 5mg/L +/- any ESR, or CRP < 5mg/L and ESR ≥ 20mm/h) were categorised as subclinical disease. Therefore, separate visits by an individual patient could be grouped into different disease activity categories.

Genotype was grouped as follows: heterozygous (heterozygous or complex heterozygous), compound heterozygous (compound heterozygous or complex-compound heterozygous) or homozygous (Table 1). Ethnicity and mutation of each patient in each disease activity group is shown in Supplementary Table 2. Additional analyses were performed according to the presence or absence of p.M694V.

The Mor and Pras modified scores (Table 1), calculated for the first year of diagnosis, are based on attack frequency, presence of erysipelas and amyloidosis, the sites affected during attacks and attack duration. (22,23) The Physician Global Assessment score (PGA), scored from 0-10 (0=no clinical activity, 10=highly active disease), recorded at the time of the visit, reflects current clinical disease activity. These scores were analysed where possible to further confirm the categorisation of patients.

Patient cohort: measurement inflammatory markers and cytokines
S100A12 in supernatants and serum were analysed using an in-house ELISA assay (normal level <150 ng/mL) as previously reported. (24) All serum S100A12 measurements were performed blinded, in a single centre (Muenster), in patient samples tracked through the AID-Net biobank. Serum CRP, ESR and SAA were prospectively measured from blood samples locally where patients attended visits. The upper limit for SAA concentration in HC was taken as < 20 mg/L, as reported in the literature.(25) Where multiple episodes for 128 study patients (n=288) fulfilled inclusion criteria within a single disease activity category, mean S100A12, CRP, ESR and SAA were analysed per patient (Table 1). Serum cytokines (IL-18 and IL-1beta) were retrospectively measured in the same patient cohort using a bead based multiplex assay (ProcartaPlex, eBioscience, San Diego, CA, USA). In contrast to the routinely measured markers and S100A12 (routinely measured in the AID-Net), cytokines were measured in one sample from each patient in each disease activity group (Table 1).

Statistical analysis
Results are presented as median (inter-quartile range, IQR) unless specified otherwise. In Figure 1, error bars indicate the standard error of the mean, and in Figures 2-5 showing scatter plots, horizontal bars indicate the median. Statistical significance is represented as: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***) or $p<0.0001$ (****). Analysis of in vitro data was performed with the Wilcoxon matched pairs test (for within patient or within HC differences) or the Mann-Whitney U test (patients versus HC). Differences in serum inflammatory markers or cytokines by disease activity or genotype were tested with the Mann Whitney U test after confirming data was non-parametric.

Ethical approval
The Ethical Commission of the Medical Faculty of the University Duisburg-Essen and Westphalian-Wilhelms University Muenster (Ref: 2009-031-f-S), the North Rhine Medical association and the University Medicine Charite, Berlin (Ref: EA2/033/09), approved the study. Written informed consent was obtained from patients on registration in AID-Net and prior to inclusion for HC. Further information regarding data safety and pseudonymisation was previously published.(21)

Table 1: Patient cohort characteristics by disease activity

	Total sample	Inactive disease	Subclinical disease	Active disease
Patients, n (% of total sample)	128 (100)*	92 (72)	57 (45)	21 (16)
Gender: male/female	69/57	52/39	28/28	12/9
Age at diagnosis, mean (range) years	6.7 (0.3-39)	6.9 (0.3-39)	6.2 (0.3-38.8)	5.5 (1.1-12.3)
Age at inclusion, mean (range), years	12 (2-47)	11 (2-37)	12 (2-47)	12 (2-19)
Ethnicity, n				
Turkish	86	61	38	12
German	1	1	0	0
Other	41	30	19	9
Colchicine dose, mean (range) mg/day	1.3 (0.5-2,5)	1.2 (0.5-2.0)	1.3 (0.5-2.0)	1.6 (0.5-2.5)
Disease Activity Severity Score, mean (n)				
Modified Mor score#	2.2 (68)	2.4 (51)	2.2 (37)	2.1 (5)
Modified Pras score#	1.7 (61)	1.8 (46)	1.7 (31)	1.7 (4)
PhGA score##	1.8 (47)	0.9 (28)	2.1 (15)	4.2 (9)
Genotype, n/total				
p.M694V-positive	91	59	45	19
Heterozygous	18	17	5	2
Compound Heterozygous	52	44	20	7
Homozygous	58	31	32	12
Serum markers of inflammation, median (IQR) unless indicated				
S100A12, ng/mL	245 (775)	193 (317)	420 (1980)	2290 (6454)
Patients measured in (n)	128	92	57	21
Visits measured in (n)	288	166	96	26
Visits per patient, mean (range)	2.3 (1-7)	1.8 (1-6)	1.7 (1-7)	1.2 (1-3)
CRP, mg/l	1.4 (10.3)	0.3 (1.1)	10.6 (16.5)	12.0 (26.5)
Patients measured in (n)	128	92	57	21
Visits measured in (n)	288	166	96	26
SAA, mg/l	7.5 (15.5)	3.9 (5.5)	24 (135)	14 (210)
Patients measured in (n)	88	56	37	14
Visits measured in (n)	166	90	59	17
ESR, mm/hr	13 (15)	8 (7)	23 (19)	22 (38)
Patients measured in (n)	121	81	54	21
Visits measured in (n)	257	141	90	26
Serum cytokines**				
Patients measured in (n)	122/128*	86/92	53/57	18/21
IL-1beta, median (IQR) pg/mL	0 (0)	0 (4.4)	0 (4.7)	0 (1.1)
IL-18, median (IQR) pg/mL	264 (637)	221 (416)	353 (801)	606 (2094)

**Patients may be included in more than one disease activity category. Individual visits are only included in one disease activity group. **Serum cytokines were measured in patients in each disease activity group in one sample from one visit each.*

#***Modified Mor and Pras score:*** mild disease = 1, moderate = 2, severe = 3.

##***PhGA:*** Physician Global Assessment score (scored 0-10 on a visual analogue scale).

Results

FMF-neutrophils secrete S100A12, IL-18 and caspase-1 in vitro

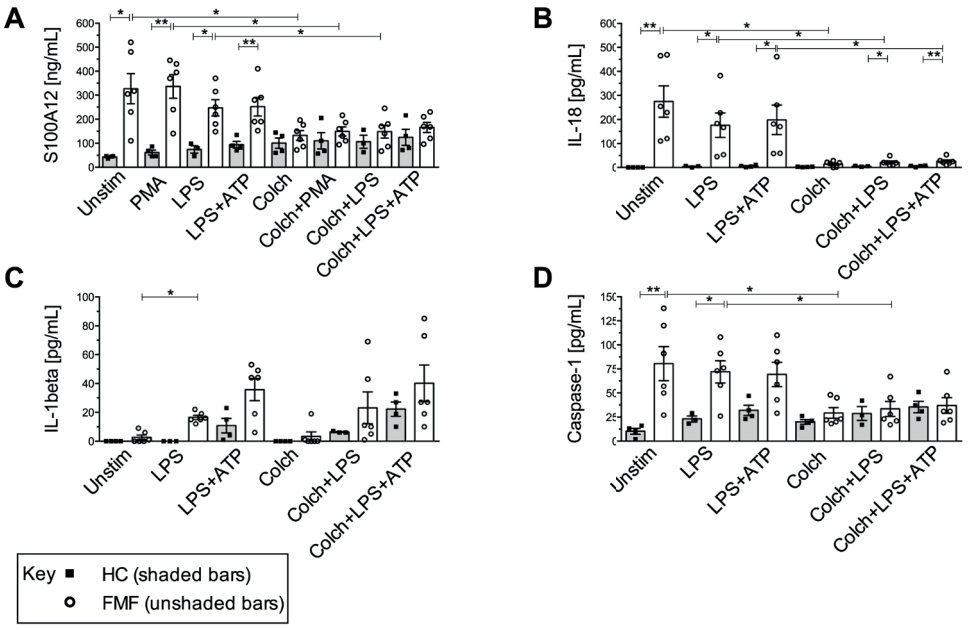
Peripheral serum levels of S100A12 are massively elevated in FMF patients with active disease, as we showed previously.(16) We investigated the source of secreted S100A12 by stimulating neutrophils of patients and HC ex vivo. Patient neutrophils had a seven times higher baseline secretion of S100A12 (median: 311, IQR: 290 ng/mL) compared to HC (45, 8 ng/mL), which did not further increase with PMA or LPS stimulation. Secretion was blocked by colchicine, an inhibitor of microtubule polymerization, restoring secreted S100A12 levels to that of HC (Figure 1). The same pattern of secretion was found for IL-18, also secreted by unconventional secretory pathways. In contrast, secretion of IL-1beta was only marginally elevated in patient neutrophils without stimulation, could not be blocked by colchicine, but increased significantly with LPS stimulation. A further increase in IL-1beta secretion was observed with LPS and ATP addition, as previously shown in monocytes, which was not seen for S100A12 or IL-18. (26–28) Interestingly, the pattern of caspase-1 secretion was identical to that of IL-18 and S100A12, but not to that of IL-1beta. LDH activity was measured in patients (n=5/6) and HC (n=3/4) supernatants of stimulated and unstimulated neutrophils. The mean (range) percentage LDH activity in stimulated neutrophils from FMF patients and HC were normalized to the activity in unstimulated HC neutrophils. In FMF: LPS-stimulated neutrophils 92% (52-114%), PMA-stimulated neutrophils 95% (54-162%). In HC: LPS-stimulated 112% (105-121%), PMA-stimulated 110%, (93-116%). The LDH positive control was 163%. In summary, LDH activity was not increased after stimulation of cells.

Disease severity in the patient cohort

Mor and Pras disease activity scores were available for n=68 and n=61 patients from the Berlin and Essen centres respectively and each indicated no differences in baseline disease severity between inactive, subclinical or active disease (Table 1). The mean PGA, available for a proportion of patients, was higher in active > subclinical > inactive disease. Mor and Pras results therefore suggested all patients, regardless of disease activity group, did not significantly differ in baseline severity, while PGA suggested correct acute disease activity categorisation (Table 1).

Figure 1: In vitro neutrophil secretion of S100A12, IL-1beta, IL-18 and caspase-1

Secretion of A: S100A12, B: IL-18, C: IL-1beta and D: caspase-1 by neutrophils after stimulation with PMA (10nM) and LPS (10ng/ml), with or without ATP (1mM), colchicine ("colch", 5 µg/ml), or left unstimulated ('unstim'). Independent measurements in six individual patients (□) and four HC donors (□) are shown. All patients were receiving recommended doses of colchicine. Within patient or HC differences were analysed using the Wilcoxon test. The Mann-Whitney U test was used to compare patients and HC. Only significant differences are shown (*P < 0.05; **P < 0.01). The error bars indicate the standard error of the mean.



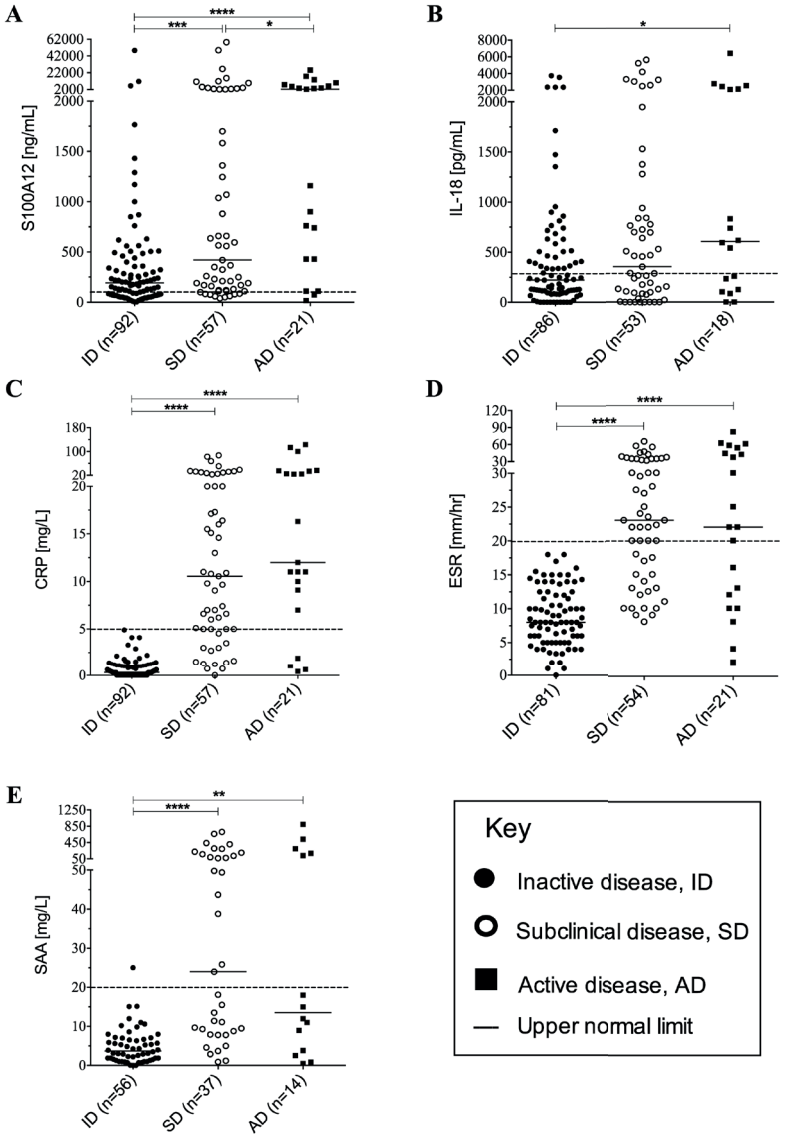
572961-L-bw-PS4U
Processed on: 25-1-2022

S100A12 serum levels correlate with disease activity

To determine their potential as biomarkers in FMF, we investigated S100A12 and IL-18 serum levels in 128 patients (Table 1). As anticipated from our previous studies, median S100A12 concentration increased with disease activity. Inactive disease: median 193 (IQR: 317) ng/mL, subclinical disease 420 (1980) ng/mL and active disease 2290 (6454) ng/mL. The difference between inactive and subclinical disease as well as between subclinical and active disease, S100A12 concentration was statistically significant ($p<0.0001$ and $p=0.049$, respectively), as was that of inactive disease and active disease ($p<0.00001$) as shown in Figure 2. (16) IL-18 levels differed significantly between inactive disease and active disease ($p=0.026$, Figure 2) while IL-1beta was unmeasurable (Table 1).

Figure 2: Inflammatory markers analysed by disease activity

Serum concentrations of A: S100A12, B: IL-18, C: CRP, D: ESR, and E: SAA during inactive (ID), subclinical (SD) and active disease (AD). The y-axes differ in range from A-E. The Mann-Whitney-U test was used to test the difference between two groups (ID vs SD or AD, and SD vs AD). Only significant differences are shown, **P < 0.01; ***P < 0.001; ****P ≤ 0.0001. The horizontal bar shows the median. The upper cut-off for each inflammatory marker (S100A12: 150 ng/mL, IL-18: 260 pg/mL, CRP: 5 mg/L, ESR: 20 mm/h and SAA: 20 mg/L) is indicated by the dotted line (---).



Serum S100A12 indicates a gene dosage effect

Analysis of genotype by disease activity revealed significantly increasing serum concentrations of S100A12 with increasing MEFV gene dosage as follows: heterozygotes < compound heterozygotes < homozygotes, in both inactive disease and subclinical disease. This was partly mirrored by IL-18 in subclinical disease, but not by conventional inflammatory markers (CRP, ESR or SAA). In active disease, in contrast, no gene dosage effect was seen for IL-18, CRP or ESR (Figure 3). Effect of genotype in inactive disease was not analysed for CRP, ESR or SAA, as these were within normal levels for all those with inactive disease by definition and SAA measurements were not always available at the time of S100A12 levels (Table 1). Too few heterozygous patients had active disease for statistical analysis (n=2). All patients were on colchicine treatment and in the subclinical disease group, 56/57 patients were confirmed to be on optimal mg/kg treatment doses by standard age-determined criteria.(19)

S100A12 analysed by p.M694V phenotype indicates disease severity

Homozygous p.M694V patients had higher serum S100A12 concentrations than other genotypes in our previous study.(16) Therefore, here we analysed serum S100A12 in a larger cohort of patients with inactive and subclinical disease according to the presence or absence of M694V mutations. Higher median serum S100A12 in p.M694V-positive versus p.M694V-negative patients was seen in both, inactive disease and subclinical disease, which was less pronounced for IL-18 (Figure 4 and 5). This is consistent with hypersecretion of these molecules in vitro in the supernatants of unstimulated p.M694V-positive patients. The specificity and sensitivity of S100A12 (cut-off: 153 ng/mL) for discriminating p.M694V-positive and negative patients was 76% and 62% respectively and for IL-18 (cut-off: 63 pg/mL) was 85% (specificity) and 23% (sensitivity). CRP was not analysed, as by definition all concentrations were < 5mg/l in the inactive disease group.

Figure 3: Inflammatory markers analysed by disease activity and genotype

Influence of genotype on serum concentrations of A: S100A12 and B: IL-18 in inactive (ID), subclinical (SD) and active disease (AD), and C: CRP, D: ESR and E: SAA in SD and AD are shown. The y-axes differ in range from A-E. The Mann-Whitney-U test was used to test the difference between two groups (*P < 0.05; **P < 0.01). (Abbreviations: het: heterozygous. comp het: compound heterozygous, hom: homozygous). The upper cut-off for each inflammatory marker (S100A12: 150 ng/mL, IL-18: 260 pg/mL, CRP: 5 mg/L, ESR: 20 mm/h and SAA: 20 mg/L) is indicated by the dotted line (---) and the horizontal bar shows the median.

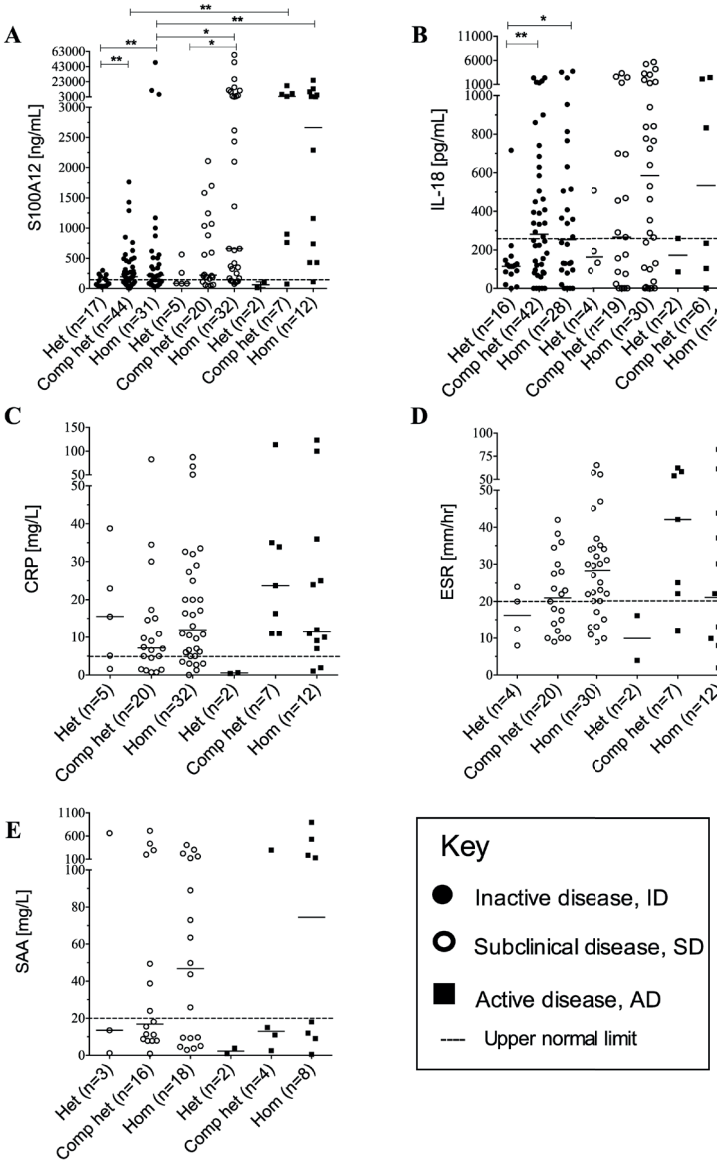


Figure 4: Inflammatory markers analysed by genotype and p.M694V presence or absence (in inactive disease)

p.M694V-positive (shaded circle) or negative patients (unshaded circle) were analysed for A: S100A12, B: IL-18, C: CRP, D: ESR and E: SAA in inactive disease (ID) for patients with heterozygous, compound heterozygous or homozygous genotype. The Mann-Whitney-U test was used to test the difference between two groups (*P < 0.05; **P < 0.01). The upper cut-off for each inflammatory marker (S100A12: 150 ng/mL, IL-18: 260 pg/mL, CRP: 5 mg/L, ESR: 20 mm/h and SAA: 20 mg/L) is indicated by the dotted line and the horizontal bar shows the median. (Abbreviations: het: heterozygous, comp het: compound heterozygous, hom: homozygous).

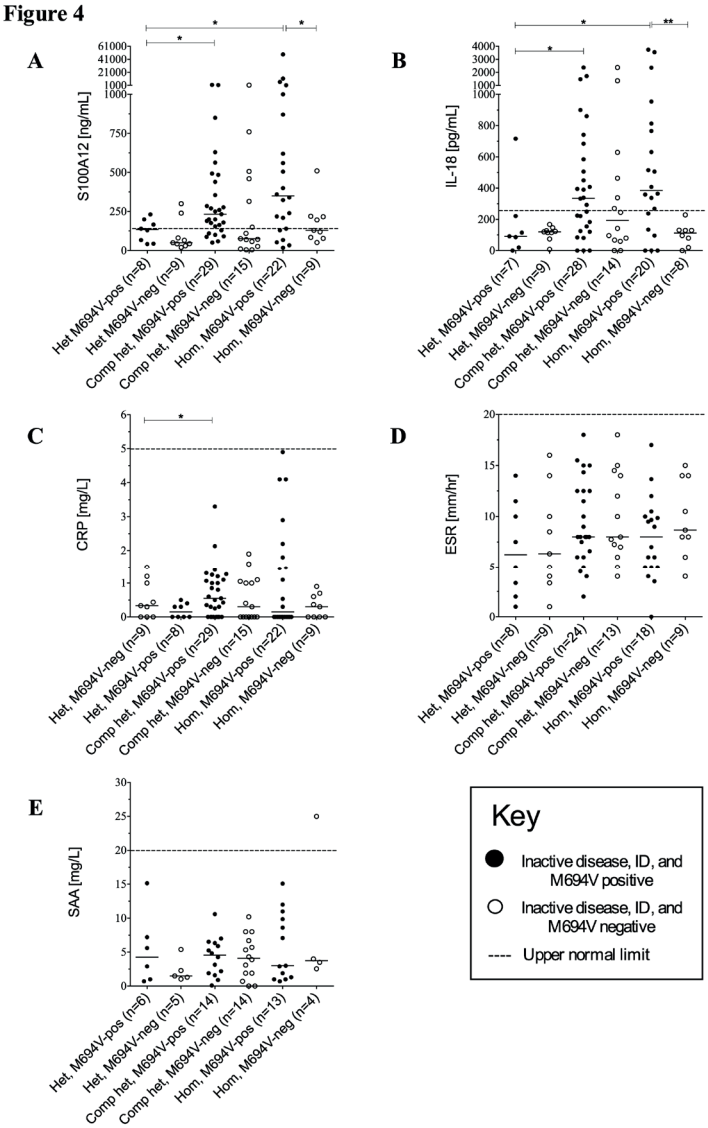
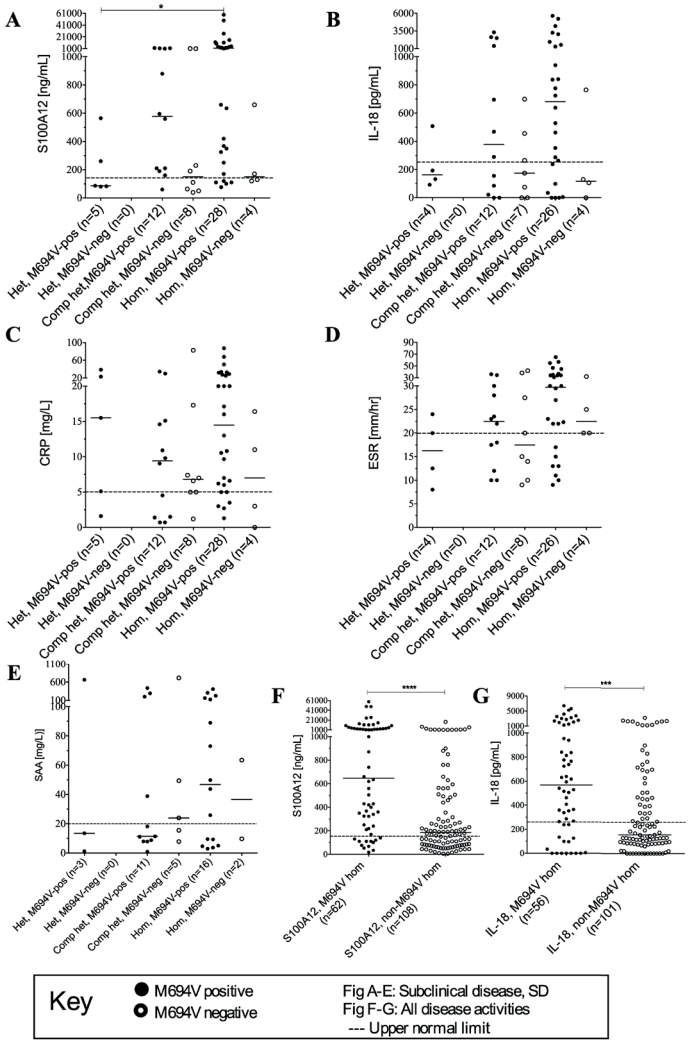


Figure 5: Inflammatory markers analysed by genotype and p.M694V presence or absence (in subclinical disease and all disease activities)
p.M694V-positive (shaded) or negative patients (unshaded) were analysed for A: S100A12, B: IL-18, C: CRP, D: ESR, and E: SAA in subclinical disease (SD) for patients with heterozygous, compound heterozygous or homozygous genotype. F: The S100A12 and G: IL-18 concentrations of patients with M694V homozygous genotype (shaded circle) were compared to those with any other genotype (unshaded circle), regardless of disease activity. The y-axes differ in range from A-E. The Mann-Whitney-U test was used to test the difference between two groups (*P < 0.05; ***P < 0.001). The upper cut-off for each inflammatory marker (S100A12: 150 ng/mL, IL-18: 260 pg/mL, CRP: 5 mg/L, ESR: 20 mm/h and SAA: 20 mg/L) is indicated by the dotted line (---) and the horizontal bar shows the median. (Abbreviations: het: heterozygous, comp het: compound heterozygous, hom: homozygous).



Discussion

Wide genetic and clinical variability is seen in FMF, leading to a need for prognostic (e.g indicates likely long-term risks such as development of amyloidosis) and predictive (e.g. who will respond better to a treatment) biomarkers.(29,30) Biomarkers are particularly required for the detection of subclinical inflammation, which is difficult to detect with conventional inflammatory markers. We characterised neutrophilic secretory activity in FMF patient neutrophils and analysed inflammatory markers and cytokines by disease activity and genotype in patient serum to investigate their potential as biomarkers.

Patient neutrophils secreted IL-1beta and showed increased caspase-1 activity in vitro. This finding is consistent with previously described elevated IL-1beta secretion in monocytes and in an FMF-mouse model that identified IL-1beta as the main cytokine driving FMF pathology.(26,27) In addition, we found highly elevated spontaneous secretion of S100A12 and IL-18, not further increased with cell stimulation, unlike for IL-1beta. This finding at the protein level has not been previously shown though elevated baseline mRNA expression of IL-1beta and caspase-1 in neutrophils and IL-1beta secretion from monocytes in inactive FMF patients versus HC has been reported.(31,32)

Schmidt et al. showed that inflammasome processing of IL-1beta or IL-18 had different requirements resulting in different patterns of secretion in their microbial stimulation of NLRP3, in line with findings from Brydges et al. mutant NLRP3 knock-in mouse model of CAPS (cryopyrin-associated periodic fever syndromes).(33,34) These studies required inflammasome stimulation before IL-18 secretion was seen, differing from the spontaneous secretory phenotype we observed. Whilst secretion of S100A12 and Il-18 in vitro was inhibited by colchicine, secretion of IL-1beta was not.

Concentrations of the circulating cleaved form of IL-1β in FMF patients has been shown to be reduced by colchicine therapy.(35) Response to colchicine is also associated with decrease in serum S100A12 levels and aids the differentiation of a diagnosis of FMF from other periodic fever syndromes.(16,36) Postulated mechanisms for this anti-inflammatory activity includes interference with pyrin, suppression of caspase-1 activity and microtubule inhibition resulting in blocking of unconventional secretion.(37–40) Most FMF patients achieve colchicine response, but the rate of unresponsiveness and

continued occurrence of flares is higher in p.M694V-homozygous patients. (7,41) Interestingly, colchicine resistant patients, 5-10% of all FMF patients, have responded well to IL-1 (anakinra or canakinumab) antagonist therapy. (42–45)

The leaderless protein S100A12 is a TLR4-ligand which amplifies monocyte activation.(46) The exact mechanism of S100A12 release is not yet established. It is suspected that S100A12, like IL-18, is stored in substantial amounts in the cytoplasm and released upon stimulation via an alternative secretory pathway.(15) The demonstrated spontaneous secretion of S100A12, IL-18 and caspase-1 by patient neutrophils without stimulation, together with the very high concentrations of S100A12 and IL-18 in patient serum, supports the hypothesis of hyper-reactive FMF neutrophils. Elevated serum IL-18 but not S100A12 is described in CAPS.(47,48) In contrast, in one patient with de novo NLRC4 mutation, elevated levels of S100 protein genes as well as IL-18 was found.(49) Similarly high serum S100A12, as found in FMF, is also found in systemic juvenile arthritis (SJIA), also a disorder with known neutrophil dysregulation, though the two disorders can eventually be differentiated by clinical signs.(17,50) Vastert et al. showed high serum levels of S100A12 and IL-18 before initiating recombinant IL-1Ra therapy in patients with SJIA which normalised rapidly in treatment responders.(51) IL-1-blocking regimes are effective in both FMF and SJIA.(42,44,52) Therefore, it is feasible that the demonstrated dysregulated neutrophil activation in FMF can result in positive secretory feedback loops with S100A12, IL-1beta and IL-18, in turn leading to the elevated serum concentrations of S100A12 found even in quiescent disease. Increased IL-1beta release upon stimulation on top of these positive-feedback secretory loops could explain the periodic phenotype of FMF.

Next, we applied the in vitro results to the clinical setting. Serum inflammatory markers of patients with FMF enrolled in the AID-Net registry were analysed by disease activity and genotype. Conventional inflammatory markers, serum S100A12 and IL-18 all correlated well with disease activity. Very few studies have investigated serum IL-18 in FMF, which demonstrated higher IL-18 in FMF disease compared to HC.(18,53–55) However, of these, only Haznedaroglu et al. and Simsek et al. compare IL-18 and disease activity in much smaller cohorts, finding no difference between inactive disease and active disease.(18,55) Yamakazi et al. were the only identified study to ana-

lyse serum IL-18 by genotype, finding higher concentrations in typical versus atypical genotype. However, this study only compared patients carrying the p.M694I mutation with those harbouring the low-penetrance mutation p.P369S/R408Q.(54)

Interestingly, S100A12 and Il-18 concentrations were very similar in their pattern of secretion by disease activity, genotype and p.M694V-positive genotype, with the gene dosage association greater for S100A12 than for IL-18. We unexpectedly found that S100A12 and IL-18 serum levels better correlated with gene dosage during quiescent disease (inactive disease and subclinical disease) than during active disease, which may reflect the inherent hypersecretion of these markers as seen in vitro.

S100A12 and IL-18 results for identifying p.M694V-positive patients in inactive disease or subclinical disease suggests these markers might represent screening tools for ongoing disease activity, though first require evaluation in longer-term prospective studies. Our findings that S100A12 and IL-18 both are more significantly elevated than CRP during subclinical disease, specifically in p.M694V-positive patients, makes them very promising markers for the clinical monitoring of these patients, in whom worse outcomes are specifically recognised. Additionally, persistent subclinical inflammation can result in the most significant complication seen in FMF, renal amyloidosis, which might be preventable by monitoring and treating subclinical inflammation.(10,16,56) However, a number of other factors associated with greater risk of developing amyloidosis should also be considered in individual patients, including family history of amyloidosis, male gender, SAA alpha/alpha genotype and the country of residence.(56–58)

Whilst our genotype-biomarker association findings are focused on the p.M694V status of patients as this was the most frequent mutation in our cohort, the use of S100A12 and IL-18 in other FMF exon 10 founder mutation combinations (M694I, M680I, V726A) should be evaluated in future studies, because these mutations are also associated with worse disease severity and SAA amyloidosis.(10) If patients with elevated S100A12 and IL-18 levels during inactive disease, despite normal inflammatory markers, require more aggressive therapeutic management, for example, with increased colchicine dose or anakinra, is presently unclear.(7, 45) Further prospective studies are required to determine whether these more aggressive treatment strategies in

this specific group of patients would improve long term disease outcomes, including reduced risk of amyloidosis.
In summary, we show S100A12 is a sensitive biomarker for active and inactive FMF as well subclinical disease. We are the first to report both increased serum S100A12 and IL-18 concentrations in FMF patients by disease activity and also by genotype. Both in vitro and clinical data show S100A12 and IL-18 hypersecretion in p.M694V-positive FMF patients. Therefore S100A12 and IL-18 measurement can identify patients at increased risk for long-term complications, to whom treatment could be targeted. Currently, clinical decisions should continue to include the patient's genetic background and long-term observations are required before determining whether successful treatment of FMF could be defined as achieving normalised S100A12 and/or IL-18 serum concentrations.

Acknowledgements
We thank everyone involved with the AID-Net network. We also thank Dr Nils Onken, Dr Rainer Berendes and Dr Klaus Tenbrock, who also contributed patients for inclusion. We are grateful to Volker Hilger and Nicole Weyandt for database assistance, Antje Hellige for clinical data assistance and Jerzy-Roch Nofer for assistance with LDH measurement. Finally, we especially thank Melanie Saers and Susanne Schleifenbaum for excellent technical assistance in performing the in vitro experiments and all the S100A12 assays for the AID-Net.

Supplementary Table 1: In vitro analysis sample characteristics

	Healthy controls	**FMF patients**
Total subjects	4	6
Age at inclusion, mean (range) years	33 (25-42)	20 (16-28)
Gender: male	2	3
Ethnicity, n	German (n=4)	Turkish (n=6)
Mutation present (n)	Nil known	M694V/M694V (n=5) M694V/E230K (n=1)
Colchicine dose, n, mean (range) mg/day	n/a	1.2 (0.5-2.0)
Any indication of currently active inflammatory disease or infection (indicated by symptoms/questionnaire answers or clinical examination which included abdominal pain, chest pain, joint or skin inflammation) at the time of blood sample?	No, for all subjects	No, for all subjects
Acute FMF episodes per yr, mean (range)	n/a	5 (1-13)
Disease activity scores		
Modified Mor score[#]	n/a	*Severe (n=6/6)*
Modified Pras score[##]	n/a	*Mild (n=3/6)* *Moderate (n=3/6)*
Serum inflammatory markers		
S100A12, mean (range) ng/ml	10 (0-15)	1664 (13-9890)
CRP, mean (range) mg/L	0.7 (0.2-1.7)	6.4 (0.7-8.8)
The Mor and Pras disease severity scores correlate with severity groups as follows: [#]***Mor score:*** mild disease < 1, intermediate = 2, severe > 3. [##]***Pras score:*** mild disease 3-5, moderate 6-9, severe > 9.		

Supplementary Table 2: Patient ethnicity and mutation by disease activity

	Total sample	**Inactive disease**	**Subclinical disease**	**Active disease**
Patients, n (%)	128 (100)*	92 (72)	57 (45)	21 (16)
Ethnicity, n (100%)				
Turkish	86 (67)	61 (66)	38 (67)	12 (57)
Unknown	19 (15)	12 (13)	4 (7)	6 (29)
Arab: Arabian/Libyan /'generic'	15 (12)	13 (14)	10 (18)	1 (5)
Armenian	3 (2)	1 (1)	3 (5)	0
Persian	2 (2)	2 (2)	2 (4)	0
German/Caucasian	1(1)	1 (1)	0	1 (5)
Albanian	1 (1)	1 (1)	0	0
Egyptian	1 (1)	1 (1)	0	1 (5)
Mutations present, n*				
Homozygous				
M694V/M694V	48	22	28	12
M680IGC/M680IGC	5	4	1	0
R761H/R761H	2	2	2	0
E148Q/E148Q	1	1	0	0
V726A/V726A	1	1	1	0
M694I/M694I	1	1	0	0
Compound Heterozygous / Complex-compound heterozygous				
M694V/V726A	11	10	3	2
M694V/M680IGC	12	10	4	3
M694V/M680IGA	1	0	0	1
M694V/R761H	4	4	1	0
E148Q/L110P	2	2	2	0
E148Q/L110P/M694V	1	1	0	0
E148Q/L110P/P369S/R408Q/R92Q	1	1	1	0
M694V/E148Q	1	1	1	0
M694V/K695R	1	1	0	0
M694V/A744S	1	1	1	0
M694V/M680L	1	1	1	0
M694I/V726A	2	2	1	0
M694I/E148Q/E230K	1	0	1	1
M694I/A744S	1	1	0	0
M680IGC/V726A	3	3	1	0

572961-L-bw-PS4U
Processed on: 25-1-2022

M680IGC/M680IGA	1	1	0	0
M680IGC/M694I	1	1	0	0
M680I/E230K	1	0	1	0
E167D/F479L/V726A	1	1	0	0
E167D/V726A	1	0	1	0
V726A/R761H	1	1	0	0
P369S/E148Q	1	1	1	0
K695R/I591T	1	1	0	0
M694V/I641F	1	0	1	0
Heterozygous/ Complex Heterozygous				
M694V	9	8	5	1
V726A	3	3	0	0
E148Q	3	3	0	0
K695R	1	1	0	1
P369S/R408Q	1	1	0	0
S242R	1	1	0	0
**Patients may be included in more than one disease activity category. Individual visits are only included in one disease activity group.*				

References

1. Livneh A, Langevitz P, Zemer D, N Z, S K, T L. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 1997;40:1879–85.
2. Ozen S. Changing concepts in familial Mediterranean fever: is it possible to have an autosomal-recessive disease with only one mutation? Arthritis Rheum 2009;60:1575–7.
3. Marek-Yagel D, Berkun Y, Padeh S, Abu A, Reznik-Wolf H, Livneh A, et al. Clinical disease among patients heterozygous for familial Mediterranean fever. Arthritis Rheum 2009;60:1862–6.
4. Zaks N, Shinar Y, Padeh S, Lidar M, Mor A, Tokov I, et al. Analysis of the three most common MEFV mutations in 412 patients with familial Mediterranean fever. Isr Med Assoc J 2003;5:585–8.
5. Jeske M, Lohse P, Kallinich T, Berger T, Rietschel C, Holzinger D, et al. Genotype-Phenotype and Genotype-Origin Correlations in Children with Mediterranean Fever in Germany – an AID-Net Study Familial Mediterranean fever. Klin Pädiatrie 2013;225:325–330.
6. Hentgen V, Grateau G, Stankovic-Stojanovic K, Amselem S, Jéru I. Familial Mediterranean fever in heterozygotes: are we able to accurately diagnose the disease in very young children? Arthritis Rheum 2013;65:1654–62.
7. Lidar M, Yonath H, Shechter N, Sikron F, Sadetzki S, Langevitz P, et al. Incomplete response to colchicine in M694V homozygote FMF patients. Autoimmun Rev 2012;12:72–6.
8. Milhavet F, Cuisset L, Hoffman HM, Slim R, El-Shanti H, Aksentijevich I, et al. The infevers autoinflammatory mutation online registry: update with new genes and functions. Hum Mutat 2008;29:803–8.
9. Booty MG, Chae JJ, Masters SL, Remmers EF, Barham B, Le JM, et al. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? Arthritis Rheum 2009;60:1851–61.
10. Lachmann HJ, Sengül B, Yavuzşen TU, Booth DR, Booth SE, Bybee A, et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford) 2006;45:746–50.
11. Jéru I, Hentgen V, Cochet E, Duquesnoy P, Borgne G Le, Grimprel E, et al. The risk of familial Mediterranean fever in MEFV heterozygotes: a statistical approach. PLoS One 2013;8:e68431.
12. Ben-Zvi I, Livneh A. Chronic inflammation in FMF: markers, risk factors, outcomes and therapy. Nat Rev Rheumatol 2011:105–12.
13. Korkmaz C, Ozdogan H, Kasapçopur O, Yazici H. Acute phase response in familial Mediterranean fever. Ann Rheum Dis 2002;61:79–81.
14. Ebrahimi-Fakhari D, Schönland SO, Hegenbart U, Lohse P, Beimler J, Wahlster L, et al. Familial Mediterranean fever in Germany: clinical presentation and amyloidosis risk. Scand J Rheumatol 2013;42:52–8.
15. Foell D, Wittkowski H, Roth J. Mechanisms of disease: a "DAMP" view of inflammatory arthritis. Nat Clin Pract Rheumatol 2007;3:382–90.
16. Kallinich T, Wittkowski H, Keitzer R, Roth J, Foell D. Neutrophil-derived S100A12 as novel biomarker of inflammation in familial Mediterranean fever. Ann Rheum Dis 2010;69:677–82.
17. Wittkowski H, Frosch M, Wulffraat N, Goldbach-Mansky R, Kallinich T, Kuemmerle-Deschner J, et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever

of unknown origin. Arthritis Rheum 2008;58:3924–31.
18. Haznedaroglu S, Oztürk MA, Sancak B, Goker B, Onat AM, Bukan N, et al. Serum interleukin 17 and interleukin 18 levels in familial Mediterranean fever. Clin Exp Rheumatol 2005;23:S77-80.
19. Kallinich T, Haffner D, Niehues T, Huss K, Lainka E, Neudorf U, et al. Colchicine use in children and adolescents with familial Mediterranean fever: literature review and consensus statement. Pediatrics 2007;119:e474-83.
20. Hjorth R, Jonsson A, Vretblad P. A Rapid Method for Purification of Human Granulocytes Using Percoll. A Comparison With Dextran Sedimentation. J Immunol Methods 1981;43:95–101.
21. Lainka E, Bielak M, Hilger V, Basu O, Neudorf U, Wittkowski H, et al. Translational research network and patient registry for auto-inflammatory diseases. Rheumatology (Oxford) 2011;50:237–42.
22. Mor A, Shinar Y, Zaks N, Langevitz P, Chetrit A, Shtrasburg S, et al. Evaluation of disease severity in familial Mediterranean fever. Semin Arthritis Rheum 2005;35:57–64.
23. Pras E, Livneh A, Balow JE, Kastner DL, Pras M, Langevitz P. Clinical differences between North African and Iraqi Jews with familial Mediterranean fever. Am J Med Genet 1998;75:216–9.
24. Gerss J, Roth J, Holzinger D, Ruperto N, Wittkowski H, Frosch M, et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. Ann Rheum Dis 2012;71:1991–7.
25. Hijmans W, Sipe JD. Levels of the serum amyloid A protein (SAA) in normal persons of different age groups. Clin Exp Immunol 1979;35:96–100.
26. Omenetti A, Carta S, Del L, Martini A, Gattorno M, Rubartelli A. Increased NLRP3-dependent interleukin 1 β secretion in patients with familial Mediterranean fever : correlation with MEFV genotype. 2014:462–469.
27. Chae JJ, Cho Y-H, Lee G-S, Cheng J, Liu PP, Feigenbaum L, et al. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. Immunity 2011;34:755–68.
28. Chae JJ, Wood G, Masters SL, Richard K, Park G, Smith BJ, et al. The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. Proc Natl Acad Sci 2006;103:9982–9987.
29. Brünner N. What is the difference between "predictive and prognostic biomarkers"? Can you give some examples. Connection 2009:2009.
30. Søreide K. Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research. J Clin Pathol 2009;62:1–5.
31. Manukyan G, Petrek M, Kriegova E, Ghazaryan K, Fillerova R, Boyajyan A. Activated phenotype of circulating neutrophils in familial Mediterranean fever. Immunobiology 2013;218:892–8.
32. Notarnicola C, Didelot MN, Seguret F, Demaille J, Touitou I, Communication B. Enhanced cytokine mRNA levels in attack-free patients with familial Mediterranean fever. Genes Immun 2002;3:43–45.
33. Schmidt RL, Lenz LL. Distinct licensing of IL-18 and IL-1β secretion in response to NLRP3 inflammasome activation. PLoS One 2012;7:e45186.
34. Brydges SD, Broderick L, McGeough MD, Pena CA, Mueller JL, Hoffman

HM. Divergence of IL-1, IL-18, and cell death in NLRP3 inflammasomopathies. J Clin Invest 2013;123:4695–705.
35. Migita K, Izumi Y, Fujikawa K, Agematsu K, Masumoto J, Jiuchi Y, et al. Dysregulated mature IL-1β production in familial Mediterranean fever. Rheumatology (Oxford) 2014:keu359-.
36. Ozen S, Hoffman HM, Frenkel J, Kastner D. Familial Mediterranean fever (FMF) and beyond: a new horizon. Fourth International Congress on the Systemic Autoinflammatory Diseases held in Bethesda, USA, 6-10 November 2005. Ann Rheum Dis 2006;65:961–4.
37. Taskiran EZ, Cetinkaya A, Balci-Peynircioglu B, Akkaya YZ, Yilmaz E. The effect of colchicine on pyrin and pyrin interacting proteins. J Cell Biochem 2012;113:3536–46.
38. Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol 2013;14:454–60.
39. O'Brien JM, Wewers MD, Moore SA, Allen JN. Taxol and colchicine increase LPS-induced pro-IL-1 beta production, but do not increase IL-1 beta secretion. A role for microtubules in the regulation of IL-1 beta production. J Immunol 1995;154:4113–22.
40. Papin S, Cuenin S, Agostini L, Martinon F, Werner S, Beer H-D, et al. The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. Cell Death Differ 2007;14:1457–1466.
41. Özçakar ZB, Elhan AH, Yalçınkaya F. Can colchicine response be predicted in familial Mediterranean fever patients? Rheumatology (Oxford) 2014;53:1767–72.
42. Soriano A, Verecchia E, Afeltra A, Landolfi R, Manna R. IL-1β Biological Treatment of Familial Mediterranean Fever. Clin Rev Allergy Immunol 2013;45:117–130.
43. Chae JJ, Aksentijevich I, Kastner DL. Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. Br J Haematol 2009;146:467–78.
44. Meinzer U, Quartier P, Alexandra J-F, Hentgen V, Retornaz F, Koné-Paut I. Interleukin-1 targeting drugs in familial Mediterranean fever: a case series and a review of the literature. Semin Arthritis Rheum 2011;41:265–71.
45. Eroglu FK, Beşbaş N, Topaloglu R, Ozen S. Treatment of colchicine-resistant Familial Mediterranean fever in children and adolescents. Rheumatol Int 2015;35:1733–1737.
46. Foell D, Wittkowski H, Kessel C, Lüken A, Weinhage T, Varga G, et al. Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4. Am J Respir Crit Care Med 2013;187:1324–34.
47. Carta S, Penco F, Lavieri R, Martini A, Dinarello CA, Gattorno M, et al. Cell stress increases ATP release in NLRP3 inflammasome-mediated autoinflammatory diseases, resulting in cytokine imbalance. Proc Natl Acad Sci U S A 2015;112:2835–40.
48. Rieber N, Gavrilov A, Hofer L, Singh A, Öz H, Endres T, et al. A functional inflammasome activation assay differentiates patients with pathogenic NLRP3 mutations and symptomatic patients with low penetrance variants. Clin Immunol 2015;157:56–64.
49. Canna SW, Jesus AA de, Gouni S, Brooks SR, Marrero B, Liu Y, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with

recurrent macrophage activation syndrome. Nat Genet 2014;46:1140–6.
50. Ruperto N, Brunner HI, Quartier P, Constantin T, Wulffraat N, Horneff G, et al. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. N Engl J Med 2012;367:2396–406.
51. Vastert SJ, Jager W de, Noordman BJ, Holzinger D, Kuis W, Prakken BJ, et al. Effectiveness of first-line treatment with recombinant interleukin-1 receptor antagonist in steroid-naive patients with new-onset systemic juvenile idiopathic arthritis: results of a prospective cohort study. Arthritis Rheumatol (Hoboken, NJ) 2014;66:1034–43.
52. Başaran Ö, Uncu N, Çelikel BA, Taktak A, Gür G, Cakar N. Interleukin-1 targeting treatment in familial Mediterranean fever: an experience of pediatric patients. Mod Rheumatol 2014:1–4.
53. Deveci K, Korkmaz S, Senel S, Deveci H, Sancakdar E, Uslu AU, et al. Do neutrophil gelatinase-associated lipocalin and interleukin-18 predict renal dysfunction in patients with familial Mediterranean fever and amyloidosis? Ren Fail 2014;36:339–344.
54. Yamazaki T, Shigemura T, Kobayashi N, Honda K, Yazaki M, Masumoto J, et al. IL-18 serum concentration is markedly elevated in typical familial Mediterranean fever with M694I mutation and can distinguish it from atypical type. 2014.
55. Simsek I, Pay S, Pekel A, Dinc A, Musabak U, Erdem H, et al. Serum proinflammatory cytokines directing T helper 1 polarization in patients with familial Mediterranean fever. Rheumatol Int 2007;27:807–811.
56. Touitou I, Sarkisian T, Medlej-Hashim M, Tunca M, Livneh A, Cattan D, et al. Country as the primary risk factor for renal amyloidosis in familial Mediterranean fever. Arthritis Rheum 2007;56:1706–12.
57. Medlej-Hashim M, Delague V, Chouery E, Salem N, Rawashdeh M, Lefranc G, et al. Amyloidosis in familial Mediterranean fever patients: correlation with MEFV genotype and SAA1 and MICA polymorphisms effects. BMC Med Genet 2004;5:4.
58. Blank N, Hegenbart U, Lohse P, Beimler J, Röcken C, Ho AD, et al. Risk factors for AA amyloidosis in Germany. Amyloid 2015.

Chapter 6

S100A12 is associated with response to therapy in juvenile idiopathic arthritis

Faekah Gohar, Janneke Anink, Halima Moncrieffe,
Lisette W A Van Suijlekom-Smit, Femke H.M. Prince,
Marion A.J. van Rossum, Koert M. Dolman,
Esther P.A.H. Hoppenreijs, Rebecca ten Cate, Simona Ursu,
Lucy R. Wedderburn, Gerd Horneff, Michael Frosch, Dirk Foell
and Dirk Holzinger

J Rheumatol. 2018;45(4):547-554

Abstract

Objectives

Around one third of patients with juvenile idiopathic arthritis (JIA) fail to respond to first line methotrexate or anti-TNF therapy, with even fewer achieving ≥ ACRpedi 70, though individual responses cannot yet be accurately predicted. As change in serum S100-protein MRP8/14 is associated with therapeutic response, we tested granulocyte-specific S100-protein S100A12 as a potential biomarker for treatment response.

Methods

S100A12 serum concentration was determined by ELISA in MTX (n=75) and anti-TNF (n=88) treated patients at baseline and follow-up. Treatment response (≥ ACRpedi 50 score), achievement of inactive disease (ID) and improvement in JADAS-10 score were recorded.

Results

Baseline S100A12 concentration was measured in patients treated with anti-TNF (etanercept n=81, adalimumab n=7, median 200, IQR 133-440 ng/ml) and MTX (median 220, IQR 100-440 ng/ml). Of the patients in the anti-TNF therapy group, 74 (84%) were receiving MTX. Responders to methotrexate (n=57/75) and anti-TNF (n=66/88) therapy had higher baseline S100A12 concentration compared to non-responders: median 240 (IQR 125-615) ng/ml versus 150 (IQR 87-233) ng/ml p=0.021 for MTX, and median 308 (IQR 150-624) ng/ml versus 151 (IQR 83-201) ng/ml p=0.002 for anti-TNF therapy. Follow-up S100A12 could be measured in 44/75 methotrexate-treated (34/44 responders) and 39/88 anti-TNF-treated (26/39 responders) patients. Responders had significantly reduced S100A12 concentration (MTX: p=0.031, anti-TNF: p<0.001) at follow-up versus baseline. Baseline serum S100A12 in both univariate and multivariate regression models for anti-TNF therapy and univariate analysis alone for MTX therapy was significantly associated with change in JADAS-10.

Conclusion

Responders to MTX or anti-TNF treatment can be identified by higher pre-treatment S100A12 serum concentration levels.

Introduction

Juvenile idiopathic arthritis (JIA) is a clinically heterogeneous condition, frequently requiring therapy with conventional disease-modifying anti-rheumatic drugs (cDMARDs) such as methotrexate (MTX). Combination therapy increasingly also includes biological DMARDs (bDMARDs) with TNF-inhibitors (e.g. etanercept and adalimumab).(1–3) However up to 40%, or even higher depending on the definition used, of patients will not respond to treatment with bDMARDs.(4–6) Using biomarkers, alongside known predictive demographic and clinical factors, could help improve the prediction of response.(1,7,8)

S100A12 and myeloid related protein complex 8/14 (MRP8/14 or S100A8/A9) are S100 protein family members. Both proteins are calcium binding proteins and phagocyte activation markers acting as pro-inflammatory ligands of Toll-like receptor-4 (TLR-4), which are constitutively expressed predominantly in phagocytic myeloid cells (i.e. granulocytes and monocytes). It is thought that both proteins are secreted in a similar mechanism, either by non-classical secretion from active cells or passively released from necrotic cells.(9) Both S100A12 and MRP8/14 are both validated predictors of relapse risk and disease activity in JIA.(10–12) S100A12 concentration measured at the time of treatment withdrawal in patients with JIA predicted the development of flare better than MRP, with the combination of S100A12 plus high-sensitivity c-reactive protein (CRP) performing best.(13) This suggests differences exist in the performance of S100A12 and MRP8/14 as biomarkers, despite their many apparent similarities. Baseline MRP8/14 has already been shown to predict response to MTX and anti-TNF treatment in JIA patients. However, the association of serum S100A12 with response to therapy in JIA has not yet been evaluated.(14,15)

Material and methods

Study population

Data were analysed from three prospective cohort studies which were designed to study either the response to starting MTX or starting anti-TNF treatment (alone or in combination with other therapy including MTX, see Table 1) in patients with JIA diagnosed according to the International League of Associations for Rheumatology (ILAR) criteria.(3) The study was open for patients with undifferentiated JIA but no patient was enrolled. The prediction of response by MRP8/14 in these cohorts has already been published in

572961-L-bw-PS4U
Processed on: 25-1-2022

detail (14,15) and here we focus on reporting the associations of S100A12. Response to MTX was analysed using data from the UK Childhood Arthritis Response to Medication Study (CHARMS, n=75 patients). Data on response to anti-TNF treatment were collected in the Dutch Arthritis and Biologicals in Children (ABC) Register (n=68), the German Registry for Biologics in Paediatric Rheumatology (BIKeR, n=12) and the CHARMS study (n=8). Each of these studies recruited patients with all subtypes of JIA who fulfilled ILAR criteria and started either new DMARDS or biologic therapy for active arthritis (CHARMS). ABC and BIKER cohort data were combined to increase statistical strength. MTX and anti-TNF therapies were prescribed at the dose according to the previously published study protocols.(4,6,14)

The BIKeR register was approved by the ethics committee of the Ärztekammer Nordrhein Duesseldorf (ref 2/2015/7441), the CHARMS study was approved by the Institute of Child Health/Great Ormond Street NHS Trust (MREC-05/Q0508/95) and the ABC Register was approved by the Medical Ethics Committee at Erasmus MC Rotterdam (MEC-225.804/2003/51). The BIKER and ABC registries as well as the CHARMS study included provision in their ethical approvals for the collection, storage and analysis of biobanked samples. All three cohorts have been previously published in full elsewhere. Subjects were recruited with fully informed consent and child assent where appropriate.(4,6,14)

Definition of treatment response

Treatment responders achieved an ACRpedi 50 or better score at follow-up, equivalent to ≥ 50% improvement in a minimum of 3 out of 6 core variables, with no worsening in >1 remaining variables by >30%. Core variables are: 1) physician's global assessment score (PGA, using VAS: range 0-10 cm, 0=best score), 2) patient/parent global assessment of wellbeing (VAS: range 0-10 cm, 0=best score), 3) Childhood Health Assessment Questionnaire (CHAQ, range 0-3, 0=best score), number of joints with 4) active arthritis 5) limited motion and 6) erythrocyte sedimentation rate (ESR).(3,16) Disease activity and response were also quantified by parent/patient pain visual analogue scale (VAS), the achievement of inactive disease and change in JADAS-10, defined as the difference between baseline and follow-up JADAS-10.(17) The JADAS-10 score is quantified in four domains, three on a continuous scale (physician global, parent/patient global and number of active joints out of 10

Table 1: Baseline demographics and characteristics of patients starting MTX and anti-TNF therapy

Baseline demographic	**MTX-treated patients (n=75)**	**Anti-TNF-treated patients (n=88)**
Age at JIA onset in years, median (IQR)	5.3 (2.5-10.5)	10.0 (3.9-12.3)
Disease duration at therapy start in years, median (IQR)	1.4 (0.5-3.8)	2.3 (0.9-6.0)
Female, n (%)	52 (69)	66 (75)
ANA positive, n/N (%)	48/72 (67)	25/76 (33)
RF positive, n/N (%)	10/71 (14)	13/80 (16)
JIA Category at MTX or anti-TNF start, n (%)		
Oligoarticular persistent	13 (17)	5 (6)
Oligoarticular extended	17 (23)	24 (27)
Polyarticular RF-	29 (39)	33 (38)
Polyarticular RF+	6 (8)	13 (15)
Enthesitis-related arthritis	6 (8)	4 (5)
Psoriatic	3 (4)	9 (10)
Not available	1 (1)	0
Undifferentiated	0	0
Clinical variables at therapy start		
Physician's VAS (0-100)	38 (22-56)	54 (30-68)
Active joints, n	5 (2-8)	10 (5-17)
Restricted joints, n	3 (2-6)	6 (2-14)
Parent/Patient VAS (0-100)	33 (14-56)	53 (5-70)
CHAQ score (0-3)	1.00 (0.25-1.75)	1.5 (0.8-2.1)
ESR (mm/h)	23 (10-63)	13 (8-27)
Concomittant therapy at therapy start		
Methotrexate	75 (100)	74 (84)
Anti-TNF therapy	0	88 (100)
Systemic prednisolone	25/61 (41)	25/88 (28)
JADAS-10 (0-40), median (IQR)	13 (8-20)	19 (14-23)
S100A12 (in-house) at start in ng/ml, median (IQR)	220 (100-440)	200 (133-440)
S100A12 (*CircuLex*) at start in ng/ml, median (IQR)	605 (318-1330)	348 (195-655)

Abbreviations: **MTX** methotrexate, **anti-TNF** anti-tumour-necrosis factor, **JIA** juvenile idiopathic arthritis, **IQR** inter-quartile range, **ANA** anti-nuclear antibodies, **RF** rheumatoid factor, **VAS** visual analogue scale, **CHAQ** Childhood Assessment Questionnaire, **ESR** erythrocyte sedimentation rate**, JADAS-10** Juvenile Arthritis Disease Activity

specified) and the fourth being the presence of a normalized ESR.(18) The modified definition of inactive disease (ID, Wallace et al.(19)) requires the absence of active arthritis, systemic features, uveitis, normal ESR (≤20 mm/h) but accepts a higher acceptable PGA ≤1.0 cm (which in practice is rarely scored as 0) compared to the standard ID definition. As all patients achieving ID also fulfil ACR50, ACR50 was used as the measure of response because if any prediction of response was found with this lower threshold, it is likely the same or a higher response would be present with the use of ID. Baseline demographics and clinical scores including JADAS-10 are shown in Table 1 and the follow up characteristics (responders and non-responders) are shown in Supplement 1.

S100A12 measurement

Serum concentrations were measured using a well described in-house ELISA assay as well as a commercial assay (CircuLex, CycLex Co.Ltd) on frozen samples.(11,13). Both assays were utilised in order to investigate whether measured concentrations were reproducible in both assays and identify a suitable commercial assay, approved for research use, for use for further studies, which do not have access to this in-house ELISA. Reference internal control sera were used in each assay. S100A12 is a stable biomarker which is reliably measurable in samples sent at room temperature as well as in repeatedly thawed and frozen samples. All reported S100A12 values refer to in–house assay results unless specified. Results using the commercial assay are shown in Supplement 2. All assays were performed blind to the clinical diagnosis and results were not reported to treating clinical staff during the study. Results are presented as median (IQR).

Statistical analysis

Categorical characteristics were tested using Chi-squared, continuous variables using Mann-Whitney U and correlations with the Spearman (rs) or Pearson (r) test. Baseline and follow-up S100A12 was compared in paired analyses using the Wilcoxon signed rank test. Baseline S100A12 concentration was assessed for its prediction of ACRpedi outcome by binary logistic regression modelling and association with change in JADAS-10 by linear regression modelling. Multivariable linear models were also fitted for change in JADAS-10, allowing correction for other potential predictors and to assess the added value of S100A12 in predicting response. For this modelling, known

predictive variables (gender, age at JIA onset, disease duration, baseline JADAS-10, baseline CHAQ, number of previously used DMARDs and ESR) were pre-specified.(7,8,20,21) Missing data were handled using the chained equations multiple imputation command ice in Stata/SE (v13.0). Anti-TNF (adalimumab or etanercept) treated patients were combined after being assessed as having identical characteristics. Cut-off values for baseline S100A12 as a predictive marker for treatment response were defined using receiver operator characteristic (ROC) analysis.(13) Other analyses were performed with SPSS (IBM for Windows V.21) and Prism (Graphpad v5).

Results

Baseline characteristics

Baseline median S100A12 concentration in patients before either therapy (MTX: n=75, anti-TNF: n=88) significantly correlated with baseline ESR (MTX rs 0.40, p<0.001; anti-TNF rs 0.38, p<0.001) and JADAS-10 (MTX rs 0.25, p=0.04; anti-TNF rs 0.22, p=0.04, Table 1). Subgroup analysis of S100A12 with number of active joints at start showed no correlation (Spearman's rho 0.19 (p=0.072). In MTX treated patients, there was no difference in baseline S100A12 among JIA subtypes (p=0.17, Kruskal Wallis test). However, in anti-TNF treated patients a difference among patients of different subtypes was seen (p=0.024), with the highest concentrations in polyarticular RF positive JIA (median 411 ng/ml, n=13) and lowest in oligoarticular persistent JIA (median 56 ng/ml, n=5).

Clinical response to therapy

Follow-up was at a median of 6.6 (IQR: 5.8-7.6) months for MTX and 3.2 (2.6-5.0) months for anti-TNF treated patients. The clinical response of each treatment group was analysed separately, therefore this difference did not impact the results shown. Based on achievement of ACRpedi 50 or better at follow-up, 57 of 75 MTX-treated patients and 66 of 88 anti-TNF treated patients were responders. Of the 66 anti-TNF treated responders, 46 had an ACRpedi 70 or better response, while 31 patients were in clinical remission. The modified criteria for ID were fulfilled by 25/75 of MTX and 31/88 of anti-TNF treated patients. JADAS-10 at follow-up was median 3 (IQR 1-8) for MTX and 4 (1-9) for anti-TNF treated patients (Supplement 1), improving from baseline (Table 1). There were no significant differences between responders

and non-responders for either treatment group in terms of baseline disease characteristics, excluding the variables included in the ID and JADAS-10 score (Supplement 1).

Baseline S100A12 and response to therapy

Baseline S100A12 concentration was higher in responders versus non-responders (Figure 1A MTX median 240 (IQR: 125-615) ng/ml versus 150 (87-233) ng/ml, p=0.02; Figure 1B anti-TNF median 308 (IQR: 150-624) ng/ml versus 151 (IQR: 83-201) ng/ml, p=0.002). Increased baseline S100A12 was associated with odds ratios >1 for the prediction of ACRpedi 50 and improvement in JADAS-10 in univariate models at follow-up, for both treatments (Table 2). For patients using anti-TNF and MTX therapy, logistic regression modelling was also performed with the additional variable "MTX at start" and the odds ratio for baseline S100A12 did not change, and concomitant MTX was not a significant factor in the combined model (OR 3,46, 95% CI 0,93-12,85). Multivariate models constructed with known predictors of response, as detailed in the statistical methods above, tested their prediction of JADAS-10. Excluding S100A12, model variables explained 70% of the variance in change in JADAS-10 at follow-up for MTX-treated patients and 50% of the variance for the anti-TNF group. Including S100A12 as a variable improved the predictive models by 2% (not significant) for MTX and 5% (p=0.004) for anti-TNF therapy (Table 2).

Follow-up S100A12

Follow-up S100A12 concentrations were determined for MTX (44/75) and anti-TNF (39/88) treated patients, limited only due to lack of serum for this analysis which was performed blinded. Of these, 34/44 (77%) MTX and 26/39 (67%) anti-TNF treated patients were responders. At follow-up, both responders and non-responders, irrespective of therapy, had comparable S100A12 concentrations: MTX responders median 165 (IQR: 113-273), non-responders 79 (46-213, p=0.08), and anti-TNF treatment responders 110 (53-254) and non-responders: 91 (42-235), p=0.55 (Figure 1). However, responders (those achieving ACRpedi 50) had significant reduction from their baseline S100A12 concentration measured by the Wilcoxon signed rank test (Supplement 1). Sensitivity, specificity and likelihood ratios for prediction of response by S100A12 using ROC analysis are shown in Table 3.

Table 2: Association of response to therapy to baseline S100A12 concentration

Logistic regression: *predicted minimum ACRpedi50 response*	**Unadjusted OR (95% CI)**	**P-value**
MTX therapy		
S100A12, per 50 unit ng/ml increase	1.213 (1.01-1.45)	0.034
Anti-TNF therapy		
S100A12, per 50 unit ng/ml increase	1.04 (1.01-1.08)	0.014
Univariate linear regression: *predicted change in JADAS-10*	**Beta (95% CI)**	**P-value**
MTX therapy		
S100A12, per 50 unit ng/ml increase	-0.453 (-0.726- -0.181)	0.002
Anti-TNF therapy		
S100A12, per 50 unit ng/ml increase	0.064 (0.025-0.102)	0.001
Multivariate linear regression: *predicted change in JADAS-10*	**Beta (95% CI)**	**P-value**
MTX therapy		
S100A12, per 50 unit ng/ml increase	0.197 (-0,397-0.003)	ns
Anti-TNF therapy		
S100A12, per 50 unit ng/ml increase	0.045 (0.015-0.076)	0.004

Abbreviations: **OR** odds ratio, **CI** confidence interval, **MTX** methotrexate, **anti-TNF** anti-tumour necrosis factor

Table 3: Sensitivity, specificity and likelihood ratios for the determined cut-off of S100A12 predicting response to MTX and anti-TNF therapy

Accuracy measure	**MTX therapy**	**Anti-TNF therapy**
Cut-off level S100A12 (ng/ml)	260	213
Sensitivity	47.4	58.6
Specificity	88.9	80.7
Positive likelihood ratio	4.3	3.0
Negative likelihood ratio	1.7	0.5
Youden index	0.363	0.392
AUC (95% CI)	0.675 (0.559-0.805)	0.734 (0.622-0.846)

Abbreviations: **AUC** area under the curve, **MTX** methotrexate, **anti-TNF** anti-tumour necrosis factor

Use of concomitant therapy

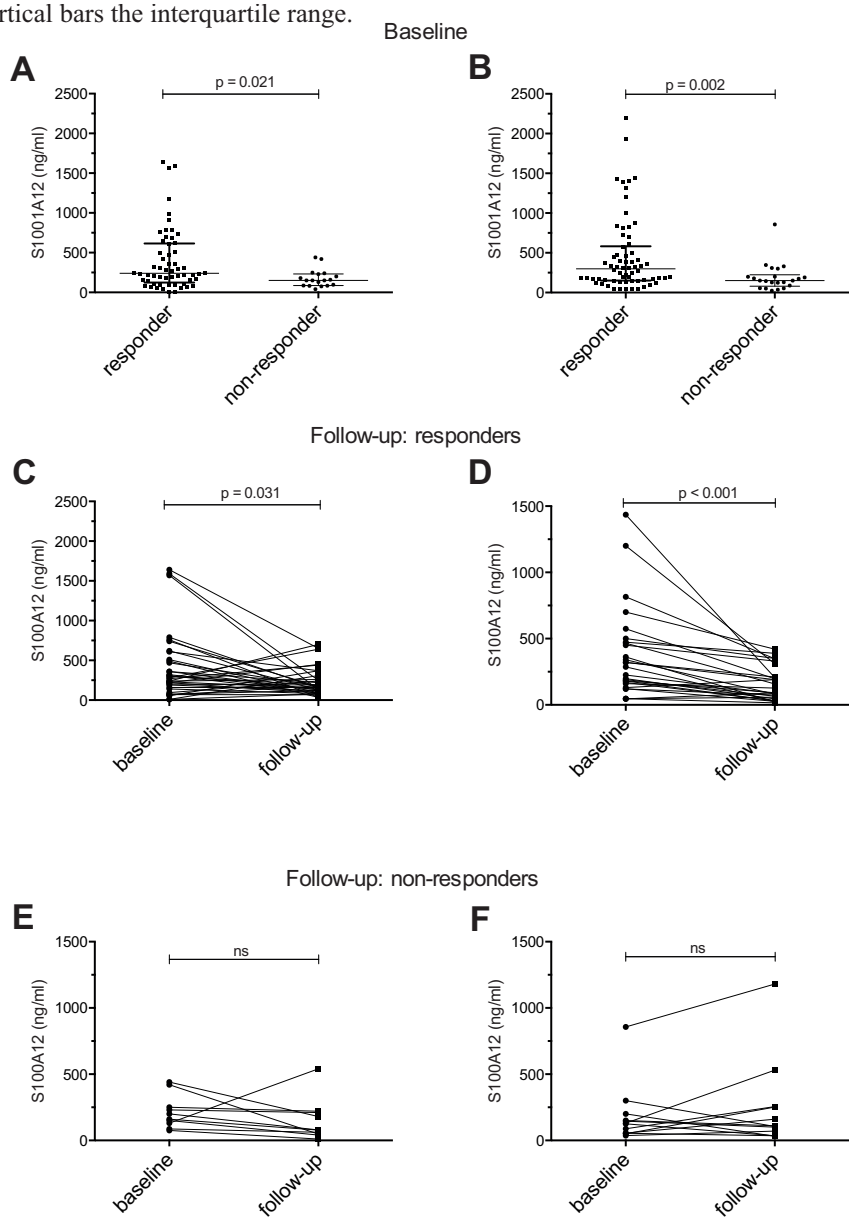
Concomitant therapy was given according to physician choice. The percentage of patients using concomitant MTX at start of anti-TNF therapy in the group of responders was 91% (60/66) and 63% (14/22) in the non-responders. Systemic corticosteroid use at the start of MTX treatment (n=25/61, 41%) was not associated with any significant differences in either baseline or follow-up S100A12. However, in the anti-TNF treatment group, those who were also receiving corticosteroids at the start of the treatment (n=25/88) had higher baseline S100A12 than those who did not (median 380 (IQR: 177-838) ng/ml versus 187 (128-331) ng/ml, p=0.006) and also greater change at follow-up (delta S100A12 -145 (-327 to -97) versus -84 (-149 to 13, p=0.034). However, there was no difference in corticosteroid use between patients characterised as responders or non-responders, therefore concomitant corticosteroid use was unlikely to be the major factor in patients reaching clinical response. Concomitant DMARD use (excluding MTX), were used by so few patients (MTX-treated= 3/66; anti-TNF-treated=3/88) that no conclusions could be drawn.

Measurement of S100A12 by commercial ELISA

S100A12 measured by commercial assay (Supplement 2) was comparable with in-house assay results and also showed significantly higher S100A12 in responders versus non-responders and higher baseline versus follow-up concentrations. However, while a good AUC was obtained for both therapy groups, this was lower with the commercial (MTX AUC 0.662, 95% CI 0.532-0.791; anti-TNF 0.675, 95% CI 0.550-0.800) versus in-house (MTX AUC 0.675, 95% CI 0.559-0.805; anti-TNF 0.734, 95% CI 0.662-0.846) assay. Sensitivity (commercial ELISA: MTX 45.6, anti-TNF 39.4; in-house ELISA: MTX 47.4, anti-TNF 58.6) and specificity (commercial ELISA: MTX 83.3, anti-TNF 86.4; in-house ELISA: MTX 88.9, anti-TNF 80.7) were also lower with the commercial ELISA. Absolute commercial assay concentrations were also higher than the in-house assay, approximately double, and the cut-off levels calculated for each therapy group were also much wider than with the in-house assay.

Figure 1: Baseline and followup S100A12 concentration by therapy used.

Differences in baseline S100A12 concentrations in responders and nonresponders to MTX (A) or anti-TNF therapy (B) measured by the in-house ELISA are shown. Change in S100A12 concentration after treatment with MTX and anti-TNF therapy is shown for responders (C–D) and nonresponders (E–F). Horizontal bars indicate the median concentration and vertical bars the interquartile range.



572961-L-bw-PS4U
Processed on: 25-1-2022

Discussion

Baseline serum S100A12 was associated with response to both MTX and anti-TNF therapy in patients with JIA who had a high baseline concentration that decreased significantly with either MTX or anti-TNF treatment. Patients with higher baseline S100A12 concentration had higher disease activity and ESR and were more likely to be treatment responders. Furthermore, the addition of S100A12 to multivariate models improved the prediction of response.

The aim of this study was not to directly compare level of response to MTX versus anti-TNF therapy, or consider their combined therapy versus individual use, but rather to determine whether S100A12 concentration can predict a response to therapy when a clinician initiates either of these medications. Further work and specific trials are needed to determine which therapy would be best initiated in which patients, and such studies would also require the availability of predictive markers of response, like S100A12 which is discussed here.

S100A12 has already been shown to correlate with disease activity and concentrations >175 ng/ml potentially predict increased risk of flare in patients who have had treatment withdrawn.(10,13,22–24) The follow-up time of patients in this study was a median of five months. Most patients would be expected to show a treatment response within three months after initiation, with S100 concentrations shown to decrease in response to effective biological treatment within four weeks of beginning treatment.(25,26)

Moncrieffe et al. and Anink et al. identified MRP8/14 as being associated with MTX- and anti-TNF therapy response, and also suggested predictive modelling could be improved by including additional variables.(14,15) S100A12, like MRP8/14 has the advantage over other cytokines, e.g. IL-1beta, in having greater temperature stability, even withstanding storage and postage at room temperature. S100A12 measurement could therefore feasibly be incorporated into the routine laboratory work-up for JIA and therefore also be incorporated into in treatment prediction models.(7,21,27,28)

Whilst a well-established experimental ELISA S100A12 protocol exists, this is not yet in routine use. The commercial ELISA has already been demonstrated to perform well in analysing patient's serum.(11,26,29) Both assays

require serial dilution of serum to obtain reliable results, due to the wide range of S100A12 concentrations in patients.(11) Therefore, while either assay can be used, results from each should not be directly compared and only used with assay-specific cut-offs. Although overall the same pattern of results were obtained with both assays, the in-house ELISA performed marginally better, as reflected by the slightly higher AUC and Youden Index values achieved for both MTX and anti-TNF treatment groups with the in-house assay compared to with the commercial assay.

Whereas S100A12 and MRP8/14 have some reported similarities in intra- and extracellular functions, the mechanism of release for each remains unknown. There are clear differences in the expression and functions between the two proteins.(9) A hallmark of MRP8/14 is its formation of a heterodimer, whilst the hexamer is thought to be the active extracellular form of S100A12.(30) Adding S100A12 into the multi-variable models (investigated for MRP8/14 by Moncrieffe et al.) did result in a further increase in explained variance, though only a relatively small percentage (2%, non-significant) for MTX, but 5% ($p < 0.005$) for the anti-TNF group.(14)

In this cohort, baseline ESR and number of active joints already differentiated well between those patients who later became responders versus non-responders, which could be one factor why the addition of S100A12 to a multi-marker model added limited benefit. Other cohorts, particularly larger clinical cohorts, are required to ascertain whether S100A12 is a clinically useful predictive marker.

It is likely that no single biomarker can be sufficiently sensitive or specific for predicting response and multi-marker panels are increasingly being sought, such as the multi-biomarker disease activity test (MBDA) for rheumatoid arthritis.(1,31) It is also important to acknowledge that there is a lack of clinically viable alternative biomarkers that could replace S100A12 or MRP8/14, or add to their prediction in such multi-variable models at present. Additionally, heterogeneity within the same subgroup of JIA could be a further factor in variation in treatment response, and would further support the use of multi-marker panels to individualize management strategies. Small cohort size also increases the chance of clinical heterogeneity leading to statistically significant outcomes, and we combined two cohorts for the anti-TNF group

572961-L-bw-PS4U
Processed on: 25-1-2022

to counter this. Larger studies would require greater multicentre collaboration and the use of inception cohorts. One factor that could be investigated is the presence and influence of TNF-alpha gene polymorphisms, which could be associated with the heterogeneity of response to anti-TNF treatment.(32)

Biological and MTX therapies are associated with potentially significant adverse effects and are expensive.(3,25,33) Most importantly, around a third of patients will show poor response to therapy.(4–7) In our study, the initiation of both MTX and anti-TNF treatment was effective and was associated with improvements in clinical disease activity measures, JADAS-10 score, attainment of ID and ACRpedi 50 responses. Due to limitations in the size of the data set, we could not perform further subgroup analyses of response by each ACRpedi level, and instead used ACRpedi 50 or better as the cut-off, using information from the JADAS score to supplement the measure of clinical improvement. Over 50 % of patients in each group reached an ACRpedi 50 or better response, in line with published literature, including the study of etanercept efficacy by Quartier et al., where over half of treated JIA patients had over a minimum 50% improvement in their core set criteria at 3 months, which alongside the baseline characteristics suggested our patient population was an average group of patients.(25,34) However, the effect of concomitant therapy use by patients (MTX plus anti-TNF therapy and/or other therapies such as corticosteroids) should be investigated specifically in more detail.

In conclusion, we have shown that high pre-treatment S100A12 serum concentrations of patients with JIA is associated with a good response to methotrexate or anti-TNF therapy. Further work to identify the ideal clinical scenarios where this biomarker could best be utilized (at onset of treatment in the absence of corticosteroid treatment for example, limited to anti-TNF treated patients or use in predicting patients who will respond to one drug rather than another or to combined therapies from the outset) should be performed. In addition, this work highlights that there is a significant clinical need for the clinical evaluation of predictive biomarkers. However, to achieve these objectives, validation cohorts with frequent longitudinal follow up is required.

Acknowledgements
The authors thank all the patients and their families for participating in the included studies, the ward and clinical staff for help collecting samples and data, and laboratory teams for sample handling and processing as well as each member of the study groups (Childhood Arthritis Response to Medication Study, CHARMS; Arthritis and Biologicals in Children, ABC, Register and the Biologics in Paediatric Rheumatology, BIKER, Registry). We especially thank M.Saers and S.Schleifenbaum for excellent technical assistance.

Funding
F.G. was supported by the European Union Seventh Framework Programme (FP7) EU-TRAIN (ref.289903) grant. L.W. is supported by the Great Ormond Street Hospital Children's Charity. D.H. and D.F. were funded by the Interdisciplinary Centre of Clinical Research at the University of Muenster (IZKF CRA04) and received an unconditional grant from Pfizer Germany for this study. The ABC register is maintained with unconditional support from the Dutch Board of Health Insurances (2003-2006), Pfizer and AbbVie. The BIKER Registry is supported by an unconditional grant from Pfizer Germany and also the FP7 grant, EC-GA 305266 MIAMI. The CHARMS study was funded by SPARKS UK (08ICH09), the Big Lottery Fund UK (RG/1/010135231), UK Medicines for Children Research Network (NIHR-funded) and NIHR Great Ormond Street Biomedical Research Centre (BRC). The funding sources had no involvement in study design, data analysis, report writing or decision to submit the article for publication.

Financial disclosures
LvS has previously received grants from the Dutch Arthritis Association, consultancy fees from Pfizer, Roche and Novartis, travel grants from Pfizer and Bristol-Myers Squibb, RtC has received research grants and consultancy fees from Pfizer, GH received research grants from AbbVie, Pfizer and Roche and speaker fees from AbbVie, Novartis, Pfizer and Roche. Other authors declare no conflicts of interest.

Supplement files
Supplement file 1 and 2 are found on the following pages.

Supplement 1

Table S1: Baseline demographics and characteristics in all responders and non-responders

	MTX-treated patients (n=75)		**Anti-TNF-treated patients (n=88)**	
	Responders (n=57)	Non-responders (n=18)	Responders (n=66)	Non-responders (n= 22)
Baseline demographic				
Age at JIA onset in years, median (IQR)	6.1 (2.6-11.0)	4.5 (2.4-10.5)	10.0 (4.2-12.3)	9.4 (3.1-13.7)
Disease duration at therapy start in years, median (IQR)	1.3 (0.4-4.7)	2.2 (0.7-3.8)	2.4 (1.1-4.9)	2.3 (0.8-7.7)
Female, n (%)	39 (68)	13 (72)	48 (73)	18 (82)
Anti-TNF therapy,				
Etanercept, n (% of all Etanercept)	n/a	n/a	61 (75)	20 (25)
Adalimumab, n (% of all Adalimumab)	n/a	n/a	5 (71)	2 (29)
JIA Category at start, n (%)				
Oligoarticular persistent	7 (12)	6 (35)	2 (3)	3 (14)
Oligoarticular extended	15 (26)	2 (12)	16 (24)	8 (36)
Polyarticular RF-	23 (40)	6 (35)	26 (39)	7 (32)
Polyarticular RF+	5 (9)	1 (6)	11 (17)	2 (9)
Enthesitis-related arthritis	5 (9)	1 (6)	3 (5)	1 (5)
Psoriatic	2 (4)	1 (6)	8 (12)	1 (5)
Clinical variables at baseline				
Active joints, n	5 (2-10)	4 (2-5)*	11 (5-18)	8 (2-16)
CHAQ score (0-3)	1 (0.31-1.75)	0.81 (0.25-2.06)	1.49 (0.75-2.13)	1.35 (0.63-1.96)
ESR (mm/h)	25 (10-69)	19 (8-35)	16 (9-30)	12 (7-18)
JADAS-10 (0-40), median (IQR)	14 (8-23)	10 (7-14)	20 (14-23)	17 (11-22)
S100A12 at baseline, median (IQR), ng/ml	240 (125-615)	150 (87-233)*	308 (150-624)	151 (83-201)**

Abbreviations: **MTX** methotrexate, **anti-TNF** anti-tumour-necrosis factor therapy, **JIA** juvenile idiopathic arthritis, **CHAQ** Childhood Assessment Questionnaire, **ESR** erythrocyte sedimentation rate**, JADAS-10** Juvenile Arthritis Disease Activity

*/** indicates significance between responders and non responder within MTX treated patients, or within anti-TNF treated patients as follows: *p< 0.05, **p< 0.005 (Mann Whitney U)

Supplement 2: S100A12 concentrations measured by commercial CircuLex ELISA

Performance of in-house assay versus Circulex assay
MTX: S100A12 concentrations measured by the in house ELISA assay significantly correlate with CircuLex measured concentrations (Spearman's rho: 0.85, p< 0.001). Anti-TNF: S100A12 concentrations measured by the in house ELISA assay significantly correlate with CircuLex measured concentrations (Spearman's rho: 0.687, p<0.001).

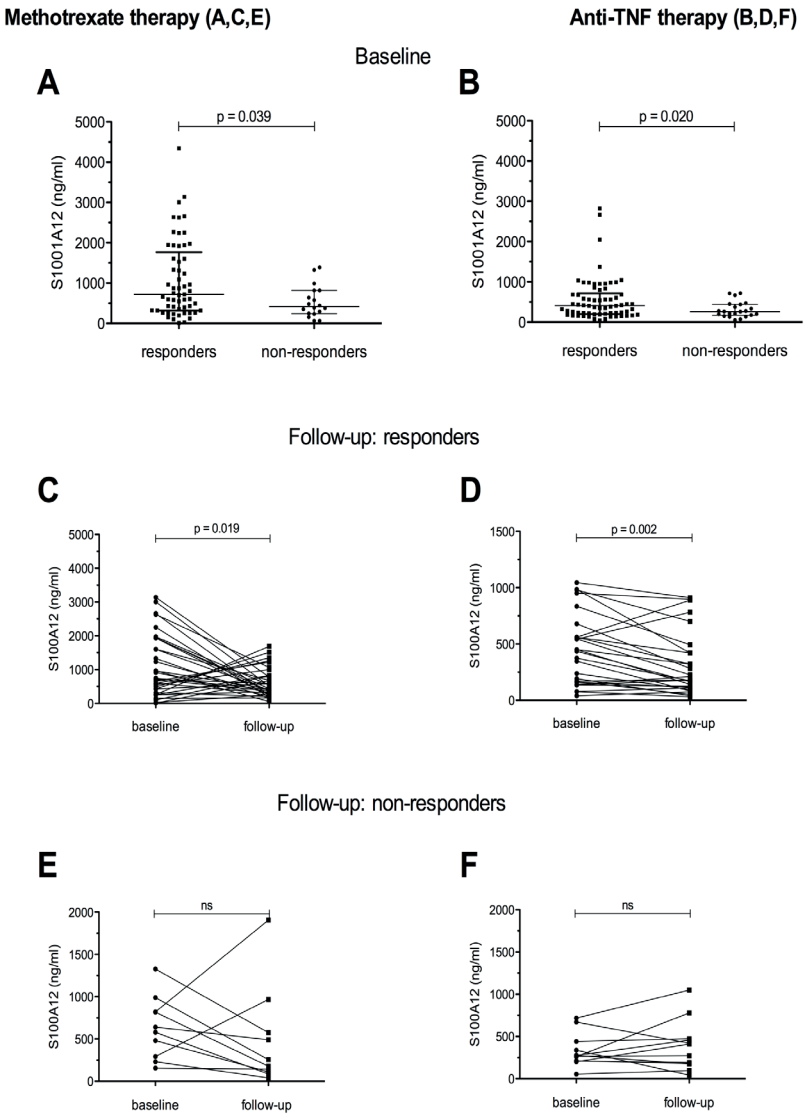
S100A12 levels at baseline and response to treatment
Baseline S100A12 serum levels were higher in responders (median 720 (IQR 320-1765) compared to non-responders (median 417, IQR 243-818, p=0.039) for MTX treated patients (Figure 1A). For anti-TNF treated patients, baseline S100A12 serum levels were also higher in responders (median 407, IQR 212-710) compared to non-responders (median 239, IQR 150-436, p=0.020). In a univariate logistic regression this resulted in an OR of 1.06 for MTX therapy (95%CI 1.004-1.115), and an OR of 1.14 (95% CI: 1.01-1.28) for achieving at least an ACRpedi 50 response per 50 units of S100A12 (CircuLex)(ng/ml) for anti-TNF therapy.

Prediction of response corrected for other variables
Baseline S100A12 serum levels were significantly associated with change in JADAS-10 in a univariate linear regression analysis (β = -0.149, 95% CI -0.298 to -0.0007, p=0.050 per 50 units change in S100A12) for anti-TNF treated patients. For MTX this was: β = -0.159 (95% CI -0.264 - -0.053) In the corrected multivariable analysis the corrected β was -0.089 per 50 units increase in ng/ml, 95% CI -0.212 to 0.034 for anti-TNF therapy. The change in explained variance was 1.4% (not significant). Multivariable analysis: corrected beta for MTX: -0.102 (95% CI: -0.139 - -0.039), the change in explained variance was 5.3% (p=0.002). Multivariate models constructed with known predictors of response as shown in the method were performed to test their association with JADAS-10 score for each treatment group. Without S100A12, the variables in the model explained 70 % (equal to that as measured by in-house ELISA) of the variance in change in JADAS-10 at follow-up for MTX-treated patients, and 50 % (also the same as with the in-house ELISA) for the anti-TNF group. Including S100A12 as a variable increased the models prediction by 5.3% (more than the 2% with the in-house ELISA) for MTX and 1.4% (vs 5% with the in-house ELISA for anti-TNF treated groups.

572961-L-bw-PS4U
Processed on: 25-1-2022

Figure 1: Baseline and follow-up S100A12 concentration by therapy used, measured by Circulex ELISA

Differences in baseline S100A12 concentrations in responders and non-responders to MTX (A) or anti-TNF therapy (B) measured by Circulex ELISA are shown. Change in S100A12 concentration after treatment with MTX and anti-TNF therapy is shown for responders (C-D) and non-responders (E-F). Horizontal bars indicate the median concentration, and vertical bars the IQR.



Use of S100A12 as a prognostic marker for response to treatment
The CircuLex ELISA was less accurate compared to the in-house ELISA for predicting response to anti-TNF treatment and MTX, shown in Table S3.

Table S3: Sensitivity, speicificty and likelihood ratios for the determined cut-off S100A12 predicting response to MX and anti-TNF treatment, *CircuLex* ELISA.

	CircuLex **ELISA: MTX**	*CircuLex* **ELISA: anti-TNF**
Cut-off level S100A12 (ng/ml)	846	508
Sensitivity	45.6	39.4
Specificity	83.3	86.4
Positive likelihood ratio	2.7	2.9
Negative likelihood ratio	0.7	0.7
Youden index	0.289	0.258
AUC	0.662 (0.532-0.791)	0.675 (0.550-0.800)

AUC= area under the curve

References

1. Hinze C, Gohar F, Foell D. Management of juvenile idiopathic arthritis: hitting the target. Nat Rev Rheumatol. 2015;11:290–300.
2. Beukelman T, Patkar NM, Saag KG, Tolleson-Rinehart S, Cron RQ, DeWitt EM, et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. Arthritis Care Res (Hoboken). 2011;63:465–82.
3. Ringold S, Weiss PF, Beukelman T, DeWitt EM, Ilowite NT, Kimura Y, et al. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis. Arthritis Rheum 2013;65:2499–512.
4. Horneff G, Schmeling H, Biedermann T, Foeldvari I, Ganser G, Girschick HJ, et al. The German etanercept registry for treatment of juvenile idiopathic arthritis. Ann Rheum Dis. 2004;63:1638–44.
5. Lovell DJ, Reiff A, Ilowite NT, Wallace CA, Chon Y, Lin S-L, et al. Safety and efficacy of up to eight years of continuous etanercept therapy in patients with juvenile rheumatoid arthritis. Arthritis Rheum. 2008;58:1496–504.
6. Prince FHM, Twilt M, ten Cate R, van Rossum MAJ, Armbrust W, Hoppenreijs EPAH, et al. Long-term follow-up on effectiveness and safety of etanercept in juvenile idiopathic arthritis: the Dutch national register. Ann Rheum Dis. 2009;68:635–41.
7. Otten MH, Prince FHM, Armbrust W, ten Cate R, Hoppenreijs EPAH, Twilt M, et al. Factors associated with treatment response to etanercept in juvenile idiopathic arthritis. JAMA. 2011;306:2340–7.
8. Geikowski T, Becker I, Horneff G. Predictors of response to etanercept in polyarticular-course juvenile idiopathic arthritis. Rheumatology (Oxford) 2014;53:1245–9.
9. Kessel C, Holzinger D, Foell D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. Clin Immunol. 2013;147:229–41.
10. Holzinger D, Frosch M, Kastrup A, Prince FHM, Otten MH, Van Suijlekom-Smit LWA, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis. 2012;71:974–80.
11. Rothmund F, Gerss J, Ruperto N, Däbritz J, Wittkowski H, Frosch M, et al. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care Res (Hoboken). 2013;66:949–55.
12. Foell D, Wulffraat N, Wedderburn LR, Wittkowski H, Frosch M, Gerss J, et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. JAMA. 2010;303:1266–73.
13. Gerss J, Roth J, Holzinger D, Ruperto N, Wittkowski H, Frosch M, et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. Ann Rheum Dis. 2012;71:1991–7.
14. Moncrieffe H, Ursu S, Holzinger D, Patrick F, Kassoumeri L, Wade A, et al. A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein. Rheumatology (Oxford). 2013;52:1467–76.

15. Anink J, Van Suijlekom-Smit LWA, Otten MH, Prince FHM, van Rossum MAJ, Dolman KM, et al. MRP8/14 serum levels as a predictor of response to starting and stopping anti-TNF treatment in juvenile idiopathic arthritis. Arthritis Res Ther. 2015;17:200.
16. Giannini EH, Ruperto N, Ravelli A, Lovell DJ, Felson DT, Martini A. Preliminary definition of improvement in juvenile arthritis. Arthritis Rheum. 1997;40:1202–9.
17. Horneff G, Becker I. Definition of improvement in juvenile idiopathic arthritis using the juvenile arthritis disease activity score. Rheumatology (Oxford). Oxford University Press; 2014;53:1229–34.
18. Consolaro A, Ruperto N, Bazso A, Pistorio A, Magni-Manzoni S, Filocamo G, et al. Development and validation of a composite disease activity score for juvenile idiopathic arthritis. Arthritis Rheum. 2009;61:658–66.
19. Wallace CA, Giannini EH, Huang B, Itert L, Ruperto N. American College of Rheumatology Provisional Criteria For Defining Clinical Inactive Disease in Select Categories of Juvenile Idiopathic Arthritis. Arthritis Care Res. 2011;63:929–36.
20. Solari N, Palmisani E, Consolaro A, Pistorio A, Viola S, Buoncompagni A, et al. Factors associated with achievement of inactive disease in children with juvenile idiopathic arthritis treated with etanercept. J Rheumatol. 2013;40:192–200.
21. Bulatovic M, Heijstek MW, Van Dijkhuizen EHP, Wulffraat NM, Pluijm SMF, de Jonge R. Prediction of clinical non-response to methotrexate treatment in juvenile idiopathic arthritis. Ann Rheum Dis. 2012;71:1484–9.
22. Bae CB, Suh CH, An JM, Jung JY, Jeon JY, Nam JY, et al. Serum S100A12 may be a useful biomarker of disease activity in adult-onset still's disease. J Rheumatol. 2014;41:2403–8.
23. Yamasaki Y, Takei S, Imanaka H, Nerome Y, Kubota T, Nonaka Y, et al. Prediction of long-term remission of oligo/polyarticular juvenile idiopathic arthritis with S100A12 and vascular endothelial growth factor. Mod Rheumatol. 2015;1–6.
24. Rahman MT, Myles A, Gaur P, Misra R, Aggarwal A. TLR4 endogenous ligand MRP8/14 level in enthesitis-related arthritis and its association with disease activity and TLR4 expression. Rheumatology (Oxford). 2014;53:270–4.
25. Quartier P, Taupin P, Bourdeaut F, Lemelle I, Pillet P, Bost M, et al. Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type. Arthritis Rheum. 2003;48:1093–101.
26. Choi IY, Gerlag DM, Herenius MJ, Thurlings RM, Wijbrandts CA, Foell D, et al. MRP8/14 serum levels as a strong predictor of response to biological treatments in patients with rheumatoid arthritis. Ann Rheum Dis. 2013;
27. Pomirleanu C, Ancuta C, Miu S, Chirieac R. A predictive model for remission and low disease activity in patients with established rheumatoid arthritis receiving TNF blockers. Clin Rheumatol. 2013;32:665–70.
28. Marotte H, Miossec P. Biomarkers for prediction of TNFalpha blockers response in rheumatoid arthritis. Joint Bone Spine. 2010;77:297–305.
29. CircuLex S100A12/EN-RAGE ELISA Kit Ver.2 | Kits | MBL Life Sience -ASIA- [Internet]. [cited 2016 May 3]. Available from: http://ruo.mbl.co.jp/bio-/g/dtl/P/?pcd=CY-8058V2#u-pub
30. Kessel C, Fühner S, Brockmeyer S, Wit-

tkowski H, Föll D. OP0194 Hexameric S100A12 is Required for Pro-Inflammatory TLR4-Signalling. Ann Rheum Dis. 2015;74:144.4-145.
31. Centola M, Cavet G, Shen Y, Ramanujan S, Knowlton N, Swan KA, et al. Development of a multi-biomarker disease activity test for rheumatoid arthritis. PLoS One. 2013;8:e60635.
32. Scardapane A, Ferrante R, Nozzi M, Savino A, Antonucci I, Dadorante V, et al. TNF-alpha gene polymorphisms and juvenile idiopathic arthritis: Influence on disease outcome and therapeutic response. Semin Arthritis Rheum. 2015;45:35–41.
33. Tynjälä P, Vähäsalo P, Tarkiainen M, Kröger L, Aalto K, Malin M, et al. Aggressive combination drug therapy in very early polyarticular juvenile idiopathic arthritis (ACUTE-JIA): a multicentre randomised open-label clinical trial. Ann Rheum Dis. 2011;70:1605–12.
34. van Riel PLCM, Taggart AJ, Sany J, Gaubitz M, Nab HW, Pedersen R, et al. Efficacy and safety of combination etanercept and methotrexate versus etanercept alone in patients with rheumatoid arthritis with an inadequate response to methotrexate: the ADORE study. Ann Rheum Dis 2006;65:1478–83.

Chapter 7

The translational medicine professional: A bridge between bench and bedside?

Faekah Gohar, Aisha Gohar, Georg Hülskamp, Otfried Debus

Front Med. 2018;5:294.

Abstract

Translational medicine (TM) can be defined as the interdisciplinary application of biomedical research for the improvement of health of patients and society. The focus of TM has so far been largely on the bench-to-bedside rather than bedside-community transition of research. Several "Valleys of Death" in this process have been described, identifying transitional failures that may halt or impede the pathway, which would otherwise lead to the development of medicines, technologies, and/or evidence based practice guidelines. In order to help bridge these gaps, increasing patient-orientated research at each stage could improve the success of projects and increase societal impact. Increasing the accessibility and involvement of patients in TM outside of traditional research centers, such as universities and teaching hospitals, is one crucial pre-requisite. For example, where clinical research units with active links to local universities have been set-up, research participation can be increased. Such non-traditional research centers (NRTCs) might include primary or secondary care services, or even social care institutions. TM professionals (TMPs) from multi-disciplinary backgrounds, with work experience in university or research centers and with experience of TM, could play a vital role in this organizational change. TMPs in NTRCs are well placed to collaborate with local universities, larger research centers and commercial research and development organizations. Exchanging information could benefit all shareholders involved. TMPs can also stimulate the education and innovative thinking that is required for TM to achieve its full societal impact.

We discuss the scope of a potential role for TMPs in NTRCs, as well as the possible barriers and difficulties they might face, along with measures that could widen the accessibility of TM outside of the traditional setting.

The European Society for Translational Medicine defines translational medicine (TM) as being an interdisciplinary branch of biomedicine supported by three pillars: bench, bedside and community. It's goal is to improve the health of society by improving disease management, e.g., with new therapies.(1)

TM has predominantly focused on the bench-to-bedside approach, with most research activities being conducted in traditional research centers such as specialist centers and universities. Several "Valleys of Death" in TM or the bench-to-bedside pathway, defined as the route between drug or technology development (the "bench") and its integration into clinical care (the "bedside"), have been described.(2–4) The valleys represent gaps that impede the pathway, impacting the development of medicines, technologies and/or evidence based practice guidelines. Until now, less focus has been on the third pillar of TM: the involvement of the wider community, or "bedside to community" phase 1. Multi-faceted organizational changes and innovation, for example in trial design, are required to bridge these valleys as success rates of products that reach the "end" clinical trial stage remain poor.(2,5,6)

Increasing patient-orientated research at all stages could improve the success of research projects and increase societal impact. Research practice often focuses on select groups of patients, for example those with rare or financially or academically "attractive" diseases, and who are primarily treated in hospitals either in or linked to traditional research centers. Such organizational factors result in an inherently biased system in many respects, including in the setting of research agendas and allocation of funding for projects. Such factors could potentially explain the limited output of the TM pathway. Optimizing the accessibility for patients outside of traditional research centers is also a crucial pre-requisite to innovating TM for the benefit of the wider society. To tackle this problem, Clinical Research Units (CRUs) to link local universities and hospitals have been set-up. Funding through the European Clinical Research Infrastructures Network (ECRIN) has further encouraged the connection of research institutions including CRUs, also referred to as CTUs (Clinical Trial Units) or CRCs (Clinical Research Centers) into hubs and networks in 14 countries across Europe.(7) Accessibility to research participation in other non-traditional research centers (NTRCs) such as primary or secondary care services, and social care institutions, should also be addressed. An onus on research funders to require evidence of early and consistent patient input beginning in the consultation phase could be an additional driver of change.

A range of professionals from basic scientists, laboratory members, regulatory agencies, educational facilities, members of ethics boards, and journals are involved in TM. Professionals with expertise in TM (Tranlational Medicine Professionals, TMPs) from multi-disciplinary backgrounds could play a central role in innovating TM (Figure 1). TMPs in NTRCs are well placed to collaborate with the traditional research centers and shareholders, and can coordinate the exchange of information as well as stimulate education and innovative thinking. While some clinical academic tracks for the training of TMPs exist, they may be informal and without a focus on TM. One example where TM and the training of future TMPs was a strong focus was the European Translational Training for Autoimmunity & Immune manipulation Network (EUTRAIN) research and training as part of the EU Marie Curie Initial Training Network programme.(8, 9) Whilst most TMPs remain based in the organizations where they are trained, i.e., university and research centers, many will spend at least some of their training time in NTRCs. Encouraging such TMPs to continue research in such sites would have a dual effect of avoiding these skills going to waste and maximize the extension of TM into NTRCs. TMPs in NTRCs may even face less constraints on their work, for example with the freedom to conduct projects for societal benefit rather than to achieve prestige in terms of high impact publications and big grants, which may be the case in specialist research centers. In NTRCs, incorporating research into daily clinical practice allows the advantages of TM, such as increased job satisfaction and professional development, to also reach a wider group of professionals. However, TMPs in NTRCs face their own challenges, such as the long held misbelief that research activities should be secondary to the provision of good patient care and limited to research centers. TMPs should engage with colleagues to widen education about TM and its fundamental tenet of incorporating society. NTRCs could themselves drive the process by changing the culture to support and nurture the process of research, for example by recruiting staff with a research interest or experience. The scope of a potential role for TMPs in NTRCs, particularly in (1) widening participation and (2) improving collaboration in TM outside of the traditional research setting will be discussed and is summarised in Table 1.

Table 1. Specific roles the Translational Medical Professional could play in shifting the focus of the translational pathway from "bench to bedside" to "bench to society" by (1) widening participation to research and (2) improving collaboration.

Widening participation

- Encourage involvement in research activities in non-traditional research centers (NTRCs) and other partners including:
  - social care institutions: e.g. hospices, rehabilitation centers, schools and care homes
  - primary care (general practitioner services)
  - secondary care centers (specialist or teaching hospitals)
  - industry partners
  - universities
  - patient groups
  - ethics research committees
- Recruit and include patients outside of NTRCs in clinical trials and monitoring of medical devices
- Encourage the relocation of research & development offices and clinical research units into NTRCs, or take up roles in such centers or work independently but collaboratively with existing centers
- Take an organisational role in sharing of research facilities such as laboratory facilities
- Support and encourage the wider inclusion of patient advocates on ethical approval boards and grant approval committees
- Encourage new grants and apply for existing grants or other benefits, such as awards of a recognition of excellence to research centers that widen participation in TM could be a focus for TMPs in NTRCs
- Participation in, and encourage new educational programmes, pre- and post-graduate as well as on-going clinical educational opportunities to address challenges facing translational medicine professionals (TMPs)

Improving collaboration

- Communication and outreach activities to connect different research partners and participants
- Organisation of collaborative forums and meetings
- Development and participation in mentorship programmes
- Setting up and maintenance of shared biobank facilities
- Sharing the use of specialist research equipment between different centers
- Mentoring and supporting non-TMP colleagues in realising the potential personal and wider benefits of TM.

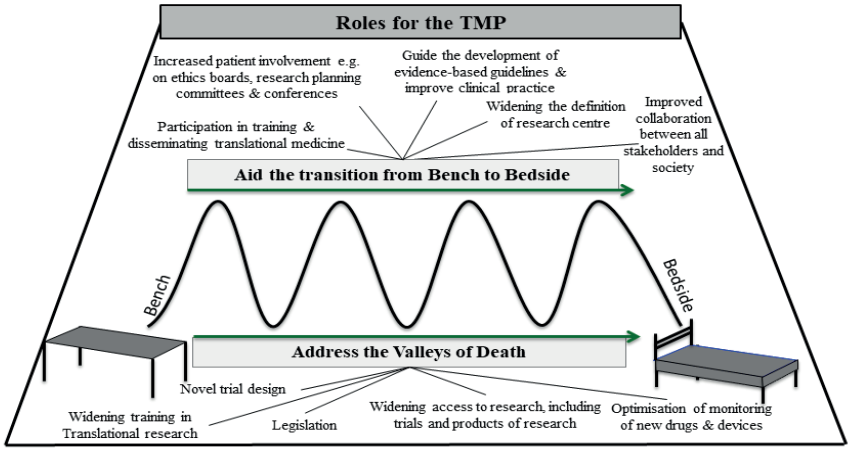
Widening participation

When research participation is excluded from the majority of NTRCs, a goal of wide societal impact and improvement of health is unlikely to be achieved. All members of society should be seen as potential research participants and receive the opportunity to take part in research.(10) All members of society will be affected by healthcare provisions at some point of their life either as recipients of health interventions, or as carers for someone else receiving health care. Therefore, NTRCs should also include social care institutions such as hospices, rehabilitation centers, schools and care homes as well as primary and secondary care centers.(10) In addition, some research questions are population based questions, and require broader patient inclusion to be adequately addressed. For this, the support of patient advocacy groups and ethical review boards is also vital, with TMPs supporting the case for widening TM participation in NTRCs.

Longer-term monitoring of drugs and product related adverse effects, for example after clinical trials are concluded or after the acute phase of a disease is over, might be better performed in NTRCs rather than in specialist centers. Whilst the reporting of drug side effects after licensing is encouraged and required in all countries, the monitoring of products is not monitored to the same extent.(6) One recent example of the failure of adequate follow-up and monitoring of devices is the mounting evidence that mesh used in the surgical management of pelvic organ prolapse has been responsible for many post-surgical complications and that the medical devices (the Mesh) was approved based on weak evidence leading to a large unexpected need for costly post-intervention care.(11)

A programme of legislative support and training initiatives is required to support the process of patient engagement.(12) Research activities are already being shifted to NTRCs, which can benefit from increased funding streams and patient access and also developing organizational links with local teaching hospitals and commercial research centers.(13) Structural changes within NTRC, such as the setting up of research and development offices and facilities for clinical research, are also vital. While their financial set-up may not be under the control of TMPs, TMPs can support their development and help staff them. Clinical research centers often include outpatient facilities with consultation rooms and treatment beds as well as access to a laboratory which can perform basic research procedures such as Real-time PCR and flow cytometry, sample preparation for DNA extraction or serum bio-banking.

Figure 1 Roles for the Translational Medical Professional in aiding the transition from bench to bedside and addressing potential points of failure, or "Valleys of Death".



To be effective, TMPs should be adequately trained and be inter-disciplinary, including laboratory staff and research coordinators as well as specialist research and clinical nurses and doctors.(14,15) Therefore, a programme of widening participation for TMPs is also required. In the UK, academic clinical fellowships (ACF) during clinical training have improved access to research programmes for trainees. In contrast to the UK, a much greater proportion of medical students in the Netherlands will undertake PhDs during their study or early in their training. In Germany, to obtain the title "Dr. med" a period of research is also usually completed during university study, much akin to intercalated degree programmes in the UK. However, ACFs and most Dr. med. or PhD and research programmes are based in research centers and include little or no focus on TM or inter-disciplinary working. Widening such programmes whether they are pre- or post-graduate based to multi-disciplinary participants and including time in the programme to develop and teach widening participation in research, novel trial design and collaboration and the inclusion of a period of training time in NTRCs is also vital. There is a general

consensus that research and TM requires specially trained professionals, and there is increasingly financial and structural support for interdisciplinarity in clinical and research settings. Many universities have developed new institutes with industry partners as well as clinicians and researchers collaborating and now also offer translational study programmes.(1,2,6) However, one of the largest challenges in widening participation in TM in NTRCs is achieving the organizational changes to support such a transition.

Improving collaboration

TMPs could foster links between NTRCs and local research centers which excel in a particular field or service by driving collaborations as well as widening research participation. Practical measures may include the organization of regular open meetings, with an open forum to present ideas and updates for new or on-going research projects that could help overcome problems or barriers that projects may be facing. This inter-disciplinary sharing of information could drive innovation and benefit all parties involved, e.g. by pooling potential research participants and sharing access to technology or specialists. Common goals and challenges could help lead to solutions such as the recruitment of a suitable control group. Collaboration between departments from different centers, or even between departments from the same center that may have been unaware of pre-existing research facilities or goals available in-house could be improved upon. Open and equal exchanges of ideas, which is the basis of inter-disciplinary research, opens the door to broader sources of funding. Traditional hierarchies of power, which still often exist in traditional research centers, may also be more effectively challenged when committees are inter-disciplinary. Collaboration between NTRCs and established research centers could also be organized in the form of "outreach programmes" which might include the development of mentorship programmes. Taking an active role in the development and running of such integration and outreach activities could provide career benefits to early-stage TMPs, providing earlier opportunities to undertake leadership roles.

Challenges facing TMPs

Some challenges facing TMPs focus around accepting the idea of TM in NTRCs. Many TMPs will have trained with a specialist focus. For their new role in NTRCs, TMPs will need to maintain this focus on detail but also develop wider research skills including novel trial design and collaborative work,

which takes public health into account. The role of a TMP will comprise many challenges, including that they must work hard in their NTRCs to be seen as effective and successful in both their clinical and research activities. TMPs must also cross barriers such as addressing common misconceptions including that research has no place in clinical training programmes and be able to engage colleagues to also drive good research practices in their workplace.(13) The main barrier will be to change perceptions so that research is seen as a part of daily practice in NTRCs and not as a supplementary or a career progress driven activity. TMPs will also need to develop time management skills as well as leadership and delegation if they are to achieve all the activities associated with TM including: teaching, publishing papers and writing research grants. Balancing expectations from colleagues, supervisors and patients will also be vital.

In order to achieve the variety of goals we have discussed as well as to excel in communication and drive innovation, TMPs must be creative - a skill which is difficult to teach and measure. This creativity is fundamental to driving new concepts in the design and practice of trials as well as of medical products and the TM pathway itself.(6) TMPs must also use their creativity to develop collaborations with research centers, universities and commercial centers. This can all be achieved with support from colleagues, mentors, and collaborative practices as discussed above.

Summary

In conclusion, greater focus on the societal aspect in TM is required to tackle the so-called "Valleys of Death." The TMP could be a potentially vital driver of innovation and the organizational processes that are required. However, whilst the focus on TM and the number of TMPs might be increasing, TMPs still face multiple challenges but there are many ways in which they can help widen access of TM and improve collaboration within TM.

Author Contributions

FG conceived the study and performed the literature review. All authors contributed to the writing of the manuscript and made substantial contributions to the content and approved the final version.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Cohrs RJ, Martin T, Ghahramani P, Bidaut L, Higgins PJ, Shahzad A. Translational Medicine definition by the European Society for Translational Medicine. New Horiz Transl Med. (2015) 2:86–8. doi: 10.1016/j.nhtm.2014.12.002
2. Waldman SA, Terzic A. Clinical and translational science: from bench-bedside to global village. Clin Transl Sci. (2010) 3:254–7. doi: 10.1111/j.1752-8062.2010.00227.x
3. Meslin EM, Blasimme A, Cambon-Thomsen A. Mapping the translational science policy "valley of death." Clin Transl Med. (2013) 2:14. doi: 10.1186/2001-1326-2-14
4. Tugwell P, Knottnerus JA. The new paradigm: from "bench to bedside" to the translational research "valleys of death." J Clin Epidemiol. (2015) 68:725–6. doi: 10.1016/j.jclinepi.2015.05.010
5. Lippi G, Plebani M, Guidi GC. The paradox in translational medicine. Clin Chem. (2007) 53:1553. doi: 10.1373/clinchem.2007.087288
6. Fernandez-Moure JS. Lost in translation: the gap in scientific advancements and clinical application. Front Bioeng Biotechnol. (2016) 4:43. doi: 10.3389/fbioe.2016.00043
7. Demotes-Mainard J, Kubiak C. A European perspective - the European clinical research infrastructures network. Ann Oncol. (2011) 22(Suppl. 7):vii44–9. doi: 10.1093/annonc/mdr425
8. EUTRAIN (2012) (Accessed January 26 2018). Available online at: http://eutrain-network.eu/about-eutrain/
9. Petrelli A, Prakken BJ, Rosenblum ND. and Eu fellows Developing translational medicine professionals : the Marie Skłodowska-Curie action model. J Transl Med. (2016) 14:329. doi: 10.1186/s12967-016-1088-1
10. Anderson M, McCleary KK. From passengers to co-pilots: patient roles expand. Sci Transl Med. (2015) 7:291fs25. doi: 10.1126/scitranslmed.aac6023
11. Heneghan CJ, Goldacre B, Onakpoya I, Aronson JK, Jefferson T, Pluddemann A, et al. Trials of transvaginal mesh devices for pelvic organ prolapse: a systematic database review of the US FDA approval process. BMJ Open (2017) 7:e017125. doi: 10.1136/bmjopen-2017-017125
12. Anderson M, Kimberly McCleary K. On the path to a science of patient input. Sci Transl Med. (2016) 8:336ps11. doi: 10.1126/scitranslmed.aaf6730
13. Moore A. Trial Blazers: The Benefits of Carrying Out Clinical Studies (2012) (Accessed January 18, 2018). Available online at: https://www.hsj.co.uk/efficiency/trial-blazers-the-benefits-of-carrying-out-clinical-studies/5046668.article
14. Molas-Gallart J, D'Este P, Llopis O, Rafols I. Towards an alternative framework for the evaluation of translational research initiatives. Res Eval. (2016) 25:235–43. doi: 10.1093/reseval/rvv027
15. Davis AM, Hull SC, Grady C, Wilfond BS, Henderson GE. The invisible hand in clinical research: the study coordinator's critical role in human subjects protection. J Law Med Ethics (2002) 30:411–9.

Footnotes

1. ^University of Glasgow - Postgraduate study - Taught Degree Programmes A-Z - Translational Medicine (Accessed April 21, 2018). Online at: https://www.gla.ac.uk/postgraduate/taught/translationalmedicine
2. ^Institute of Translational Medicine - University of Liverpool (Accessed April 21, 2018). Online at: https://www.liverpool.ac.uk/translational-medicine/about-us/

CHAPTER 8

Driving medical innovation through interdisciplinarity: Unique opportunities and challenges

Faekah Gohar, Patrick Maschmeyer, Bechara Mfarrej, Mathieu Lemaire, Lucy R Wedderburn, Maria Grazia Roncarolo, Annet van Royen-Kerkhof

Front Med. 2019;6:35.

Introduction

Many health problems facing society are multifactorial and often require social and political input as well as interventions from medical and technological experts. For example, the treatment of chronic pain requires expertise from multiple disciplines: imaging technology, cellular electrophysiology, neurochemistry, genetics, social, psychological, and cultural studies.(1) While these activities are coordinated by the treating physician, they usually remain parallel and are never fully integrated to create an innovative therapy for the patient. From a research standpoint, we argue that for these new solutions to emerge, there needs to be a concerted effort to move from multidisciplinarity to interdisciplinarity.

Multidisciplinary research is defined as work involving researchers from different fields who "remain conceptually and methodologically anchored in their respective fields".(2) In contrast, interdisciplinary research is defined as "a mode of research by teams or individuals that integrates information, data, techniques, tools, perspectives, concepts, and/or theories from two or more disciplines or bodies of specialized knowledge, to advance fundamental understanding or to solve problems whose solutions are beyond the scope of a single discipline or area of research practice".(3) It may lead to the creation of a new scientific field, such as environmental humanities.(4–6)

The major difference between the two types of research is that while interdisciplinarity involves deep and robust integration of distinct disciplines, multidisciplinarity implicates juxtaposition of a variety of expertises.(5) By these definitions, both research types are clearly valuable, but interdisciplinary research should drive more impactful results for complicated problems. These advances come at a cost for researchers because interdisciplinarity has its own set of unique challenges, ranging from communication issues to allocation of credits among a team. In this article, we discuss these hurdles and potential solutions to raise awareness amongst researchers keen to lead a successful interdisciplinary project.

Characteristics of an interdisciplinary team

Collaborative teams consist of individuals from different fields working toward a common goal that transcends the borders of a single discipline. Exactly who will comprise the members of an interdisciplinary and multidisciplinary team must be individually determined for each project according to the specific needs. It is almost a certainty in research projects that individuals will face hurdles that can only be solved with group support, leading to a widespread feeling among members of being out of one's comfort zone.(7) Communication can be challenging when a team involves members from a variety of disciplines. A classic strategy employed to dominate the discourse and decision-making process is to use highly technical language specific to one's field of expertise. Bammer proposed the creation of a new role for integration and implementation scientists.(8) Such experts would contribute to teams tackling complex problems by assessing the problems and their interconnections, and by identifying strategies for approaching them. These implementation scientists could define the level of involvement of the different stakeholders and strategize how to incorporate the various disciplines and stakeholder objectives. Furthermore, they can identify knowledge gaps and predict evolving problems, whilst providing support throughout the process. Two major hurdles can be identified: first in identifying a universal requirement for experts in this role, and secondly establishing a clear identity for scientists in this role with a clear consensus on methods and processes to be used for example in training for such a role.(9)

In the same direction, a new field of research is developing, which was first termed “the science of team science” or SciTS in 2006. This field focuses on systematic efforts to overcome barriers in collaborative work, and how to achieve the targeted research outcomes. Other goals of SciTS are to support scientists in creating and working effectively within a team. However, above a certain team size (different for each research setting and question) output decreases and bureaucracy increases, with potential conflicts arising within teams. Therefore, in a world of limited resources, important questions for researchers also include the question of resource allocation i.e., when to decide if external collaborators or cross-disciplinary support is required and how to fund this adequately.(10)

Efficient coordination of project tasks is vital for progress to occur. In large teams, a power struggle for the “lead” role may emerge when several indi-

viduals have equal seniority or leadership experience. The team leader must match responsibilities to expertise and time commitment, to plan a schedule that is realistic yet ambitious, and to provide ample opportunities for team members to share updates and knowledge. The team leader also often plays a key role in designing the research plan and in identifying potential team members with complementary knowledge and skills. "The science of team science" is a new field of research that aims to provide evidence to support scientists responsible for these tasks and helps them to overcome barriers.(11) A survey of researchers revealed that successful interdisciplinary work often includes mutual respect, comfort, or already established positive relationships.(12) These concepts gave rise to a new ethical framework known as relational ethics, stemming from the fact that all ethics are grounded in relations, interdependency, engagement and the importance of community.(13) This framework suggests that a climate of safety, trust, respect and equality is necessary to effectively challenge the status quo.(14,15)
Successful solutions to complex problems can be achieved when a team is comprised of individuals with complementary expertises, interests, ideas, and/or professional goals. An example is the creation of arterio-venous fistulas for hemodialysis access using an innovative endovascular catheter-based system: this system was conceived and implemented by a team of interventional radiologists, vascular surgeons, biomedical, and industrial engineers.(16) Another example is the invention of a blood-resistant biodegradable surgical glue by a team of pediatric cardiologists, cardiac surgeons, biomedical, biological, and chemical engineers.(12) In both cases, long-identified unmet medical needs became solvable because of well-directed interdisciplinary efforts over many years.

Advantages and hurdles of working in interdisciplinary projects
The interpretation of the concept interdisciplinarity varies among individuals. It is reported that researchers face challenges in justifying the benefit of interdisciplinary interactions against their perception of increased time and resource requirements. In a study by Roy et al. both natural and social scientists identified departmental or institutional difficulties, communication difficulties and differing disciplinary approaches as significant challenges.(17)

In another descriptive study, 19 researchers indicated that they conducted interdisciplinary research specifically because of their individual lack of know-

ledge in some sectors.(7) Other benefits were the generation of new knowledge, exposure to new methods or theories, and the opportunity to make a bigger impact. However, the respondents also indicated caveats to performing interdisciplinary work, such as the need to allocate more time compared to their usual line of research as well as limited credits for academic promotion. Other issues highlighted included the significantly greater effort needed to understand interpersonal dynamics, to clarify leadership roles, and to determine the contributions of each team member. Finally, some researchers noted that some individuals may be marginalized as a result of power imbalances.(18)
Funding agencies have traditionally rewarded independent scientists proposing research in their field of expertise rather than teams of researchers offering to conduct interdisciplinary projects. Over time, complex problems such as climate change led to increased funding for inter- or multidisciplinary research teams. Some researchers have argued that efforts to make research funding contingent on inclusion of interdisciplinarity leads to inefficiency.(7) How successful such interdisciplinary focused funding approaches are remains unclear: the US National Institute of Health (NIH) reports slightly better outcomes for funding fostering interdisciplinary funded programmes vs. conventional, projects of independent research, whereas the opposite is true for the European Research Council (ERC).(18) Funding for collaborative projects are increasingly available and are internationally well supported. For example, the European Framework Program for Research and Innovation, which includes the "Horizon 2020" (H2020) program, is the world's largest interdisciplinary funding program.(19) In the USA, the National Science Foundation (NSF) (20) and the Clinical and Translational Science Awards (CTSA) Program supports national networks of medical research institutions that collaborate to improve the efficiency of translational research, promoting the integration of underserved populations, and train future translational researchers.(21)

In summary, many researchers hold negative perceptions about interdisciplinary research. However, these perceptions could be overcome by adopting strategies such as advanced planning of the study, including whether a project is to be multi- or interdisciplinary (see Figure 1), and by including a balanced team with the abilities required for the project (see Table 1).

572961-L-bw-PS4U
Processed on: 25-1-2022

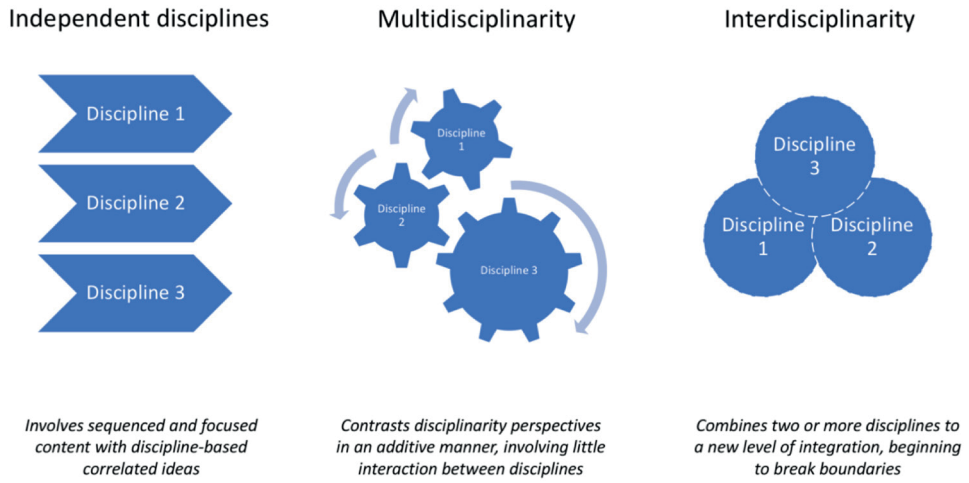
Table 1 Recommendations to stimulate sustainable interdisciplinary research environments.

Pre-project
- Include a trainee or have a future team member seek additional training in a program with a focus on interdisciplinary research.
- Determine the extent of collaboration wished (inter- vs. multi-disciplinary).
- Plan the team composition, the balance of abilities and role delegation. Consider including a scientist in an integration and implementation role.

During the project
- Allocate the supervisor role to someone with experience of interdisciplinary project supervision, not necessarily the most senior.
- Plan early for potential project hurdles, such as funding issues, allocation of funds, credits etc.
- Plan the allocation of credits, such as the authorship order, early.
- Focus on the training of inexperienced project members.
- Consider the implementation of a team-based mentoring program and integrate team-based evaluation.

Post-project
- Ask for anonymous feedback from all team members on what worked well and what could be done better to provide helpful hints to improve the team performance.
- Consider success of the project to be not only based upon achievement of publication in high-impact journals, but rather achievement of societal goals and wider translational objectives.
- All team members actively engage in knowledge translation to promote the project in their own field, including considering the use of "newer" resources or publication modes such as interactive journals or Social Media.

Figure 1. Definitions and illustrations of independent, multidisciplinary and interdisciplinary working.



Interdisciplinary research in early career stages and for career progression

The World Health Organization (WHO) has recently concluded that effective interdisciplinary education facilitates later collaborative practice.(22) Introducing the interdisciplinarity concept early in a scientist's career promotes the later unconscious incorporation of it into their future research.(23) As a result, this early practical exposure ensures the new generation of researchers is better equipped to manage the challenges of interdisciplinarity. The integration of interdisciplinarity into higher education could be driven by educational institutions, the UK Research Excellence Framework being a good model.(24)

A more structured approach is the formation of multidisciplinary translational teams (MTT) as a training and mentoring approach focusing on translational innovation by research capacity building, interprofessional integration, and team-based mentoring approaches. This methodology enhances the development of translational research competencies and productivity in terms of collaborative publications.(25, 26) Another innovative structured approach is industry-based studentships, as recommended by the Canadian Academy of Health Sciences (CAHS) after an in-depth assessment of interdisciplinary health research.(27) An argument against this model of training is that it increases pressure and constraints placed on trainees by adding an additional layer of training and evaluation to their portfolio.

For challenging topics with dedicated grants and that require interdisciplinary approaches, evaluation of teams supersedes the evaluation of individuals. Yet, the coordinator carries most of the evaluation pressure, since their track record needs to show they have coordinated interdisciplinary teams and trained next-generation scientists to implement interdisciplinary research. It is true that progression from early stage to established scientists requires continuous evaluation with the “expertise” binoculars, yet one needs to start somewhere. The pressure is on early-stage researchers to acquire “expertise” in order to progress, yet, be open to learning and implementing interdisciplinary methods in preparation for the tackling of complex problems.

Throughout their careers, scientists are traditionally evaluated based on the quality of their output. Articles only “count” in the academic tally if the scientist is first or last author. Middle authorships are reflexively disqualified irrespective of the nature of the contribution or the importance of the discovery. Scientists interested to work as part of interdisciplinary teams may be discouraged to do so when realizing that they will be at a significant disadvantage compared to others who prefer “flying solo.” McLeish and Strang identify

"Individual Career Progression" as one of the crucial levels at which there is an immediate need for an effective evaluation method for interdisciplinarity. (28)

Furthermore, from their experience as evaluators, the authors report enormous pressures on researchers to establish a distinct identity, fueling the claim that career progression is hampered by interdisciplinary research and potentiated by single-discipline work. Nevertheless, some successful interdisciplinary translational researchers counter-argue that their aim is impact, a goal favored by several institutions. "Resisting the concept of focusing in research meant to surround myself with collaborators of different skills to fill the gaps in knowledge and exploring constantly new areas. One's focus gets defined by products (29) and technologies they put on the market that have large impact on patients' lives" - personal communication, Dr. Jeffrey Karp from Brigham and Women's Hospital, Boston (MA).(30)

What is the Best Approach for Training Future Scientists?

While it is critical to continue training scientists who are highly knowledgeable in one specific field, it is important to expose them early on to the notions of multi- and inter-disciplinarity. Ideally, this exposure would be an integral part of their didactic and practical training. It is also critical to strive to train individuals with broader interests by allowing them to straddle a few fields during their training, with the understanding that their training is likely to be substantially longer than usual (and thus will require unusually long periods of support from funding bodies). The clinician-scientist training model is an example of this approach since it generates a workforce that is conversant in the language of both clinical and basic science. This will facilitate the dialogue between the disciplines and render a deeper mutual understanding. There are now a large number of training programs for non-physicians that aim to specifically train researchers focused on interdisciplinarity in a given discipline such as cancer or cardiovascular diseases, although no specific standards for training exist to which these programs can be evaluated by.

Independent vs. Team vs. Interdisciplinary Science

It is important to emphasize that our goal is not to dismiss independent or team science. These two approaches, which rely on work within a more narrow scientific perspective, are distinguished by the number of independent teams involved. There are many important research questions that are best addressed using either of these traditional approaches. For example, assessing the impact of a genetic deficiency on human physiology using genetically engineered cellular or animal models. Reductionism is often a critical heuristic device to solve these scientific problems. In contrast, interdisciplinary science is most useful to answer research questions nested in complex structures. By definition, they cannot be answered by relying only on reductionistic methods but rather require integrated, multi-pronged approaches. For example, multifactorial conditions that are caused by the confluence of multiple genetic and environmental factors have been notoriously difficult to study. This has long been a frustrating situation since many diseases under this banner are prime public health problems (e.g. diabetes, atheroembolism, hypertension, or dementia). While there is no guarantee of success, the fresh look provided by interdisciplinary science is likely to yield insights and breakthroughs that may not be otherwise possible.

Conclusion

Whilst remembering the overarching goal of interdisciplinarity research is impact, research teams should be carefully constructed, led, and organized to allow for the fulfillment of individual objectives required for personal development, as well as for overall project success and achievement of the project aims. Effective collaborative practices are enabled by effective interdisciplinary education and can be promoted by the active provision of funding streams, in order to drive creative interdisciplinarity in academia.

Author Contributions:
FG, PM, BM, and ML made equal contributions in writing the paper. LW, MR, and AvR-K also wrote the manuscript and supervised the project.

Conflict of Interest Statement
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The handling editor declared a shared affiliation, though no other collaboration, with one of the authors ML.

Acknowledgments
LW is supported by Arthritis Research UK (21953), Great Ormond Street Children's Charity and the NIHR Biomedical Research Centres at Great Ormond Street Hospital. ML is supported by New Investigators Awards from the CIHR/KRESCENT and the Canadian Child Health Clinician Scientist Program (CCHCSP).

References

1. Institute of Medicine. Bridging Disciplines in the Brain, Behavioral, and Clinical Sciences. National Academies Press (2000). Available online at: https://market.android.com/details?id=-book-_NSXrR_5sTcC
2. Stokols D, Hall KL, Taylor BK, Moser RP. The science of team science: overview of the field and introduction to the supplement. Am J Prev Med. (2008) 35:S77–89.
3. Institute of Medicine National Academy of Engineering National Academy of Sciences Committee on Science Engineering and Public Policy Committee on Facilitating Interdisciplinary Research. Facilitating Interdisciplinary Research. National Academies Press (2005). Available online at: https://market.android.com/details?id=-book-mPronXxGcboC
4. Klein JT. Creating Interdisciplinary Campus Cultures: A Model for Strength and Sustainability. John Wiley & Sons (2009). Available online at: https://market.android.com/details?id=-book-U84svxR3H6oC
5. Klein JT. Evaluation of interdisciplinary and transdisciplinary research: a literature review. Am J Prev Med. (2008) 35:S116–23.
6. Wagner CS, Roessner JD, Bobb K, Klein JT, Boyack KW, Keyton J, et al. Approaches to understanding and measuring interdisciplinary scientific research (IDR): A review of the literature. J Informetr. (2011) 5:14–26.
7. Nair KM, Dolovich L, Brazil K, Raina P. It's all about relationships: a qualitative study of health researchers' perspectives of conducting interdisciplinary health research. BMC Health Serv Res. (2008) 8:110.
8. Bammer G. Toward a New Discipline of Integration and Implementation Sciences. In: Frodeman R, editor. The Oxford Handbook of Interdisciplinarity, 2nd Edn. (2017). Oxford, UK: Oxford University Press.
9. Bammer G. Should we discipline interdisciplinarity? Palgrave Commun. (2017) 3:30.
10. Stokols D, Misra S, Moser RP, Hall KL, Taylor BK. The ecology of team science: understanding contextual influences on transdisciplinary collaboration. Am J Prev Med. (2008) 35:S96–115.
11. Baker B. The science of team science. Bioscience (2015) 35:S96–115.
12. Lang N, Pereira MJ, Lee Y, Friehs I, Vasilyev NV, Feins EN, et al. A blood-resistant surgical glue for minimally invasive repair of vessels and heart defects. Sci Transl Med. (2014) 6:218ra6.
13. Bergum V, Dossetor JB. Relational Ethics: The Full Meaning of Respect. Hagerstown, MD: University Publishing Group (2005).
14. Brown J, Isaacs D. Conversation as a core business process. Syst Thinker

(1996) 7:1–6.
15. Schunn CD, Crowley K, Okada T. The growth of multidisciplinarity in the cognitive science society. Cogn Sci (1998) 22:107–30.
16. Rajan DK, Ebner A, Desai SB, Rios JM, Cohn WE. Percutaneous creation of an arteriovenous fistula for hemodialysis access. J Vasc Interv Radiol. (2015) 26:484–90.
17. Roy ED, Morzillo AT, Seijo F, Reddy SMW, Rhemtulla JM, Milder JC, et al. The elusive pursuit of interdisciplinarity at the human—environment interface. Bioscience (2013) 63:745–53.
18. Ledford H. How to solve the world's biggest problems. Nature (2015) 525:308–11.
19. Quests for Interdisciplinarity: A Challenge for the ERA and HORIZON 2020. Publications Office (2015). Available online at: https://www.nap.edu/catalog/9942/bridging-disciplines-in-the-brain-behavioral-and-clinical-sciences
20. Introduction to Interdisciplinary Research. NSF - National Science Foundation (2004). Available online at: https://www.nsf.gov/od/oia/additional_resources/interdisciplinary_research/index.jsp (Accessed July 12, 2018).
21. About the CTSA Program. National Center for Advancing Translational Sciences (2015). Available online at: https://ncats.nih.gov/ctsa/about (Accessed July 14, 2018).
22. Gilbert JHV, Yan J, Hoffman SJ. A WHO report: framework for action on interprofessional education and collaborative practice. J Allied Health (2010) 39(Suppl. 1):196–7.
23. Carey GE, Smith JA. Jack-of-all-trades, master of none: postgraduate perspectives on interdisciplinary health research in Australia. BMC Health Serv Res. (2007) 7:48.
24. Higher Education Funding Council for England. Interdisciplinary research - Higher Education Funding Council for England. Available online at: http://www.hefce.ac.uk/rsrch/interdisciplinary/ (Accessed July 13, 2018).
25. Ameredes BT, Hellmich MR, Cestone CM, Wooten KC, Ottenbacher KJ, Chonmaitree T, et al. The Multidisciplinary Translational Team (MTT) Model for Training and Development of Translational Research Investigators. Clin Transl Sci. (2015) 8:533–41.
26. Hager K, St Hill C, Prunuske J, Swanoski M, Anderson G, Lutfiyya MN. Development of an interprofessional and interdisciplinary collaborative research practice for clinical faculty. J Interprof Care (2016) 30:265–7.
27. Hall JG, Bainbridge L, Buchan A, Cribb A, Drummond J, Gyles C, et al. A meeting of minds: interdisciplinary research in the health sciences in Canada. CMAJ (2006) 175:763–71.
28. McLeish T, Strang V. Evaluating interdisciplinary research: the elephant in the peer-reviewers' room. Palgrave Commun. (2016) 2:16055.
29. Fernández CR. Gecko Biomedical's Bio-inspired Surgical Glue Gets EU Approval. Labiotech.eu (2017) Available online at: https://labiotech.eu/gecko-biomedical-setalum-ce-mark/ (Accessed July 15, 2018).
30. Jeff Karp - Partners HealthCare. Partners HealthCare Available online at: https://innovation.partners.org/council-member/jeff-karp (Accessed July 15, 2018).

CHAPTER 9

Summary and Discussion

572961-L-bw-PS4U
Processed on: 25-1-2022

Summary

An insight into the need for biomarkers for the diagnosis and management of paediatric rheumatology disorders has been presented in this thesis, including a broad range of aspects from discovery to clinical translation.

Part I, Biomarker Need, included two review articles which set the scene of current treatment strategies for JIA and limited availability of validated biomarkers. In Chapter 2, the review of management of JIA, concepts which form the foundation of management strategies such as 'window of opportunity', 'treat-to-target' and 'immunological remission' were defined and discussed. Current treatment strategies and guidelines were summarized along with their relevance for targeted, patient-centered care. The systematic review in Chapter 3 was performed to identify the range of serum diagnostic or prognostic biomarkers and their level of clinical utility according to their availability, measurability and level of validation for SJIA. Not unexpectedly, most of the biomarkers had limited evidence for their current clinical use. However, a range of markers were identified that could be further validated including: IL-6, IL-12, IL-18, osteoprotegerin, S100A12 and S100A8/A9.

In Part II, Biomarker Discovery, Chapter 4 described a translational multi-platform biomarker discovery study performed to identify novel molecular signatures of patients with two phenotypes of SJIA compared to patients with infection. Validation with the targeted proteomic method MRM confirmed the results of the discovery study performed with LC-MS/MS. 91% of SJIA patients were correctly identified as having the systematic versus the polyarticular SJIA disease phenotype. The differing molecular signatures provided further evidence for the biphasic model of SJIA. Chapter 5 focused on the in vitro and in vivo characterization of neutrophil secretory activity in patients with FMF. In vitro, neutrophils from M694V homozygous patients spontaneously secreted more S100A12, IL-18 and caspase-1 compared to healthy controls. In serum, S100A12 concentrations correlated with disease activity and genotype, being highest in M694V homozygous patients. In patients with inactive disease both S100A12 and IL-18 concentrations were higher in M694V-positive versus M694V-negative patients reflecting higher levels of subclinical disease activity.

In Part III of Biomarker Translation, in Chapter 6, serum S100A12 was measured in patients treated with methotrexate or anti-TNF medications (adalimumab or etanercept) at baseline and at follow-up and treatment response was recorded. Treatment responders could be identified by higher pre-treatment S100A12 serum concentration levels. Chapter 7 described the potential role for translational medical professionals (TMPs), focusing on their role in non-traditional research centers, such as non-university hospitals and primary care. Practical considerations for widening participation, improving collaboration and addressing the challenges facing TMPs were discussed to identify methods of improving collaboration within translational medicine. In Chapter 8 the opportunities and challenges of driving medical innovation through interdisciplinarity were discussed. Fundamental characteristics of an interdisciplinary team include efficient coordination of tasks and choice of team leader. Strategies to address hurdles and advantages of interdisciplinarity were addressed in detail, including newly developing fields of research.

Samenvatting in het Nederlands

Deel I, De noodzaak van biomarkers, omvat twee overzichtsartikelen die het kader aangeven voor de huidige behandelstrategieën voor JIA en de beperkte beschikbaarheid van gevalideerde biomarkers. In Hoofdstuk 2, overzicht van de behandeling van JIA, worden concepten die de basis vormen voor behandelstrategieën, zoals 'window of opportunity', 'treat-to-target' en 'immunologische remissie' gedefinieerd en besproken. De huidige behandelstrategieën en richtlijnen worden samengevat, evenals hun relevantie voor gerichte, patiëntgerichte zorg. De systematische evaluatie in Hoofdstuk 3 werd uitgevoerd om het scala aan serum diagnostische of prognostische biomarkers te identificeren en de klinische toepasbaarheid ervan op basis van hun beschikbaarheid, meetbaarheid en validatieniveau voor SJIA. Niet geheel onverwacht was er voor de meeste biomarkers slechts beperkt bewijs voor hun huidige klinische toepasbaarheid. Er werd echter een scala aan markers geïdentificeerd die verder gevalideerd kunnen worden, waaronder: IL-6, IL-12, IL-18, osteoprotegerine, S100A12 en S100A8/A9.

In Deel II, De Ontdekking van Biomarkers, beschrijft Hoofdstuk 4 een translationeel, multiplatform onderzoek naar de ontdekking van biomarkers dat uitgevoerd werd om vernieuwende moleculaire handtekeningen te identificeren van patiënten met twee fenotypes van SJIA in vergelijking met patiënten met een infectie. Validering met de gerichte proteomische methode MRM bevestigde de resultaten van de onderzoeksstudie die uitgevoerd werd met LC-MS/MS. 91 % van de patiënten met SJIA werd correct geïdentificeerd als lijdend aan het systemische t.o.v. het polyarticulaire fenotype van SJIA . De verschillende moleculaire handtekeningen leverden verdere bewijzen voor het bifasische model van SJIA. Hoofdstuk 5 richt zich op de in vitro en in vivo karakterisering van neutrofiel secretoire activiteit bij patiënten met FMF. In vitro scheidden neutrofielen van M694V homozygote patiënten spontaan meer S100A12, IL-18 en caspase-1 uit ten opzichte van de gezonde controlegroep. In serum was er een correlatie tussen S100A12 concentraties en ziekteactiviteit en genotype, deze was het hoogst bij M694V homozygote patiënten. Bij patiënten waarbij de ziekte inactief was, waren zowel de concentraties van S100A12 als IL-18 hoger bij M694V-positieve patiënten t.o.v. M694V-negatieve patiënten, wat wijst op hogere niveaus van subklinische ziekteactiviteit.

In Deel III, Biomarker translatie, werd in Hoofdstuk 6 S100A12 serum gemeten bij patiënten die behandeld werden met methotrexaat of TNF-remmers (adalimumab of etanercept) bij de baseline en werd de follow-up en behandelingsrespons vastgelegd. Behandelingsresponders konden geïdentificeerd worden door hogere concentraties van S100A12 serum voor de behandeling. Hoofdstuk 7 beschrijft de rol die potentieel weggelegd is voor translationele medische professionals (TMP's), waarbij de focus ligt op hun rol in niet-traditionele onderzoekscentra, zoals niet-universitaire ziekenhuizen en in de eerstelijnszorg. Praktische overwegingen voor het uitbreiden van de participatie, het verbeteren van de samenwerking en aanpakken van uitdagingen waar TMP's mee geconfronteerd worden, worden besproken om methoden te identificeren om de samenwerking binnen de translationele geneeskunde te verbeteren. In Hoofdstuk 8 worden de mogelijkheden en uitdagingen om medische innovaties te bevorderen behandeld. Fundamentele kenmerken van een interdisciplinair team omvatten het efficiënt coördineren van taken en de keuze van een teamleider. Strategieën om obstakels aan te pakken en de voordelen van interdisciplinariteit worden in detail behandeld, met inbegrip van opkomende onderzoeksgebieden.

Discussion

Uncertainties still line the patient journey

Whilst the development of bDMARDs revolutionised the management of paediatric rheumatological diseases, they also led to new insights into disease pathogenesis and treatment targets. With good management, communication and patient/family education guided by an inter-disciplinary team, the best possible outcomes can be achieved. However, there still remains room for more effective and individualised diagnostic and therapeutic tools. From delayed diagnosis to differential diagnoses or treatment decisions, the patient's journey is lined with uncertainty and difficult decisions – for both patients and clinicians. Involvement of patients and their families could do much to direct the research towards addressing this. Patient orientated research is rightly receiving more interest in recent years, with multiple studies investigating how patient engagement both in the design phase as well as downstream can be best achieved.(1–4) In this thesis entitled Biomarker need in paediatric rheumatology: from discovery to clinical translation, the central theme is biomarker research, which can both lead to a deeper understanding of the molecular basis of paediatric rheumatological disorders as well as help direct individualised management decisions. Reoccurring themes included the need for improved diagnostic and classification criteria, as well as a need for new patient and immunologically relevant definitions of disease remission. A selection of these themes pertinent to the future interdisciplinary management of paediatric rheumatology will be discussed in this chapter.

Rethinking diagnostic and classification criteria

A need for updated criteria

Limitations and criticisms of the initial and revised ILAR classification of JIA have existed since its conception. Major criticisms focus on the subgrouping of patients according to the number of joints involved at disease onset and the lack of inclusion of newer immunological data which have increased calls for further revisions or new criteria.(5) ILAR-criteria were originally defined for use in a research setting, but also have clinical consequences including that therapy options may be limited according to the assigned diagnosis.(5–9) An international group is working within the Pediatric Rheumatology International Trials Organisation (PRINTO) group on a 4-step initiative to achieve an evidence-based validated JIA Classification consensus.(8) Recent insights

into the underlying causes and pathology of JIA have moved the question further to the fore, with new criteria expected to be built on new clinical and molecular knowledge and data, such as response to medication, disease progression, pathogenesis and relevant biomarkers.(7,10) For example, as described in the introduction and seen in Chapters 4 and 5, SJIA is classified as an autoinflammatory condition and has more in common with FMF than with other subtypes of JIA. SJIA is associated with inflammasome activation, with oversecretion of IL-18, IL-1 and S100A8/A9 and S100A12 proteins. Additionally, there are also significant variations in clinical and molecular phenotypes within the SJIA subtypes. In Chapter 4, despite fulfilling ILAR criteria for diagnosis, patients with the clinical 'classical' autoinflammatory systemic phenotype had a different molecular subphenotype to those patients with an 'articular-dominant' polyarthritis.

New candidates for inclusion

The main limitation to the inclusion of new markers to an updated diagnostic classification has been the persisting lack of validated biomarkers and molecular profiles for a precision medicine management strategy. Much of this thesis has centered around the testing of serum biomarkers, in particular the S100 proteins. Patients with SJIA and FMF have markedly higher serum levels of S100A8/A9 and S100A12 compared to other autoinflammatory and fever syndromes. Several studies have shown S100 levels to outperform other existing biomarkers for the diagnosis of SJIA and FMF.(11–13) Chapter 4 additionally identified S100A12 as correlating with FMF genotype and disease activity.(14)

Both S100A8/A9 and S100A12 have also shown themselves to be useful markers of subclinical inflammation and therefore for monitoring disease activity, inflammation and response to medication.(13,15,16) Chapter 5 found that patients with JIA who responded to anti-TNF or methotrexate therapy had higher baseline S100A12 concentrations.(17) This followed a prospective study and randomized control trial by Foell et al. who showed that normal S100A8/A9 (MRP8/14) serum concentration as well as clinical inactivity, could identify patients with JIA where methotrexate treatment could be safely withdrawal.(15,16) Since then, another prospective multi-center study, S100A8/A9 did not predict disease flare in the eight months after withdrawal of anti-TNF medication, whilst S100A12 had modest predictive value at

one, two and three month evaluation.(12) Barendregt et al. also showed no prediction of treatment response by MRP8/14 in their Dutch cohort.(18) A number of factors could account for these contrasting findings including, that medication was not withdrawn at inclusion, medication use was more mixed, composition of the JIA subtypes varied greatly as did the definitions for flare and relapse used, which likely accounts for the lack of reproducibility. Such inconsistencies have been argued as reasons for the continued exclusion of biomarkers from classification criteria until further evidence.
Since completion of this thesis, the measurement of S100A8/A9 has become a routine laboratory test performed in the University of Münster for the measurement of subclinical activity in patients with JIA before ending therapy and a useful test for the diagnosis of SJIA in cases of fever without focus.(19,20) Genome-wide association studies (GWAS) have indicated HLA profiles and gene loci which are associated with paediatric arthritis. Such genetic insights have only partially supported the grouping of JIA categories and therefore other factors are likely to play a bigger role.(21) The routine rheumatological markers HLA-B27 and rheumatoid factor are acknowledged as having clinical relevant diagnostic and prognostic features and help define subcategories of JIA in the ILAR classification (Chapter 1, Figure 1). ANA-positivity is not included in the criteria, though ANA-negativity has been associated with a different pattern of joint disease, including a greater frequency of shoulder and hip involvement, higher frequency of symmetrical disease and a greater cumulative number of affected joints over time with less frequent iridocyclitis compared to patients who are ANA-positive.(22,23)

Musculoskeletal ultrasound and immunological markers as biomarkers
Musculoskeletal ultrasound (MSUS) could also be included into future JIA diagnostic criteria and management standards. MSUS detects the involvement of more joints than clinical examination alone and therefore may be another method of identifying subclinical inflammation.(24) Features that are often overlooked in clinical examination are entheseal involvement and tenosynovitis. Ultrasound based studies have shown that in particularly complex joints like the ankle, tenosynovitis is significantly under-diagnosed by clinical examination alone.(24–27) However, before the possible inclusion of MSUS related findings into classification criteria, a scoring system to define disease activity and damage is required, for which international efforts are underway.(28–30)

A strategy of combining immunological biomarkers, high quality MSUS and clinical as well as patient-derived disease activity indicators is an area of interest for the future treat-to-target management of paediatric rheumatological disorders. Serum markers of angiogenesis, which have so far not been useful as clinical biomarkers of JIA disease activity, have shown promise in MSUS studies, showing correlation with vascular changes indicated by power doppler activity.(39,40) The serum biomarkers associated with JIA disease activity, S100A8/A9 (MRP8/14) and IL-6, described in Chapters 3, 4 and 6, have been shown to correlate with MSUS defined synovitis in studies in adults with rheumatoid arthritis.(41–43) Therefore, immunological markers which alone or in multi-marker panels are insensitive for subclinical activity, may show more promise when combined with MSUS within a multi-dimensional evaluation, though prospective studies are required (Figure 1).(44) The relevance of MSUS in clinical evaluation and management is therefore increasingly important.

An individualised approach

Patient and public involvement in research

A fundamental pillar of patient-centric management is the involvement of patients (and families) in all aspects of treatment from defining standards of care, to research design and treatment choices. While major shifts in the right direction have occurred, for example with translational medicine driving more clinically focused projects and many funding opportunities being tied to public and patient involvement, this is not always routinely and fully integrated into research processes. In Chapter 7 the role of the translational medical professional (TMP) describes the role clinical researchers can play in prioritising patient-centered research, whether by improving patient involvement or in collaborative efforts. The focus of Chapter 8 was to discuss implementation and optimisation of research methodology, focusing on interdisciplinary working to optimise the impact of research.

Biobanking of patient samples

As paediatric rheumatological diseases are rare, collaboration and innovation is of particular importance. In this thesis, these aspects were key in sample collection and methodology. Chapters 4, 5 and 6 all used samples from several biobanks, illustrating the importance of multi-centre studies to obtain statistically valid sample numbers, and as validation cohorts. In Chapter 3,

multiple primarily single-centre studies identified multiple biomarkers for the diagnosis, disease activity and risk of macrophage-activation syndrome in SJIA, but also revealed a significant lack of success in biomarkers reaching validation and clinical use. Technological developments have led to improvements in the sensitivity and reproducibility of immune assays as well as the ability to perform high throughput analyses with ever decreasing sample volumes. New methods of validation of biomarkers might include using more statistical modelling, or modifying methods or the way validation is defined, however, combined and specific biobanks currently remain vital for performing many biomarker studies.

Patient orientated models of management

Standards can be defined as statements of the minimal quality of care that patients should receive, usually agreed by experts in the field on a local, national or international level. In Chapter 2, consensus statements, guidelines and standards of care in JIA were discussed in detail, alongside evolving strategies to personalize or individualize therapy. An aspect of management which requires more attention are the biopsychosocial aspects of rheumatic diseases. This includes patients' health beliefs, social aspects such as school attendance and sport participation, as well as the general well-being and the psychological impact of chronic disease and pain. The paediatric rheumatology multi-disciplinary team comprising of physiotherapists, occupational therapists, orthotic specialists, psychologists and clinicians are well placed to integrate these aspects into management during clinical care. However, it could be argued, that the majority of work in defining standards of care and aims of management have focused predominantly on clinically measurable outcomes rather than on the biopsychosocial goals of treatment.

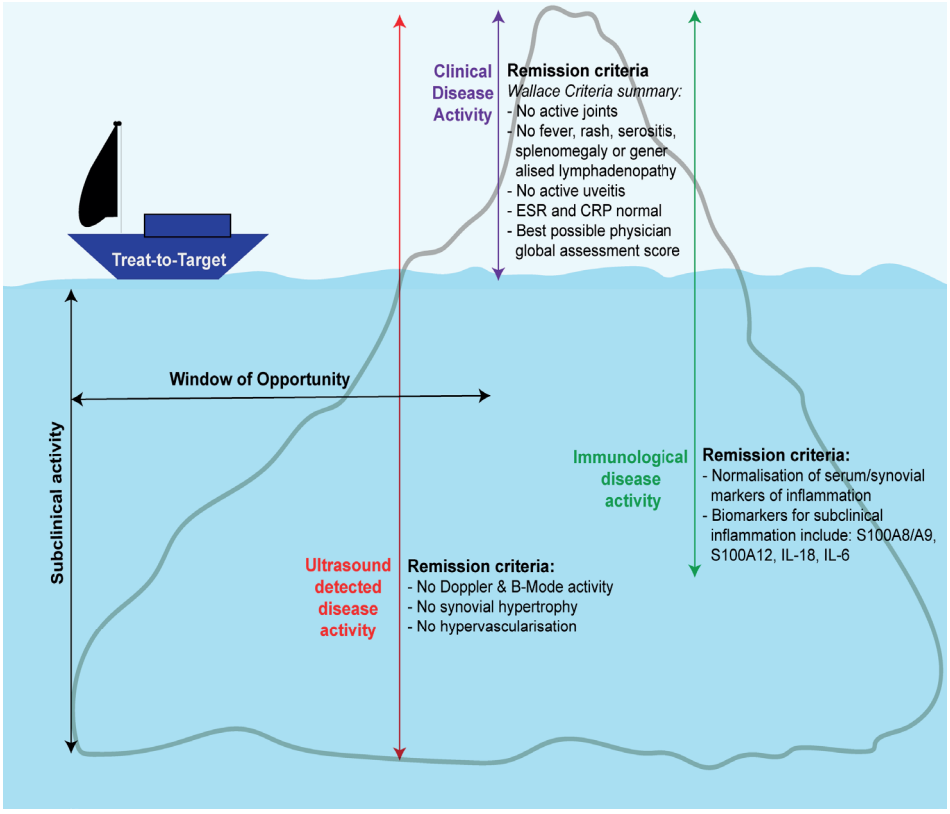
Colchicine non-compliance and resistance

In Chapter 5, elevated levels of IL-18 and S100A12 were measured in patients with FMF in clinically inactive as well as active disease. The study was not a prospective longitudinal study, and did not test if treating immunologically detected subclinical disease could reduce the risk of disease complications. Colchicine is the primary treatment for FMF and is almost always recommended as a lifelong treatment. Though generally well tolerated, colchicine side effects include diarrhoea, nausea, cytopenia and neuromyopathy. The starting dose is titrated until clinical symptoms and laboratory markers

of inflammation are controlled. The European League Against Rheumatism (EULAR) Guideline for the management of FMF recommends a six monthly assessment of colchicine response, toxicity and compliance.(45,46) Knieper et al. investigated parameters predicting need for colchicine dose increase were male gender, early age at diagnosis and the presence of an M694V genotype. The study also demonstrated a role for S100 proteins to guide colchicine dose increases.(47) Non-response to colchicine has been reported to be around 15 %. Colchicine non-compliance has been reported in up to 40 % of patients in FMF cohorts and is associated with more frequent proteinuria and higher SAA concentrations.(48,49) Therefore non-compliance in FMF has potentially serious and silent disease consequences if not addressed. Patient education is central to improving compliance. However, in cases of colchicine resistance, intolerance or persistent non-compliance despite intervention, IL-1 inhibitors (e.g. anakinra or canakinumab) are effective therapy options which should be considered.(50)

Figure 1: The iceberg model of subclinical disease adapted to show the role of ultrasound and immunological markers in the Treat-to-Target management of juvenile idiopathic arthritis

(adapted from Gohar F, Windschall D. The new role of musculoskeletal ultrasound in the treat-to-target management of juvenile idiopathic arthritis. Rheumatology (Oxford). 2021 Jan 25)



References

1. van Dijkhuizen, E. H. P. et al. Patient's experiences with the care for juvenile idiopathic arthritis across Europe. Pediatr. Rheumatol. 16, 10 (2018).
2. van der Scheer, L., Garcia, E., van der Laan, A. L., van der Burg, S. & Boenink, M. The Benefits of Pa-tient Involvement for Translational Research. Heal. Care Anal. 25, 225–241 (2017).
3. Ocloo, J. & Matthews, R. From tokenism to empowerment: Progressing patient and public involve-ment in healthcare improvement. BMJ Qual. Saf. 25, 626–632 (2016).
4. Maccarthy, J., Guerin, S., Wilson, A. G. & Dorris, E. R. Facilitating public and patient involvement in basic and preclinical health research. PLoS One 14, 1–16 (2019).
5. Martini, A. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. Ann. Rheum. Dis. 71, 1437 LP – 1439 (2012).
6. Nigrovic, P. A. Autoinflammation and autoimmunity in systemic juvenile idiopathic arthritis. Proc. Natl. Acad. Sci. 112, 15785–15786 (2015).
7. Beukelman, T. & Nigrovic, P. A. Juvenile idiopathic arthritis: An idea whose time has gone? J. Rheu-matol. 46, 124–126 (2019).
8. Martini, A. et al. Toward new classification criteria for juvenile idiopathic arthritis: First steps, pedi-atric rheumatology international trials organization international consensus. J. Rheumatol. 46, 190–197 (2019).
9. Rosenberg, A. M. Do we need a new classification of juvenile idiopathic arthritis? Clin. Immunol. 211, 108298 (2020).
10. Rumsey, D. G. & Laxer, R. M. The Challenges and Opportunities of Classifying Childhood Arthritis. Curr. Rheumatol. Rep. 22, (2020).
11. Wittkowski, H. et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum. 58, 3924–31 (2008).
12. Aljaberi, N. et al. The use of S100 proteins testing in juvenile idiopathic arthritis and autoinflamma-tory diseases in a pediatric clinical setting: A retrospective analysis. Pediatr. Rheumatol. 18, 1–6 (2020).
13. Kallinich, T., Wittkowski, H., Keitzer, R., Roth, J. & Foell, D. Neutrophil-derived S100A12 as novel bi-omarker of inflammation in familial Mediterranean fever. Ann. Rheum. Dis. 69, 677–82 (2010).
14. Gohar, F. et al. Correlation of Secretory Activity of Neutrophils With Genotype in Patients With Fa-milial Mediterranean Fever. Arthritis Rheumatol. 68, 3010–3022 (2016).
15. Foell, D. et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remis-sion: a randomized clinical trial. JAMA 303, 1266–73 (2010).
16. Foell, D. Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? Ann. Rheum. Dis. 63, 206–208 (2004).
17. Gohar, F. et al. S100A12 Is Associated with Response to Therapy in Juvenile Idiopathic Arthritis. J Rheumatol. 45, 547–554 (2019).
18. Barendregt, A. M. et al. MRP8/14 and neutrophil elastase for predicting treatment response and occurrence of flare in patients with juvenile idiopathic arthritis. Rheumatology 59, 2392–2401 (2020).
19. Frosch, M. et al. Myeloid-related proteins 8 and 14 are specifically secreted

during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum. 43, 628–37 (2000).
20. Wittkowski, H., Kallinich, T., Keitzer, R., Roth, J. & Foell, D. The endogenous TLR-4 ligands MRP8/14 as biomarkers of inflammation in Familial Mediterranean Fever (FMF). Pediatr. Rheumatol. 6, P208 (2008).
21. Moncrieffe, H. et al. Generation of novel pharmacogenomic candidates in response to methotrexate in juvenile idiopathic arthritis: correlation between gene expression and genotype. Pharmacogenet. Genomics 20, 665–76 (2010).
22. Ravelli, A. et al. Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. Arthritis Rheum. 52, 826–32 (2005).
23. Nordal, E., Songstad, N. T. & Rygg, M. Difficulties in defining antinuclear antibody-positive patients as a separate category in the classification of juvenile idiopathic arthritis: Comment on the article by Ravelli et al. Arthritis Rheum. 63, 2835–2835 (2011).
24. Guzman, J., Oen, K. & Loughin, T. Predicting disease severity and remission in juvenile idiopathic ar-thritis: Are we getting closer? Curr. Opin. Rheumatol. 31, 436–449 (2019).
25. Jousse-Joulin, S. et al. Ultrasonography for detecting enthesitis in juvenile idiopathic arthritis. Arthri-tis Care Res. 63, 849–855 (2011).
26. Pascoli, L., Wright, S., McAllister, C. & Rooney, M. Prospective Evaluation of Clinical and Ultrasound Findings in Ankle Disease in Juvenile Idiopathic Arthritis: Importance of Ankle Ultrasound. J. Rheu-matol. 37, 2409 LP – 2414 (2010).
27. Rooney, M. E., McAllister, C. & Burns, J. F. T. Ankle Disease in Juvenile Idiopathic Arthritis: Ultra-sound Findings in Clinically Swollen Ankles. J. Rheumatol. 36, 1725 LP – 1729 (2009).
28. Collado, P. et al. Amendment of the OMERACT Ultrasound Definitions of Joints' Features in Healthy Children When Using the Doppler Technique. Pediatr. Rheumatol. Online J. 16, 23 (2018).
29. Weiss, P. F. et al. Detection of enthesitis in children with Enthesitis-related arthritis: dolorimeter ex-amination compared to ultrasonography. Arthritis Rheumatol. 66, 218–227 (2014).
30. Windschall, D. et al. Pediatric musculoskeletal ultrasound: age- and sex-related normal B-mode find-ings of the knee. Rheumatol. Int. 36, 1569–1577 (2016).
31. Malattia, C., Rinaldi, M. & Martini, A. The role of imaging in juvenile idiopathic arthritis. Clin. Immu-nol. 14, 681–694 (2018).
32. Chauvin, N. A. & Khwaja, A. Imaging of Inflammatory Arthritis in Children: Status and Perspectives on the Use of Ultrasound, Radiographs, and Magnetic Resonance Imaging. Rheum. Dis. Clin. North Am. 42, 587–606 (2016).
33. Rebollo-Polo, M. et al. Ultrasound findings on patients with juvenile idiopathic arthritis in clinical remission. Arthritis Care Res. 63, 1013–1019 (2011).
34. Damasio, M., Malattia, C., Martini, A. & Toma, P. Synovial and inflammatory diseases in childhood: role of new imaging modalities in the assessment of patients with juvenile idiopathic arthritis. Pedi-atr Radiol. 40, 985–998 (2010).
35. Roth, J. et al. Preliminary Definitions

for the Sonographic Features of Synovitis in Children. Arthritis Care Res. 69, 1217–1223 (2017).
36. Collado, P. et al. Toward Standardized Musculoskeletal Ultrasound in Pediatric Rheumatology: Nor-mal Age-Related Ultrasound Findings. Arthritis Care Res. 68, 348–356 (2016).
37. Roth, J. et al. Definitions for the sonographic features of joints in healthy children. Arthritis Care Res. 67, 136–142 (2015).
38. Lanni, S. et al. Ultrasound changes in synovial abnormalities induced by treatment in juvenile idio-pathic arthritis. Clin. Exp. Rheumatol. 36, 329–334 (2018).
39. Misra, S. et al. Association of Angiogenic and Inflammatory Markers with Power Doppler Ultrasound Vascularity Grade and DAS28-CRP in Early Rheumatoid Arthritis: A Comparative Analysis. Biomed Res. Int. 2018, (2018).
40. Ramírez, J. et al. Patients with rheumatoid arthritis in clinical remission and ultrasound-defined ac-tive synovitis exhibit higher disease activity and increased serum levels of angiogenic biomarkers. Arthritis Res. Ther. 16, (2014).
41. Gullick, N. J. et al. Linking power doppler ultrasound to the presence of Th17 cells in the rheumatoid arthritis joint. PLoS One 5, 1–11 (2010).
42. do Prado, A. D. et al. Ultrasound power Doppler synovitis is associated with plasma IL-6 in estab-lished rheumatoid arthritis. Cytokine 83, 27–32 (2016).
43. Kang, K. Y., Woo, J. W. & Park, S. H. S100A8/A9 as a biomarker for synovial inflammation and joint damage in patients with rheumatoid arthritis. Korean J. Intern. Med. 29, 12–19 (2014).
44. Gohar, F. & Windschall, D. The new role of musculoskeletal ultrasound in the treat-to-target man-agement of juvenile idiopathic arthritis. Rheumatology (2021). doi:10.1093/rheumatology/keab004
45. Ozen, S. et al. EULAR recommendations for the management of familial Mediterranean fever. Ann. Rheum. Dis. 75, 644–651 (2016).
46. Lidar, M. et al. Incomplete response to colchicine in M694V homozygote FMF patients. Autoimmun. Rev. 12, 72–6 (2012).
47. Knieper, A. M. et al. Familial Mediterranean fever in children and adolescents: Factors for colchicine dosage and predicting parameters for dose increase. Rheumatol. (United Kingdom) 56, 1597–1606 (2017).
48. Zemer, D. et al. Colchicine in the Prevention and Treatment of the Amyloidosis of Familial Mediter-ranean Fever. N. Engl. J. Med. 314, 1001–1005 (1986).
49. Berkun, Y. et al. A Single Testing of Serum Amyloid A Levels as a Tool for Diagnosis and Treatment Dilemmas in Familial Mediterranean Fever. Semin. Arthritis Rheum. 37, 182–188 (2007).
50. De Benedetti, F. et al. Canakinumab for the treatment of autoinflammatory recurrent fever syn-dromes. N. Engl. J. Med. 378, 1908–1919 (2018).

10

572961-L-bw-PS4U
Processed on: 25-1-2022

CHAPTER 10

Appendix

i List of abbreviations
ii Co-author affiliations
iii Curriculum vitae
iv List of publications
v Acknowledgements

572961-L-bw-PS4U
Processed on: 25-1-2022

List of abbreviations

ACPA	Anti-citrullinated protein antibodies
A1AG1 / AGP1	Alpha-1-acid glycoprotein
A2GL	Leucine-rich alpha-2-glycoprotein
A2M / A2GL	Alpha-2-macroglobulin
AACT	Alpha-1-antichymotrypsin
ABC Register	Arthritis and Biologicals in Children Register, the Netherlands
ACAN	Aggrecan core protein cartilage-specific core protein
ACCP	Anti-cyclic citrullinated peptide
ACR	American College of Rheumatology
ACRpedi50	American College of Rheumatology Pediatric 50% criteria for response
ACT	Alpha-1-antichymotrypsin
AD	Active disease
ADA	Adalimumab
AECA	Anti-endothelial cell antibodies
AID-net	Auto-Inflammatory Disease network registry
ANA	Antinuclear antibodies
Anti-BiP	Anti-immunoglobulin binding protein/glucose regulated protein 78 (GRP78)
Anti-TNF	Anti-tumour necrosis factor
AOSD	Adult-onset Still's disease
APO	Apolipoprotein
APRIL	A proliferation-inducing ligand
AUC	Area under the curve
B2M	Beta -2-microglobulin
BAFF	B-cell activating factor
bDMARD	Biological disease-modifying antirheumatic drug
BIKeR	Register for Biologics in Paediatric Rheumatology
BMS	Biomarker scoring system
BPS	Biopsychosocial model
CAHS	Canadian Academy of Health Sciences
CAPS	Cryopyrin-associated autoinflammatory syndromes;
CARRA	Childhood Arthritis and Rheumatology Research Alliance
cDMARD	Conventional disease-modifying antirheumatic drug
CHAQ	Childhood assessment questionnaire
CHARMS	Childhood Arthritis Response to Medication Study, UK
CID	Clinically inactive disease
CLARITY	Childhood Arthritis Risk factor Identification Study
COMP	Cartilage oligomeric matrix protein
Comp het	Compound heterozygous
CRCs	Clinical research centres

572961-L-bw-PS4U
Processed on: 25-1-2022

CRP	C-reactive protein
CRUs	Clinical research units
CTSA	Clinical and Translational Science Awards
CTUs	Clinical trial units
CVD	Collagen vascular disorders (SLE/scleroderma)
DAMP	Damage associated molecular pattern
DAS28	Disease Activity Score in 28 joints
DMARD	Disease modifying arthritis drugs
E oJIA	Extended oligoarticular juvenile idiopathic arthritis
EBV-HLH	Epstein-Barr virus haemophagocytic lymphohistiocytosis
ECRIN	European clinical research infrastructures network
ELISA	Enzyme-linked immunosorbent assay
EMA	European medicines agency
EMA	European medicines agency
E-oJIA	Extended oligoarticular JIA
ERA	Enthesitis-related arthritis
ERA	Enthesitis-related arthritis
ERC	European research council
ESR	Erythrocyte sedimentation rate
ETN	Etanercept
EULAR	European league against rheumatism
EUTRAIN	European Translational Training for Autoimmunity& Immune manipulation Network
FHL	Familial hemophagocytic lymphohistiocytosis
FMF	Familial Mediterranean fever
FSTL-1	Follistatin-like protein 1
GELS / GSN	Gelsolin
HC	Healthy controls
Het	Heterozygous
HO-1	Heme oxygenase-1
Hom	Homozygous
ID	Inactive disease
IFN	Interferon
IL1	Interleukin-1
IL1a	Interleukin 1apha
IL1RA	IL 1 receptor antagonist
IL-2R	Interleukin 2 receptor
IL6	Interleukin 6
IL8	Interleukin 8
IL10	Interleukin 10
IL18	Interleukin 18
IL18BP	Interleukin-18 binding protein
IL20	Interleukin 20
IL21	Interleukin 21
IL23	Interleukin 23

ILAR	International League of Associations for Rheumatology
IQR	Interquartile range
JADAS-10	Juvenile arthritis disease activity score
JIA	Juvenile idiopathic arthritis (non-systemic)
KD	Kawasaki disease
KLKB1	Kininogenin
LC-MS/MS	Label-free liquid mass spectrometry
LC-MS/MS	Label-free liquid chromatography mass spectrometry
LDH	Lactate dehydrogenase
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
MAS	Macrophage activation syndrome
MBDA	Multibiomarker assessment of disease activity
MCP-2	Monocyte chemotactic protein 2
M-CSF	Macrophage colony stimulating factor
MEFV	Familial Mediterranean fever gene, pyrin-encoded
MIG	Monokine induced by gamma interferon
MMP	Matrix metalloproteinase
MOES	Moesin
MRM	Multiple reaction monitoring
MRP8/14	Myeloid related protein 8/14
MS	Mass spectrometry
MTCD	Mixed connective tissue disease
MTT	Multidisciplinary translational teams
MTX	Methotrexate
NIH	National Institutes of Health
NPV	Negative predictive value
NTRCs	Non-traditional research centres
Ns	Non-significant
NSAID	Non-steroidal anti-inflammatory drugs
NSF	National science foundation
OA	Oligoarthritis
oJIA	Oligoarticular juvenile idiopathic arthritis
ONP	Osteopontin
OPG	Osteoprotegerin
OSA	Osteoarthritis
PA	Polyarthritis
PBMC	Peripheral blood mononuclear cells
PGA	Physicians global assessment
pJIA	Polyarticular juvenile idiopathic arthritis
pJIA	Polyarticular JIA
PPV	Positive predictive value
PRCSG	Pediatric rheumatology collaborative study group
PRINTO	Paediatric Rheumatology International trials Organisation
PsA	Psoriatic arthritis

RA	Rheumatoid arthritis
RCTs	Randomised control trials
ReACCH-Out	Research in Arthritis in Canadian Children emphasising Outcomes
RF	Rheumatoid factor
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
S100A12	S100 calcium-binding protein A12
S100A8/A9	S100 calcium-binding protein A8/A9 (MRP8/14)
SAA	Serum amyloid A
SAP	Serum amyloid P
sCD163	Soluble Cluster of Differentiation 163
SciTS	Science of team science
SD	Subclinical disease
SHBG	Sex hormone-binding globulin
sICAM-1	Soluble intracellular adhesion molecule-1
SJIA	Systemic juvenile idiopathic arthritis, or Still's Disease
SJIA syst	Classical auto-inflammatory SJIA
SJIA-MAS	MAS in patients with SJIA
SJIApoly	Chronic articular-dominant SJIA
SLE	Systemic lupus erythematosus
SRM	Selected reaction monitoring
sST2	Soluble ST2/IL-1 receptor-like 1
STRING	Search tool for the retrieval of interacting genes/proteins platform
T2T	Treat to Target
TIMP	Tissue inhibitors of metalloproteinase
TLR4-ligand	Toll-like receptor 4 ligand
TM	Translational medicine
TMP	Translational medical professionals
TNF	Tumour necrosis factor
TNFi	Tumour necrosis factor inhibitor
TSLP	Thymic stromal lymphopoietin
TTr	Transthyretin
TWEAK	TNF-like weak inducer of apoptosis
VA-HLH	Virus-associated haemophagocytic lymphohistiocytosis
VAS	Visual analog scale
vs	Versus
WCC	White cell count
WHO	World health organisation

Co-author affiliations

Anink, Janneke
Department of Pediatrics/Pediatric Rheumatology, Erasmus MC Sophia Children's Hospital Rotterdam, The Netherlands

Callan, Niamh
UCD Conway Institute of Biomolecular and Biomedical Research, School of Medicine, University College Dublin, Dublin, Ireland.

de Roock, Sytze
University Medical Center Utrecht, Wilhelmina Children's Hospital and Utrecht University, Utrecht, The Netherlands

Debus, Otfried
Department of Paediatrics, Clemenshospital, Münster, Germany

Dolman, Koert M.
Department of Pediatrics/Pediatric Rheumatology, Onze Lieve Vrouwe Gasthuis, The Netherlands

Dressler, Frank
Hannover Medical School, Hannover, Germany

FitzGerald, Oliver
1) UCD Conway Institute of Biomolecular and Biomedical Research, School of Medicine, University College Dublin, Dublin, Ireland.2) Department of Rheumatology, St Vincent's University Hospital, Dublin, Ireland

Foell, Dirk
Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany

Frosch, Michael
German Pediatric Pain Centre, Children's and Adolescents' Hospital

Giese, Arnd
St. Josef-Hospital, Ruhr University, Bochum, Germany

572961-L-bw-PS4U
Processed on: 25-1-2022

Gohar, Aisha
Department of Experimental Cardiology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

Haas, Johannes-Peter
German Centre for Child and Adolescent Rheumatology, Garmisch-Partenkirchen, Germany

Hernandez, Belinda
TILDA (The Irish Longitudinal Study on Ageing), Trinity College Dublin, Ireland

Hinze, Claas
Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany

Holzinger, Dirk
1)Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany. 2) Klinik für Kinderheilkunde III, Zentrum für Kinder- und Jugendmedizin, Universitätsklinikum Essen

Hoppenreijs, Esther P.A.H.
Department of Pediatrics/Pediatric Rheumatology, St. Maartenskliniek and Radboud University Medical Centre, Nijmegen, The Netherlands

Horneff, Gerd
Centre of Pediatric Rheumatology, Department of General Pediatrics, Asklepios Clinic Sankt Augustin;

Hülskamp, Georg
Department of Paediatrics, Clemenshospital, Münster, Germany

Jeske, Marion
Paediatric Rheumatology, University of Duisburg-Essen, Essen, Germany

Jones, Melissa
UCD Conway Institute of Biomolecular and Biomedical Research, School of Medicine, University College Dublin, Dublin, Ireland.

Kallinich, Tilmann
Department of Paediatric Pneumology and Immunology, Charité University Medicine, Berlin, Germany

Kessel, Christoph
Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany

Lainka, Elke
Department of Paediatrics, University Hospital Essen, Essen, Germany

Lavric, Miha
1)Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany. 2) 2Department Industrial Engineering and Business, Information Systems, University of Twente, Enschede, The Netherlands

Lemaire, Mathieu
Department of Paediatrics, Division of Nephrology, The Hospital for Sick Children, University of Toronto, Toronto, Canada

Lieber, Mareike
Department of Paediatric Pneumology and Immunology, Charité University Medicine, Berlin, Germany

Lohse, Peter
Center for Genomics and Transcriptomics, Tuebingen, Germany

Maschmeyer, Patrick
Therapeutic Gene Regulation, German Rheumatism Research Center (DRFZ) Berlin, Berlin, Germany

Masjosthusmann, Katja
Department of General Paediatrics, University Children's Hospital Münster, Germany

McArdle, Angela
UCD Conway Institute of Biomolecular and Biomedical Research, School of Medicine, University College Dublin, Dublin, Ireland.

Miranda-Garcia, Maria
1)Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany. 2)Paul Ehrlich Institute, Langen, Germany

Mfarrej, Bechara
Institut Paoli-Calmettes, Marseille, France

Moncrieffe, Halima
Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, and Department of Pediatrics, University of Cincinnati, USA

Neudorf, Ulrich
Paediatric Rheumatology, University of Duisburg-Essen, Essen, Germany

Orak, Banu
Department of Paediatric Pneumology and Immunology, Charité University Medicine, Berlin, Germany

Pennington, Stephen R.
UCD Conway Institute of Biomolecular and Biomedical Research, School of Medicine, University College Dublin, Dublin, Ireland.

Pretzer, Carolin
Department of Paediatric Rheumatology and Immunology, University of Muenster, Muenster, Germany

Prince, Femke H.M.
Department of Pediatrics/Pediatric Rheumatology, Erasmus MC Sophia Children's Hospital Rotterdam, The Netherlands

Roncarolo, Maria Grazia
Institute for Stem Cell Biology and Regenerative Medicine. Stanford University, USA

ten Cate, Rebecca
Leiden University Medical Centre, The Netherlands

Ursu, Simona
School of Biological Sciences, Royal Holloway, University of London, UK

572961-L-bw-PS4U
Processed on: 25-1-2022

van Rossum, Marion A.J.
Emma Children's Hospital, Academic Medical Centre and Amsterdam Rheumatology and Immunology Centre, Reade location, Jan van Breemen Institute, The Netherlands

van Royen-Kerkhof, Annet
Department of Pediatric Immunology and Rheumatology, Wilhelmina Children's Hospital Utrecht, the Netherlands

Van Suijlekom-Smit, Lisette W.A.
Department of Pediatrics/Pediatric Rheumatology, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands

Vastert, Sebastiaan J.
University Medical Center Utrecht, Wilhelmina Children's Hospital and Utrecht University, Utrecht, The Netherlands

von Bernuth, Horst
1) Department of Paediatric Pneumology and Immunology, Charité University Medicine, Berlin, 2) Department of Immunology, Labor Berlin, Charité Vivantes GmbH; Berlin Center for Regenerative Therapies (BRCT), Charité University Medicine; Sozialpädiatrisches Zentrum, Charité University Medicine.

Wedderburn, Lucy R.
Infection, Immunity, Inflammation Programme, UCL GOS Institute of Child Health, UCL

Weinhage, Toni
Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany

Weissbarth-Riedel, Elisabeth
University Hospital Hamburg-Eppendorf, Hamburg, Germany

Wittkowski, Helmut
Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Germany

Curriculum vitae

Name	Faekah Gohar
E-Mail	faekahgohar@hotmail.com
Birthdate	9. Juni 1984
Place of Birth	England
Nationality	English and German

Paediatric and rheumatology clinical training

01/2020-	Assistenzärztin: St. Josef-Stift, Sendenhorst: Paediatric rheumatology
03/2017-12/2019	Assistenzärztin: Clemenshospital, Münster: Paediatrics
02/2012-11/2012	Countess of Chester Hospital: Paediatrics & Neonatology
08/2011-02/2012	Alder Hey Children's Hospital, Liverpool: Paediatric rheumatology
02/2011-05/2011	Liverpool Women's Hospital, Liverpool: Neonatology
08/2010-02/2011	Alder Hey Children's Hospital: A&E and endocrinology
08/2010-12/2012	Alder Hey Children's Hospital: Academic Clinical Fellowship (Paediatric rheumatology)
08/2008-08/2010	Wigan Infirmary, 4 mth placements in Breast Surgery, Paediatrics, Obstetrics & Gynaecology, Geriatrics, Orthopoedics and General Practice.

Academic training and titles

12/2012-2016	Marie Curie Fellow, Early Stage Researcher, EUTRAIN (EUropean Translational tRaining for Autoimmunity & Immune manipulation Network)
09/11/2019	Fachärztin für Kinder- und Jugendmedizin
25/11/2016	Ärztliche Approbation, Bezirksregierung Münster
09/2002-07/2008	Bachelor of Medicine and Bachelor of Surgery (MBChB), Universität Birmingham
09/2005-07/2006	Bachelor of Medical Science (BMedSc): Public Health & Epidemiology, Universität Birmingham

List of publications

Gohar F, Windschall D. Gelenksonografie im Treat-to-Target-Konzept bei der juvenilen idiopathischen Arthritis. Arthritis und Rheuma. 2021;41(01):2046-2053.

Gohar F, Windschall D. The new role of musculoskeletal ultrasound in the treat-to-target management of juvenile idiopathic arthritis. Rheumatology. 2021;60:2046-2053.

Gohar F, McArdle A, Jones M, Callan N, Hernández B, Kessel C, Miranda-Garcia M, Lavric M, Holzinger D, Pretzer C, Lainka E, Vastert SJ, de Roock S, FitzGerald O, Pennington SR and Foell D. Molecular characterization of different inflammatory phenotypes of systemic juvenile idiopathic arthritis. Ann Rheum Dis. 2019;78:1107-1113.

Gohar F, Maschmeyer P, Mfarrej B, Lemaire M, Wedderburn LR, Roncarolo MG and van Royen-Kerkhof A (2019) Driving Medical Innovation Through Interdisciplinarity: Unique Opportunities and Challenges. Front. Med. 2019: 6:35.

Gohar F, Gohar A, Hülskamp G and Debus O. The Translational Medicine Professional: A bridge between bench and bedside? Front. Med. 2018: 5:294.

Gohar F, Anink J, Moncrieffe H, Van Sijlekom-Smit LWA, Prince FHM, van Rossum MAJ, Dolman KM, Hoppenreijs EPAH, ten Cate R, Ursu S, Wedderburn LR, Horneff G, Frosch M, Foell D and Holzinger D. S100A12 is associated with response to therapy in Juvenile Idiopathic Arthritis. J of Rheum 2018:45:3.

Brückner M, Heidemann J, Nowacki TM, Cordes F, Stypmann J, Lenz P, **Gohar F**, Lügering A, Bettenworth D. Detection and Characterization of Murine Colitis and carcinogenesis by Molecularly Targeted Contrast-Enhanced Ultrasound. World J Gastroenterol 2017;23(16):2899-2911.

Gohar F, Kessel C, Lavric M, Holzinger D and Foell D. Review of biomarkers in systemic juvenile idiopathic arthritis: Helpful tools or just playing tricks? Arthritis Research & Therapy 2016:18:163.

Gohar F, Orak B, Kallinich T, Jeske M, Lieber M, von Bernuth H, Giese A, Weissbarth-Riedel E, Haas JP, Dressler F, Holzinger D, Lohse P, Neudorf U, Lainka E, Hinze C, Masjosthusmann K, Kessel C, Weinhage T, Foell D and Wittkowski H. Correlation of secretory activity of neutrophils with genotype in Patients With Familial Mediterranean Fever. Arthritis and Rheumatology 2016:68(12):3010-3022.

Petrelli A, Prakken ABJ, Rosenblum ND and **EUtrain fellows**: Developing translational medicine professionals: the Marie Skłodowska-Curie action model. J Transl Med 2016: 14;329.

572961-L-bw-PS4U
Processed on: 25-1-2022

Ward T, Voss J, Yuwen W, Foll D, **Gohar F**, Ringold S. Sleep Fragmentation and Biomarkers in Juvenile Idiopathic Arthritis. Biol Res Nurs 2016: 18(3):299-306.

Brückner M, Lenz P, Mücke M, **Gohar F**, Willeke P, Domagk D and Bettenworth D. Diagnostic Imaging Advances in Murine Models of Colitis. World J Gastroenterol. 2016:996-1007.

Anink J, van Suijlekom-Smit LWA, Otten MH, Prince FHM, van Rossum MAJ, Dolman KM, Hoppenreijs EPAH, Ten Cate R, Ursu S, Wedderburn LR, Horneff G, Frosch M, Vogl T, **Gohar F**, Foell D, Roth J, Holzinger D. MRP8/14 serum levels as a predictor of response to starting and stopping anti-TNF treatment in juvenile idiopathic arthritis. Arthritis Res Ther. 2015: 17(1):200.

Hinze C, **Gohar F**, Foell D. Management of juvenile idiopathic arthritis: Hitting the target. Nat Rev Rheumatol. 2015:11(5):290-300.

Giese A, Ornek A, Kurucay M, Kara K, Wittkowski H, **Gohar F**, Menge BA, Schmidt WE, Zeidler C. P Wave Dispersion and QT Dispersion in Adult Turkish Migrants with Familial Mediterranean Fever Living in Germany. Int J med sci. 2014: 11(11):1140-1146.

Rothmund F, Gerss J, Ruperto N, Däbritz J, Wittkowski H, Frosch M, Wulfraat N, Wedderburn LR, Holzinger H, **Gohar F,** SJ Vastert, Brik R, Deslandre CJ, Melo-Gomes JA, Magalhães CS, Barcellona R, Russo R, Gattorno M, Martini A, Roth J, Foell D. Validation of relapse risk biomarkers for routine use in patients with juvenile idiopathic arthritis. Arthritis Care & Res 2014: 66(6):949-55.

van Bon L, Cossu M, Loof A, **Gohar F**, Wittkowski H, Vonk M, Roth J, van den Berg W, van Heerde W, Broen JCA, Radstake TRDJ: Proteomic analysis of plasma identifies the Toll-like receptor agonists S100A8/A9 as a novel possible marker for systemic sclerosis phenotype. Ann Rheum Dis 2014; 73(8):1585-9.

Foell D, Wittkowski H, Kessel C, Lüken A, Weinhage T, Varga G, Vogl T, Wirth T, Viemann D, Björk P, van Zoelen MA, **Gohar F**, Srikrishna G, Kraft M, Roth J. Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4. Am J Respir Crit Care Med. 2013:15;187(12):1324-34.

Watson L, **Gohar F**, Beresford MW. Diagnosis and management of juvenile-onset SLE. Paediatrics and Child Health, 2011:21(12):539-545.

Gohar F. et al. Self-care and adherence to medication: a survey in the hypertension outpatient clinic.BMC Complement Alt Med. 2008 Feb 8;8:4.

572961-L-bw-PS4U
Processed on: 25-1-2022

Acknowledgements

There have been several pivotal time-points that led me into paediatric rheumatology. I would like to thank everyone who supported me. Several people at Birmingham Children's Hospital encouraged me into reserch during my university training: Dr Henderson, Dr Chakrapani and Dr Giessen in the Department of Inherited metabolic diseases, Dr Solanki, Department of Paediatric Neurosurgery and Professor Moss, Dermatology. I also need to thank the paediatric consultants in Wigan, Wrightington & Leigh: Dr Farrier, Dr Robinson, Dr Sharma, Dr Shenoy and Dr Vel. You all were instrumental in encouraging me to pursue a specialisation in paediatrics. I also thoroughly enjoyed my academic clinical fellowship time with the rheumatology team at Alder Hey. Without the support and supervision of Dr Beresford, I wouldn't have experienced laboratory research, which led me to making the move from Liverpool to Germany at the end of 2012 to join the Eutrain program.

A special thank-you goes to Dirk Foell and Berent Prakken. You have been so supportive of the whole process. I also wish to thank Christoph, Dirk H, Helmut and Claas for their supervision of projects and lab work. Working in Münster has been a fantastic experience. I have been extremely happy to be a part of your team and really felt part of a 'work family'. A special thank you of course to Melanie, Sabrina, Susanne and Toni for helping me learn lab skills, and also integrate in Germany.

I want to thank Berent, Bas, Dirk, Lucy and the rest of the Eutrain Board for the wonderful collaborative and innovative project you designed. Thanks also to all my fellow 'Eutrainers' who kept the job fun. I'm lucky to have made some life-long friends. A special thank you to Bechara and family for their friendship, and Patrick for helping me get started with the boardgaming.

For helping me have the confidence to make the move to Germany, I must thank the paediatric team at the Countess of Chester hospital, both consultants (Dr Brearey, Dr Gibbs, Dr Jayaram, Dr Jamal, Dr Newby and Dr Saladi) and fellow trainees who helped with my application and encouraged me to leave (!), as did several close friends, most notably Lisa, Nina and Amy (thank you so much for the push). The follow up support from Chester after I

left has also been amazing too, thank you for having m back in the years that followed. I was always amazed at the level of support and encouragement I received there.

Meinen großen Dank muss ich Dr. Debus und Dr. Hülskamp aussprechen, die mir die Möglichkeit eröffnet haben meine Weiterbildung zur Fachärztin für Kinder- und Jugendmedizin in Münster fortzusetzen. Es war eine herausfordernde, intensive Zeit und gleichzeitig großartige Erfahrung, die ich immer in guter Erinnerung behalten werde. Ich habe so viel von ihrem hoch motivierten und erfahrenen Team gelernt (Oberärzte Peter Küster, Holger Schültingkemper, Wolfgang Tebbe, Sonja Kampschulte, Martin Dercks und den Assistenten)! Danke Euch allen für die Unterstützung mein Deutsch und meine medizinische Arbeit zu verbessern. Das ganze Team – Ärzte, Krankenpflege und Sekretariat – war so viel hilfsbereiter, als ich es mir je hätte wünschen können. Letztlich bin ich in der Zeit bei Euch nicht nur zur Fachärztin für Kinder- und Jugendmedizin geworden, sondern habe das Clemenshospital Ende 2019 auch als deutsche Staatsbürgerin und verheiratete Frau verlassen – zwei Dinge die ich bei meinem Start am 13.03.2017 noch gar nicht ins Auge gefasst hatte!

Dankeschön an Gerd Ganser und Daniel Windschall, den ehemaligen und den aktuellen Chefarzt am St. Josef-Stift Sendenhorst, wo ich meine Zusatzweiterbildung im Bereich Kinder- und Jugendrheumatologie beginnen konnte. Ich fühle mich geehrt Ihre Unterstützung zu haben, sowie die Freiheit translationale klinische Forschung fortführen zu dürfen. Natürlich sind auch die klinischen Lernerfahrungen von unschätzbarem Wert für mich und ich hoffe es werden noch viele folgen.

Next, thank you to all my friends/substitute family all over Europe who have supported me and encouraged me in different ways. There are to many people too name, but I must mention the ‘Queen’s girlies’: Alex, Emma, Laura, Rachael and Lisa), for always being there; the ‘Birmingham people’ Jenny, Nina and Navpreet who always find time for me to visit when I contact them at the last minute; members of ‘team boob’ who helped me get through that first medical job, and the ‘LWH A-team’ who made a seriously hard job really fun.

Thank you to my ‘German learning’ friends, Mercedes and Jochem, who made the VHS classes (sometimes) worth attending, the ‘native english speakers’ who were my first support group in Münster. Thank you to all my former flatmates, and everyone who helped me move flat each time (and there were so many times!). Thank you to Melanie, Sabrina, Nadine, Janek, Manuel and Andreas for just being really great friends, from making sure I didn’t spend my first 'Silvester' alone to supporting my language learning. And thank you to Nikola, who was a the perfect last 'WG' flatmate. Living so happily in Germany wouldn’t have been possible without you all.

One of the greatest surprises of my PhD time was finding my husband during it. I couldn’t have completed the final part of this thesis process without your help and encouragement (and your front cover!). You have supported me through numerous difficult processes from German language exams, job searches, publication rejections, gaining double citizenship due to Brexit uncertainty, paediatric and then rheumatology examinations and now writing this thesis. Thanks for always being available for a game as well as proofreading my German. I am so fortunate to have your patience to make up for my own lack thereof. I also want to thank Bernhard, Bärbel, Carolin and Christian for their support of us and my work.

Finally, I thank my family. I am extremely lucky to have parents who appreciate the value of education and the importance of good health, and therefore always encouraged me to study further and help as many patients as possible. Your help in storing my many belongings, being on-call for the house and dealing with my UK post and problems has been immeasurable. I also specially thank my siblings, Aisha, Hannah and Hassan, for always keeping me on my toes, for example by usually beating me at scrabble with a ridiculous frequency of 7-letter bonus words (Hannah!). Aisha, you won the who-gets-the-PhD-first competition, and Hannah, you had to move even further away to Dubai. We are all so competitive, but I know this energetic spirit has helped us all achieve so much.