EMJ EUROPEAN MEDICAL JOURNAL

RHEUMATOLOGY

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EDITORIAL BOARD

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RHEUMATOLOGY 4.1
AUGUST 2017

Staff members attend medical congresses as reporters when required.

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Welcome

Hello, and a warm welcome to this year's edition of *EMJ Rheumatology*, which we hope proves an interesting and informative read, and piques the interest of all those involved in the rheumatology field. Herein, we provide a well-rounded summary of this summer's European League Against Rheumatism (EULAR) congress held in Madrid, Spain, which attracted >14,000 participants from 120 countries worldwide. This is then followed by a number of high-quality, peer-reviewed papers from prominent rheumatology figures.

The 4-day EULAR congress was held at the impressive IFEMA conference centre and covered a vast array of current, innovative topics during the 180 sessions. The congress also saw the launch of EULAR's campaign of: "Don't Delay, Connect Today!", which focussed on early recognition and treatment of rheumatic and musculoskeletal diseases. As expected, arthritis, in its many forms, featured heavily in the >3,500 abstracts. New drug classes for the treatment of psoriatic arthritis and the importance of nurse-lead vaccination programmes for vulnerable rheumatic patients are just two of the many informative topics covered in our Abstract Reviews section, as part of our congress coverage.

The eJournal also features inspiring interviews from four of our *EMJ Rheumatology 4.1* Editorial Board. The interviews give a personal account of their inspirations to pursue specialisms in rheumatology, their current work, and their view of the field and its possible future directions.

Our Editor's Pick for this edition of *EMJ Rheumatology* was written by Clarke and is entitled: 'Why do Diseases Start One Sided? Clues from HLA-B27 Acute Anterior Uveitis'. This thought-provoking paper investigates the mystery of uveitis and its tendency towards unilateral presentation in one eye, leaving the other totally unaffected. Uveitis is attributed to up to 10% of all legal blindness cases in the USA, but the mechanisms behind this unique difference are still unknown. This paper reviews the disease alongside other systemically associated diseases and the mechanisms behind the left–right asymmetry. Furthermore, the intriguing interplay of epigenetics and disease is covered by Hui-Yuen et al. Systemic lupus, a chronic, multiorgan disease, predominantly affects women of childbearing age. This article reviews data from novel methodologies with the aim of contributing to a more accurate diagnosis and the development of therapies to improve patient outcome. These two outstanding articles represent just a snippet of the high-quality science presented in this edition of *EMJ Rheumatology*.

Producing this journal has been an absolute pleasure. We hope that you enjoy the read, and that it will not only prove to be a fascinating read but also help you in your clinical practice and research. We look forward to seeing you at next year's EULAR congress!

Spencer Gore

Director, European Medical Journal

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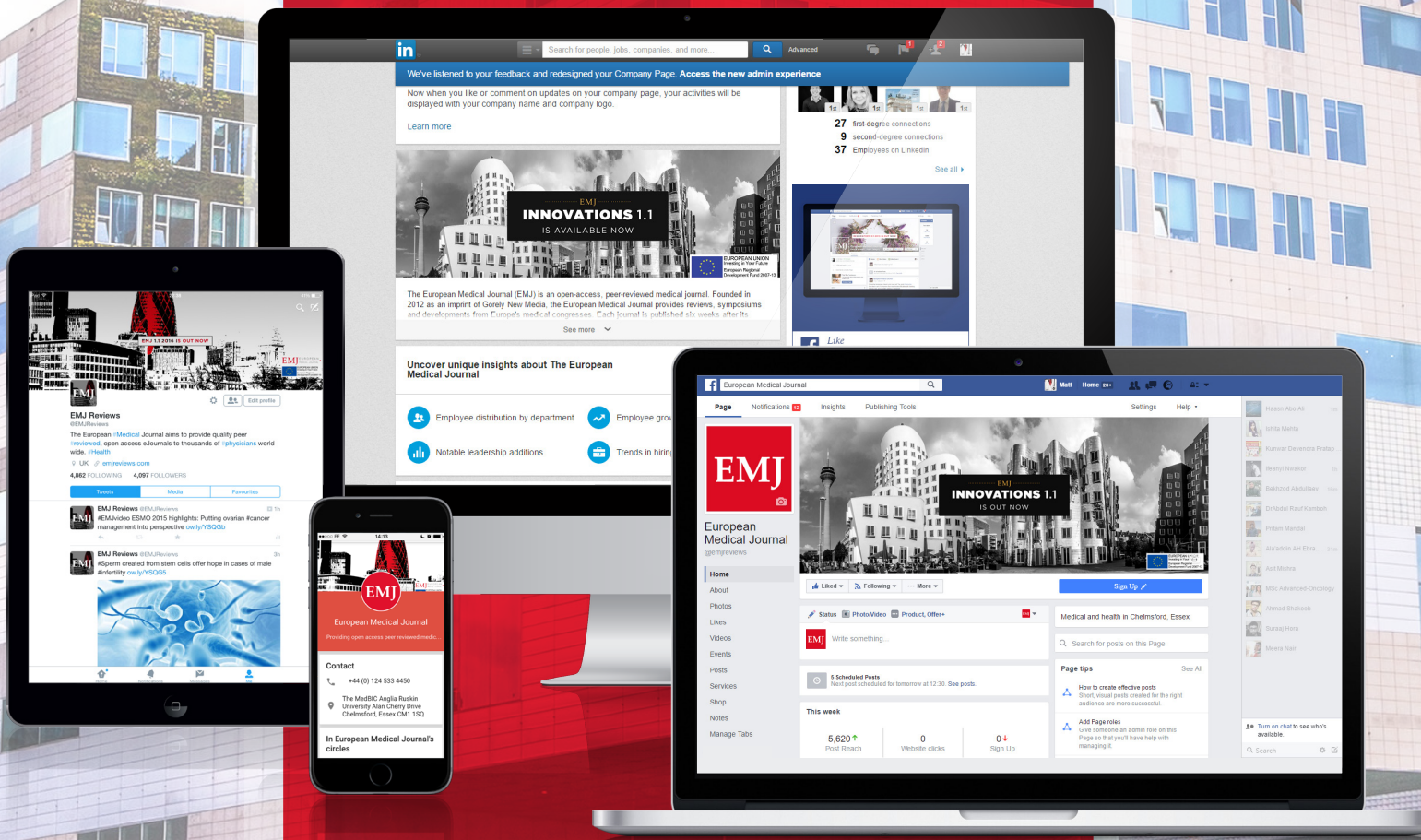

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Foreword

Dr João Fonseca

Lisbon Academic Medical Centre,
Lisbon, Portugal.

Dear Friends and Colleagues,

I would like to welcome you to the new issue of *EMJ Rheumatology* 2017.

This exciting eJournal contains a range of impressive peer-reviewed papers, bringing you up to date with the recent developments in the field of rheumatology. These include an investigation into HLA-B27 acute anterior uveitis and a study using osmolytes as mediators of the inflammatory response by muscle tissues in myositis; however, I am pleased to present several more to you. I believe they will be of great interest to you and will spark discussions and debates amongst colleagues.

For those unable to attend, or simply wanting to refresh their memories, this edition provides an overview of the themes and highlights from the European League Against Rheumatism (EULAR) Annual European Congress of Rheumatology, which took place over 4 inspiring days in June. There is also a selection of reviews contributed by abstract presenters from the congress, detailing their latest rheumatology research, which I encourage you to read to learn more about the very latest advances in this fast-moving field of medicine.

> “It is a great pleasure to present this new issue to you and I would like to take this opportunity to thank you all for submitting your work to *EMJ Rheumatology*.”

Finally, you will find a selection of detailed interviews from some of my colleagues who are specialists in their fields. They answer questions relating to their past experiences, current research, and future aspirations for rheumatology patients, and I am sure you will welcome this opportunity to learn more about the opinions, experiences, and future goals of these experts.

It is a great pleasure to present this new issue to you and I would like to take this opportunity to thank you all for submitting your work to *EMJ Rheumatology*. I hope you enjoy reading this latest edition and I look forward to seeing many of you at the next EULAR congress in June 2018, in Amsterdam, Netherlands.

Kind regards,

João Eurico Cabral da Fonseca

Department of Rheumatology, Santa Maria Hospital, North Lisbon Hospital Centre (CHLN); Rheumatology Research Group, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon Academic Medical Centre, Lisbon, Portugal.

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Includes a selection of the latest articles for rheumatology, urology, interventional cardiology, and more.

Featured inside:

FEATURE

Climate Change: What Can Doctors Do?

- Thomas Micklewright

ARTICLES

Editor's Pick: 3D Printing for Biomedical Applications: Where Are We Now?

- Carlos Miguel Chiesa Estomba et al.

Hide and Seek: The Game Between Chronic Lymphocytic Leukaemia Cells and B Cell Receptor Signalling Inhibitors

- Kumudha Balakrishnan et al.

Haemangioma: A Review of the Literature and an Unusual Case Report

- Ajaya Kumar

Transcatheter Repair of Congenital Heart Defects in the Young

- Sonia A. El-Saiedi et al.

Anaesthesia Techniques in Transfemoral Transcatheter Aortic Valve Implantation: A Brief Review

- Mehmet Aksoy et al.

New Drug Treatments for Osteoarthritis: What is on the Horizon?

- Fiona E. Watt, Malvika Gulati

Contemporary Drug-Eluting Stents and Vascular Response

- Anwer Habib et al.

Does Race/Ethnicity Have a Role in a Link Between Lower Urinary Tract Symptoms and Metabolic Syndrome?

- Seong Ho Lee, Sang Kon Lee

Antiphospholipid Syndrome and the Lungs

- Süreyya Yılmaz, Zülfükar Yılmaz

Cardiovascular Risk Factor Control in Type 2 Diabetes Mellitus and New Trial Evidence

- Peter M. Nilsson

State-of-the-Art Advances in Duchenne Muscular Dystrophy

- Henriette Van Ruiten et al.

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EULAR ANNUAL CONGRESS 2017

IFEMA
MADRID, SPAIN
14TH–17TH JUNE 2017

Welcome to the European Medical Journal review of the 18th EULAR Annual European Congress of Rheumatology

Madrid, Spain's magnificent capital city, was the location for the 18th European League Against Rheumatism (EULAR) Annual European Congress of Rheumatology, held from 14th–17th June 2017. Welcoming attendees from 120 countries worldwide, EULAR was held in the impressive IFEMA conference centre in close proximity to the vibrant city centre, bursting with history, culture, and impressive architecture. The 2017 event marked the 70th anniversary of the EULAR society, and the organising committee ensured that the celebrations did not go unnoticed!

The scale of this year's event was evident; >14,000 participants flooded the congress centre, an impressive 180 scientific sessions and interactive poster tours were available, and >4,850 abstracts were submitted. Of these abstracts, 74% were either accepted for presentation or publication. Once again, EULAR provided a unique and exciting opportunity for the exchange of clinical and scientific information, and the perfect occasion to recognise the importance of health professionals and patient organisation collaboration, for advancement within the field.

There was a heavy emphasis on the exciting launch of the general EULAR campaign, "Don't Delay, Connect Today!", which is centred around early recognition and treatment of rheumatic and musculoskeletal diseases. Attendees could efficiently schedule which sessions to attend using the official EULAR Congress App, which also allowed access to a plethora of scientific content following the event.

In our congress review section, we present you with the highlights and key discussion topics, as a welcoming reminder for those present, as well as a concise and informative overview for those who were unable to attend. During the opening ceremony, EULAR's President, Prof Gerd R. Burmester, spoke of EULAR's past, present, and future goals. To commemorate the 70 years of EULAR, attendees were guided through the timeline, at each time point reflecting on the most prominent achievement. Prof Burmester emphasised: "The World Health Organization (WHO) recognised the fundamental importance of the medical muscular skeletal

diseases, in this year 2016, and you all know without the recognition of WHO, it is very difficult to tell the politicians that these diseases are very important."

Looking towards the future, Prof Burmester provided a 2020 vision update, and discussed the seven EULAR objectives for 2017. When discussing the objectives, he commented: "Most of them have been reached, one was, we wanted to be the central platform to facilitate and stimulate innovative basic and clinical research projects." He continued: "The next one was education, we want to be the provider and facilitator of high-quality educational events, and what about the congress, we are here, we want to make it the top congress for rheumatic and musculoskeletal diseases."

The opening ceremony also featured the award winners of the congress in order to honour their outstanding contributions to the field of rheumatology. The Health Professionals in Rheumatology and PARE award winners included Mr Huang Zhengping (China) for his feasibility study for use of telemedicine in patients with ankylosing spondylitis, Dr Wilfred Peter (Netherlands) for his role as study lead for the reliability, responsiveness, and interpretability of the Animated Activity Questionnaire, designed to measure the limitations in hip and knee osteoarthritis, as well as Ellen M. H. Selten (Netherlands) and Karl Cattelaens (Germany) for their research. The awardees of the basic science and clinical abstracts were also recognised.

The presentation and symposium topics were extensive and all-encompassing, with something for every attendee from across the board. In the following review highlights, we summarise the very latest cutting-edge research and clinical data from across Europe. With EULAR's unshaken reputation as one of the best events for professional development for the practising physician and health professional, I am confident we will see many of you at next year's congress, due to be held in the thriving city of Amsterdam, Netherlands.

We hope you will enjoy reading the EULAR highlights, and I am sure you will all join us here at EMJ in wishing EULAR a very happy 70th anniversary!

> "...we want to be the provider and facilitator of high-quality educational events, and what about the congress, we are here, we want to make it the top congress for rheumatic and musculoskeletal diseases."

Dramatic Increase in Uptake of Pneumococcal Vaccination

THOUSANDS of lives could be saved using a nurse-led pneumococcal vaccination programme for vulnerable rheumatic patients, according to a EULAR press release dated 15th June 2017. As a population with previously low pneumococcal vaccination rates, this is a crucial development for many of these high-risk patients.

Dr Tiphaine Goulenok and colleagues at the Bichat Hospital, Paris, France, successfully implemented a vaccination programme run by nursing staff to improve pneumococcal vaccination coverage amongst susceptible rheumatic patients. Dr Goulenok explained: "Patients with chronic inflammatory rheumatic diseases and receiving immunosuppressive therapies are at increased risk of dying from infections compared with the general population."

The group screened 126 adult patients with a chronic inflammatory rheumatic disease admitted to the day hospital unit at Bichat Hospital over a 4-month period. Overall, 76 patients were suitable for pneumococcal vaccination, based on French national recommendations, because they were receiving prednisone, immunosuppressive drugs, and/or biotherapy. Among the 63 of these patients who had not previously received the pneumococcal vaccination but were eligible, 56 patients were successfully identified by nursing staff as requiring the vaccination, and subsequently 46 of these patients agreed to be vaccinated. The study confirmed that there was a significant improvement ($p<0.001$) in the rate of pneumococcal vaccination after the introduction of the nurse-led programme, compared to before the programme was implemented.

> "Our study has shown that nurses can play an important role in improving the uptake of pneumococcal vaccination in these vulnerable patients."

Dr Goulenok commented: "Our study has shown that nurses can play an important role in improving the uptake of pneumococcal vaccination in these vulnerable patients." Indeed, since this population has a low rate of pneumococcal vaccination, nurses have a key role in potentially saving thousands of lives.

New Prediction Model for Relapse in Rheumatoid Arthritis Patients

A COMBINATION of two measurements has been reported to enable tapering of disease-modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis (RA) patients: multiple-biomarker disease activity (MBDA) score and anti-citrullinated protein (ACPA) status. This study was discussed in a EULAR press release dated 16th June 2017. Currently, ~50% of patients with RA achieve disease remission with the use of DMARDs. Once a patient is in remission, the question for rheumatologists is whether treatment can be tapered or stopped completely. Additionally, it is important to determine patients at particular risk of relapse as a result of tapering treatment.

The RETRO study had previously found that >50% of patients remain in remission after tapering or stopping DMARD treatment. Furthermore, the presence of ACPA was found to be associated with relapses. This study tested a risk-stratified tapering model to predict relapse rates; MBDA and ACPA status were used as predictive markers.

One hundred and forty-six patients with RA were randomised into three study arms: one arm tapered their DMARD dose by 50%, one arm ceased DMARD treatment after 6 months of treatment, and the third arm continued DMARD treatment. After an observation period of 1 year, it was determined that the patients at the lowest risk of relapse (19%) were those with a negative ACPA status and a low MBDA score of <30. A relapse risk of 61%, the highest in this study, was found in patients with double-positive ACPA.

This finding is expected to have great utility in terms of economic savings, with the authors estimating that stopping DMARD treatment after tapering resulted in a 75% reduction in drug costs over the 1-year period. The study's lead author, Dr Melanie Hagen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, expanded: "Having shown in the RETRO study that those RA patients who relapse after tapering their DMARDs respond well to their reintroduction, a structured tapering and stopping of DMARDs is not only a cost economic strategy but also clinically feasible."

"Having shown in the RETRO study that those RA patients who relapse after tapering their DMARDs respond well to their reintroduction, a structured tapering and stopping of DMARDs is not only a cost economic strategy but also clinically feasible."

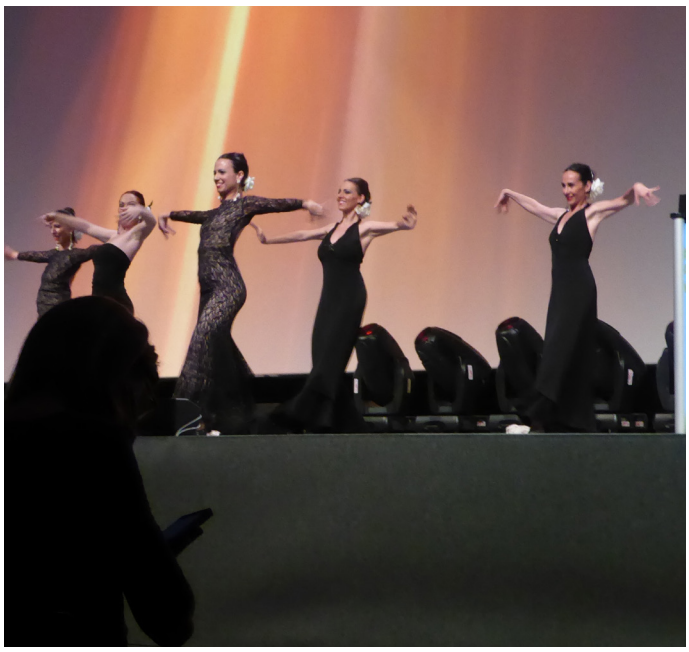
New Drug Classes for the Treatment of Psoriatic Arthritis

PROMISING new data supports two new drug classes for the treatment of psoriatic arthritis (PsA), as suggested by the results of studies reported in a EULAR press release dated 16th June 2017.

In a Phase III study, 422 patients with PsA were randomised and treated with either twice-daily tofacitinib tablets (either 5 mg or 10 mg), a subcutaneous adalimumab injection every 2 weeks (40 mg), or placebo. Tofacitinib, an oral Janus kinase inhibitor, was shown to be superior to placebo in terms of American College of Rheumatology 20 criteria (ACR20) response rates as early as 2 weeks into treatment, and this was maintained for 12 months ($p<0.001$ [5 mg]; $p<0.0001$ [10 mg]). "Since tofacitinib is a tablet and not an injection, once it receives regulatory approval, it is likely to be popular with both physicians and patients," noted Prof Philip J. Mease, Swedish-Providence St. Joseph Health Systems and University of Washington School of Medicine, Seattle, Washington, USA. Additionally, no new safety risks were recorded with this treatment.

Likewise, a Phase IIa study yielded positive results for the drug guselkumab, a fully human monoclonal antibody that targets interleukin (IL)-23. This study examined patients with active PsA and >3% of their body's surface area affected by plaque psoriasis, despite prior or current treatment. Following the administration of guselkumab, nearly 40% of the group achieved Psoriasis Area Severity Index 100 (completely clear skin) after 24 weeks, compared to just 6.3% in the accompanying placebo group. "Guselkumab, which targets IL-23, appears to be a promising new treatment of PsA," said Prof Atul Deodhar, Oregon Health and Science University, Portland, Oregon, USA. Phase III trials are now underway following these encouraging results.

> "Although anti-tumour necrosis factor treatments have revolutionised the management of PsA, new next-generation therapies are needed in the treatment of this disease."



Further research is required to verify the efficacy of these drugs, but these discoveries represent a positive step towards treating PsA. "Although anti-tumour necrosis factor treatments have revolutionised the management of PsA, new next-generation therapies are needed in the treatment of this disease," said Prof Deodhar.

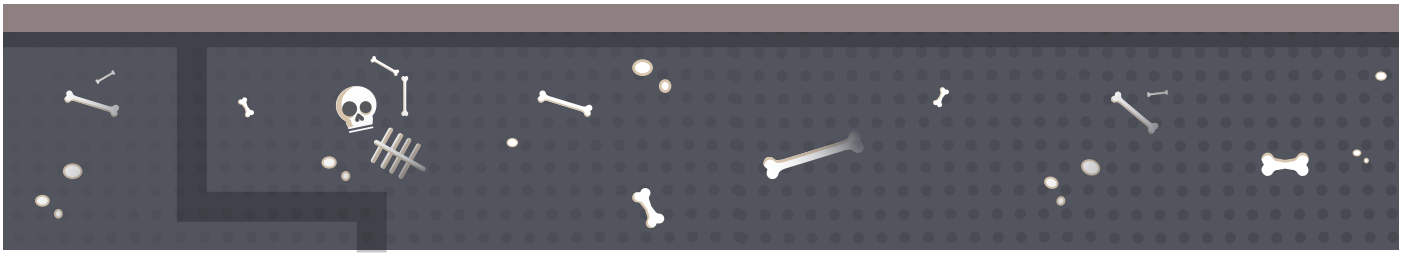
Cognitive Behaviour Therapy Benefits Chronic Pain Patients

COGNITIVE behaviour therapy (CBT), in the form of Acceptance and Commitment Therapy (ACT), improved the symptoms of depression and anxiety in chronic pain patients in a recent study, reported in a EULAR press release dated 15th June 2017. The therapy is based around psychological flexibility and behavioural changes that aim to facilitate improvements in the mental wellbeing of patients.

The benefits of ACT for chronic pain patients had been documented in an earlier study, which suggested that ACT had a positive impact on physical function and distress for adult chronic pain sufferers who attended a pain rehabilitation programme. This has contributed to a growing body of evidence pointing towards the advantages of ACT for chronic pain patients.

> "To further validate the role of ACT in the treatment of chronic pain, specifically in a rheumatology context, a randomised controlled clinical trial that includes measures of physical and social functioning within a rheumatology service would be desirable."

The study utilised data from >100 patients who had been enrolled on the ACT programme on the recommendation of three consultant rheumatologists over a 5-year period. Researchers used the Chronic Pain Acceptance Questionnaire to assess reports of pain acceptance and activity engagement in a group of chronic pain patients who were enrolled on an 8-week programme of ACT. Alongside this, the team used the Hospital Anxiety and Depression Scale to analyse psychological distress at initial assessment, at the end of the programme, and at the 6-month follow-up. They noticed statistically significant improvement in all measures for patients who had recorded values at each of the time points. This included the change in mean score of depression, anxiety, self-efficacy, activity engagement, and pain willingness ($p<0.001$).



Dr Nealon Lennox, University of Limerick, Limerick, Ireland, commented: "To further validate the role of ACT in the treatment of chronic pain, specifically in a rheumatology context, a randomised controlled clinical trial that includes measures of physical and social functioning within a rheumatology service would be desirable."

Biomarker to Identify Cardiovascular Risk in Lupus Patients

LUPUS (systemic lupus erythematosus) is a complex immune mediated rheumatic disease. Research by Dr Karim Sacre, Bichat Hospital, Paris, France was presented in a EULAR press release dated 15th June 2017, indicating that a biomarker in lupus patients could predict the presence of plaques and therefore an increased risk of cardiovascular disease (CVD).

> "The results of our study raise the possibility that this easily measured biomarker could be introduced into clinical practice as a more reliable way of evaluating CVD risk in lupus patients."

Lupus is an inflammatory disease affecting a variety of tissues and predominantly affects women. Lupus is particularly difficult to diagnose, as it has high variability between individuals. Due to improvements in treatment, affected individuals are living longer and premature CVD is becoming a significant threat to their health. Indeed, premature CVD is far more common in young premenopausal women with lupus than their healthy counterparts.

A total of 63 lupus patients and 18 controls were studied, all of whom had no symptoms of CVD. Vascular ultrasounds were carried out on all individuals; 23 (36.5%) lupus patients and 2 (11.1%) controls were identified as having signs of carotid plaques. Patients were assessed for the biomarker High Sensitivity Cardiac Troponin (HS-cTnT). It was reported that 54.5% of lupus patients had detectable HS-cTnT. Additionally, 87% of lupus patients with carotid plaques had detectable HS-cTnT but of the patients without plaques, 42.5% had detectable HS-cTnT ($p<0.001$). Only 11.5% of lupus patients with undetectable HS-cTnT had carotid plaques ($p<0.001$).

Overall, the risk of having carotid plaques due to atherosclerosis was eight-times greater in lupus patients who had detectable HS-cTnT in their blood. The lead study author, Dr Sacre, commented: "The results of our study raise the possibility that this easily measured biomarker could be introduced into clinical practice as a more reliable way of evaluating CVD risk in lupus patients." Further research needs to be carried out on larger cohorts with longer follow-up periods to assess other major cardiovascular events.

New Tools Improve Early Diagnosis of Systemic Sclerosis

INNOVATIVE tools have the potential to play a vital role in the early diagnosis of systemic sclerosis (SSc), according to a EULAR press release dated 14th June 2017. With a 10-year survival rate of ~50% once pulmonary fibrosis and pulmonary arterial hypertension begins, new tools for the diagnosis of SSc in very early diagnosis of systemic sclerosis (VEDOSS) patients are essential to facilitate the discovery of disease treatment targets.

Researchers led by Prof Vanessa Smith, Ghent University Hospital, Ghent, Belgium, studied early vascular damage in 1,085 VEDOSS patients with Raynaud's phenomenon, using a highly magnified microscope in combination with a digital video camera and specific software, a technique known as nailfold videocapillaroscopy.

Overall, this tool was successful in showing that the capillaries of VEDOSS patients displayed giant capillaries and haemorrhages, a distinct characteristic of SSc, more frequently in anti-nuclear antibody positive VEDOSS patients than in anti-nuclear antibody negative patients (40% versus 13%). This allowed quantitative assessment of the capillary changes and associated damage.

A second study identified an 'immunodominant peptide' from a peptide library of a previously discovered epitope (PGGFR-α) to develop a blood test; this peptide is recognised by immunoglobulin G in the blood of SSc patients but not controls. The test enabled researchers to distinguish between reactive samples that were taken from patients with active/progressive SSc and non-reactive samples that were taken from patients with a less active form of the disease.

Although currently this test can only determine if the patient has an active or inactive form of SSc, the lead study author, Dr Gianluca Moroncini, Università Politecnica Marche, Ancona, Italy, commented: "We propose using this assay for the prospective screening of large groups of patients affected by, or suspected of suffering from SSc to properly validate it as a tool for disease activity assessment and/or the early diagnosis of SSc." He added: "The VEDOSS cohort would be an ideal target for assessing the utility of this new test in the early identification of active/progressive forms of SSc."

> "We propose using this assay for the prospective screening of large groups of patients affected by, or suspected of suffering from SSc to properly validate it as a tool for disease activity assessment and/or the early diagnosis of SSc."

Negligible Placental Transfer of Certolizumab Pegol

NEGLIGIBLE or no placental transfer of certolizumab pegol (CZP), an anti-tumour necrosis factor (TNF) drug, was discerned from pregnant mothers to infants. This finding was from a pharmacokinetic study that was reported on in a EULAR press release dated 14th June 2017.

> “We therefore believe these data will have a significant impact on clinical practice by providing robust information for women who need treatment to keep their disease under control during pregnancy.”

Researchers studied 16 pregnant women (≥30 weeks gestation) who were already receiving maintenance CZP and received their last CZP dose within 35 days of delivery. At delivery, blood samples were taken from the infants, mothers, and umbilical cords. Further blood samples were collected at 4 and 8 weeks after delivery. A CZP-specific electrochemiluminescence immunoassay was used to measure CZP levels. Results showed that all maternal CZP levels were within the expected range (5.0–49.4 µg/mL). At birth, 13 out of 14 infant blood samples had CZP levels <0.032 µg/mL, which was the assay’s lower limit of quantification. By follow-up at Week 4 and Week 8, no infant blood samples had quantifiable CZP levels.

It is important to determine efficacious and safe treatment modalities for use in pregnant women with chronic active inflammatory diseases in order to reduce adverse pregnancy outcomes and maintain the best possible fetal and maternal health. For rheumatologists, there is often a need to strike a balance between the management of disease activity and the withdrawal of certain drugs. As most anti-TNF drugs that are an effective treatment option for rheumatoid arthritis and spondyloarthritis do transfer across the placenta, they are typically ceased in pregnancy. Therefore, this finding in regard to CZP was of importance. Building on this, the study’s lead author, Prof Xavier Mariette, Hôpitaux Universitaires Paris Sud, Paris, France, further explained the significance of these results: “The results of this study support the continuation of CZP treatment during pregnancy when considered necessary to control disease activity. We therefore believe these data will have a significant impact on clinical practice by providing robust information for women who need treatment to keep their disease under control during pregnancy.”

Fluorescence Optical Imaging Outperforms Ultrasound at Identifying Joint Inflammation

MONITORING response to treatment in juvenile idiopathic arthritis through the use of fluorescence optical imaging (FOI) has proved as effective as the use of ultrasound with power Doppler (US/PD), suggests a new study presented in a EULAR press release dated 14th June 2017.

> “FOI may be used in clinical practice to accurately identify joint inflammation earlier and with greater confidence. It should be particularly useful in identifying those children with clinically non-apparent joint inflammation of the hands and/or wrists who need to start on anti-rheumatic drug treatment.”

This study assessed 37 patients with polyarticular juvenile idiopathic arthritis. Twenty-four of those patients were treated with methotrexate and 13 were placed on a biologic. Three methods of joint examination were then compared: FOI, US/PD, and clinical examination. Measurements took place at baseline, Week 12, and Week 24. Overall, of the three methods, FOI detected the highest number of signals suggesting active inflammation of joints (32%), compared to US/PD and clinical examination (20.7% and 17.5%, respectively).

The potential benefits of FOI are considerable. The efficacy of US/PD is somewhat operator dependent, and is potentially limited in the level of information it can provide when visualising very detailed inflammatory changes, particularly in very small figure joints. On the other hand, FOI can be performed by nurses or non-medically qualified personnel, as well as potentially providing a greater level of detail regarding microcirculation. “FOI may be used in clinical practice to accurately identify joint inflammation earlier and with greater confidence. It should be particularly useful in identifying those children with clinically

non-apparent joint inflammation of the hands and/or wrists who need to start on anti-rheumatic drug treatment," said the study's lead author, Prof Gerd Horneff, Asklepios Children's Clinic, Sankt Augustin, Germany.

The results suggest that the use of FOI will not only greatly increase overall inflammation detection, but also increase diagnostic precision. "Being able to discriminate between painful but uninflamed joints and those with inflammation will avoid unnecessary treatment with conventional disease-modifying anti-rheumatic drugs or biologics in the former," concluded Prof Horneff.

Optimism for New Class of Drug in Postmenopausal Female Osteoporosis Patients

POSTMENOPAUSAL women with osteoporosis had a significantly reduced risk of vertebral fracture following 12 months of treatment with romosozumab compared with placebo, a EULAR press release dated 14th June 2017 reported. In an international, randomised, double-blind, placebo-controlled, parallel-group trial that analysed data on 7,180 patients, participants received monthly doses of romosozumab (n=3,589) or placebo (n=3,591) over the course of 1 year.

> "These results support this new class of drug as a highly effective treatment for postmenopausal women with osteoporosis with established bone mineral density deficit who are at increased risk of fracture."

Initially, the FRAME study demonstrated that romosozumab was associated with a reduced risk of new vertebral fractures compared to placebo at 12 months. The drug exerted a quick influence on patients, with 14 vertebral fractures during the first 6 months and 2 during the second 6 months for the romosozumab group. The most recent data were concerned with the incidence of clinical vertebral fractures in the patients who developed back pain consistent with such a diagnosis. X-rays were used to diagnose the fractures.

Out of a total of 119 patients who reported back pain during the trial, 20 were diagnosed with new or worsening fractures. The romosozumab arm of the study saw 3 clinical vertebral fractures, which all occurred during the first 2 months of the trial, compared to 17 in the placebo arm. At 12 months, the risk of developing clinical vertebral fractures was 83% lower in the romosozumab arm than placebo. More severe osteoporosis was noted in women who did develop the fractures, as measured by bone mineral density.

Commenting on the results, lead study author, Prof Piet Geusens, Department of Internal Medicine, Maastricht University, Maastricht, Netherlands explained: "These results support this new class of drug as a highly effective treatment for postmenopausal women with osteoporosis with established bone mineral density deficit who are at increased risk of fracture." Continuing on from this, he stated: "The rapid and large reduction in clinical vertebral fracture risk is an important and highly relevant clinical outcome."

Bacterial Infection Increases Risk of Newly-Diagnosed Sjögren's Syndrome

RESEARCH demonstrating a link between newly-diagnosed Sjögren's syndrome (SjS) and previous infection with non-tuberculous mycobacteria (NTM), was reported in a EULAR press release dated 14th June 2017.

SjS is an immune-mediated chronic inflammatory disease that affects fluid secreting glands and causes painful burning in the eyes, dry mouth, and sometimes dryness in the nasal passages. SjS can affect individuals at any age, but symptoms usually present between the ages of 45 and 65 years. Additionally, it affects 10-times as many women as men. Primary SjS occurs in people with no other rheumatic disease and secondary SjS occurs in people with other rheumatic diseases, such as lupus.

During this study, SjS patients with rheumatoid arthritis and lupus were excluded, which left 5,751 newly-diagnosed SjS patients and 86,265 non-SjS patients matched for age, sex, and year of first diagnosis of NTM. There was an association found between SjS and NTM infection, which was quantified after adjusting for the Charlson comorbidity index and bronchiectasis (odds ratio: 11.24; 95% confidence intervals: 2.37–53.24). Of the 7 patients with NTM infection followed later by diagnosis of SjS, 3 were diagnosed within 3 months of NTM infection, indicating a potential coexistence of the two diseases. Overall, patients newly-diagnosed with SjS were ~11-times more likely to have had prior NTM infection than a control group. Patients between the ages of 45 and 65 years had the biggest association between NTM and SjS. No association was found between SjS and previous tuberculosis infection.

As a result of the association highlighted by this research, screening for the presence of SjS in patients previously infected with NTM could allow prompt diagnosis and treatment of SjS. The lead study author, Dr Hsin-Hua Chen, Taichung Veterans General Hospital, Taiwan, Province of China, commented: "Identifying NTM as one of the triggers will hopefully provide a clue to the future development of a targeted therapy for these patients."

Disease-Modifying Anti-Rheumatic Drugs Reduce Knee and Hip Replacements

TOTAL knee replacements (TKR) carried out on rheumatoid arthritis (RA) patients began to decrease after the introduction of biological disease-modifying anti-rheumatic drugs (bDMARDs) in accordance with national treatment guidelines as reported by a Danish study in a EULAR press release dated 15th June 2017. This study analysed the trends in TKR and total hip replacements (THP) from 1996–2016.

In 2002, the new bDMARDs were incorporated into the national treatment guidelines for Denmark. Before this introduction, TKR had been increasing among RA patients. The baseline incidence rate of TKR among RA patients in Denmark is 5.87 per 1,000 person years in RA patients, and before 2002 had been increasing at a rate of 0.19 per year. After the introduction of bDMARDs, TKR began to decrease in RA patients at a rate of -0.20 per year. TKR continued to rise among non-RA patients throughout the study period.

Researchers then analysed the incidence of THR during the study period. THR maintained a steady decrease in incidences in RA patients, with a baseline incidence rate of 8.72 per 1,000 person years in Denmark with a -0.38 reduction (1996–2002; 2003–2016). However, there was an unexpected increase of +2.23 in 2003.

Lead author Dr Lene Dreyer, Centre for Rheumatology and Spine Diseases, Copenhagen, Denmark commented: "Our findings show a clear downward trend in these two operations in RA patients in Denmark since the addition of bDMARDs to treatment protocols." Dr Dreyer also mentioned the findings were "in line with those recently reported from England and Wales" and that a "...more widespread use of conventional DMARDs and the treat-to-target strategy may have contributed to this positive development."

Low-Dose Computed Tomography Improves Ankylosing Spondylitis Assessment

LOW-DOSE computed tomography (LD-CT) is more sensitive than conventional radiographs (X-rays) in monitoring progression of ankylosing spondylitis (AS) in affected patients. This study, aiming to further validate LD-CT, was reported on in a EULAR press release dated 15th June 2017. Comparisons of LD-CT's ability to demonstrate the formation of new bony growths (syndesmophytes) and/or an increase in size of syndesmophytes revealed that LD-CT routinely identified more AS patients with signs of disease progression than conventional X-rays.

Caused by chronic inflammation of the spine and with varying prevalence across the globe (~23.8 and ~31.9 per 10,000 in Europe and the USA, respectively), AS is an infamously painful, progressive, and disabling form of arthritis.

Fifty AS patients were recruited from the Sensitive Imaging of Axial Spondyloarthritis (SIAS) cohort from Leiden, Netherlands and Herne, Germany. Conventional X-rays of the lateral cervical and lumbar spine, and LD-CT of the entire spine at baseline and 2 years, were performed on each patient. Two investigators independently assessed the resulting images in separate sessions, blinded to time order and the result of the other imaging technique. The percentage of patients with newly formed syndesmophytes, growth of existing syndesmophytes, and a combination of both were compared. LD-CT was found to detect more patients with progression in all comparisons, which was especially apparent when there were a higher number of new or growing syndesmophytes per patient. A total of 30% of patients showed bony proliferations at ≥3 sites on LD-CT, versus just 6% by conventional X-ray.

Lead author Dr Anoek de Koning, Leiden University Medical Centre, Leiden, Netherlands, commented: "Our findings support the use of LD-CT as a sensitive method for the assessment of new or growing syndesmophytes in future clinical research without exposing patients to high doses of radiation."

Helena Canhão

Invited Full Professor, Epidemiology and Clinical Research, NOVA Medical School, National School of Public Health, NOVA University of Lisbon; Head, EpiDoC Unit, Center for the Study of Chronic Diseases (CEDOC), NOVA Medical School; Rheumatology Senior Consultant, Santa Maria Hospital, North Lisbon Hospital Centre (CHLN), Lisbon, Portugal.

Q: What piqued your interest in the field of rheumatology and inspired you to pursue it as your career?

A: From the intellectual point of view, rheumatology is a very stimulating specialty. Diagnoses are most frequently based on clinical grounds with challenging differential diagnosis between pathologies from distinct areas, such as immune-mediated, malignancies, neurological, genetic, and infectious diseases. Another aspect is chronicity, with which we are able to establish long-term relationships with patients. It is also a field where clinical, translational research, and innovation are hallmarks.

This field combines clinical practice, teaching, and research, offering a career that is undoubtedly rich with plenty of fulfilment.

Q: You are Principal Investigator at the Nova Medical School, Nova University, Lisbon. Could you tell us what this involves and why it is important to you?

A: I am EpiDoC Unit's Principal Investigator at CEDOC, NOVA Medical School. Our research unit focusses on the epidemiology of non-communicable chronic diseases, large databases and registries, clinical research through large observational studies, interventional studies, patient-reported outcomes, active and healthy ageing, inequities, lifestyles, and outcomes research. We have collaborations and partnerships with several research units and hospital departments participating in different national and international projects. Successful clinical research requires multidisciplinary knowledge, exchange of ideas, collaborations, as well as human resources with different backgrounds to address societal challenges and real-life problems (from the individual level to population level), to have net impact in health, and social outcomes. I embrace all of these activities with true joy and enthusiasm.

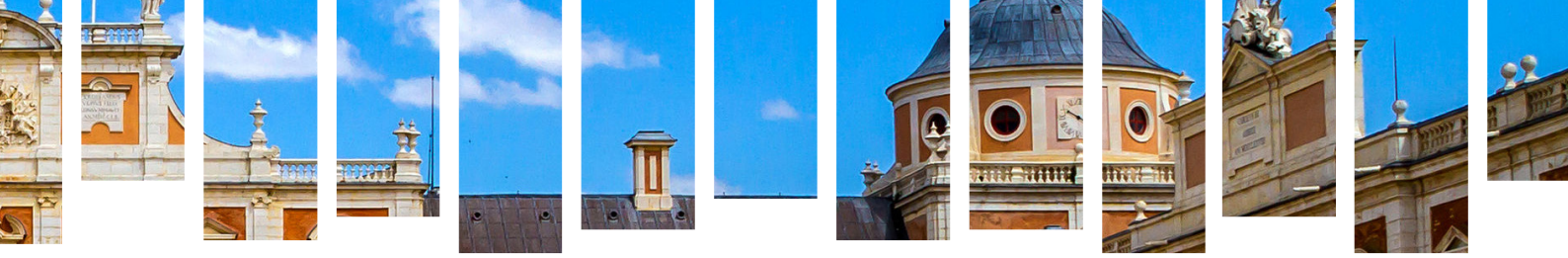
Q: Could you share with us what you believe to be the most pressing matters that rheumatologists face at present?

A: Rheumatologists face common problems, but some of them are more specific, related to the national organisation of the specialty. In the last 20 years, we have observed a huge increase in knowledge about physiopathology, pathways, targets, and new therapies for combatting inflammatory rheumatic diseases. The clinical practice has changed, and has become more demanding and rigorous in early diagnosis by monitoring tools, criteria, and guidelines for use in conjunction with new therapies. This has raised costs, however quality of life and damage prevention have increased, with less disability. Another challenge is the increasing age of the population, with more osteoporosis, osteoarthritis, frailty, falls, loss of function, and autonomy.

At the national level, the pressure varies. In Portugal, we need more specialists (there are only currently around 130 in the entire country), more rheumatology centres, better site distribution, and earlier patient referral to specialists.

Q: How would you like to see the field of rheumatology progress over the next 5 years, and how do you think this will be achieved?

A: As I previously mentioned, the great evolution in recent years has been in the field of inflammatory rheumatic diseases. As a result, all rheumatologists are enthusiastic about rheumatoid arthritis and spondylarthropathies. Of course, I am too. But as



I also mentioned, in the future we must look very carefully at our ageing population; osteoarthritis, osteoporosis, sarcopenia, and frailty should attract rheumatologists as preferential specialties. At the same time, researchers and companies are very alert to these areas and are already working on new targets and therapies. I think these fields will evolve, even explode, with novel advances in following years. Another field is of course personalised medicine, with approaches tailored to individuals, including rheumatic diseases.

Q: You are a long-standing member of the Paediatric Rheumatology Study Group of the Portuguese Society of Rheumatology (PSR). What developments have been made since you started with the study group in 2000?

A: In Portugal, paediatric rheumatology is not a speciality. Some rheumatologists and paediatricians have training and are more dedicated to following the path of specialising in paediatrics. In the last few years, the relationship between the two specialities has improved and we organise joint courses and meetings. Also, the PSR has developed Reuma.pt, the Portuguese register of rheumatic diseases, where both specialities enrolled paediatric patients. We developed and published national recommendations for treating children with biological therapies and we have also improved research in this area, with national and international publications.

Q: Could you give us an insight into your research in bone biology and pathology?

A: Bone biology and pathology is one of our areas of interest which mainly focusses on translational research and trying to understand mechanisms of bone loss in early phases of arthritis using cells, animal models, and patients with early arthritis. We also established a protocol with the orthopaedics department and for >5 years we have collected and sent to the lab all femoral heads resulting from surgeries (osteoarthritis and fractures) allowing the development of biomechanical tests, histomorphometry, micro computed tomography (micro-CT), among others. This has given interesting new knowledge, and we have doctoral theses and several papers based on these subjects.

Q: We understand that you have a particular interest in health innovation. Are there any innovations you have seen that you are looking forward to being introduced in the future? How might these impact research or patient care in the future?

A: Over the past 3 years I've been working as part of the Patient Innovation Project (https://patient-innovation.com), developed by Catolica-Lisbon School of Business and Economics in partnership with medical schools. I am the Chief Medical Officer. We established an open, free, non-profit platform to share innovations developed by patients and non-professional caregivers. It is amazing how innovative patients can be, when motivated by their own needs. The solutions are published online after medical screening, and drugs and topical products etc. are not allowed. We have around 700 solutions from 50 countries and the worldwide impact of the project and its initiatives are impressive. User-innovation in the field of health is a new paradigm, and this will change in the future due to innovation and entrepreneurship in healthcare.

Q: How important is an interdisciplinary approach in rheumatology? Are there any other branches of medicine that rheumatologists can learn from?

A: Rheumatology must be interdisciplinary. Patients have multimorbid conditions and must be seen as a collective. Neurology, orthopaedics, primary care, rehabilitation, paediatrics, dermatology, and ophthalmology are some of the medical specialities involved, but also, other health professionals such as nurses, pharmacists, social workers, therapists, and nutritionists, as well as families and the patients, must be involved in the disease management.

> "This field combines clinical practice, teaching, and research, offering a career that is undoubtedly rich with plenty of fulfilment."

Q: What do you feel has been your greatest achievement during your career?

A: This question is difficult because there are so many important achievements! Of course, some are easy to recall, such as graduating in medicine, being awarded the rheumatology board certification, my PhD, the habillitation (academic grade after PhD). But others are probably more distinctive, such as the senior clinical award from the Harvard-Portugal Programme, allowing me to live and conduct research in Boston for 2 years, or the Grande Premio Bial, the most prestigious Portuguese prize in medicine. I also consider the development of two structural projects in Portugal to be very important: Reuma.pt, the Portuguese Register of Rheumatic Diseases, and EpiReumaPt, the epidemiological study of rheumatic diseases. Reuma.pt and EpiReumaPt are the basis that we are now using to develop robust research in the field of rheumatology. I'm very proud to have been a part of the team since the beginning. Similar to what I said before, patient innovation is a changing paradigm in healthcare and I really appreciate being part of it.

Q: Drawing on your experience, what advice do you feel would be most helpful to pass on to medical students hoping to specialise in rheumatology?

A: Rheumatology is really a very complete and interesting speciality, even more so if one combines the clinic, teaching, and research. It is not the best medical speciality to earn money, nor to have the stress of acute patients (we have it sometimes, but fortunately it is not the rule), or to experience technical challenges, like in surgery's specialities. But it is a challenging medical speciality where clinical history and observation matter, differential diagnosis is not always easy, and patients come from all ages and economic status. The relationship with the patient is a very important piece for disease management success, research, continuous study, and updates are truly important.

João Fonseca

Department of Rheumatology, Santa Maria Hospital, North Lisbon Hospital Centre (CHLN);
Rheumatology Research Group, Institute of Molecular Medicine, Faculty of Medicine,
University of Lisbon, Lisbon Academic Medical Centre, Lisbon, Portugal.

Q: How did you first become interested in the study of rheumatology?

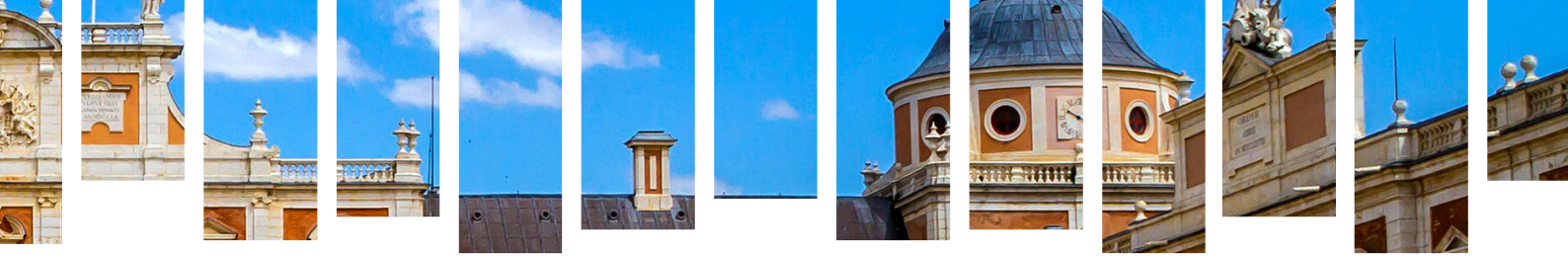
A: It started quite early in my medical school career. During my 3rd grade, I had the opportunity to work in a cell biology lab and worked with anti-Sjögren's syndrome type A and anti-Sjögren's syndrome type B antibodies as a way of gaining insight into the mechanisms of nucleus–cytoplasm communication. Three years later, in my final undergraduate year, I worked intensively with an animal model of arthritis, exploring the innervation of the synovial tissue.

Q: Would you say that the field of rheumatology has changed significantly since you began your career? What are some of the major changes you have seen?

A: Immensely! When I started as a resident in 1995, we had many wheelchair-bound patients in the waiting room of our academic hospital rheumatology clinic and that is now extremely rare. We came from gold salts and very shy doses of methotrexate into an era of aggressive early treatment, including early introduction of biologics for those in need of it.

Q: Of the achievements in your career, which are you most proud of?

A: Giving my contribution to the development of higher organisational standards for rheumatology in my home country gives me a sense of true satisfaction. No one works alone, and major developments are only achievable by teams. In Portugal, rheumatologists worked as a 100-strong



team, which led to the PubMed indexing and impact factor of our national journal, the creation of the National Register of Rheumatic Patients (Reuma.pt), guidelines, a rheumatology research funding system, and, above all, a leap forward in clinical care and scientific production.

Q: Are there any areas in rheumatology you believe would benefit from greater awareness within the general population? How could this be achieved?

A: Yes. A new approach has to be sought for osteoarthritis and generalised pain/fibromyalgia. Information and education are key, more so than clinical appointments and medication.

Q: As someone who is involved in clinical trials, what do you think are the most important factors that must be taken into account in their design?

A: The sample is a key factor. We should be as close as possible to real-life patients.

Q: Your research group works with the Portuguese Society of Rheumatology (PSR) to develop registries. What, for you, is the primary aim of such registries?

A: Registries are key tools for the development of any medical field. They provide guidance for excellent care, particularly if they are also electronic clinical charts, like Reuma.pt, that help in the training of residents and promote pharmacovigilance and clinical research. These are absolutely a central piece for high-standard medical care.

Q: How important is it for rheumatologists to adopt an interdisciplinary approach?

A: Most of our diseases have frontiers with other medical fields and rheumatologists have to be able to orchestrate these multiple collaborations through multidisciplinary clinics.

Q: What issues in rheumatology do you believe the gathering of 'big data' could assist with?

A: Big data is crucial for the detection of rare adverse effects and can give useful information on comparative effectiveness of medical interpretation.

Q: If you could cure one rheumatological condition, which would it be and why?

A: Systemic sclerosis, no doubt. It is a dreadful intractable disease.

Q: What advice would you give to a medical student considering specialising in rheumatology?

A: Be resilient, enthusiastic, and generous.

> "Registries are key tools for the development of any medical field. They provide guidance for excellent care..."

Lucía Silva-Fernández

Consultant Rheumatologist, Rheumatology Department,
Complexo Hospitalario Universitario de Ferrol (CHUF), A Coruña, Spain.

Q: What inspired you to pursue a career in rheumatology? Was there something in particular that initially piqued your interest?

A: I only started becoming interested in rheumatology once I finished my degree in medicine. When preparing for my exams to access the speciality training, I had the opportunity to study rheumatology in more depth and I discovered my interest in it. One of the things that looked more appealing to me about rheumatology was the wide range of diseases that a rheumatologist deals with. We can treat small musculoskeletal problems affecting soft tissues through to systemic diseases that require strong immunosuppression. Being a rheumatologist requires a profound knowledge of

the immunological mechanisms of the diseases and at the same time being highly skilled in performing manual techniques.

Q: Could you tell us more about your daily roles and responsibilities as a consultant rheumatologist at your institution?

A: My role as a consultant rheumatologist includes mainly clinical work in outpatient clinics and also with inpatients. We also have some teaching responsibilities with doctors in training and students. Currently, research represents only about 20% of my work and this is focussed on clinical research.

Q: How has the field of rheumatology evolved since the beginning of your career?

A: I have been working as a rheumatologist for about 15 years now. The main change in this field has been the development of multiple new therapies to treat rheumatic diseases. Following the availability of effective therapies to treat rheumatic diseases in their very early stages, many of the diseases have been redefined. In the last few years, a number of new classification criteria have been published for several diseases to help clinicians diagnose these conditions earlier and treat them while progression can still be avoided.

Q: Have you noticed an increase in the incidence of any particular rheumatic diseases since you began your career? What do you attribute this increase to, and how could it be combatted in the future?

A: I have not noticed any increase in the incidence of any particular disease, except for those where the classification criteria have changed. However, this is not a real change in incidence but a consequence of having better methods to detect diseases earlier. I have worked in three different countries and in different areas within those countries. What I have observed is the different prevalence of some diseases like vasculitis or systemic lupus erythematosus (SLE) between the different geographical areas.

Q: Are there any novel therapies or emerging technologies that you feel may have the capability of revolutionising the field in the near future?

A: The range of effective therapies we have for diseases like SLE, scleroderma, or vasculitis is not as wide as for arthritis. I think that the development of new molecules to treat these diseases can bring to rheumatology a revolution similar to that which biologics brought to the treatment of rheumatoid arthritis >15 years ago.

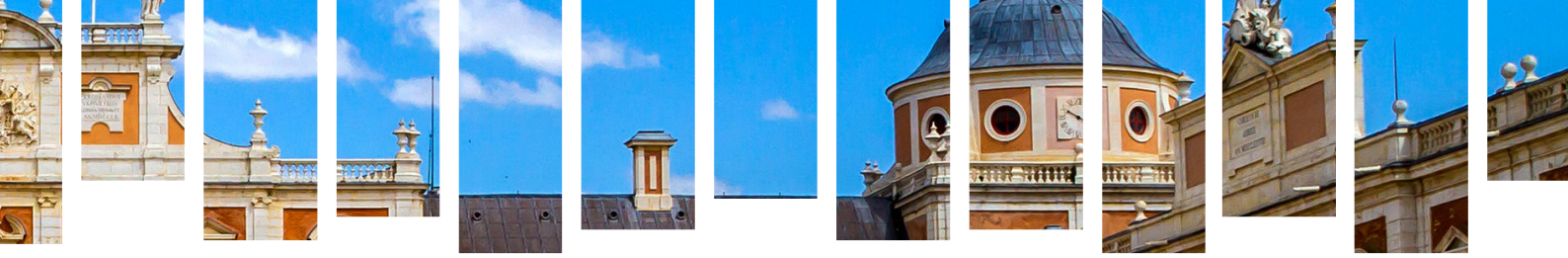
Q: Several of your recent publications are focussed on SLE, specifically analysing data from large cohort studies and/or registries. Could you tell us more about this research? How important are registries and large cohort studies for the characterisation of conditions such as SLE?

A: Those publications are part of the Spanish Register of SLE (RELESSER). A register like this facilitates large-scale research of the characteristics of the disease and more importantly, of treatment outcomes. New drugs for SLE are changing the natural history of the disease, so there is a need to re-evaluate the current prognosis of the disease and assess the impact of these drugs on the quality of life of patients with SLE. In this sense, registers and cohort studies allow the evaluation of the impact of these new drugs in real life. The effect of the drugs can be studied in unselected patients and for much longer periods than a clinical trial allows.

Q: It appears that another of your research interests involves the association between incidence of cancer in patients who are prescribed biologics for rheumatic conditions. Could you provide us with more information about this area of research?

A: The development of cancer has a long latency, which means that a clinical trial is not ideal to study this type of outcome. On the other hand, patients with a prior malignancy are systematically excluded from clinical trials, so the information we have about the effect of biologics or any other drug on patients with a previous cancer is very scarce.

> “Being a rheumatologist requires a profound knowledge of the immunological mechanisms of the diseases and at the same time being highly skilled...”



Therefore, the best scenario to study the association between cancer and biologics is a cohort study with a long follow-up where patients are not selected under strict criteria and the follow-up is long enough for cancer to develop. My research in this field has focussed on the recurrence of cancer in patients receiving biologics from the British Biologics Register, which is a large prospective cohort with a long follow-up. The results show that the use of biologics in patients with a prior malignancy is not increasing the incidence of recurrence. Previous research has not found an overall increase of cancer in patients receiving biologics. Nevertheless, with the development of new drugs and the changing profile of patients receiving biologics, there is still a need for new research on this topic.

> “... I feel more proud of my clinical work than my research. But I would not be able to point out any specific work as the greatest achievement. The reward of knowing that we help people to improve their quality of life is for sure the best part of this profession.”

Q: Are there any areas of research you would like to pursue in your career that you have not yet had an opportunity to explore?

A: Until now, I have exclusively done clinical research. Basic research is very relevant in rheumatic diseases to understand the immunological mechanisms of the conditions. To date, I have not had the opportunity to learn about it.

Q: Of all your professional accomplishments to date, which would you consider to be your greatest achievement?

A: In general, I feel more proud of my clinical work than my research. But I would not be able to point out any specific work as the greatest achievement. The reward of knowing that we help people to improve their quality of life is for sure the best part of this profession.

Q: What words of wisdom would you give to emerging clinicians or researchers with an interest in rheumatology?

A: I believe that in rheumatology, and medicine in general, one should never forget that the patient is the centre of our work/research. All efforts should aim to improve the patient’s life.

Jessica Bertrand

Professor, Experimental Orthopaedics, Department of Orthopaedic Surgery,
Otto von Guericke University Magdeburg (OvGU), Magdeburg, Germany.

Q: What was it that initially piqued your interest in the subject of orthopaedic surgery, and, in particular, experimental orthopaedics? Was there an event or a mentor that particularly inspired you?

A: I did my PhD thesis on cardiac arrhythmias that were caused by mutations in a potassium ion channel. When I was nearly finished with my PhD thesis, I wanted to do something different, as I felt that patch-clamping would not be my favourite technique for the rest of my life. At this time, I got to know Thomas Pap, who had just moved from Magdeburg to Münster, Germany. I was fascinated by his research and his hypotheses on cartilage degradation in osteoarthritis (OA). He offered me a postdoc position and asked me to take over the OA research in his lab. We started off with two projects at this time, which further piqued my interest in understanding the pathways that are activated during the disease. We discussed different ideas and came up with the theory that OA is in some way related to endochondral bone formation, which remains one of my working hypotheses.

> “...cartilage is not like a tyre on a car which gets run down by time and use, but is in fact an active tissue building its own matrix.”

I went to London, UK to Francesco Dell'Accio's lab for my second postdoc. He inspired me to investigate the Wnt signalling-induced loss of chondrocyte phenotype in OA. During our discussions, we came to the idea that there must be something like cartilage homeostasis in healthy cartilage, which is disturbed during OA. This is also still one of my working hypotheses. Both mentors shaped my research ideas and I still enjoy discussing my research and their projects with them.

Q: Could you give us a brief understanding of the roles and responsibilities involved in your position as Professor of Experimental Orthopaedics?

A: As a Professor of Experimental Orthopaedics and working at a university hospital, I do some teaching in orthopaedics. But, as I am a biologist by training, I mainly give lectures on the cellular and molecular basics of musculoskeletal diseases. Furthermore, I give medical students the opportunity to get involved in lab work in a practical course, where they learn basic lab techniques hands-on. I also give medical PhD students the possibility to write their PhD thesis in my lab, which can be done in Germany during their study time. Besides my teaching in the medical faculty, I also teach on the master's module of Immunology at the university and have different PhD students with biology, molecular biology, and medical engineering backgrounds working in my lab. Because my lab is also interested in the testing of hip implants and improving the current materials, as well as understanding the effects of wear particles, it is very interdisciplinary and I promote discussions between engineers, clinicians, and scientists.

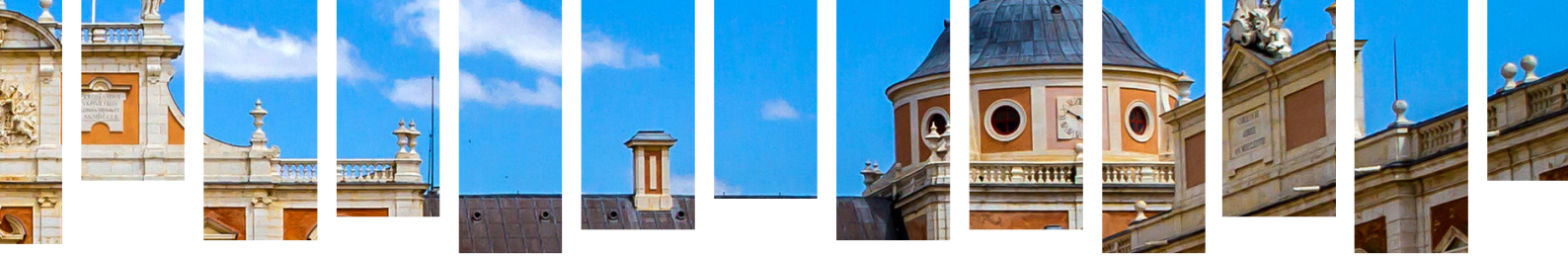
Beside my teaching activities, I am a member of different boards at the medical faculty, and I try to foster co-operation between the technical campus and medical campus in Magdeburg. Like every other principle investigator in research, I am also in charge of writing grant applications and leading the research activities in my lab, as well as writing papers and going to conferences. I try to give all my students the opportunity to go to conferences to present their data, as well as to go abroad on a short fellowship to get to know other labs and learn new techniques.

Q: You have recently been researching the molecular mechanisms of cartilage remodelling under physiological and pathological conditions. What advances have been made in this area lately, and what developments do you hope to see in the future?

A: In my opinion, the biggest advance in the past 10 years in this field is the understanding that cartilage is not like a tyre on a car which gets run down by time and use, but is in fact an active tissue building its own matrix. There have been many excellent publications on the involvement of different pathways in the regulation of the chondrocyte phenotype, and many of these have shown to be able to delay or inhibit OA onset in mice, although only few of them could be transferred to human OA, and even now there is still no effective treatment for end-stage OA. The past 5 years have shown that treating OA means also to treat pain and that these two conditions do not always correlate very well. The correlation between structural cartilage damage and pain reception varies between different patients and there is no proof that restoration of cartilage structure will reduce the pain. In my opinion, the investigation of pain and how pain reception correlates with cartilage damage will be one of the main topics of future OA research. Pain is the main complaint of patients with OA; they do not ask for healthy cartilage, but to be able to walk without pain.

Q: Is there a specific area of research that you have not had a chance to explore thus far that you would like the chance to investigate in the future?

A: I do not feel that I have missed out on any specific field of research. There are certainly trends in the field that I did not follow, like specific methods or hypotheses. I try to focus on my own



understandings of cartilage remodelling and follow these hypotheses. Sometimes I need a specific technique and ask for help and co-operation from the society. On the other hand, sometimes people ask me to help them study their ideas and need my help. In my opinion, everybody can do everything with the right partner to lend their expertise, and therefore I never felt that I was not able to do something.

Q: In 2012, you were the recipient of the German Academic Exchange Service (DAAD) Travel Grant. How do international collaborations impact research in rheumatology and the wider medical community?

A: As I have stated in my answer above, co-operation is very important to get new ideas or the right technique to investigate the problem you are looking at. I cannot be the expert in every pathway or specific technique applied to investigate OA, but I can certainly find the experts in the society and try to co-operate with these people. In my opinion, it is very important to co-operate with other scientists to understand some research questions quicker and sometimes to interpret the gathered data correctly. I am still working very closely with Francesco Dell'Accio and the DAAD grant was a great chance for me to get to know another lab and bring new influences to my understanding of OA. This is the reason I also try to give this opportunity to my PhD students by sending them to a collaboration partner abroad and experience this for themselves.

Q: How do events such as the European League Against Rheumatism (EULAR) congress benefit attendees? What advice do you have for delegates attending the congress about how to make the most of the opportunities on offer?

A: The EULAR congress is a great chance to get to know the people in the field and get to know their research. Whenever I am looking for a co-operation partner for a specific question, I find these people at conferences like the EULAR. At conferences, you have the chance to discuss with the leading scientist in the field of your own data. These are also the future reviewers of your manuscripts, so you have to listen very closely to their suggestions and ideas, because these might be the requirements to get your research published. You also receive direct feedback on your research, which is very important, because sometimes you are routine-blinded. My advice for delegates attending the conference is to use the opportunity to discuss your own data with other scientists and if you need help with a specific technique or research question, try to find the expert in the field and ask for help.

Q: Of your professional achievements thus far in your career, which are you most proud of and why?

A: I am most proud of my publication on how blocking syndecan-4 protects mice from OA. I did this work together with Frank Echtermeyer in Thomas Pap's lab in Münster, Germany. I enjoyed the scientific discussions at this time and the way we thought of experiments to prove our hypotheses. At this time, I was intrigued by how blocking just one protein in the extracellular matrix could block cartilage degradation. This is most likely the reason why Syndecan-4 is still one of my favourite proteins to study, and I am still investigating the mechanisms of how the blockade of Syndecan-4 blocks cartilage degradation. Syndecan-4 is a very exciting protein with lots of different functions and roles in the extracellular matrix.

“The EULAR congress is a great chance to get to know the people in the field and get to know their research. Whenever I am looking for a co-operation partner for a specific question, I find these people at conferences like the EULAR.”

BIOSIMILARS AND SWITCHING: WHAT IS YOUR PERSPECTIVE?

This satellite symposium took place on 16th June 2017 as a part of the European League Against Rheumatism (EULAR) Congress in Madrid, Spain

Chairperson

John Isaacs[1]

Speakers

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MEETING SUMMARY

The licensing of biosimilars heralds the start of a new era for physicians treating immune and inflammatory diseases. This symposium provided an update on biosimilar drugs and dealt with questions and concerns around switching from a reference biological drug to its biosimilar.

Prof Isaacs presented the physician's perspective, describing the regulatory process that is designed to provide reassurance regarding clinical equivalence for biosimilars alongside comparable safety and immunogenicity data. A current consequence of a range of different clinical trial designs is that biosimilars cannot be compared. As more biosimilars enter the market, he made the case for the standardisation of clinical trial designs to simplify comparisons between the different biosimilars. Dr Goll gave an overview of the NOR-SWITCH study. The Norwegian government-funded study showed that switching from reference infliximab (INX) to the biosimilar CT-P13 was not inferior to continued treatment with INX.

Prof Gonçalves shared the pharmacist's perspective and explained that post-approval pharmacovigilance is crucial for consolidating confidence in biosimilars. He presented studies showing that there was no evidence for biosimilar-related immunogenicity beyond the reference molecule. He concluded that in pharmacovigilance all switching information obtained in registries should be pooled with voluntarily reported and suspected adverse-drug reactions.

Ms Bosworth focussed on the views and needs of patients with regard to key issues associated with switching to biosimilar drugs. She stated honesty and transparency were required when explaining the reasons for switching and that healthcare staff should not hide the fact that saving money is the reason for switching. Financial savings resulting from introducing biosimilars, she stressed, should be shared between commissioners, hospital units, and rheumatology teams. A range of resources

on biosimilars for both health professionals and patients are available from the National Rheumatoid Arthritis Society (NRAS).

A New Wave of Biosimilar Drugs

Professor John Isaacs

Welcoming delegates, Prof Isaacs quoted the World Health Organization (WHO)'s definition of biosimilars as a "biotherapeutic product which is similar in terms of quality, safety, and efficacy to an already licensed reference biotherapeutic product".[1] The US Food and Drug Administration (FDA) and European Medicines Agency (EMA), he said, use different definitions for biosimilarity, with the FDA focussing on 'safety, purity, and potency' and the EMA 'quality, safety, and efficacy'. In reality, he said, they are referring to the same concept of using the totality of evidence to confirm bioequivalence of the reference medicine and the biosimilar.

Currently, there is a great deal of activity in the field of biosimilars, with development of biosimilars underway for adalimumab, INX, etanercept, tocilizumab, and rituximab.[2] Key issues facing physicians working with this 'new wave' of biosimilar drugs, are efficacy and safety (regulation), immunogenicity, pharmacovigilance, extrapolation, working with multiple biosimilars, and switching and substitution.

Biosimilar Studies Show Equivalence

Professor John Isaacs

In comparison with small molecule drugs, biologics represent 'very sophisticated and complicated molecules'. It is well known that monoclonal antibodies have typical Y-shaped structures, with one end binding antigen and the other having effector functions. What is less well known, said Prof Isaacs, is that many of the antibody functions depend on the fine structure, which can be affected by modifications to the protein sequence. Such modifications (including glycosylation, methylation, deamidation, and oxidation) occur post-translationally, once the protein has been synthesised from RNA.[3]

As a result, the final monoclonal antibody is not just a product of the DNA/amino acid sequence but 'critically influenced' by proprietary factors such as cell lines, the way cells are cultured, and antibodies purified. Just as it may not be possible to make identical bread, wine, and beer (which are also manufactured from living organisms), Prof Isaacs explained, it is not possible to exactly replicate reference drugs.

With biosimilars, Prof Isaacs said, regulators were most interested in whether the drug looks the same as the bio-originator, i.e. whether their structures, including post-translational modifications, are highly similar. They must also behave similarly in *in vitro* assays, for example, in terms of antigen binding and effector function. Consequently, and in contrast to conventional drugs, regulators require less evidence from clinical studies for biosimilars, and instead place greater emphasis on analytical dossiers. Nonetheless, efficacy and pharmacokinetics (PK) have to be equivalent, and safety and immunogenicity must be shown to be comparable to the reference, with immunogenicity continuing to be assessed post-marketing. Provided the above criteria are met, there is no requirement for formal efficacy trials of biosimilars; if similarity is established with the bio-originator, it is assumed that the biosimilar will be effective in the same range of indications.[4]

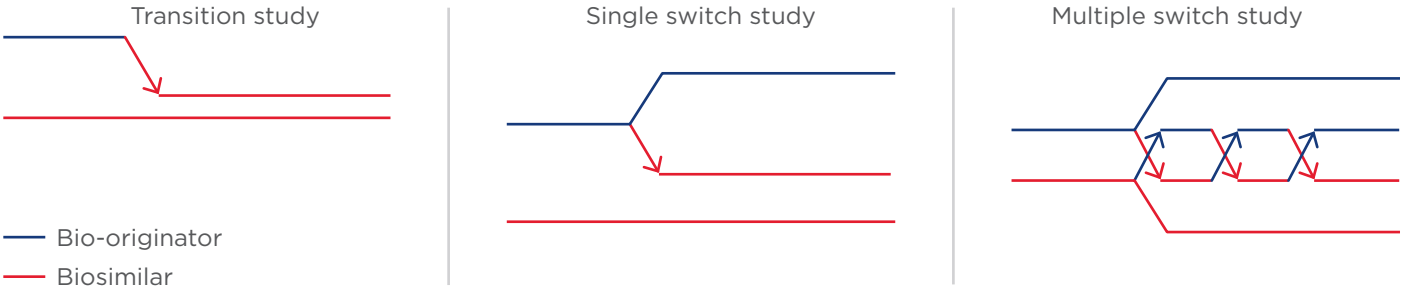

Figure 1: Examples of biosimilar switching studies.[2]

Prof Issacs provided the example of CT-P13 development, an INX biosimilar that was the first biosimilar to be licensed by the EMA. The Phase I PLANETAS study[5] showed that, at the fifth dose, the geometric mean area under the concentration–time curve was 32765.8 µgh/mL for CT-P13 versus 31359.3 µgh/mL for INX with a ratio of geometric means of 104.5%. Furthermore, the serum C_{max} geometric mean was 147.0 for CT-P13 versus 144.8 for INX (ratio of geometric means of 101.5%). Both of these measures fell within the pre-defined equivalence margins.

In the Phase III efficacy study, at Week 30, the American College of Rheumatology (ACR) Criteria ACR20 responses were 60.9% for CT-P13 versus 58.6% for INX in the intention-to-treat population response rate, giving a treatment difference of 2% (95% confidence interval [CI]: -6%, 10%), again well within the equivalence margin for the trial.[6]

Extrapolation describes the situation where a biosimilar which was considered equivalent in one indication is also considered to work in a second indication (without demonstrating this in an additional clinical trial).[7] To illustrate the concept, Prof Isaacs gave an example. If a biosimilar is equivalent to the reference medicine in disease X, and if the reference is effective in diseases X, Y, and Z, then the biosimilar can be considered effective in all three conditions. In fact, the reference clinical trial data becomes incorporated into the biosimilar summary of product characteristics.

It should be noted that, for extrapolation to be accepted, therapeutic efficacy must rely on a similar mechanism of action for both the original indication where the confirmatory study was conducted in and the extrapolated indication, e.g. INX in rheumatoid arthritis and psoriatic arthritis.

Switching and substitution, said Prof Isaacs, represented one of the most controversial topics. Switching, which refers to a clinician deciding to change from a reference to a biosimilar, represented a very different situation from substitution, which involves a third party (such as a pharmacist) making that decision. This is not currently allowed anywhere in the world, although the FDA are considering a category of 'interchangeability' that would allow substitution, subject to rigorous clinical trial data involving multiple switches between reference and biosimilar.

For pharmacovigilance, product identification is of paramount importance. In Europe, biosimilars use the same international non-proprietary name e.g. INX, while in the USA they are given distinct names with the international non-proprietary name modified by a suffix (e.g. etanercept-szzs, INX-dyab). Prof Isaacs noted the danger that the European system could inappropriately suggest interchangeability, underlining the need to use brand names within Europe. He provided an overview of the different trial designs used in biosimilar switching studies to confirm clinical bioequivalence for biosimilar approval (Figure 1).[2] The difference in design does not allow for direct comparison of efficacy and safety data of different biosimilars.

Transition Studies

In a transition study, half the subjects receive reference drug and half the subjects receive biosimilar, and the reference group is subsequently switched to the biosimilar. The idea is to address whether the transition has, for example, precipitated immunogenicity. Examples of transition studies are PLANETRA and PLANETAS, which compare CT-P13 and INX.[5,6,8,9]

Single Switch Studies

In a single switch study, half the patients receive the reference and half receive the biosimilar, and then half the reference group is switched to the biosimilar and half remains on the same treatment. This approach allows comparison of the two arms to see if there are any differences in outcome. An example is the ABP 501 study exploring an adalimumab biosimilar in psoriasis.[10]

Multiple Switch Studies

Multiple switch studies involve switching therapies multiple times (alternating) between the reference drug and a biosimilar. The objective is to look at the PK after each switch, as well as efficacy and immunogenicity. An example of a multiple switch study is EGALITY comparing the etanercept biosimilar GP2015 with its reference in psoriasis.[11]

With a future prospect of multiple biosimilars, Prof Isaacs questioned what would happen if formularies were to subsequently adopt multiple biosimilars (A, B, C, D, or E) and whether physicians could feel confident switching between these biosimilars and back to reference. At present, a difficulty is that biosimilar trial designs are heterogeneous, he said, making indirect comparisons problematic.

In a recent editorial that he wrote with Jonathan Kay,[4] Prof Isaacs argued that as more biosimilars enter the market, standardisation of clinical trial design would allow for indirect comparisons between them, adding confidence to decision-making around switching. Standardisation, they suggested, might include studying healthy subjects versus patients (in Phase I), specific inclusion and exclusion criteria, equivalence margins, primary endpoints (including timing of assessment), secondary endpoints (including timing of assessment), PK assays (endpoints compared and timing of assessment), immunogenicity (assays used and timing of testing), analysis of effects of immunogenicity on PK, efficacy and safety, definition of adverse events, statistical analyses, and crossover designs beyond the primary endpoint (in Phase III). If adopted, standardisation would simplify indirect comparison between biosimilars and help clinicians to feel more comfortable about switching.

Clinical Evidence for Biosimilars and Switching: The Nor-Switch Study

Doctor Guro Løvik Goll

Dr Goll gave a lucid introduction into the Norwegian healthcare system, where 100% of healthcare costs are covered by the government. With increasing use of biologics over the past 7 or 8 years, pharma companies in Norway have been invited to tender for providing treatments, with cost calculations proving a key consideration for choosing the winning options. It was important to stress that the tendering process only applies to patients starting their first biologic, and furthermore, if physicians feel there are 'good reasons' to choose different drugs they can make a special case for individual patients.

A substantial discount in the price of biosimilar INX in early 2015 led to a dramatic increase in its use in Norway and highlighted the key clinical question of what to do with patients who were already stable on the reference biologic Remicade® (INX). Questions included whether it was safe or even ethical to switch them to the biosimilar? The NOR-SWITCH study,[12] funded by the Norwegian government, set out to assess if CT-P13 was non-inferior to innovator INX with regard to disease worsening in patients who had been on stable INX. The study took place across 40 centres in Norway, involving 16 rheumatology departments, 19 gastroenterology departments, and 5 dermatology departments.

Inclusion criteria for NOR-SWITCH were clinical diagnoses of either rheumatoid arthritis (n=78), spondyloarthritis (n=91), psoriatic arthritis (n=30), ulcerative colitis (n=93), Crohn's disease (n=155), or chronic plaque psoriasis (n=35). To be eligible, patients needed to have undergone stable treatment with INX for the last 6 months.

Enrolled patients (N=482) were randomised 1:1 to continue treatment with INX (n=241) or to be switched from INX to CT-P13 (n=241). Additionally, 380 patients from both arms were entered into an open label extension study with all patients treated with CT-P13. For this group additional assessment was scheduled for Week 78. See Figure 2 for the NOR-SWITCH study design.

From power calculations, using a 15% non-inferiority margin with 30% disease worsening at Week 48 (based on a power of 90% and alpha 2.5%) it had been estimated that the NOR-SWITCH study would need to treat 394 patients.

Results showed the primary endpoint (disease worsening assessed at Week 52 using different definitions for each of the six diseases) occurred in 26.2% of patients continued on INX compared to 29.6% switched to CT-P13 (rate difference: -4.4%; 95% CI: -12.7 to -3.9). Although results fell within the 15% prespecified non-inferiority margin for CT-P13 to INX, data for Crohn's disease fell close to the non-inferiority margin. Dr Goll said that, investigators have been cautious about drawing conclusions based on the individual diagnoses, and it was thought likely that Crohn's disease was a 'spurious' finding since they did not see any signals for C-reactive protein or disease remission. Serum drug trough levels for INX and CT-P13 were similar from baseline to Week 52. Additionally, incidence of anti-drug antibodies occurred in 7.1% of patients taking INX versus 7.9% taking CT-P13.

The strengths of NOR-SWITCH, said Dr Goll, included the randomised controlled trial design, comprehensive data collection, inclusion of sufficient numbers of patients according to power calculations, having patient representatives in the project group, government finance (no industry involvement), and that drugs were provided through the regular payment schedule. Limitations included the finding that it was not powered for non-inferiority within each diagnostic group and the absence of data on patients who declined participation.

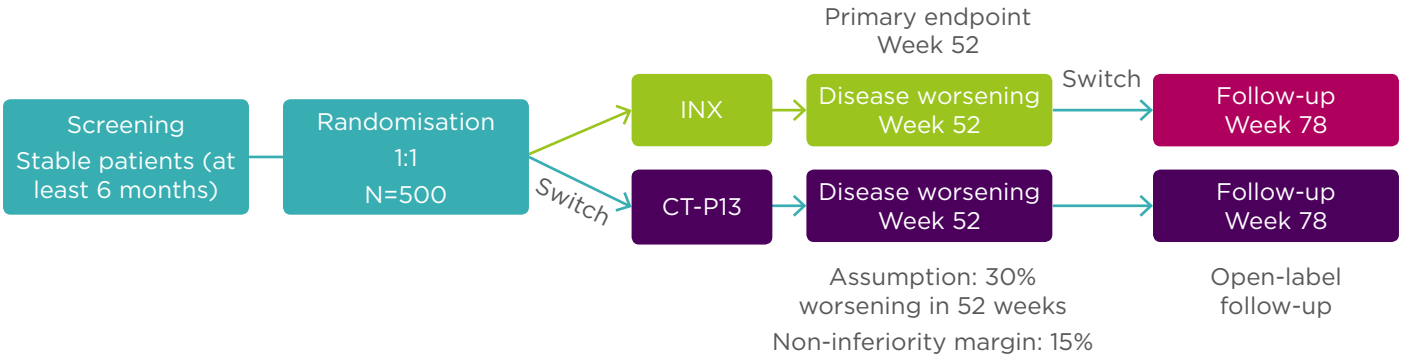

Figure 2: NOR-SWITCH study design.[12]
A randomised, double-blind, parallel-group study to evaluate the safety and efficacy of switching from innovator infliximab to biosimilar infliximab compared with continued treatment with innovator infliximab in patients with rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, ulcerative colitis, Crohn's disease, and chronic plaque psoriasis.
INX: infliximab.

The NOR-SWITCH trial, said Dr Goll, showed that switching from INX to CT-P13 was not inferior to continued INX treatment and supported switching from INX to CT-P13 for non-medical reasons.

Safety and Immunogenicity in Switching

Professor João Gonçalves

Currently, said Prof Gonçalves, there are around 50 biosimilars in clinical development. Biosimilars, he explained, have stringent assessment criteria that include clinical trials and functional and analytical testing.

The goal for companies is to show consistent quality, which in turn delivers consistent safety and efficacy. It was important to pay close attention to critical quality and safety variables including:[13]

- Immunogenicity (aggregates, impurities)
- Safety/toxicity (antibody purity, antibody glycosylation, antibody modifications)
- PK (antibody structure, antibody glycosylation)
- Efficacy (antibody glycosylation mechanism of action in all indications)

For biosimilars, extrapolation to other unstudied indications is possible, based on all data generated with the biosimilar (totality of evidence concept). For example, biosimilar INX CT-P13 was first studied in rheumatoid arthritis and ankylosing spondylitis, but data have been extrapolated to allow use in psoriatic arthritis, rheumatoid arthritis, ulcerative colitis, Crohn's disease, psoriasis, and ankylosing spondylitis. Prof Gonçalves gave the example of seven approved biosimilars studied in seven indications and then used in 22 different indications.[14]

Data from Prof Gonçalves' laboratory (unpublished) found fewer adverse events for CT-P13 when the number of adverse events occurring in the first 3 years after the launch of INX were compared to the number of adverse events occurring in the first 3 years after launch of CT-P13.[15] What is crucial for consolidating confidence in biosimilars, said Prof Gonçalves, is continuation of active pharmacovigilance after biosimilars come to market.

The uncomfortable reality of all biological treatments is that manufacturing changes happen frequently, which can result in structure and function differences. It is important that regulators and companies know how to manage such variability, said Prof Gonçalves. 'Critical quality attribute' testing can be used to ensure biological drugs act in similar ways to previous batches. He explained that safety issues more often arise with the reference medicine than in the biosimilar.

A further concern is the immunogenic sensitivity of inflammatory diseases, which are sensitive to aggregated proteins. Therapeutic proteins have a propensity for aggregation (during manufacture, shipping, and storage) and the presence of aggregates may induce adverse immune responses in patients that may affect safety and efficacy.[16]

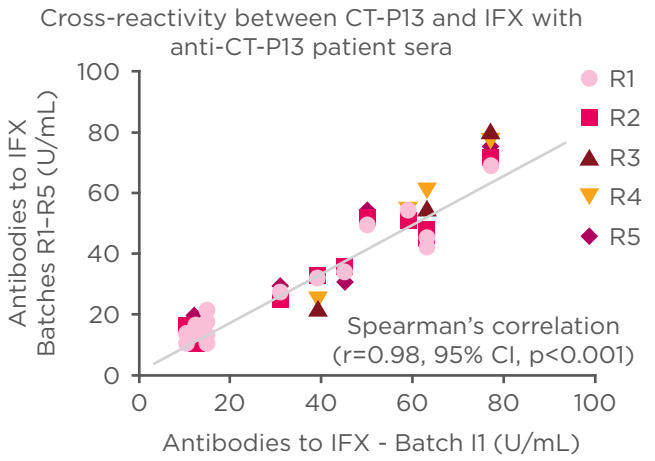

Figure 3: Studies showing that the immunogenic epitopes show no difference between CT-P13 and infliximab.

No evidence of new epitopes in biosimilar IFX.

IFX: infliximab; CI: confidence interval.

Aggregates are believed to result in amplification of anti-drug immune responses including enhancing T cell responses and activation of dendritic cells.

The risk of anti-drug immune responses is known, said Prof Gonçalves, and its main consequence is that patients who are anti-drug antibody positive might achieve lower drug response rates. One meta-analysis assessing the effects of anti-drug antibodies on response to INX, adalimumab, and etanercept showed that patients with rheumatoid arthritis, spondyloarthritis, psoriasis, and inflammatory bowel disease who were anti-drug antibody positive achieved lower response rates than patients who were anti-drug antibody negative (risk ratio: 0.32; 95% CI: 0.22 to -0.48).[17]

The probability of developing immunogenicity does not depend on the drug alone. A number of factors influence the probability of immunogenicity.[18,19] Those associated with the product include whether self or non-self, the presence of T cell epitopes, formulation (including impurities and aggregates), and post-translational modifications. Those not associated with the product include routes of administration (intravenous versus subcutaneous), whether used for an acute or chronic disease, PK, and whether the target was cellular or soluble. Finally, factors associated with patients include haplotype, tolerance to protein, immunosuppression, and pathology.

An interesting question, said Prof Gonçalves, was whether the immune system identifies the biosimilar as different from the reference medicine. The factor that is often forgotten, he said, was that biosimilars and reference biologic have exactly the same amino acid sequences leading to low risks of B cell activation. It would only be triggered by protein aggregation. Recently, when Prof Gonçalves and colleagues undertook a cross reactivity assessment between CT-P13 and IFX using anti-CT-P13 patient sera, they showed strong correlations suggesting no evidence of new epitopes in CT-P13.[20] (Figure 3)

When investigators went on to identify every monoclonal antibody produced by patients for both the original drug and biosimilar, they found no differences between the Fc and Fab regions. Such findings, said Prof Gonçalves, suggest the immune system recognises the two drugs in the same way. A range of studies exploring immunogenicity in INX, adalimumab, and etanercept biosimilars showed that the risk of switching was minimal for both switching to biosimilars and also using different biosimilar batches.[8,21-23]

Emphasising the importance of pharmacovigilance around immunogenicity for both original biologics and biosimilars, Prof Gonçalves said measures should be introduced to ensure traceability of batches and products. He suggested there should also be possibilities to define and monitor clinical endpoints relevant to potential risk of immunogenicity.[24]

It was important, he added, to establish immunogenicity endpoints that would be measured during a managed biosimilar switch. They could include drug trough levels and immunogenicity testing, adverse events (which could be included in a biologic registry), patient-reported side effects, patient-reported outcome measures, disease activity assessments, laboratory tests of inflammation (C-reactive protein and erythrocyte sedimentation rates), other blood tests, and economic endpoints. The frequency of follow-up and the member of the healthcare team administering the test would also need to be decided.

An integrated approach is needed. Prof Gonçalves added that all switching information obtained from registries should be pooled with voluntarily reported and suspected adverse drug reactions in pharmacovigilance. Furthermore, to eliminate drug-related causes of concerns the pharmacy should undertake a risk assessment model to

consider unsafe handling of biological agents that could lead to altered immunogenicity.

The Patient's Voice in Biosimilar Use and Switching Decisions

Ms Ailsa Bosworth

Patients, said Ms Bosworth, require reassurance and explanations about the differences between biologics and biosimilars in language and terminology that makes sense to them. The experience of the NRAS has been that patients accept switching when reasons are explained to them in 'accessible' ways that they can relate to.

Honesty, said Ms Bosworth, is required when explaining reasons for switching. Health staff should not hide the fact that saving money is the reason for switching. In economies where health systems are under strain from ageing populations it is right to be careful about use of resources. However, Ms Bosworth said, she had come across examples of units saying that switching would mean that more patients would benefit from new treatments at earlier stages in their disease pathway. In the UK, she said, this was not the case since the National Institute for Health and Care Excellence (NICE) had strict eligibility criteria. NRAS, she said, has just launched a new booklet, 'Medicines in Rheumatoid Arthritis', covering such issues.

When considering switching, not all patients are suitable candidates. Ms Bosworth said that patients who do well on biologics will most probably do well with biosimilars, but patients who have reacted badly to biologics or had difficulty becoming stable may not be suitable to switch. Healthcare teams must review their patients carefully before making decisions to 'switch all'.

NRAS believes when decisions are taken to switch patients to biosimilars, that stakeholder groups should be established for discussions around the switch programmes with representatives from all parties involved, including at least two patients. The rationale for including two patients, she explained, was to prevent individuals from feeling isolated. Sadly, there are examples of switching programmes that have been implemented without consulting patients. In the UK, patients are most often advised by letter that they are going to be switched. This, said Ms Bosworth, can work if such letters are accurate, appropriately worded, and give patients the opportunity to contact members of their healthcare teams to discuss any concerns.

NRAS resources on biosimilars include its position paper, a video interview with the NRAS Chief Medical Advisor Prof Peter Taylor, an NHS England publication "What is a Biosimilar", a stakeholder review, and a report of the NHS England Biosimilar Medicines Workshop. NRAS, said Ms Bosworth, is happy to advise units on the wording for patients' letters about switching.

Ms Bosworth explained that data collection should be considered important by everyone, with manufacturers investing in registries and patients providing data for them. Registry data on biologics collected in registries across Europe has provided enormous reassurance around safety. Now, she said, it is vital to collect similar data on biosimilars. While currently the numbers of patients on biosimilars are most likely insufficient to identify rare side effects, the increasing number of patients who will in future be prescribed biosimilars should enable this.

When it comes to sharing the savings resulting from switching patients, NRAS believes they should be split in an equitable way between the commissioners, the hospital units, and the rheumatology teams. When rheumatology teams (who implement the work of switching) receive a share they can invest in patient services. In the UK, there have been examples where rheumatology teams have invested their share of savings into appointing additional nurse specialists. However, Ms Bosworth cautioned that in the UK there have been delays lasting >12 months while stakeholders argue about who receives the savings. It was important, she stressed, to settle such discussions quickly since as prices shift downwards the current levels of gain will not last.

To illustrate comprehensible educational materials in patient-appropriate language as provided by NRAS, Ms Bosworth closed her presentation with a clip from an educational interview on the issues of biosimilars she had undertaken with NRAS' Chief Medical Advisor Prof Peter Taylor.[25] Patient organisations are partners of choice for patients as well as healthcare professionals to optimise the shared decision-making process in biosimilar use and switching.

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SWITCHING PATIENTS FROM ORIGINATOR TO BIOSIMILAR MEDICATIONS IN RHEUMATOID ARTHRITIS: LIMITING THE 'NOCEBO' EFFECT

This symposium took place on 16th June as part of the European League Against Rheumatism (EULAR) Congress 2017 in Madrid, Spain

Moderator

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MEETING SUMMARY

Biosimilars have been available in Europe since 2006, and biosimilars of monoclonal antibodies since 2013, and are now a widespread clinical reality. Since their introduction, various sources of data have become available to help physicians make knowledgeable decisions about their use. For example, randomised clinical trials can demonstrate the comparable efficacy and safety between the biosimilar and its reference biologic. Real-world evidence from registries and individual clinical centres provide additional data on the actual use of biosimilars across different therapeutic indications and broader patient populations, including those who have switched from the reference biologic to the biosimilar, while offering additional understanding of the long-term safety and effectiveness. Well-informed decisions based on a solid understanding of these data are important and can help the physician guide the patient to their own well-informed decision, thereby reducing the possibility of a nocebo effect. Here we review available data sources and look at best practice examples of communicating with patients.

Introduction

Professor Chris Edwards

Over 20 biosimilar products are licensed in Europe, including two infliximab (INF) biosimilars (CT-P13 and SB2) and two etanercept (ETN) biosimilars (SB4 and GP2015). These biosimilars increase patient access to effective biologic treatment and offer opportunities for cost savings to healthcare organisations. For these benefits to be realised, healthcare professionals (HCPs) must be confident that biosimilars have similar efficacy and safety to their reference products. Furthermore, HCPs should consider how transferring this confidence

in treatment to patients influences the likelihood of optimising adherence and limiting unwanted nocebo effects.

The different sources of information available to HCPs to gain that confidence and then use it to make informed shared treatment decisions with their patients were reviewed by international experts at a Biogen-sponsored interactive symposium at the European League Against Rheumatism (EULAR) 2017 congress. Additionally, the expert faculty and audience discussed the process, practicalities, and best practice when switching patients to biosimilar treatment. Prof Edwards commented: "[For me] we are thinking more broadly than biosimilars because there are messages and lessons within what we are going to talk about that influence how we treat patients, how we manage change, and how we work as individuals and departments."

Randomised Clinical Trials: Laying the Foundation

Professor Chris Edwards

Prof Edwards stated: "There are different layers of information required to make a confident decision to change treatment, what do you need to know? We need to look at the Phase III studies, specifically the extension studies, to look at the switching of patients from one biologic to another."

For novel biologics, the goal of the development process is to demonstrate *de novo* the risk-benefit profile of the candidate product, thus emphasising the role of clinical trials in this process. However, in biosimilar development, the reverse is true, as the aim is to demonstrate that the biosimilar is similar to the reference product, and to leverage the risk-benefit profile that has previously been established. Following comprehensive analytical comparability exercises and a Phase I pharmacokinetic comparability study, a Phase III randomised controlled trial (RCT) is conducted to demonstrate equivalent efficacy and comparable safety of the biosimilar with the reference product. Data from extension studies are available to demonstrate clinical outcomes following switching between reference products and biosimilars.

For example, the development programme for SB4 included a Phase III, 52-week, randomised, double-blind trial, where patients with moderate-to-severe rheumatoid arthritis (RA) despite methotrexate (MTX) therapy received either subcutaneous SB4 or ETN 50 mg every week. After 52 weeks of treatment, patients in the Czech Republic and Poland were enrolled into an open-label extension period for an additional 48 weeks and received SB4. Long-term safety and efficacy of SB4 were compared between patients who continued SB4 (SB4/SB4; n=126) and those who switched from ETN to SB4 (ETN/SB4; n=119).[1] The mean disease activity score based on 28 joints and simplified disease activity index were found to be comparable between the two groups during the extension period, suggesting efficacy is sustained after switching from ETN to SB4. Switching from ETN to SB4 also resulted in no treatment emergent issues, suggesting SB4 was well-tolerated and effective over 2 years in patients with RA.[2]

"Overlap of confidence intervals suggests there is no evidence of difference in disease activity scores achieved with these agents. Safety, being of great importance whenever starting a new drug, was also similar between the two groups regardless of whether they continued on SB4 treatment or switched from ETN to SB4," noted Prof Edwards.

Another Phase III study investigated the safety and sustained efficacy in patients with moderate-to-severe RA who were randomised to receive either SB2 or INF until Week 46. At Week 54, patients previously receiving INF were re-randomised to either receive SB2 (INF/SB2; n=94) or continue INF (INF/INF; n=101) up to Week 70; those patients initially receiving SB2 continued to receive SB2 (SB2/SB2; n=201).[3] Efficacy, safety, and immunogenicity were comparable between the three treatment groups up to Week 78, suggesting SB2 was well-tolerated and effective even in patients who switched from INF to SB2.

Cross-Reactivity

Additional analysis to confirm cross-reactivity between the biosimilar and reference product can provide further support of the similarity of these agents and provide additional confidence when considering switching patients. For example, a recent study aimed to determine whether antibodies to INF (ATI) developed in patients with inflammatory bowel disease treated with INF or CT-P13 or, having switched from INF to CT-P13, cross-reacted with the biosimilars CT-P13 and SB2. Three bridging enzyme-linked immunosorbent assays were used to measure ATI levels and

results suggested that similar immunodominant epitopes on the reference and biosimilar drugs are responsible for the same degree of reactivity, and that the Promonitor®-ANTI-INF (Grifols, Barcelona, Spain) test can be used to detect antibodies to CT-P13 or SB2 in patients treated with biosimilars.[4]

"Thinking about immunogenicity, does switching patients between reference product and biosimilars mean these monoclonal antibodies are similarly recognised by the immune system in these patients? Do the anti-drug antibodies bind to similar, immune epitopes, of these drugs? The same levels of anti-drug antibodies were seen in these patient groups, suggesting antibodies against one type of INF antibodies will bind to other INF-type molecules in the form of CT-P13 and SB2 as well," mused Prof Edwards.

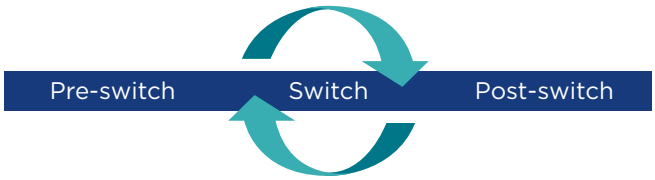
Registry Data: Framing the Knowledge-Base

Professor Merete Lund Hetland

Additional evidence that complements RCT data is derived from registries. DANBIO, a mandatory nationwide registry in Denmark, is capturing routine practice across the country. In 2015, according to guidelines, a switch for non-medical reasons (non-medical switch) from INF to CT-P13 was implemented in Denmark. Following the approval of SB4 in 2016, patients receiving ETN also experienced a non-medical switch to SB4.

Prof Hetland explained: "In Denmark, the originators and biosimilars are treated as equals by the authorities. The HCPs know what the patient is receiving, whether it's an originator or a biosimilar, and can therefore monitor their response to the individual treatments in DANBIO, enabling us to study effectiveness of the biosimilar drugs."

Using data from DANBIO, the following observational study aimed to investigate the impact of a nationwide switch from INF to CT-P13 and from ETN to SB4 on disease activity and flare rates 3 months before, during, and 3 months after switching patients to CT-P13 or SB4. In patients treated with CT-P13, reasons for withdrawal were analysed and the CT-P13 retention rate was compared with an historic cohort of INF-treated patients.[5,6] Patients who switched from INF to CT-P13 had prior INF treatment duration of 6.3–7.3 years. Results showed no evidence of clinically relevant differences in disease activity across indications (RA, psoriatic arthritis [PsA], and axial spondyloarthritis) (Figure 1) and similar flare rates were seen pre and post-switch.



	Pre-switch	Switch	Post-switch
RA (DAS28)			
INF → CT-P13 (n=403)	2.2 (1.7–2.9)	2.2 (1.8–3.0)	2.2 (1.8–3.2)
ETN → SB4 (n=891)	2.1 (1.7–2.9)	2.1 (1.6–2.9)	2.2 (1.6–3.1)
PsA (DAS28)			
INF → CT-P13 (n=120)	2.5 (1.7–3.1)	2.3 (1.7–3.1)	2.4 (1.8–3.3)
ETN → SB4 (n=335)	2.0 (1.5–2.6)	2.0 (1.6–2.8)	2.1 (1.5–3.0)
AxSpA (BASDAI, mm)			
INF → CT-P13 (n=279)	23.0 (8.0–40.0)	24.0 (10.0–40.0)	25.0 (11.0–45.0)
ETN → SB4 (n=322)	33.0 (15.0–51.0)	27.0 (12.0–48.0)	29.0 (16.0–47.0)

Figure 1: Disease activity 3 months prior to versus 3 months after the switch stratified by diagnosis.
AxSpA: axial spondyloarthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; DAS28: disease activity score in 28 joints; ETN: etanercept; INF: infliximab; PsA: psoriatic arthritis; RA: rheumatoid arthritis.

Of the 132 patients (16%) who discontinued CT-P13 treatment within a year, lack of efficacy and adverse events (AEs) were registered as reasons for withdrawal in 71 and 37 patients, respectively. As all patients were switched to biosimilar treatment, there was no control group to compare retention rates against and, therefore, historic cohort group data were used. This adjusted analysis (Multivariable Cox regression analysis adjusted for the following baseline variables: age, sex, diagnosis, MTX treatment [yes/no], comorbidities [number], and patients' global score) found the absolute retention rate of CT-P13 was significantly different by 3.4% from that of INF in the comparison cohort, suggesting CT-P13-treated patients had a slightly higher risk of withdrawal than INF-treated patients.

Patients who switched from ETN to SB4 had received prior ETN treatment for an average of 5.2 years, and a large proportion of patients with RA (60%) and PsA (49%) received concomitant MTX. In this population, 3-month disease activity and flare rates were largely unaffected by switching patients to SB4 (Figure 1). Discontinuation of SB4 (n=129; 9%), during 5 months treatment, was mainly reported as lack of effect or AEs. A higher patient global score (hazard ratio [HR]: 1.12/cm; 95% confidence interval [CI]: 1.05–1.21; p=0.002) and no concomitant MTX (HR: 2.28; 95% CI: 1.48–3.52; p<0.001) at baseline were associated with higher discontinuation rates.

These observational nationwide studies of 2,350 patients with RA, PsA, or axial spondyloarthritis who undertook a non-medical switch to biosimilars found disease activity and flare rates were largely unaffected. Biologic-naïve patients are also being prescribed biosimilars in Denmark due to national guidelines recommending the use of the most economical options for a biologic.

Patient Perspective: Transfer of Knowledge

Associate Professor Lars Erik Kristensen

Assoc Prof Kristensen quoted Bernard Lown: "Words are the most powerful tool a doctor possesses, but words, like a two-edged sword, can maim as well as heal."

Although RCT and real-world data detailing the efficacy/effectiveness and safety of a medication are important when making treatment decisions, HCP interaction with their patients, and how it fundamentally influences the likelihood of success or failure of a treatment, is also vitally important.

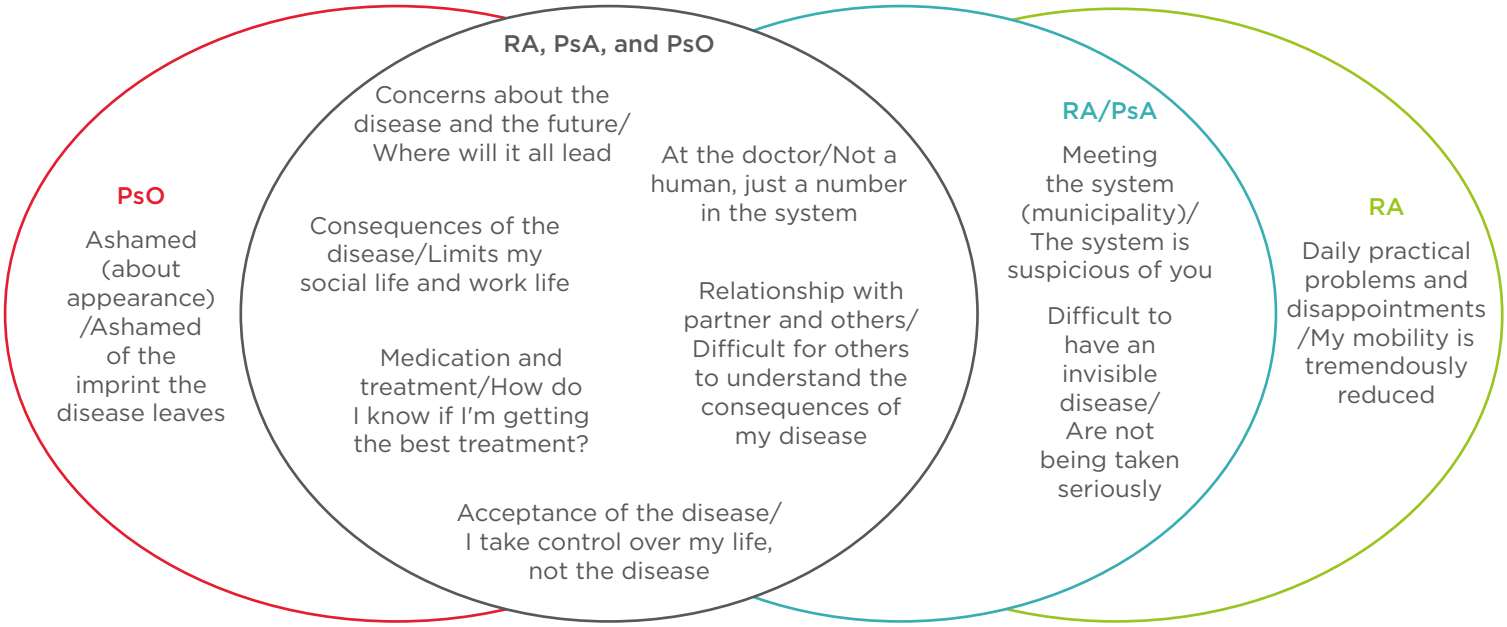


Figure 2: Disease-specific concepts generated during patient workshops.
PsA: psoriatic arthritis; PsO: psoriasis; RA: rheumatoid arthritis.
Jørgensen TS et al. Identifying Generic and Specific Patients' Perspectives on Disease and Treatment Related Issues in Rheumatoid Arthritis, Psoriatic Arthritis, and Psoriasis: A Qualitative Concept Mapping Study. Arthritis Rheum. 2016;68(Suppl. S10):Abstract 1422. Copyright © 2016. Reproduced with permission of John Wiley & Sons, Inc.

A recent example of how communication can strongly influence treatment outcomes was published.[7] In this study, both patients and physicians were blinded to treatment; patients were randomised to receive either statin or placebo. During the double-blind phase, statin-treated patients reported similar AEs to that of the placebo group (298 in the treatment group versus 283 in the placebo group; HR: 1.03; 95% CI: 0.88–1.21; p=0.72). However, a significant excess in muscle-related AEs was reported during the open-label phase when patients and physicians both knew statin treatment was being administered (161 versus 124; HR: 1.41; 95% CI: 1.10–1.79; p=0.006). This was reported to be due to a nocebo effect, the negative equivalent to a placebo effect that can lead to the induction or the worsening of symptoms, which likely resulted from negative perceptions about statin use or due to poor understanding of statin side effects. This highlights the importance patient perspective plays in promoting patient empowerment and adherence to treatment, which leads to optimal disease management.

The likelihood of a nocebo effect is influenced by different factors, including patient-related factors (e.g. psychiatric illness or personality traits), verbal and non-verbal communication, which may unintentionally contain negative suggestions or psychological factors (negative expectations and suggestibility), and neurobiological factors. Research at the Parker Institute, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark, is advancing this field by qualitatively exploring disease and treatment-related issues and concerns experienced by patients with RA, PsA, and psoriasis, and trying to understand the clinical importance of these concerns.[8] The Parker model consisted of three stages: 1) ***concept mapping***, a structured focus group process, which was used to identify and organise disease and treatment-related issues and concerns. Data were organised using participants' themes, multidimensional scaling, cluster analysis, participant validation, rating of clinical importance, and thematic analyses, to generate a conceptual model of disease-related concerns experienced by patients (Figure 2); 2) ***participatory design***, which consisted of consecutive iterative sessions with four patients to achieve four final prototypes that answered the question: "How would your ideal communication work and which elements would you need?"; and 3) ***stakeholder evaluation***, which consisted of individual and group interviews and was carried out to explore the applicability and relevance of introducing these findings into the clinic.

Results from this study suggest a three-step approach should be taken when switching patients from a reference biologic to a biosimilar: 1) patients should be informed about the switch well in advance; 2) an appointment with the physician should ideally take place to allow time with the patient to gauge their level of anxiety regarding the switch; 3) a clinical visit to administer the drug under supervision should occur. The chosen wording (i.e. biosimilar/copy/generic/identical) is sensitive and important for patient understanding and empowerment when informing about switching. These qualitative data highlight the importance patient–physician communication has in RA, PsA, and psoriasis. Although the nocebo effect can impair drug performance, effective patient–physician interaction can minimise this.

"This highlights the importance of how physicians communicate with patients, how topics are discussed and the information provided to the patient: balancing the positive and the negative information correctly. This way of communicating may be very important when switching a patient from one drug to another," stated Prof Edwards.

Implementing Change: Real-Life Experiences of Introducing Biosimilars to Patients

Professor Chris Edwards and Doctor Maria Cuadrado

Transitioning patients from a reference biologic to a biosimilar is an important topic, partly due to cost savings. Since this is now a clinical reality, practical guidance on step-by-step implementation of switching is increasingly sought and can be gained most effectively by sharing best practice from institutions that have already made the change. For example, in the UK, two large teaching hospitals, University Hospital Southampton, Southampton and Guy's and St Thomas', London, have switched patients from reference biologics to biosimilars and reported outcomes.[9-11]

At the University Hospital Southampton, all patients with rheumatic diseases were switched from ETN to SB4. Previous involvement in biosimilar trials, working with colleagues with experience of switching patients in other indications, and previous experience of switching patients from INF

to CT-P13 provided physicians with the confidence to switch all their patients simultaneously.[9,10]

The strategy that was implemented focussed on educating both patients and colleagues regarding biosimilar development, treatment, and recent advances. All stakeholders had to agree on the approach taken and if >10% of patients failed on the biosimilar they agreed to stop switching. Patients were assured that they could switch back to ETN if they were unhappy for any reason. Information provided to patients included a letter accompanied with an information sheet detailing the switch, followed by a face-to-face discussion with a consultant rheumatologist and specialist rheumatology nurse. A telephone helpline was also available, which was operated by rheumatology nurses, to provide support to patients and allow them to make an appointment with a rheumatologist and report any AEs. A routine clinical review appointment was also arranged at 3 months after switching and then 6-monthly thereafter or more frequently if required. Following the education and support programme, 99% of patients (n=92) with RA, PsA, and AS, agreed to switch from ETN to SB4. Six months after the switch, 91% of patients had continued treatment with SB4 with sustained efficacy outcomes. Those patients (n=8) who discontinued treatment reported inefficacy or AEs, and it should be noted that these discontinuation rates were comparable to those seen with ETN in the control period 6 months prior to switch. "In the process we went through to make change, we made sure we were giving as much information as possible to patients, we discussed in advance what we were going to do as well," noted Prof Edwards. From this experience, it is clear that informed patient choice, education, and agreement from all stakeholders is vital to effectively switch therapies. It is also important that patients do not feel under pressure and are given the option to revert to previous therapy.[11]

A similar approach to switching patients to biosimilars was taken at Guy's and St Thomas' Foundation Trust (Figure 3). A multidisciplinary team consisting of managers, rheumatologists, and pharmacists discussed the option to switch patients from their reference biologic to a biosimilar.

Key information provided:

- What brands of ETN are available?
- What is a biosimilar?
- Which version of ETN will be used?
- Why is the switch being made?
- When will the switch be made?
- Does the switch affect how medicines are obtained?
- Is the biosimilar as safe/effective as the orginator?
- Side effects associated with the biosimilar
- Storage of treatment
- Additional tests required
- Contact details to discuss switch

Figure 3: Process and informative letter used to switch patients from ETN to SB4.
ETN: etanercept; HCP: healthcare professional.

After extensive research and in-depth analysis of potential cost savings and the positive impact this could have, the decision was taken to switch all patients from ETN to SB4 and also prescribe SB4 to biologic-naïve patients. "We have a fixed budget for drugs, so if we save money we can treat more patients with biologics and can also treat other patients with other drugs that are expensive where we have limitations. So, the money saved will go back into the system to treat other patients," commented Dr Cuadrado.

A key feature of the letter was that it was signed by every member of the multidisciplinary team to highlight to the patient that this was an informed shared decision and therefore provide the patient with confidence. After receiving the letter, only 5 out of 112 patients requested to speak with their physician before consenting to switching. From April 2016–October 2016, 112 patients were switched from ETN to SB4 and 110 biologic-naïve patients were successfully initiated on SB4. "Sometimes we are rushing, so we have to implement a system that does not disrupt us and saving money can help provide extra resources to do that. If patients, after reading the letter, wanted to speak with somebody, they could go to the clinic and ask [the specialist nurse or pharmacist] whatever they wanted," declared Dr Cuadrado.

Conclusions

Dr Cuadrado concluded: "When we started using anti-tumour necrosis factors, we did not know what was going to happen, we did not have long-term data, but we used it and continue to use it despite safety concerns we had/have, this is because the risks do not overweigh the benefits and we have seen the lives of our patients change with anti-tumour necrosis factors. So, I now think the same approach should be taken with biosimilars."

Given the positive impact biosimilars can have on patients, individual departments, and healthcare systems as a whole, it is important that HCPs have access to information from multiple sources to develop the confidence to successfully implement their use. Additionally, a well-informed HCP is better placed to transfer confidence to patients and reduce the possibility of a nocebo effect.

"How do you get information to make you comfortable about the treatments you use in all different situations? We usually look at the RCTs, we look at the information presented at meetings like this, then we look at real-world registry data, and we learn from our own experience in our clinical centres where all these layers of information build up the pieces of a jigsaw... we keep testing and questioning all the time and remain slightly uncertain until we have a long duration of experience with any medication," said Prof Edwards.

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THE PATIENT'S PERSPECTIVE ON PSORIATIC ARTHRITIS: WHAT MORE CAN RHEUMATOLOGISTS DO TO OPTIMISE DISEASE MANAGEMENT?

This symposium took place on 16th June 2017 as a part of the European League Against Rheumatism (EULAR) Congress in Madrid, Spain

Speakers

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Disclosure: Prof Juan Gomez-Reino has served on advisory boards for AbbVie, Biogen, Bristol-Myers Squibb, Lilly, Pfizer, Roche, R-Pharma, Sanofi, and Regeneron; has been a part of a speaker's bureau for AbbVie, Bristol-Myers Squibb, Janssen, MSD, Pfizer, Roche, and Sanofi; has received grants from MSD, Pfizer, Roche, and UCB. Dr Ana-Maria Orbai has served on advisory boards for Janssen, Eli Lilly, and UCB, as a consultant for Janssen and Pfizer; has been a part of clinical trials as an investigator for Lilly and Novartis. Prof Laure Gossec has received research grants from UCB, Lilly, Pfizer, and Bristol-Myers Squibb; has received consulting fees from AbbVie, Bristol-Myers Squibb, Celgene, Janssen, Novartis, Pfizer, Roche, and UCB; and is a board member of the Executive Committees of EULAR and OMERACT.

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MEETING SUMMARY

The symposium at the European League Against Rheumatism (EULAR) 2017 congress aimed to provide insights into the burden of psoriatic arthritis (PsA) on patients' daily lives, including the significant impact of unresolved musculoskeletal symptoms, and explore the current guidelines for treatment, with a view to identifying strategies to optimise disease management. Case studies were used to evaluate current strategies in PsA management and highlight the critical role of the rheumatologist in patient care. The presentations emphasised that, while patient and clinician priorities for the management of PsA may differ, wider reporting of patient perspectives in studies and patient education may aid in aligning priorities and ensuring the best quality of life (QoL) for patients. The importance of tailoring treatment to the individual was reinforced, and the need to take into account all aspects of disease, including comorbidities and patient relevant outcomes, highlighted.

UNDERSTANDING PSORIATIC ARTHRITIS FROM THE PATIENT'S PERSPECTIVE

The Psoriatic Arthritis Patient Journey: From Diagnosis Through to Clinical Management

Doctor Juan Gomez-Reino

PsA is a progressive disease that is often preceded by dermatological symptoms, with 72% of patients developing skin lesions prior to joint disease.[1] In some cases, skin manifestations can appear ≤10 years before a diagnosis of PsA is made, with patients commonly presenting initially to a primary care physician before being seen by a dermatologist. Joint symptoms, such as pain, stiffness (in 60% of patients), dactylitis (45%), and enthesitis (31%), are often not recognised as related to the skin symptoms by the physician or the patient, and referral to a rheumatologist and diagnosis of PsA

is often delayed. There is no distinctive pattern of skin and joint symptoms, and the absence of a biomarker for PsA also contributes to the delay in diagnosis; ≤50% of patients with both psoriasis (PsO) and PsA remain undiagnosed.[2] Speed of diagnosis is dependent on the primary care physician or dermatologist having the necessary understanding of the condition to promptly refer to a rheumatologist for assessment.[3] Without appropriate treatment, 58% of patients will have erosions in more than five joints after 10 years,[4] impacting both QoL and function. Management of patients with PsA is made more challenging by the development of certain comorbidities requiring additional treatments, which may also restrict the therapeutic options for controlling PsA.[1,3]

Burden of Psoriatic Arthritis on the Patient's Quality of Life

Doctor Ana-Maria Orbai

The challenges faced by patients with PsA are diverse and include physical, psychological, social, and economic factors.[5] Pain associated with the disease is a critical issue for patients with PsA,[1] with fatigue,[6] emotional distress, and depression also contributing significantly to disease burden.[7]

Chronic rheumatic diseases have an increased inflammatory burden that is associated with the development of comorbidities including obesity, hypertension, metabolic syndrome, and infection, with 42% of patients experiencing three or more comorbid conditions.[8] Depression or anxiety occur in approximately one in four patients.[9] Multiple studies have shown that patients with PsA have a decreased QoL compared with patients with PsO alone.[10] There is also an interplay between factors influencing QoL, with emotional aspects impacting on physical and social function.[8] Depression and anxiety impair the ability of patients to self-manage and cope with PsA, leading to a cycle of lack of sleep, low energy levels, and withdrawal from personal relationships.[3]

PsA impacts on multiple aspects of a patient's daily life; patients with worse musculoskeletal symptoms or musculoskeletal damage from the disease have difficulty with many daily tasks. In a recent survey, >20% of patients reported struggling or being unable to bend down to pick up clothing from the floor, and approximately 15% reported difficulty or inability to walk outside on flat ground (either 'unable to do' or 'with much difficulty').[1]

How Can we Improve Alignment with Our Patients?

Professor Laure Gossec

Being able to continue with daily activities is of critical importance to patients and is also a key consideration for physicians when setting goals of treatment. As inflammation is typically driving joint damage in patients and the development of comorbidities, physicians commonly focus on targeting this aspect with drug therapy, with the goal to maintain function and normal daily activities.[11] For patients, improving QoL is their primary goal.[7,12]

A comparison of the impact of disease on QoL using the 36-item short form health survey (SF-36) across rheumatoid arthritis (RA), PsO, and PsA indicates that there is a greater impact on bodily pain, general health, and physical function in patients with PsA and RA, than in PsO (Figure 1).[12] A systemic literature review of 11 studies highlighted the wide range of symptoms experienced by patients with PsA and ranked their importance.[13] Pain was the highest priority physical symptom for patients, with social interaction the highest priority social symptom. Their findings were reinforced in a survey, in which pain was ranked as the highest priority by 84% of respondents.[11]

In order to achieve a more patient-centric approach to management, greater use of patient activity scores and numerical rating scales should be used to evaluate not only pain, but also other factors of importance to patients, such as fatigue. Such tools include the health assessment questionnaire (HAQ)[14] and the PsA impact of disease (PsAID) questionnaire,[15] which is available from the EULAR website in 40 languages. Greater use of such tools in recent years has improved the evaluation of a wider range of factors important to patients.[16,17]

Patient education may help individuals to more clearly communicate about their specific concerns and expectations to healthcare professionals. Recommendations have been published by EULAR, which strongly recommend patient education forms an integral part of patient care and should be tailored and needs based, as well as presented in a variety of formats.[18] Educational resources are available to aid clinicians with this, including written information, self-management programmes, and resources to aid the sharing of experiences between patients.[18]

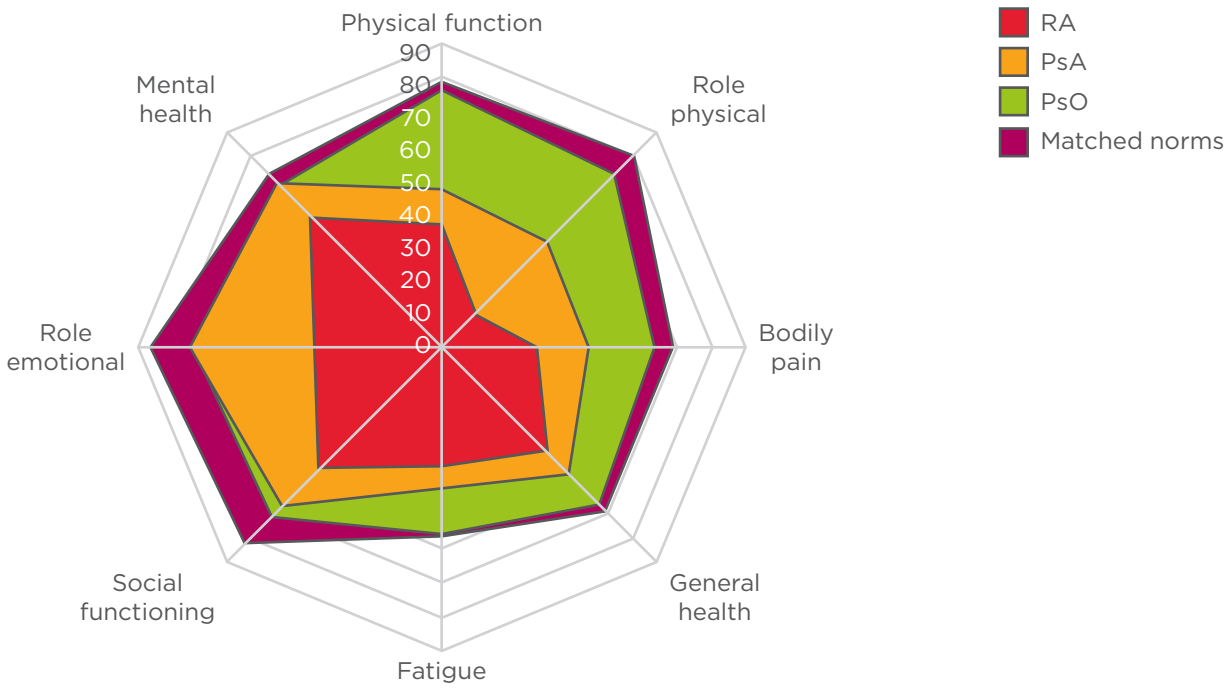

Figure 1: Comparison of health-related quality of life in rheumatoid arthritis, psoriatic arthritis, and psoriasis.

PsA: psoriatic arthritis; PsO: psoriasis; RA: rheumatoid arthritis.

Adapted from Strand et al.[12]

OPTIMISING PSORIATIC ARTHRITIS CARE IN CLINICAL PRACTICE

Integrating the Patient Perspective: Best Practices in Psoriatic Arthritis

Professor Laure Gossec

Two sets of guidelines for the treatment of PsA were published in 2016: the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) guidelines[19] and the EULAR recommendations.[20]

GRAPPA recommendations use the initial symptom, such as peripheral arthritis, enthesitis, or skin or nail disease, to determine treatment approach, although they do not include a specific treatment target.[19] Recommendations state that patients with peripheral joint involvement may be treated with either non-steroidal anti-inflammatory drugs (NSAIDs), with or without glucocorticoid injections, a conventional synthetic disease-modifying anti-rheumatic drug (DMARD), a tumour necrosis factor (TNF) inhibitor, or a phosphodiesterase-4 (PDE-4) inhibitor. As a second step, TNF inhibitors, interleukin (IL)-12/23, IL-17, or PDE-4 inhibitors can be considered.[19]

The EULAR recommendations state that the main treatment target should be clinical remission, or low disease activity where remission is not achievable, based on both patient and physician considerations.[20] NSAIDs and/or local glucocorticoid injections are recommended for peripheral joint involvement, followed by a conventional synthetic DMARD, preferentially methotrexate. Subsequent treatment depends on the presence of adverse prognostic factors, including number of joints involved, acute-phase reactants, and previous joint damage. Patients without adverse prognostic factors can begin a second conventional synthetic DMARD, while patients with such factors may be recommended TNF, IL-12/23, or IL-17 inhibitors.

Treat-to-target is well established in RA, but less well studied in PsA. However, the recent randomised controlled TICOPA trial in patients with PsA revealed that such an approach resulted in significant improvements in both clinical and patient-reported outcomes.[20,21]

The perception of remission differs between patient and physician. The EULAR definition is stable remission with no acute-phase reactants, namely disease activity, pain, patient global function, QoL, fatigue, and systemic inflammation.[22,23] Physicians

will commonly base remission status on either the EULAR principle of no active joints and normal acute phase reactants, but scores can also be used such as the Disease Activity Score (DAS28), though it is not optimal in PsA, the Disease Activity of Psoriatic Arthritis (DAPSA) score,[24] or minimal disease activity (MDA). Patients, on the other hand, are more focussed on the resolution of symptoms, return to pre-disease normality, and other patient-related outcomes. This disparity between patient and physician perception of remission requires alignment, and a combination of endpoints that are important to both parties should be considered.[25]

The Rheumatologist at the Centre of Comprehensive Care

Doctor Juan Gomez-Reino

The heterogeneous nature of PsA makes it a challenging condition to diagnose and treat. Currently, the primary outcome required for US Food and Drug Administration (FDA) approval of new PsA therapies is the American College of Rheumatology 20 criteria (ACR20), which assesses disease activity in the joints.[23] However, ACR20 does not include the assessment of other domains known to affect patients with PsA, including enthesitis, dactylitis, axial involvement, skin disease, nail disease, or peripheral arthritis.[23,25,26]

Composite outcomes for disease assessment, which integrate a number of patient and clinician measures, have been developed to allow a more comprehensive evaluation of treatment response.[25] Current rheumatological practice estimates show that 82% of clinicians include joint counts when assessing the disease, but as few as 14% employ a treat-to-target approach to patient management.[19] Implementing treat-to-target in practice requires a common target to be determined by both the patient and clinician, which also ensures that all PsA domains are assessed using validated methods. To date, MDA criteria have been suggested to be the best compromise between comprehensive assessments and clinical feasibility. Additionally, this assessment should take no more than 5–10 minutes to complete, further simplifying its integration into routine clinical practice.[19,27]

Comorbidities in patients with PsA are also an important consideration in the management of this condition. Beyond management of joint disease, rheumatologists need to become more active in addressing the wider aspects of PsA. EULAR recommendations reinforce the importance of the rheumatologist in taking on the comprehensive comorbidity management of the patient, highlighting the need for participation in systematic and routine assessment, data collection, and treatment.

Maximising Communication Among Stakeholders

Doctor Ana-Maria Orbai

A multidisciplinary approach is essential to ensuring comprehensive care to patients with PsA, with rheumatologists ideally positioned to facilitate collaborative care and communication with general practitioners, patient advocacy groups, dermatologists, psychologists, and other specialists critical to the design and delivery of an effective treatment strategy.

A recent systematic review of collaborative care (N=3 studies) demonstrated improvements in skin and joint symptoms in 56–82% of patients after treatment changes under dermatology–rheumatology management. Up to 94% of patients were reported to be 'very satisfied' with multidisciplinary care.[28] Patients' satisfaction ratings were 4.9 out of 5 for collaborative consultation, compared with 2.9 out of 5 for separate, routine consultations.

Both EULAR and GRAPPA recommend shared treatment decision-making between the patient and physician, with consideration of efficacy, safety, and costs, as well as patient preferences and expectations.[3,19,20] Furthermore, patients should be encouraged to take a more active role in their care, including self-management of pain, involving training by qualified personnel to aid both management and assessment, and to regularly feedback to their rheumatologist. To facilitate this process, channels of communication between the patient and physician should always remain open.[3] Patients involved in the decision-making process appear to have improved outcomes and higher satisfaction with their healthcare.[29]

Effective communication is key in optimising the management and care of patients with PsA. There are several suggested methods for improving and/or maintaining effective patient-physician communication, which include providing education

on the disease, its progression, and its long-term consequences; being supportive of patients recording their symptoms and communicating these to clinicians for appropriate and efficient referral; providing small amounts of information regularly over time, in combination with goal setting and action planning during disease progression; and empowering patients to feel comfortable requesting information about their condition at any point in time.[3]

A fundamental barrier to improving patient–physician communication is a declining number of rheumatologists. Based on recent trends, the number of rheumatologists is predicted to decline by 30% between 2015 and 2030.[30] Innovative approaches are needed to ensure access to care.

Greater use of technology may help overcome challenges associated with reduced access to rheumatologists; however, tools to facilitate efficient disease management are currently underutilised, and there is no effective resource to aid patient awareness and self-management of their condition.[31,32] Electronic data capture, which would allow individuals to input patient-reported outcomes into a digital tool prior to visits, can improve communication through completion of patient-reported outcomes prior to the clinic visit and at the same time facilitate a treat-to-target approach. Using the same tool, rheumatologists would input physical examination findings during the visit.[19] Specialist software can then be used to calculate composite targets based on patient and physician-reported outcomes and determine disease activity status; and also suggest follow-up times based on data history.[19]

An example of the potential value that technology can bring to shared care is exemplified by a smartphone application for RA, based on a DAS28 predictive model consisting of subjective measures and a performance measure (Figure 2). The application was used by nine patients with RA to record patient-reported outcomes over a 3-month period and register their physical activity using their smartphone accelerometer. DAS28 scores predicted by the calculator were compared with DAS28 calculated by a physician. A good correlation was found between the physician and application-calculated DAS28, and indicates that such a model shows promise for integrating routine self-assessments of rheumatologic disease in clinical practice.

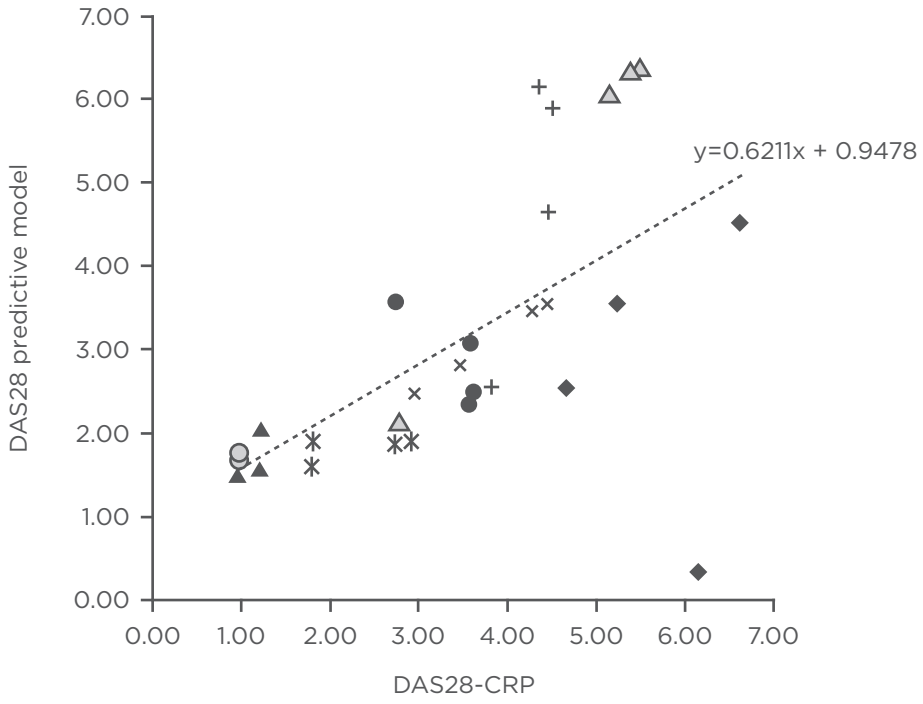

Figure 2: Self-assessed disease activity measured via smartphone correlated well with patient's actual disease.

DAS28: Disease Activity Score 28; CRP: C-reactive protein.

Adapted from Nishiguchi et al.[33]

RISING TO THE CHALLENGE: PATIENT CASE STUDIES

Case 1: Managing a Patient with Refractory Joint Issues Despite Resolution of Other Symptoms

Doctor Ana-Maria Orbai

Prior to a PsA diagnosis, patients will often experience joint pain, stiffness, and fatigue.[34] As many as 47% of patients can continue to experience erosive disease, despite clinical improvement, and 65% of patients with power Doppler ultrasound synovitis at baseline will experience a flare compared with patients without.[35,36]

Patient Case 1

Patient file:

Jeremy is a white, 28-year-old male, with a 5-year history of PsA, and a positive family history of the disease. His BMI is 32 kg/m^2, with a blood pressure of 150/90 mmHg. In addition to PsA, Jeremy's comorbidities include hypercholesterolaemia and hypertension, which are managed through diet and exercise. Jeremy's skin symptoms are currently well managed but pain/stiffness in the hand and ankle joints persist. Jeremy also experiences 1–2 flares a year where the metacarpophalangeal, proximal interphalangeal joints, ankle, and knee joints become tender and swollen. Jeremy prefers to cope with these flare ups on his own.

Initial Treatment Response and Assessment Approaches

Jeremy was started and maintained on a conventional synthetic DMARD (csDMARD) for 18 months before being switched to a TNF inhibitor. Despite 3.5 years of anti-TNF therapy, he has seven swollen joints, nine tender joints, and a HAQ disability score of 1.5, none of which met the MDA criteria cut-offs. Jeremy's clinical DAPSA score, a clinical score used to determine disease activity state based on tender and swollen joint counts, patient global assessment, and a pain scale, was 18.5, which categorises Jeremy in moderate disease activity state. Jeremy's PsAID score was 4.15, which is slightly above the patient-acceptable symptom state (cut-off of ≤4).[37]

Considerations for Case Study 1

Jeremy's active psoriatic disease combined with under-reporting of flare symptoms can make management of the disease using a treat-to-target approach especially challenging. Data collection of 66/68 joint counts, which include upper and lower extremity joints, use of ultrasound imaging data if needed, radiographs of the joints to screen for damage and inflammatory markers are recommended in the management of this specific patient. If axial disease is suspected, a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is recommended. An open discussion of the findings and their implications is encouraged with the patient to help facilitate shared decision making.

Effect of Weight

Jeremy's BMI of 32 presents a challenge in the effective treatment of his PsA. Recent studies have shown that MDA is difficult to achieve in patients with a BMI >30 compared with patients with a BMI <30. Furthermore, weight loss of >5% has been shown to increase the chances of achieving MDA in patients with PsA.[38,39]

Case 2: What is the Best Target for Patients with Multiple Comorbidities?

Doctor Juan Gomez-Reino

Patient Case 2

Patient file:

Luis is a white, 40-year-old male diagnosed with PsO in 2012, and then PsA in 2015. His BMI is 39 with a blood pressure of 160/110 mmHg. He has a family history of PsO. Luis's comorbidities include hypertension and coronary artery disease, obesity, hyperlipidaemia, and Type 2 diabetes mellitus. Luis admits to struggling with treatment adherence due to polypharmacy. Luis has experienced a recent worsening of pain/swelling in several digits, and PsO of the scalp. His history of symptoms includes pain, stiffness, and swelling around joints of the lower extremities and distal joints, a swollen right knee, and erythematous papulosquamous plaques over the extensor surfaces. Luis was started on local glucocorticoid injections, csDMARD, and folic acid for 6 months, then switched to csDMARD, biologic DMARD, and folic acid for 1.5 years.

Patients with PsA will often suffer from many comorbidities,[8] with the increased inflammatory burden commonly associated with increased comorbid conditions, including cardiovascular disease (CVD).[40] Up to 20% of patients seen by rheumatologists do not have a primary

care practitioner[41] and EULAR recommends rheumatologists are responsible for CVD management.[42] Accordingly, rheumatologists can help patients with inflammatory joint disorders, allowing them to manage and reduce the risk of CVD.[43]

Considerations for Case Study 2

NSAIDs can be used to control RA-specific inflammation, which can improve CVD risk.[43] However, NSAIDs must be used with caution in patients with multiple comorbidities, especially for patients with documented CVD or in the presence of CVD risk factors, e.g. hypertension.[42] Glucocorticoids can be used with caution, but are recommended at the lowest effective dose, for the shortest duration, and they should be tapered as rapidly as clinically feasible. Systemic glucocorticoids would not be recommended in this case. Patients such as Luis seldom reach a target of remission; therefore, it is more reasonable to set a target of MDA.

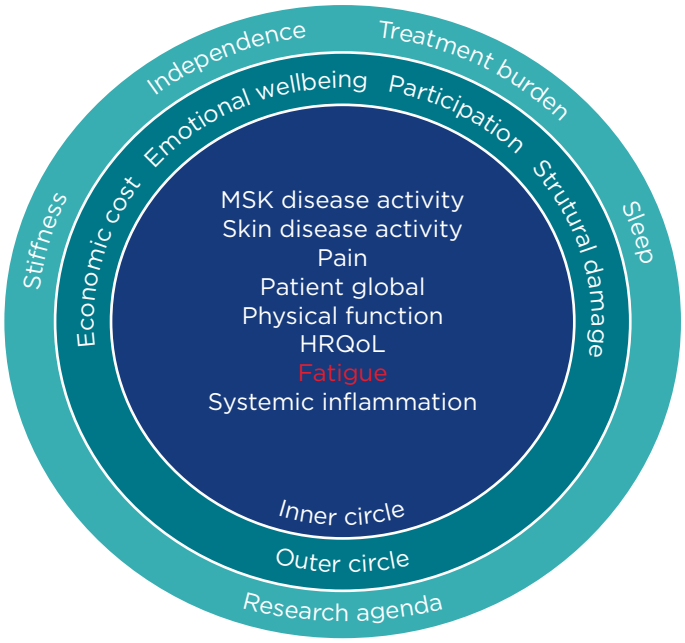

Figure 3: Updated psoriatic arthritis core domain set.
HRQoL: health-related quality of life;
MSK: musculoskeletal.
Adapted from Orbai et al.[22]

Where comorbidities exist, challenges with compliance need to be borne in mind. Failure to adhere to treatment can occur due to a variety of reasons, including medication type, number of daily doses, doubts about treatment necessity, concerns about side effects, and low satisfaction in the physician–patient relationship.[44] Luis has admitted that he struggles with treatment adherence owing to multiple medications, and treatment options that reduce the number of daily doses of treatments he is taking each day should be considered. Concerns regarding side effects associated with individual therapies also need to be addressed.

Case 3: How does Patient-Physician Discordance Influence Disease Management?

Professor Laure Gossec

Patient Case 3

Patient file:

Kim is a 39-year-old female with skin and joint symptoms that have been well-managed for 2.5 years. Kim discussed her experiences with other patients with PsA via an online forum and approached her doctor due to feeling very tired. Although she has been treated with csDMARDs for 2.5 years, current symptoms at assessment included fatigue, joint pain/swelling, and enthesitis. In order to reduce fatigue, a biologic was started alongside a recommendation of exercise, which may aid fatigue.

Patient Priorities

Fatigue reduction is a priority for patients due to its effects on daily life (Figure 3).[22] There is often misalignment between a patient's disease assessment and their physicians due to the difference in priorities; almost one-third of patients with PsA are not aligned with their physicians regarding disease activity, with patients often believing they are doing worse than their physicians.[7]

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PREVALENCE AND SIGNIFICANCE OF ANTI-*SACCHAROMYCES CEREVISIAE* ANTIBODIES IN PRIMARY SJÖGREN'S SYNDROME

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Keywords: Sjögren's syndrome, anti-*Saccharomyces cerevisiae* antibodies (ASCA), anti-Ro/SSA antibodies.

Saccharomyces cerevisiae is a common yeast used in the food industry. The integrity of the intestinal mucosal barrier is a crucial feature that prevents dietary antigens, such as *S. cerevisiae*, from coming into contact with resident immune cells and triggering an immune response. In specific conditions, for example as a consequence of a local inflammatory response, the integrity of the intestinal mucosal barrier is partially lost and subjects develop antibodies against these microbial dietary antigens, such as those against the phosphopeptidomannan of *S. cerevisiae*, termed anti-*Saccharomyces cerevisiae* antibodies (ASCA).[1] Inflammatory bowel diseases are the prototype of diseases characterised by gut inflammation and development of antibodies against microbial dietary antigens. ASCA are detectable in ≤70% of patients with Crohn's disease, highlighting them as a very specific marker for this condition.[2] It has been recently speculated that, in genetically predisposed individuals, microbial dietary antigens may trigger the development of an aberrant autoimmune response through molecular mimicry of autoantigens.[3] In this regard, ASCA have been investigated in several autoimmune diseases, and studies have reported prevalence ranging from 4–>30%.[4] Since no data are currently available for primary Sjögren's syndrome (pSS), we aimed to investigate the prevalence and significance of ASCA in a large cohort of pSS patients, using a highly specific immune-blot assay, and to identify the similarity between ASCA target molecule and pSS specific autoantigens. A total of 104 patients with pSS according to the American-European Consensus criteria were enrolled.[5] The prevalence of ASCA in our pSS cohort was 4.8%.[4] None of the patients had a personal or family history of inflammatory bowel diseases or other autoimmune conditions that could account for ASCA positivity. Since none of the 30 control subjects enrolled in our study displayed ASCA, the specificity of the test in pSS patients was shown to be 100%, despite an extremely low sensitivity. Therefore, the positive predictive value was 100% and the negative predictive value was 23%.

A comparison between the amino acid sequence of *S. cerevisiae* mannan and well characterised auto-antigens peculiar of pSS (52 kD and 60 kD Ro/SSA, La/SSB) was performed with the Basic Local Alignment Search Tool (BLAST). This analysis revealed that the similarity of the 60 kD Ro/SSA ribonucleoprotein was higher than that of both 52 kD Ro/SSA and La/SSB autoantigens when compared to *S. cerevisiae* mannan (identities: 7/11 [64%]; positives: 8/11 [72%]; E-value 2.2), supporting the molecular mimicry hypothesis in pSS. Finally, to identify possible differences in the serological and/or clinical picture, we subdivided our patients according to ASCA status, observing that ASCA positivity is associated with pSS-specific clinical and serological features. In particular, ASCA+ and pSS patients displayed a triple combination of circulating anti-Ro52/SSA, anti-Ro60/SSA,

and anti-La/SSB antibodies, associated with low complement and cutaneous involvement. Regarding the latter, we confirmed this association with binary logistic regression, as ASCA+ pSS patients display an odds ratio of 14 (95% confidence interval: 2.1–97.4; p=0.006) to have cutaneous manifestations of pSS. Conversely, ASCA+ and ASCA- patients did not differ with regard to other serological features such as rheumatoid factor, leukopenia, and hypergammaglobulinaemia, or demographic data such as age, age at diagnosis, and disease duration. In conclusion, our data suggest a possible pathogenic/prognostic significance of ASCA in pSS.

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DEVELOPMENT OF A 37-CHANNEL MASS CYTOMETRY (CYTOF) PANEL TO PREDICT TREATMENT RESPONSE IN RHEUMATOID ARTHRITIS

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Keywords: Cytometry using time-of-flight mass spectrometry (CyTOF), cluster identification, characterisation, and regression (CITRUS), T cell, proteome, immunology, biologic drugs, rheumatology.

Treating the inflammation of rheumatoid arthritis (RA) early leads to less disability and improved patient outcomes.[1] However, only 30–70% of patients will have a good response to the first biologic drug tried,[2] and currently there is no way to predict which drug will be effective in each individual patient. We hypothesise that the RA immunophenotype will be informative in selecting the treatment most likely to be effective first time around. Cytometry using time-of-flight mass spectrometry (CyTOF) greatly increases the number of markers measurable on single cells and will allow much deeper phenotyping of the proteome along with fewer compensation issues as is inherent with fluorescent (flow) cytometry. Simultaneously, advances in non-biased clustering algorithms and their accessibility has allowed multidimensional datasets to be analysed much more comprehensively.

This project optimised a CyTOF T cell panel and tested a novel non-biased clustering algorithm against conventional biaxial gating. Ten healthy controls (HC) and 10 RA patients were included. T cells were stimulated for 4 hours using anti-CD3/anti-CD28 beads, then stained with a 37-channel mass cytometry panel including surface markers, intracellular antigens, and transcription factors (Figure 1). Analysis was performed by biaxial gating and by cluster identification, characterisation, and regression (CITRUS).[3] The CITRUS algorithm compared the two groups: stimulated HC versus stimulated RA T cells.

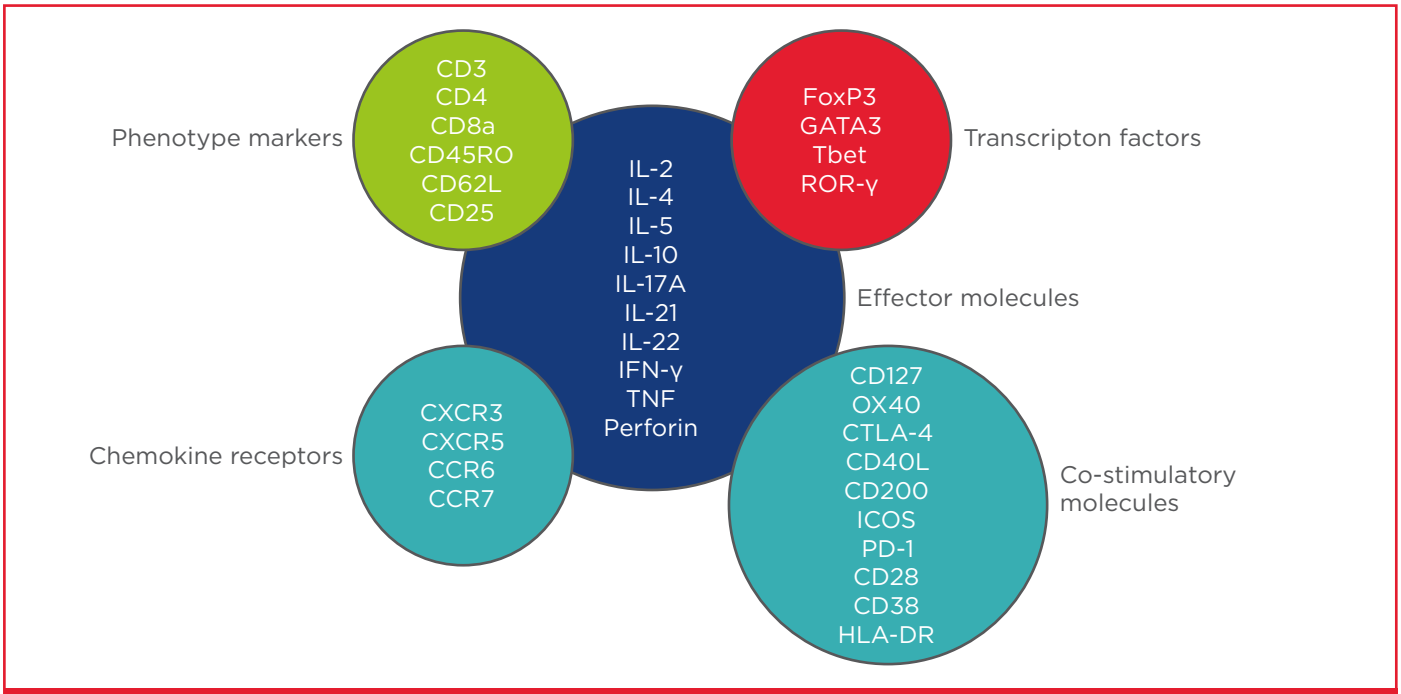

Figure 1: CyTOF T cell panel.
CyTOF: cytometry using time-of-flight mass spectrometry; HLA-DR: human leukocyte antigen-D related; IFN-γ: interferon gamma; IL: interleukin; PD-1: programmed cell death protein 1; TNF: tumour necrosis factor.

To compare abundances of cell clusters, the predictive association model 'Prediction Analysis for Microarrays in R' was used with a minimum cluster size of 2.2%, five cross-validation folds, and a false discovery rate of 1%.

Conventional gating showed that cytokine expression was highly variable within and between HC and RA samples, but no significant differences were found when comparing HC and RA samples. However, using CITRUS, we identified three clusters of cells which were significantly different in abundance between HC and RA. Cluster 1 was $CD4^{+}CD38^{+}$, had characteristics of regulatory T cells, and was less abundant in RA. Cluster 2 was $CD28^{null}CD8^{+}$ expressing perforin and Tbet, and Cluster 3 was a $CD4^{-}CD8$ $CD127^{high}CCR6^{+}$ population, both of which were more abundant in RA.

To conclude, although cytokine expression of *ex vivo* stimulated T cells from RA and HC is highly variable, no differences were found between the two groups with conventional gating and this may have been due to small sample numbers. Using the fully automated clustering algorithm CITRUS, differences in the abundance of three novel cell clusters between the RA and HC groups were revealed and the phenotype of these clusters ascertained. These clusters would have been missed using biaxial gating alone.

Our next step is to compare RA responders to non-responders using an automatic clustering algorithm. By doing so, novel cell clusters might be identified which differ between responders and non-responders, and may go some way to predict treatment response. Furthermore, the function of novel clusters might be further characterised by isolation using fluorescence-activated cell sorting and functional assays. Finally, although in this study biaxial gating did not reveal any significant differences, we believe that automatic clustering algorithms should be used in conjunction with conventional gating when analysing high-dimensional datasets to maximise the scientific output of the data.

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RHEUMATOSPHERE: REACH NEW HEIGHTS IN DIAGNOSIS AND TREATMENT OF ARTHRITIS BY ENGAGING, EMPOWERING, AND INSPIRING

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Keywords: Public engagement, arthritis, Rheumatosphere, inspiring.

Rheumatosphere is an initiative started in Glasgow, UK, in 2012; its aim is to raise awareness about arthritis and arthritis-related research. We have a dedicated team of rheumatologists, basic scientists, PhD students, and nurses, all of whom believe that engagement is a key element of the work that we do.

We strongly believe that engaging with the public is an essential part of scientific life, as the majority of work carried out by researchers is publicly funded. Therefore, we have a responsibility to take the conclusions of our work to the public and make it as easily accessible as possible. Further to this, we feel it is also essential to engage and inspire the next generation of scientists and healthcare professionals. If we do not take the time to engage with the next generation, then the work we start today will not be continued in the coming years.

Another important aspect that Rheumatosphere is planning to focus on is engagement with patients living with rheumatic disease. In order to achieve this, we will provide them with relevant information, highlighting key advances in our knowledge of pathology and treatment. We must then listen to the patients' unmet needs and concerns, and use this information, both clinically and at the bench, in order to guide care and research effectively. This two-way relationship leads to valuable outcomes for both parties.

Rheumatosphere has already developed several engagement modalities for dissemination of information, as well as the collection of information to better improve the engagement we provide. Our activities include musculoskeletal ultrasound, poster material related to immunology (such as 'superhero cells' and 'cell anatomy biscuits'), and our 'cell café'. Throughout all of these activities, we aim to talk to the public and find out what they already know about arthritis. We then use these opportunities to both expand their knowledge and challenge their opinions. During our interactions with the public, we also wish to teach some basic biology and immunology, and we try to link that discussion to the development of arthritis or other rheumatic diseases. We use hand ultrasound machines as a relatively easy and pleasant way to look at the basic anatomy of the hand, and to show how the tendons that attach to the bones and muscle facilitate movement. By teaching these basic concepts, we can then open a dialogue about changes that may occur to these structures during disease processes and then transition into the work that we are able to conduct in the laboratory to try to prevent these pathological changes from occurring.

We have also been collaborating with the Glasgow Science Centre for the past 3 years. We give children the chance to see their body working using an ultrasound machine and highlight how we can

move our fingers. Through these interactions in the schools we also hope to raise awareness about careers in the healthcare profession and science, and provide a fun and interactive introduction that we hope will resonate with the children. During both 2015 and 2016, the Rheumatosphere team attended both Explorathon and the Middle of Scotland Science Festival. Over 2 years at these events, we were able to interact with >3,000 members of the public, and over half of the individuals that we spoke to lived with or knew someone who lives with rheumatoid arthritis. Encouragingly, >80% of these individuals wanted to engage more with Rheumatosphere.

Moving forward, Rheumatosphere is looking to engage with wider audiences. Currently, the majority of the events that we attend are scientific in nature. We wish to go to non-science based community events and talk to a more general audience, in order to further raise awareness about rheumatic disease. We wish to talk to the public about the advantages of early diagnosis and highlight the importance of seeking the advice of the primary healthcare provider if early symptoms of arthritis are present. We plan to do this in conjunction with EULAR's 'Don't Delay Connect Today!' programme that was launched at this year's congress. One of our other upcoming projects is the development of a patient questionnaire and, with the help of the National Rheumatoid Arthritis Society (NRAS), we wish to gather information in order to create a patient-tailored engagement programme. This programme will address the concerns of the patients and will be a fluid programme that will change in accordance with the needs of the patients we engage with.

Rheumatosphere has a firm belief that engaging with the public, children, and patients is an essential part of our vocation as scientists and clinicians, and only by engaging, inspiring, and empowering them will we be able to deliver fully on the many promising developments in arthritic research. We believe our approach also emphasises that no one is fighting arthritis and rheumatic diseases alone, and that we are all part of one big team.

LONGITUDINAL ANALYSIS OF THE GASTROINTESTINAL MICROBIOTA IN SYSTEMIC SCLEROSIS

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Keywords: Systemic sclerosis (SSc), microbiome, gastrointestinal tract (GIT).

Understanding the interplay between the immune system and gastrointestinal tract (GIT) microbiota is an evolving area of research. Emerging evidence suggests that GIT microbiota play a key role in the development of innate and adaptive immunity,[1,2] and may induce or perpetuate aberrant immune function in autoimmune diseases, such as inflammatory bowel disease[3] and rheumatoid arthritis.[4]

Systemic sclerosis (SSc) is a devastating autoimmune disease, which profoundly affects GIT function. While the cause of GIT dysfunction in SSc is unknown, our group recently characterised the lower GIT microbiota in a small, unselected cohort of patients with SSc.[5] We found significant microbial community differences in SSc patients versus healthy controls, in the caecum and sigmoid regions, in patients undergoing colonoscopy.[5] We also observed significant genus-level differences between SSc patients and healthy controls, including decreased beneficial commensal genera such as *Faecalibacterium, Clostridium,* and *Rikenella*, as well as increased potentially pathobiont genera, including *Fusobacterium, Prevotella, Ruminococcus, Akkermansia,* and the uncommon γ-Proteobacteria, *Erwinia* and *Trabulsiella*.[5]

Table 1: Summary of increased and decreased microbial taxa in SSc patients versus controls.

Study	Region	Sample type	N	Increased in SSc*	Decreased in SSc*
Volkmann et al.[5]	Los Angeles, California, USA	Colonic lavage sample	17†	*Lactobacillus; Bifidobacterium; Fusobacterium; Erwinia; Ruminococcus Prevotella*	*Faecalibacterium; Clostridium; Rikenella*
Volkmann et al.[6]	Oslo, Norway	Faecal sample	17	*Lactobacillus*	*Clostridium; Bacteroides*
Volkmann et al.[6]	Los Angeles, California, USA	Faecal sample	17†	*Lactobacillus; Fusobacterium; Erwinia; Ruminococcus*	*Faecalibacterium; Bacteroides*

*Relative to healthy controls.
†These are the same SSc subjects. In Volkmann et al.,[5] we collected lavage specimens from the cecum and sigmoid colon during colonoscopy; in Volkmann et al.,[6] we collected faecal specimens. However, the healthy control groups comprised different individuals in the two studies.[5,6]
SSc: systemic sclerosis.

Our subsequent study, analysing faecal samples of SSc patients in two independent SSc cohorts, identified similar microbial community differences in SSc patients and healthy controls (Table 1).[6] Furthermore, we identified specific microbial genera associated with GIT symptom severity.[6]

However, these studies were cross-sectional; therefore, it is unclear whether the relationships observed between specific genera and GIT symptoms are causational and/or persist with time. To address this limitation, in the present study, we collected faecal specimens and measured GIT symptom severity in SSc patients every 3 months, over a 12-month period. The microbiota from the stool specimens were profiled by multiplex sequencing for bacterial rRNA genes, using an Illumina HiSeq 2500 (Illumina Inc., San Diego, California, USA) sequencing technique. All samples were analysed simultaneously to avoid any batch effects. We assessed GIT symptoms using the GIT 2.0, a valid measure of GIT symptom severity in SSc patients.[7] The questionnaire consists of seven domains, and has been translated and validated in several languages. Scores on the GIT 2.0 can indicate self-rated severity (i.e. none/mild versus moderate versus severe/very severe disease) of GIT involvement based on previously published score thresholds.[7]

We discovered that the absolute and relative abundances of specific genera did not significantly change within individual SSc patients over a 12-month period. In addition, GIT 2.0 scores did not significantly change over the course of the study within each SSc patient. However, we did find that patients with longer disease durations had increased GIT symptoms over time. In addition, patients who had a lower abundance of *Bacteroides* throughout the study had increased GIT symptoms over time, even after controlling for age, sex, ethnicity, disease duration, and SSc subtype (i.e. limited versus diffuse cutaneous disease).

These findings provide further evidence that specific microbial genera may contribute to the GIT phenotype in SSc. Furthermore, these results suggest that efforts to replete these key genera (e.g. supplemental probiotics, faecal transplantation) may help to restore GIT microbiota homeostasis and potentially improve symptoms in SSc.

The present research likely represents the tip of the iceberg in SSc microbiome research. Future research efforts in this area are greatly needed

to better understand the relationships between GIT microbiota, immune responses, and clinical outcomes in SSc.

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DRUG TROUGH LEVELS AND ANTIDRUG ANTIBODIES IN NONSELECTED ANKYLOSING SPONDYLITIS PATIENTS USING SUBCUTANEOUS ANTI-TNF DRUGS

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Keywords: Biological therapy, ankylosing spondylitis (AS), anti-drug antibodies (ADAb).

INTRODUCTION

Immunisation of biological drugs can result in reduced efficacy of treatment and an increased risk of adverse effects. The immunisation of biological drugs has been extensively studied in patients with rheumatoid arthritis, but less is known about the role of immunisation in the treatment of patients with ankylosing spondylitis (AS). In some studies, the development of anti-drug-antibodies (ADAb) has resulted in reduced efficacy, whereas other studies show that no correlation between the presence of ADAb and disease activity has been observed.[1,2] Our aim was to study what proportion of the non-selected AS-patients attending Finnish rheumatological outpatient clinics had been immunised to the subcutaneous anti-tumour necrosis factor (TNF) drug they were using, while also studying the effect of concomitant medication on the risk of immunisation.

METHODS

A total of 313 patients with AS were recruited. Blood samples were taken from 273 patients 1–2 days prior to the injection of the anti-TNF drug. Trough concentration of the anti-TNF drugs were measured with capture-ELISA (Sanquin Laboratories, Amsterdam, Netherlands), the levels of ADAb with radioimmunoassay (Sanquin Laboratories), and the residual serum TNF-blocking capacity by using reporter gene assay set up in house (United Medix Laboratories, Espoo, Finland). The clinical activity of AS was assessed using the Bath AS Disease Activity Index (BASDAI), the Bath AS Functional Index (BASFI), and the Maastricht AS Enthesitis Score (MASES).

RESULTS

ADAbs were observed in 21% of patients on adalimumab (n=99), in 0% of those on etanercept (n=83), in 3% of those on golimumab (n=79), and in 50% of those on certolizumab pegol (n=12). Factors affecting the immunisation of biological drugs could be further analysed in patients using adalimumab. Of adalimumab users, the drug concentration was in the target range (5–10 mg/L) in 33% of patients. Drug trough concentrations of adalimumab correlated significantly with the presence of ADAb (r: -0.54; p<0.0001). In adalimumab users, higher BMI was associated with the presence of ADAb (p=0.019, adjusted for sex, age, and the time of biological use). Methotrexate (MTX) reduced the risk of developing ADAb. Of the patients who used MTX, 12% were ADAb positive, whereas, of those who did not use MTX, 28% were ADAb positive (p=0.048 adjusted for sex, age, weight, and the duration of biological use). The use of sulfasalazine was not associated with a lower number of ADAb positive patients. The presence of ADAb resulted in lower drug trough levels, yet there was no significant correlation between the disease activity and the presence of ADAb. Of adalimumab users with ADAb+, the mean BASDAI was 1.2 (standard deviation [SD]: 1.4) and of those without ADAb 1.9 (SD: 1.9) (p=0.091). Furthermore, no significant correlation was observed between the presence of ADAb and serum erythrocyte sedimentation rate or C-reactive protein.

CONCLUSION

The disease activity of AS patients using subcutaneous anti-TNF drugs was low. The immunisation of adalimumab was relatively common in the non-selected AS patient population. The presence of ADAb resulted in lower drug trough levels, but no clear association was observed between the presence of ADAb and the disease activity. The routine monitoring of drug trough levels and/or ADAb may be indicated.

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SLEEP DISORDERS IN FIBROMYALGIA PATIENTS: THE ROLE OF AUTONOMIC NERVOUS SYSTEM DYSFUNCTION

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Keywords: Sleep disorders, fibromyalgia (FM), autonomic nervous system (ANS).

INTRODUCTION

Fibromyalgia (FM) is characterised by chronic musculoskeletal pain, autonomic nervous system (ANS) dysfunction, and disturbed sleep. In 2010, the American College of Rheumatology (ACR) included a patient-reported measure of unrefreshing sleep in its preliminary diagnostic criteria for FM and measurement of symptom severity.[1]

The ANS plays an important role in co-ordinating many bodily functions during sleep. Patients with untreated sleep disorders often describe symptoms of ANS impairment, such as morning stiffness, fatigue, depression, non-restorative sleep, and reduced cognitive performance,[2] and the majority of patients with autonomic impairment have some form of sleep disorder.[3] As the cause of sleep disorders in FM patients is still unclear, the aim of this study was to evaluate the influence of ANS dysfunction on the genesis of their sleep disorders.

METHODS

We enrolled 50 female FM patients and 45 female healthy subjects matched by age, sex, and BMI. All of the subjects underwent an 18 tender point examination[1] and described the intensity of their somatic pain using a 100 mm visual analogue scale, before being administered a sleep questionnaire (to evaluate sleeping complaints)[4] and the Epworth Sleepiness Scale.[5] The sleep profile was evaluated by using a polysomnography comprising an electroencephalogram, a 6-channel electrocardiogram, and an electro-oculogram. Submental right and left tibialis anterior muscle electromyograms were recorded using surface electrodes and standard techniques.[6] The respiratory disturbance index, the desaturation event frequency, periodic breathing (PB), and arousals index were scored in accordance with the criteria of the American Sleep Disorders Association (ASDA).[6] The cyclic alternating pattern (CAP) is a marker of sleep instability composed of a Phase A (lumps of sleep phasic events) followed by a Phase B (return to electroencephalography background) and was identified using the scoring rules.[7] CAP sequences include at least two consecutive CAP cycles.

The autonomic profile was evaluated at rest and during a tilt test to determine muscle sympathetic nerve activity (MSNA) using the microneurography technique,[8] plasma catecholamine levels, and the spectral indices of cardiac sympathetic (low frequency component of RR variability [LF_{RR}]) and vagal (high frequency component of RR variability [HF_{RR}]) modulation computed by spectrum analysis of RR during sleep.

RESULTS

The FM patients had a higher heart rate (HR), more MSNA, a higher LF/HF ratio, and lower

HF_{RR} values at rest (p<0.05), showing no increase in MSNA, a smaller decrease in HF_{RR}, and an excessive rate of syncope (46%) during the tilt test. Their sleep was less efficient (p<0.01), and they had a higher proportion of Stage 1 non-rapid eye movement (REM) sleep (p<0.001), experienced many arousals and periodic limb movements (PLM) per hour of sleep (p<0.001), and a high proportion of PB (p<0.0001). Their CAP rate was significantly increased (p<0.001) (Table 1). During sleep, they had a higher HR and LF/HF ratio, and a lower HF_{RR} (p<0.001) (Table 2). The number of tender points, CAP rate, PB, and PLM index correlated positively with HR and the LF/HF ratio, and negatively with HF_{RR} during sleep.

CONCLUSION

These data confirm that the FM patients have an ANS dysfunction that is consistent with sympathetic over-activity due to the intensity of chronic pain when awake and during sleep. These findings explain the excessive rate of syncope observed in the FM population during wakefulness and the increased presence of CAP, PB, and PLM during sleep.

As PB increases baroceptor gain and sympathetic outflow, it creates a vicious circle: the painful exacerbation increases sympathetic cardiovascular activation and reduces sleep efficiency, thus increasing light sleep, CAP rate, arousals, PLM, and the occurrence of PB, which in turn causes abnormalities in cardiovascular neural control and exaggerated pain sensitivity.

Table 1: Sleep parameters in fibromyalgia patients and healthy controls.

	FM (n=50)	Controls (n=45)	p-value
Sleep efficiency (% of sleep time)	78.0±10.0	89.0±6.0	0.01
Stage 1 non-REM sleep (% of sleep time)	20.0±5.0	12.0±5.0	0.001
Stage 2 non-REM sleep (% of sleep time)	36.0±10.0	36.0±3.0	n.s.
Slow-wave non-REM sleep (% of sleep time)	6.0±2.0	18.0±3.0	0.001
REM sleep (% of sleep time)	17.0±9.0	18.0±8.0	n.s.
Average SaO^2 during sleep (%)	94.8±1.6	95.0±1.7	n.s.
RDI (events/hour)	3.0±1.0	3.0±0.7	n.s.
DEF (events/hour)	9.0±6.0	3.2±2.0	0.01
PB (% of sleep time)	15.3±8.0	1.0±2.0	0.0001
Arousal/index (events/hour)	10.0±3.0	4.0±2.0	0.001
PLMI (events/hour)	22.0±7.0	5.0±1.0	0.0001
CAP rate	69.0±6.0	44.0±11.0	0.001
CAP cycle duration (secs)	46.0±3.0	27.0±2.0	0.001
Phase A duration (secs)	20.0±4.0	10.0±1.0	0.001
Phase B duration (secs)	25.0±2.8	16.0±3.0	0.001

Phases A and B are components of the CAP. Variations during CAP involve different degrees of muscle tone, heart rate, and respiratory activity, which increase during Phase A and decrease during Phase B.
Data expressed as mean±standard deviation; significance threshold p<0.05.
DEF: desaturation event frequency; PB: periodic breathing; PLMI: periodic limb movement index; REM: rapid eye movement; RDI: respiratory disturbance index; SaO^2: oxygen saturation; CAP: cyclic alternating pattern. FM: fibromyalgia; n.s.: not significant.

Table 2: Autonomic nerve system parameters variations during non-rapid eye movement (REM) and REM sleep in fibromyalgia patients and in controls.

	FM (n=50)	Controls (n=45)	p-value
HR (bpm) non-REM	70.0±3.0	58.0±3.0	0.001
HR (bpm) REM	63.0±3.0	62.0±3.0	n.s.
LF_{RR} (ms^2) non-REM	368.0±130.0	348.0±129.0	n.s.
LF_{RR} (ms^2) REM	342.0±140.0	345.0±148.0	n.s.
LF_{RR} NU non-REM	50.0±12.0	46.0±6.0	n.s.
LF_{RR} NU REM	46.0±8.0	44.0±4.0	n.s.
HF_{RR}(ms^2) non-REM	138.0±40.0	648.0±316.0	0.00001
HF_{RR}(ms^2) REM	313.0±140.0	393.0±320.0	0.01
HF_{RR}NU non-REM	10.0±6.0	31.0±8.0	0.0001
HF_{RR}NU REM	15.0±8.0	27.0±7.0	0.001
LF/HF non-REM	3.0±0.6	1.58±0.5	0.0001
LF/HF REM	1.7±0.6	1.68±0.8	n.s.

Data expressed as mean±standard deviation; significance threshold $p<0.05$.
HF_{RR}: high frequency component of RR variability; HR: heart rate; bpm: beats per minute; LF_{RR}: low frequency component of RR variability; NU: normalised units; FM: fibromyalgia; ms^2: metre per second squared; n.s.: not significant.

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SERUM URATE AS A SURROGATE ENDPOINT FOR FLARES IN PEOPLE WITH GOUT: A SYSTEMATIC REVIEW AND META-REGRESSION ANALYSIS

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Keywords: Gout, serum urate (SU), biomarker, systematic review, meta-analysis.

The OMERACT (Outcome Measures in Rheumatology) gout group has, for several years, been working towards a model for evaluating a biomarker as surrogate endpoint for clinically relevant outcomes. Surrogate measurements are often used in clinical trials when it is difficult or impractical to use clinically relevant or patient-reported outcomes as endpoints. Substituting a target event with a surrogate measurement (for example a soluble biomarker) allows for the conduction of shorter and smaller trials. To evaluate the usability of a biomarker as a surrogate, Maksymowych et al.[1] published a model for evaluating a proposed biomarker for surrogacy in 2009. This was followed by Stamp et al.[2] applying the OMERACT model to evaluate whether serum urate (SU) fulfilled the OMERACT biomarker criteria. They concluded that, with the exception of its effects on outcome measures, SU met the criteria.[2] The need for analysis of existing data to determine whether a reduction in SU predicts a reduction in gout flares, the number/size of tophi, and patient-reported outcomes, was then the next approach in the process of validating SU as a surrogate endpoint for clinically relevant outcomes in people with gout. With this in mind, we conducted a systematic review and meta-analysis, and part of the results were presented at the European League Against Rheumatism (EULAR) congress, 2017, as an oral presentation. For a detailed description of the background and our methods, we refer you to the published protocol.[3]

A systematic literature review was undertaken to identify all relevant studies. Randomised controlled trials (RCTs) comparing any urate-lowering therapy in people with gout with any control or placebo for ≥3 months duration were included. The search resulted in 234 abstracts for screening. Subsequently, 82 trials were scrutinised, of which

9 trials (with 16 comparisons) met our inclusion criteria. A total of 5,696 people with gout were entered into the meta-regression model. The longest RCT included was only 12 months in duration. The pooled odds ratio (OR) suggested a small, but statistically significant, favourable association between the active and comparator urate lowering therapies and flare frequency (OR: 0.83; 95% confidence interval: 0.70–0.99). Substantial heterogeneity was present (between trial variance: 0.07; 0.03–0.30). Meta-regression analysis did not reveal any statistically significant association between the proportion of individuals who achieved target SU and the observed flare rate (p=0.82); the model fit did not improve after inclusion of the covariate into the model (between trial variance: 0.08; 0.03–0.33).

In conclusion, substituting surrogate endpoints (proportion achieving target SU) for the important clinical outcome (gout flares) allows for the conduction of shorter, smaller trials. However, based on aggregate trial-level data (meta-regression), an anticipated association between SU and gout flare could not be confirmed. The heterogenicity of the studies and the difference in clinical outcome data reported, as well as the trial duration, is likely to have influenced the results. It is important to note that the longest trial duration was only 12 months and it is likely that this was too short to observe any reduction in gout flares. Additional analysis using data from long-term open label extensions is currently underway.

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WEIGHT LOSS FOR OVERWEIGHT AND OBESE INDIVIDUALS WITH GOUT: A SYSTEMATIC REVIEW OF LONGITUDINAL STUDIES

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Roche, Rottapharm-Madaus, Sobi, and Wyeth), grants from payment for manuscript preparation (Axellus, Bristol-Myers Squibb, Cambridge Weight Plan, and Aleris-Hamlet [via Norpharma]), non-financial support from patents (planned, pending, or issued), non-financial support from royalties, grants from payment for development of educational presentations (Bristol-Myers Squibb, MSD, and Pfizer), non-financial support from stock/stock options, grants from travel/accommodations/meeting expenses unrelated to activities listed (Abbott, AbbVie, Axellus, Bristol-Myers Squibb, Cambridge Weight Plan, Celgene, Laboratoires Expanscience, Norpharma, Novartis, Pfizer, Roche, Rottapharm-Madaus, and Wyeth), and is involved in many healthcare initiatives and research that could benefit from wide uptake of this publication (including Cochrane, OMERACT, IDEOM, RADS, and the GRADE Working Group). Musculoskeletal Statistics Unit, The Parker Institute is grateful for the financial support received from public and private foundations, companies and private individuals over the years. The Parker Institute is supported by a core grant from the Oak Foundation; the Oak Foundation is a group of philanthropic organisations that, since its establishment in 1983, has given grants to not-for-profit organisations around the world.

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Keywords: Systematic review, hyperuricaemia, serum uric acid, weight reduction, BMI, gout.

It has been estimated that almost half of gout patients in Europe are obese.[1] BMI is strongly correlated with serum urate (SU),[2] and weight loss in people without gout has been shown to decrease SU.[3] Hyperuricaemia is a critical factor in the development of gout, which is caused by deposition of monosodium urate crystals in the joints and tissues. Hence, lowering SU is considered central in the long-term management of gout. The recommended target for SU is <360 µmol/L (<6 mg/dL), and if this is sustained over time, it leads to dissolution of monosodium urate crystals, reduction in gout flares, and resolution of tophi.

Even though weight loss is commonly recommended for gout,[4] no one has previously conducted a systematic review investigating the effects of weight loss. Therefore, our objective was to determine the benefits and harms associated with weight loss in overweight and obese gout patients.

Following our protocol, we searched four electronic databases and two trial registries. We included longitudinal studies with ≥10 overweight or obese gout patients, where the effects following weight loss (intentionally or unintentionally) were quantitatively estimated. We specified 11 outcomes for data-extraction. During the process, we realised that the planned meta-analyses were not possible, because the studies were too heterogeneous. Thus, we decided to summarise the results from each study. The internal validity and the quality of evidence were assessed using the ROBINS-I tool[5] and the GRADE approach,[6] respectively.

We included 10 eligible studies. Only sparse data were available, and the most frequently reported outcomes were SU, achieving SU target (<360 µmol/L), and gout flares. Only one of the included studies was a randomised controlled trial,

and four of the studies had no comparison group. Interventions included intentional weight loss from dietary changes with or without increased physical activity, bariatric surgery, and unintentional weight loss from a high protein diet, metformin, and diuretics. Three studies stratified cohorts according to weight change. Follow-up ranged from 4 weeks to 7 years, and mean weight loss ranged from 3–34 kg. In the risk of bias assessment, none of the studies were rated low risk for all seven bias domains, and four of the studies were rated critical risk for the first bias domain (bias due to confounding issues).

At the latest follow-up, the studies reported a change in SU ranging from -168 to +30 µmol/L. For those with SU above target at baseline, between 0 and 60% achieved target SU. Gout flares were reported in different ways, but overall, six out of eight studies reported beneficial effects. For all three outcomes (SU, achieving SU target, and gout flares) dose-response relationships were reported. However, it should be noted, that in the short term, two studies reported a temporary increase in SU and gout flares following bariatric surgery. We rated the quality of evidence for the three outcomes to be low, moderate, and low, respectively, because we downgraded for study types, risk of bias, and upgraded for dose response relationship, and large reported effects. In conclusion, the available evidence indicated beneficial effects of weight loss for overweight and obese gout patients, although short-term, unfavourable effects may occur. Since the current evidence consists of only a few studies (mostly observational) of low methodological quality, there is an urgent need to initiate rigorous randomised controlled trials.

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RHEUMATOID ARTHRITIS AND POLYMYALGIA RHEUMATICA AFTER IMMUNE CHECKPOINT INHIBITOR TREATMENT

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Keywords: Rheumatoid arthritis (RA), polymyalgia rheumatica (PMR), immune checkpoint inhibitors (ICI), multidisciplinary care.

INTRODUCTION

Immune checkpoint inhibitors (ICI) targeting cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) have demonstrated improved survival for multiple cancers.[1] However, these new drug classes have led to increased immune-related adverse events (IrAE). Little is known about rheumatic adverse events with ICI therapy.[2] Indeed, in Phase III studies, arthralgia, which includes all musculoskeletal disorders, was present in ~5% of patients receiving ipilimumab for melanoma, 9–20% receiving pembrolizumab, and 5–16% receiving nivolumab for melanoma or non-small cell lung cancer (NSCLC) versus <1% with placebo.[3] However,

these adverse events may be underestimated, and no clinical description was provided. Recently, one series of 13 patients with non-classified rheumatic IrAE was published;[4] non-specific inflammatory arthritis developed in 9 patients without auto-antibodies and 4 presented sicca symptoms but did not fulfil the criteria for Sjögren's syndrome. We report here cases of rheumatoid arthritis (RA) and polymyalgia rheumatica (PMR) occurring after ICI treatment.

METHODS

We conducted a retrospective study on a collection of patients receiving an ICI in whom symptoms of arthritis or arthralgia developed and revealed a diagnosis of RA or PMR through the Gustave Roussy Cancer Center (Villejuif, France), which has established a national pharmacovigilance registry. A retrospective multicentre collection of observations through the Club Rhumatismes et Inflammation (CRI) network was performed, which is a section of the French Society of Rheumatology. Between September 2016 and January 2017, all rheumatologists and internal medicine practitioners registered on the CRI website, comprising almost 2,400 physicians all over France, were contacted via successive newsletters over 6 months to report cases.

RESULTS

In 10 patients who received ICI therapy (anti-PD-1 or anti-PDL1 antibodies), RA or PMR developed at a median time of 1 month after exposure. The mean age of patients was 64 years and 60% were male. No patient had pre-existing rheumatic or autoimmune disease. RA developed in six patients; all were positive for anti-cyclic citrullinated peptide (anti-CCP) antibodies and four for rheumatoid factor (RF). Anti-CCP antibodies were detected in two of the three patients that could be tested before immunotherapy.

Table 1: Characteristics of patients with rheumatoid arthritis after immune-checkpoint inhibitor treatment for cancer.

Sex/ age, years	Type of cancer	ICI	Date of first ICI exposure	Date of IrAE	IrAE response to treatment	Autoantibody results
F 55	Squamous cell carcinoma of the vagina	nivolumab	October 2015	October 2015	Resolution with NSAID	CCP: 671 U/mL RF: 18 UI/mL
F 66	Endometrial adenocarcinoma	pembrolizumab	March 2016	April 2016	Resolution with prednisone 10 mg/d	CCP: 233 U/mL RF: 180 UI/mL
M 59	Lung adenocarcinoma	nivolumab	May 2016	July 2016	Resolution with prednisone 10 mg/d	CCP: 61 U/mL RF: 47 UI/mL
F 56	Metastatic melanoma	pembrolizumab	August 2015	September 2015	NSAID and HCQ: 400 mg/d: good response	CCP: 18 U/mL RF <15 UI/mL
M 80	Metastatic melanoma	nivolumab	April 2016	April 2016	Prednisone 15 mg/d and HCQ 200 mg/d: good response	CCP: 42 U/mL RF <15 UI/mL
M 68	Lung adenocarcinoma	nivolumab	June 2015	July 2015	NSAID: no effect. stopping nivolumab and MTX 10 mg/w: good response	CCP: >300 U/mL RF: 246 UI/mL

F: Female; M: Male; RF: rheumatoid factor; HCQ: hydroxychloroquine; MTX: methotrexate; NSAID: non-steroidal anti-inflammatory drugs; RA: rheumatoid arthritis; IrAE: immune-related adverse event; CCP: cyclic citrullinated peptide; ICI: immune-checkpoint inhibitor.

Table 2: Characteristics of patients with polymyalgia rheumatica after immune-checkpoint inhibitor treatment for cancer.

Sex/ age, years	Type of cancer	ICI	Date of first ICI exposure	Date of IrAE	IrAE response to treatment
F 76	Mesothelioma	anti-PDL1	June 2014	March 2015	Resolution with prednisone 20 mg/d then tapered
M 69	Gastric adenocarcinoma	pembrolizumab	September 2016	October 2016	Resolution with prednisone 20 mg/d then tapered
M 62	Colon adenocarcinoma	nivolumab + ipilimumab (4 cycles) then nivolumab alone	June 2015	October 2015	Resolution with prednisone 60 mg/d then tapered
M 68	Metastatic melanoma	nivolumab	August 2016	August 2016	Resolution with prednisone 40 mg/d then tapered

F: Female; M: Male; RF: rheumatoid factor; PDL1: programmed cell death ligand protein 1; IrAE: immune-related adverse event; ICI: immune-checkpoint inhibitor.

Disease-modifying anti-rheumatic drugs were needed for 3 patients (hydroxychloroquine, methotrexate); the 3 others received corticosteroids or non-steroid anti-inflammatory drugs (Table 1). PMR was diagnosed in 4 patients, all responded to corticosteroids (Table 2). Despite these IrAEs, immunotherapy was pursued for all but one patient until cancer progression.

CONCLUSION

This is the first description of RA occurring after ICI therapy for cancer. PMR can also occur after ICI, particularly after anti-PD-1 therapy. All cases responded to corticosteroids or with immunosuppressive therapy.

In our study, the 6 RA patients fulfilled the 2010 ACR/EULAR criteria and were seropositive (anti-CCP positivity in all 6 and RF in 4). Anti-CCP antibodies were detected in 2 of the 3 patients (Patients 1, 4, and 5) with available serum samples before immunotherapy. Three patients were negative for RF before ICI therapy and 1 showed weak positivity (18 U/mL) afterward. The short time between ICI treatment and the development of joint symptoms and anti-CCP positivity before ICI therapy in 2 of the 3 patients suggested that some of these patients had a pre-RA status and that the treatment with ICI may have triggered the clinical disease. Indeed, studies have shown that antibodies (anti-CCP and RF) may be present several years before RA onset.[5] Nevertheless, no patient presented arthralgia before ICI treatment. In conclusion, collaboration between rheumatologists and oncologists is crucial and could lead to better recognition and care of these patients.

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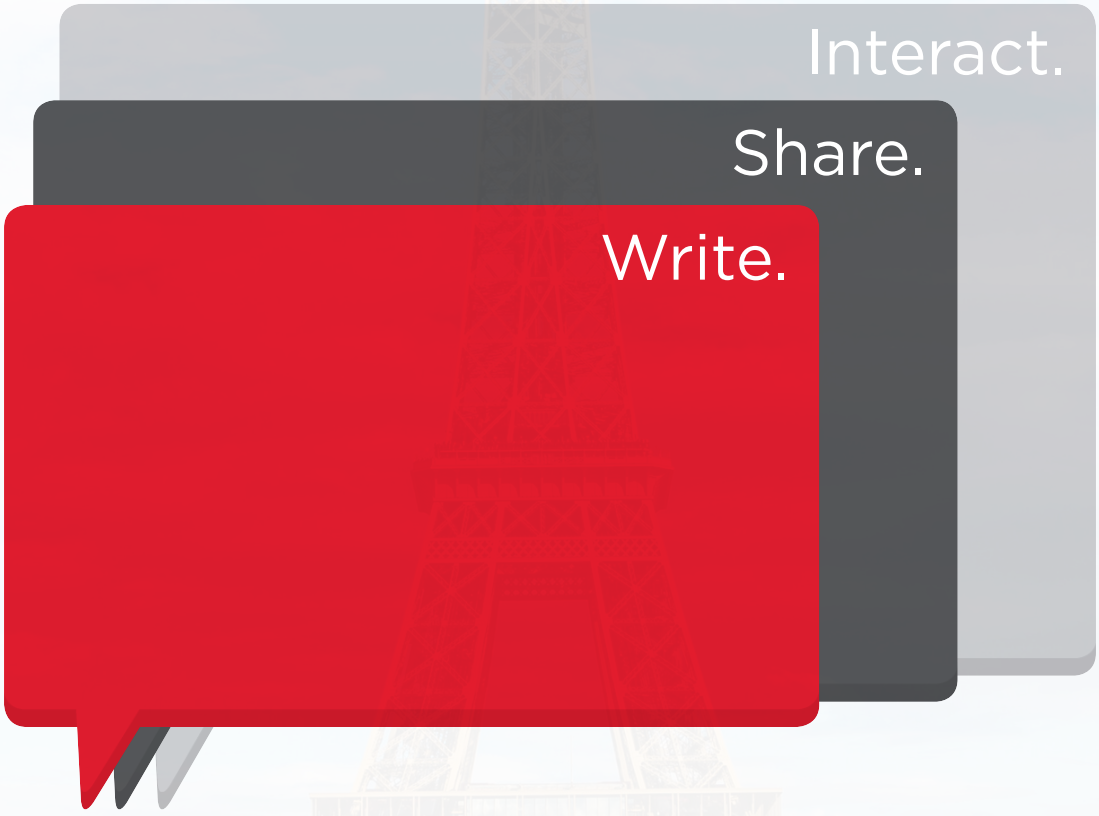

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EDITOR'S PICK

In this intriguing article, Margo S. Clarke explores the fascinating topic of disease left–right asymmetry, putting HLA-B27 acute anterior uveitis under the spotlight. She explores the immune system's exquisite selective ability to react to molecular variance and prompts further discussion on how a deeper understanding of lateralisation could impact the medical world at large.

WHY DO DISEASES START ONE SIDED? CLUES FROM HLA-B27 ACUTE ANTERIOR UVEITIS

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ABSTRACT

Uveitis is an inflammatory disease with significant disease burden, as it causes ≤10% of legal blindness in the USA. Patients are usually affected in their prime working years. Even in those with good treatment response, quality of life is substantially compromised. The most common form of uveitis is acute anterior uveitis, and approximately half of these cases are associated with human leukocyte antigen B27 (HLA-B27). The typical clinical presentation is sudden onset of a red sore eye with white cells and protein leaking into the anterior chamber. There is inter-individual variance in clinical signs, with the most severe cell response appearing like a snowstorm in the anterior chamber, causing cells to pile up in a snowbank appearance called a hypopyon. One of the truly curious, yet pathognomonic, features is the tendency for the inflammatory response to have a unilateral presentation. Either the right or left eye can manifest obvious inflammation, yet the other eye is completely unaffected. Also, subsequent attacks may occur on the same or contralateral side. Clearly, the immune system is capable of distinguishing a molecular variance between the two eyes, but what this difference is remains a mystery. This article will review HLA-B27 uveitis plus its associated systemic diseases; additionally, various mechanisms that play a role in determining left–right disease asymmetry will be discussed. Establishing how the immune system makes this left–right decision will have relevance to understanding causes of asymmetry in other inflammatory, degenerative, and malignant disorders.

Keywords: HLA-B27 uveitis, left-right asymmetry, differential protein expression, immunology, genetics, somatic mutation.

INTRODUCTION

Acute anterior uveitis (AAU) presents with the sudden onset of inflammation centred on the iris and ciliary body. At slit lamp exam, there are white cells and flare in the anterior chamber, fine cell precipitates on the corneal endothelium (nongranulomatous keratic precipitates), variable adhesion to lens or spanning trabecular meshwork (synechiae), and variable anterior vitreous cells. Typically, the episode

lasts 6–8 weeks with reoccurrence being common. More than 90% of human leukocyte antigen B27 (HLA-B27) positive AAU are unilateral,[1] and nearly 70% of recurrences involve the same eye.[2] It is the predictable lateralisation that assists in establishing the diagnosis of HLA-B27 uveitis since other forms of uveitis typically present bilaterally.[3] In a busy practice we do not stop to question why one eye is vulnerable while the other is spared. However, understanding the mechanism behind left–right selection could be an important step towards definitive treatment that stops recurrences.

DISEASE ASSOCIATIONS

HLA-B27 uveitis patients have associated spondyloarthritis at a frequency reported to vary from 55–78%.[1,4,5] Ankylosing spondylitis (AS) is the most common, but other forms of spondyloarthritis include reactive arthritis, arthritis associated with inflammatory bowel disease, psoriatic arthritis, undifferentiated spondyloarthropathy, and juvenile spondyloarthritis. Curiously, patients with HLA-B27 co-associated reactive arthritis or inflammatory bowel disease are at greater risk for more severe sudden-onset AAU that can persist to become chronic AAU.[6]

GENETICS AND ACUTE ANTERIOR UVEITIS

The strongest genetic risk factor for AAU remains the associated HLA-B27 genotype.[7,8] Approximately 55% of AAU patients are HLA-B27 positive, with this rising to 70% of patients with recurrent acute iritis episodes.[9] Notably, the prevalence of HLA-B27 varies markedly in the general population and the frequency of AAU and spondyloarthritis corresponds directly.[8] For example, 0.1–0.5% of Japanese, 4% of North Africans, 2–9% of Chinese, 8% of Caucasians,[10] and 50% of Haida Indians[11] are HLA-B27 positive.

The lifetime cumulative incidence of AAU in the general population is about 0.4%.[12] In a Dutch population with 1% HLA-B27 positive individuals, the prevalence of AAU in HLA-B27 positive relatives of HLA-B27 positive AAU patients was 13%.[13] However, the vast majority of those who are HLA-B27 positive do not develop uveitis or ankylosing spondyloarthritis.

Although 90% of AS patients are HLA-B27 positive, as opposed to 55% of patients with AAU, ≤78% of HLA-B27 with AAU have other B27-associated diseases,[1,4,5] while only 20–30% of patients with AS or reactive arthritis develop AAU. Hence, although there is overlap in risk factors there are clearly separate susceptibility modifiers. Having the HLA-B27 genotype starts one down a risky road but additional genetic variances determine which path is more likely.

In the search for both shared and separate genetic risks, association studies for AAU have identified HLA-A*02, HLA-DRB1*08:03, HLA-B*58, *MICA*, *LMP2*, *CYP27B1*, interleukin (IL)-10, complement components CFB, CFH and C22, TNF, the killer immunoglobulin receptor region, and the chromosome 9p region. None of these associations achieved genome-wide significance.[8]

A recent genome-wide study[14] detected that IL-23R, region 2p15, and *ERAP1* were associated with both AAU and AS ($p<5x10^{8}$). At a lower level of significance ($p<5x10^{6}$) IL-6R, EYS, the chromosome 1q32 harbouring KIF21B, IL-18R-IL-1R1, and region IL-10-IL 19 may play a role. Interestingly, IL-10, IL-18R, and IL-23R are shared with inflammatory bowel disease patients. The listed genetic associations relate to receptor polymorphisms that can amplify or diminish response to interleukins. The *ERPA* polymorphisms affect the efficiency of packaging peptides into the HLA-B27 groove.[15] By altering the magnitude or speed of responsiveness, the immune system becomes susceptible to dysregulation. There appear to be both organ and topographic specific differences in the relative importance of messages relayed through different interleukin receptors and their associated downstream signalling system.

ENVIRONMENTAL FACTORS AND PATHOGENESIS

Despite intensive research, the true cause of HLA-B27 diseases remains unclear. The dominant hypothesis is the arthrogenic/uveitogenetic peptides hypothesis.[16] Specifically, HLA-B27 has the unique ability to bind peptides from a microbe that activate CD8 T cells that cross-react with a HLA-B27/self-peptide. The molecular mimicry hypothesis then relates this cross-reactivity as a turning point in breaking tolerance that results in autoimmunity. The microbial culprits identified included yersinia, salmonella,[17] chlamydia,[18] and *Helicobacter pylori*.[19] Finding a clear and direct correlation of exposure to these organisms and causality in AAU is currently awaiting data.

Notably, there are now >105 recognised polymorphisms of HLA-B27.[20] The HLA B*2705 is most common and associated with both AAU and AS. These polymorphisms affect the binding strength of each candidate peptide to subtle variances within the groove which will impact the signal strength received by the T cell. Certain positions along the groove are responsible for the majority of the binding strength and the most significant site in the HLA B*2705 is an arginine at the P2 position.[21,22] Interestingly, if the arginine is switched to a histidine this polymorphism is designated as a HLA B*2709 molecule and is resistant to disease association.[23] This fascinating observation resulted in eluting peptides bound to HLA B*2705 versus HLA B*2709 and comparing their sequences. One study found a more restricted spectrum of peptides[24] in the HLA B*2709 and another group found no quantitative variance but perhaps a quantitative difference.[25] Despite extensive research, the target antigens in AAU and AS remained unknown.

Mear et al.[26] proposed another mechanism after demonstrating that the HLA-B2705 has a unique property to misfold. Misfolded proteins then trigger an internal cell stress response that can lead to increased production of cytokines that are pro-inflammatory, such as TNF-α, IL-1, and IL-6.[27] This may be sufficient to tip the balance toward autoimmunity in tissues responsive to these signals.

A third hypothesis is the homodimer formation hypothesis. HLA-B27 heavy chains tend to pair as homodimers and this can activate the intracellular stress cycle.[28] The dimers can also bind receptors on killer cells and alter their responsiveness leading to a pro-inflammatory state.

ANIMAL MODELS

Rats transgenic for human HLA-B27 develop spontaneous inflammatory arthropathy,[29] supporting that HLA-B27 is a disease associated gene; however, they did not develop AAU. Germ-free environments prevented development of inflammatory joint and gut disease, suggesting the important role for microbes as initiators. Additionally, overexpressing IL-23 in a mouse model[30] resulted in psoriasis, aortitis, and uveitis, confirming the significance of this cytokine in immune regulation for specific locations. Intra-peritoneal β-1,3 glucan, which is present in fungal cell walls, bacteria, and plants, caused arthritis, uveitis, ileitis, and enthesitis.[31] This was associated with elevated IL-12 and IL-23 (which is upstream from IL-17) and reaffirmed the importance of the IL-23 pathway. Currently, there is no ideal animal model that replicates the recurrent, unilateral, 6–8-week duration pattern that is classic for AAU.

ADVANCES IN THERAPY

Over 90% of patients with AAU respond well to topical steroids and cycloplegics. When the disease is particularly severe or poorly responsive, subconjunctival steroids and/or oral steroids are used. Some patients develop high intraocular pressure while on steroids, necessitating glaucoma treatment simultaneous to their uveitis management.

In recalcitrant uveitis, immunosuppressive therapy with methotrexate, cyclosporine, azathioprine, and mycophenolate plus biologics are options.[32] Paradoxical uveitis has been reported with etanercept for treatment of spondyloarthropathies. Hence, guidelines recommend infliximab or adalimumab before etanercept for uveitis management.[33] Fortunately, the majority of AAU patients do not require these more aggressive therapies.

PERSPECTIVES

AAU, like all autoimmune diseases, has significant inter-individual heterogeneity, with each patient following their unique path. This variance should be no surprise, since the 1000 Genomes Project determined a typical genome varies from the reference human genome by 4.1–5 million sites. Although the majority of these polymorphisms do not adversely affect function, the average person has 2,111–2,500 structural variants.[34] Fortunately, by having two parental alleles for each gene and redundant pathways, we often have a workable system. However, epigenomic imprinting affects >80 human genes and silences one allele. Further complexity is added since the allele chosen for inactivation can 'flip flop', in different body sites[35] and be modified based on age, creating mosaic patterns of variability. It was hoped with genome-wide association studies that the genes responsible for AAU and AS would be clearly identified and remediable pathways amenable to new therapies would then be developed. What has emerged however is an increasingly complex puzzle, with each identified gene only increasing the odds ratio to develop the disease by a factor of 1.2–1.5.[36]

Since we each have thousands of at risk variants, it is likely a specific combination of variants may provide a compounding effect. Specific combinations may present at a select body site at disease onset or evolve in a specific sequence, or have unique combinations of phenotypic features. The highest risk in AAU remains the HLA-B27 peptide packaging system, which implies there is one or more antigen and that there will need to be one or more triggering environmental event that sparks clonal expansion and drives the immune cycle sufficiently towards the threshold to precipitate clinical disease. Another intriguing aspect of the genome-wide association studies was that most of the polymorphisms were not in coding parts of the DNA that determine the quality of the protein, but rather in long, short, and micro non-coding segments that determine where, when, and how much of a protein is expressed under different circumstances. Non-coding DNA variances affect large numbers of genes, rather like modules, so to tease out causality necessitates sophisticated bioinformatics and systems biology. The capacity of genetic research to find new answers is strongly dependent on comparing a group of affected individuals that are distinctly homogeneous and consequently diseases are being further subdivided based on clinical features. To date, the topographic site of onset and the initial side of presentation has not been perceived to be a unique phenotype. Perhaps left–right asymmetry is molecularly meaningful (not random) and the side of onset could be useful both as a phenotype for genetic studies and in differential analysis of tissue at the genomic, epigenomic, and at micro, small, and long non-coding RNA levels. Hence, the following section introduces concepts of how lateralisation of lesion site can be determined based on left–right molecular differences.

ASYMMETRICAL PROTEIN EXPRESSION LEFT VERSUS RIGHT: EXPLAINING THE FIRST ATTACK

Autoimmune diseases are initiated by recognition of one or more antigens by T and/or B cells. For AAU to select one eye, there may be quantitative or qualitative left versus right differences in uveal tissue antigen(s), molecular vascular barrier complex, resident tissue signal modifiers, or signalling and receptor pairs that sense, measure, and generate a response detectable to the incoming immune system. Additionally, the immune tolerance and immune suppressive intraocular microenvironment may generate unequal differences in protein expression on each side that differentially impact the immune privilege, resulting in unilateral disease.

Left–right body axis determination[37,38] starts as early as the first cell division after fertilisation and asymmetrical protein expression is consistently present from the single cell zygote throughout development. Co-ordination of sidedness continues with specified proteins (Nodal and Lefty) being more strongly expressed on the left side which co-ordinates proper position of the heart and other internal organs. Protein variances have been identified in left and right sides of the human brain.[39] Curiously, insects exhibit brain lateralisation with hundreds of genes differentially expressed on left versus right sides of bee brains.[40] Additionally, paired organs, such as left and right breast tissue[41] and muscle myotomes[42] have been identified to have left–right protein differences.

From a clinical perspective, pseudoexfoliation syndrome demonstrates clinically visible differential protein production in left versus right eye. Pseudoexfoliation syndrome is a common ocular disease strongly associated with glaucoma. A genetic defect in the enzyme LOXL1[43] leads to defective elastin cross-linkage causing elastin debris to be released into the anterior chamber of the eye, where it settles onto the lens surface. With iris movement, the deposits are pushed like a snowplough, forming a layered ring appearance at slit lamp exam. It is fascinating that, like HLA-B27 uveitis, there is almost always left–right asymmetry. In some patients, the debris is robust in one eye and barely noticeable in the other. It is not known what causes this asymmetry.

A study of 23 post-mortem anterior optic nerve specimens found differences in neurofilament protein expression in each sector, plus surprisingly a consistently higher neurofilament expression in the right optic nerve compared to the left.[44] Jonas et al.[45] found that in 72 normal post-mortem eyes, the number of ganglion nerve fibres ranged from 777,000–1,679,000. When comparing right versus left eyes from the same donor, differences of >300,000 ganglion nerve fibres between left and right eyes were present in 19% of subjects.

An intriguing study of patterns of X-linked inactivation[46] showed that the mosaic pattern generated was individual-specific, but there were select regions, such as the tongue, where essentially

all one side of the tongue expressed the paternal X chromosome, while the opposite side expressed the maternal X chromosome. Hence, a single epigenomic event can induce left–right protein expression difference.

RANDOM SOMATIC MUTATION: AN ALTERNATIVE MECHANISM FOR LOCALISED DISEASE

Although somatic mutations are well known to cause cancer, somatic mutations can arise *de novo* developmentally and the mutation may be restricted to specific tissue. Mutations have been identified in several genes that are associated with enlargement of one hemisphere of the brain that manifest with epilepsy.[47] These mutations are only evident in tissue samples, not in blood. Patients can show dysfunction of essentially an entire half of their cerebral cortex, while only 8–35% of the brain cells carry the mutation. Similarly, somatic mutation has been found to be the cause for Sturge–Weber syndrome, a condition that presents unilaterally with a capillary malformation that follows the distribution of the ophthalmic branch of the trigeminal nerve.[48] Single cell tissue genomics assessing for tissue somatic mutations is relatively new and, to date, tissue somatic mutations have been correlated with diseases associated with structural defects that are often present at birth. However, somatic mutation rates throughout our bodies are incredibly high[49] and it is conceivable that the cell progeny of a somatic uveal mutation could be perceived as aberrant during routine immune surveillance, with the consequence of uveitis being triggered. Curiously, polymorphisms have recently been associated with volumetric differences in select paired right versus left brain regions.[50,51] This may suggest single nucleotide polymorphisms as a mechanism for generating laterality variance.

INNERVATION AND LATERALISATION

When unilateral ocular injury is experimentally induced in one eye in animal models, there is evidence that molecular and cellular changes are induced in the contralateral eye.[52-54] Also, in unilateral ocular infections with herpes simplex[55] or herpes zoster[56] that cause corneal endothelial cell loss or corneal nerve loss, respectively, changes have been detected not only in the previously infected eye but similar less profound changes have been observed in the contralateral eye. It appears that each eye is not working independently and brain sensing of insults are relayed to both eyes. Whether this provides a warning response that may prevent the inflammatory response from being bilateral in HLA-B27 uveitis is currently unknown. Additionally, the right and left brain hemispheres may not provide equivalent responses.[57] The left brain hemisphere may be immunopotentiating while the right is immunosuppressing.[58]

SHIFTING SIDES: BEYOND THE TIME OF INITIAL PRESENTATION

HLA-B27 uveitis may occur repetitively in the same eye in some patients, while in others the attacks can flip flop between left versus right sides. The two eyes are almost never involved synchronously. It is not uncommon with autoimmune diseases where the target antigen is known (such as myasthenia gravis-acetylcholine receptor or NMO-aquaporin 4 water channel) that the quantity of the antigen decreases as the disease progresses.[59,60] Hence, if there is a left–right quantitative variance, the side with more antigens may be initially selected, but with a subsequent attack, when antigen dose has plummeted, then the immune reaction may jump to the opposite side. A somatic tissue mutation is more likely to consistently have one side targeted. As an immune response matures epitope shift often occurs and if the second epitope has preferential left–right lateralisation this may further decide whether the left versus right side is more vulnerable. Hence, both the side of initial attack and whether it remains same sided or flip flops may provide clues regarding causality.

CONCLUSION

When diseases present unilaterally, it strikes us as odd that this should occur. If the disease that occurs is equally commonly on the right side as the left then we are inclined to conclude that the cause is random. However, asymmetry left versus right permeates all structures in our body and multiple molecular variables create that asymmetry. There is no doubt that unplanned environmental events trigger immune activation. Additionally, there will always be a component of chance added since the naïve B and T cell army, with T and B cell receptors generated by random recombination, is changing daily. However, the fact that inflammation can be profound in one eye while the other is remarkably unaffected suggests that the immune system has the exquisite selective capacity to detect

molecular variances that exist in one eye and not in the other. If we could determine how this occurs, we may have an opportunity to understand not only how to better treat uveitis but additionally move closer to directed targeted immunotherapy to one side of the body. Since there are many inflammatory and degenerative diseases that have unilateral presentation, determining this mechanism would have broad implications. This, however, requires a paradigm shift. Perhaps the dogma that sidedness is random needs to be reassessed. Currently, more intensive immunotherapy involves biologicals with associated high costs, variable responsiveness, and risks associated with generalised immunosuppression. Future ideal treatment will be patient-specific and focally directed. Perhaps a deeper understanding of lateralisation may be a key step in that direction.

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OSMOLYTES AS MEDIATORS OF THE MUSCLE TISSUE'S RESPONSES TO INFLAMMATION: EMERGING REGULATORS OF MYOSITIS WITH THERAPEUTIC POTENTIAL

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ABSTRACT

Chronic inflammation of skeletal muscle tissues, termed myositis, is associated with inherited muscular dystrophy and acquired inflammatory myopathy. In this review, the role of the organic osmolytes taurine, betaine, myo-inositol, and sorbitol in skeletal muscles in general, and in myositis in particular, is discussed. Evidence indicates that regulated osmolyte pathway activation contributes to normal muscle functioning, which becomes further activated in myositis as part of the tissue's programme of damage control. On the one hand, osmolytes seem to act as protein stabilisers in muscle fibres counteracting tissue injury but, on the other hand, these compounds also regulate immune cell function. The possibilities for treating myositis through boosting of beneficial or targeting of adversary effects are explored.

Keywords: Betaine, dermatomyositis (DM), immune-mediated necrotising myopathy (IMNM), inflammatory myopathy, muscular dystrophy (MD), myo-inositol, osmolytes, polymyositis (PM), sorbitol, sporadic inclusion body myositis (IBM), taurine.

THE MULTIPLE FACES OF MYOSITIS

Chronic inflammation of skeletal muscle tissues, termed myositis, can have various origins. It can result from infection, tissue damage caused by inherited diseases, or an acquired autoimmune disease. In the muscular dystrophies, muscle inflammation is secondary, yet it represents a hefty pathogenic factor that contributes to deterioration of the muscle tissue's integrity. The most common type is Duchenne muscular dystrophy (DMD); other subtypes include Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, and myotonic dystrophy.[1] DMD is a severe, and still incurable, X-linked muscle disease caused by protein-disruptive mutations in the dystrophin gene. The absence of dystrophin leads to increased vulnerability to contraction-induced sarcolemmal damage, resulting in cycles of muscle fibre necrosis and failing regeneration. Necrotising myofibres are attacked by macrophages; a few T cells, B cells, and dendritic cells are also found within the inflammatory areas.[2] The build-up of inflammation is complexly regulated by an interplay of soluble factors and adhesion molecules. Chemotactic cytokines, termed chemokines, are key players in the inflammatory response associated with DMD, as these diffusible proteins orchestrate the activation and directed migration of leukocytes.[3] Our understanding of DMD disease progression has benefited from studies in the murine mdx model, though considerable differences exist between animal and human diseases.[4]

The idiopathic inflammatory myopathies on the other hand are autoimmune muscle diseases and comprise four main entities: dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), and immune-mediated necrotising myopathy (IMNM). These different disease subgroups present with distinct clinical and myopathological characteristics, with either

blood vessels or muscle fibres acting as the primary immune target. In DM, complement-mediated blood vessel destruction and perifascicular muscle fibre damage and inflammation develop. PM and IBM are characterised by invasion of non-necrotic muscle fibres by auto-aggressive cytotoxic T cells and macrophages, and inflammation builds up mostly at endomysial sites.[5] In IBM muscle fibres, additional degenerative phenomena occur, with rimmed vacuoles and inclusions that contain aggregates of ectopic proteins.[6] IMNM is an increasingly recognised autoimmune myopathy in subgroups of patients triggered by statin use and associated with autoantibodies directed against 3-hydroxy-3-methylglutaryl-coenzyme A reductase,[7] or associated with anti-signal recognition particle autoantibodies.[8]

THE OSMOLYTE PATHWAY AS A PROTECTOR OF MUSCLE FUNCTION

To anticipate perturbations in volume and osmotic imbalance, cells possess a variety of channels and transporters that enable them to accumulate or release osmotically active substances. In addition to inorganic ions, cells can count on a wide range of organic osmolytes, which include free amino acids and their derivatives, methyl ammonium compounds, and polyols. The intracellular osmolytic pool generally consists of a complex mixture of compounds and, in response to stress, pathway members become activated and complexly regulate each other's activities.[9] The transient regulation of osmolyte transporters in response to changing osmotic conditions stabilises intracellular protein function. Regulation occurs both at the transcriptional and translational level, and on a relatively short time-scale (within minutes).[10] The central regulator of the expression of osmolyte pathway genes is the transcription factor tonicity enhancer binding protein, also called nuclear factor of activated T cells 5 (NFAT5).[11] Among the NFAT5 target genes are the organic osmolyte carriers for taurine, betaine, myo-inositol, and the enzyme that catalyses sorbitol synthesis. This osmo-protective mechanism represents a universal system in mammalian cells ubiquitously expressed in human tissues.

The muscle is a highly adaptive tissue, capable of increasing its mass in response to exercise, and of restoring damage caused by injury. These processes require hypertrophy and regeneration, respectively, and for that purpose, resident muscle precursor cells, termed satellite cells, are present within the tissue in a quiescent state. Myogenesis is a highly regulated process co-ordinated by regulatory factors both in favour and opposed to differentiation, which become active in an orderly and sequential fashion.[12] Interestingly, the NFAT5 pathway is an important regulator of the differentiation of immature myoblasts to mature multinucleate myotubes,[13] with NFAT5 levels increasing in the regenerating fibres of mice exposed to experimental muscle tissue injury.[14] The important role played by osmolytes in muscle functioning has been made clear in taurine transporter knockout mice, which display muscle fibre atrophy and necrosis, and reduced exercise endurance.[15,16] It is well known that in DMD muscle the osmotic balance is perturbed, probably due mostly to passive efflux of osmolytes through the leaky plasma membranes,[17] and recent evidence points to an involvement of the osmolyte pathway, also in inflammatory myopathies.

THE OSMOLYTE PATHWAY AS A REGULATOR OF MYOSITIS

In addition to an involvement in damage control and in tissue regeneration, osmolytes have been shown to be potent immune regulators. Both hyperosmotic and inflammatory conditions are able to induce NFAT5 expression and activation in muscle cells *in vitro*.[18] This illustrates how the NFAT5 pathway acts as a more general stress-induced mechanism, equally involved in the muscle tissue's responses to hypertonicity and to inflammation. In addition, the NFAT5 pathway has been firmly linked to nuclear factor κB (NFκB) activity and subsequent pro-inflammatory gene expression.[19] Both the osmolyte pathway members and NFκB p65 subunit protein levels are increased in muscle from DMD and PM/IBM patients.[20] In addition, NFAT5 is expressed in inflammatory cells recruited to sites of tissue injury.[21] Osmolytes are involved in immune cell function, regulating cell volume as an important aspect of phagocytic activity. Taurine is the predominant free amino acid in granulocytes and in lymphocytes.[22] Betaine[23] and myo-inositol[24] accumulation and transporter expression are potent regulators of phagocytosis in liver macrophages, instigated by NFAT5 activation.[25]

TAURINE

The semi-essential amino acid 2-aminoethane sulphonic acid (taurine) is the most abundant

free amino acid in human tissues. It can either be synthesised in the liver from cysteine by cysteine deoxygenase and cysteine sulphinate decarboxylase, or imported in the cell through its sodium and chloride ion-dependent taurine transporter SLC6A6. In addition to its role as an osmolyte, taurine possesses antioxidant and anti-inflammatory properties, and is important for skeletal muscle function and exercise capacity.[15] It has been used as a supplement in energy drinks for athletes for some time. In subjects in their 50s, a multi-nutrient supplement containing taurine has been reported to improve physical function and reduce the inflammation marker interleukin (IL)-6.[26] A cell organelle protective effect has also been observed, preserving mitochondrial function[27] and counteracting endoplasmic reticulum stress.[28] Taurine appears to possess anti-ageing effects, protecting muscle fibres specifically against ageing-associated damage. The content of amino acids in the skeletal muscle alters with age, with taurine levels decreasing, and *SLC6A6* knockout mice display accelerated senescence.[29]

Dystrophin deficiency perturbs taurine metabolism in the muscle, and vice versa, *SLC6A6* knockout mice display pathological changes that mimic those observed in the mdx disease model. In mdx mice, taurine content fluctuates in relation to the disease phase. At the onset of the active dystrophic phase at age 4 weeks, reduced taurine and SLC6A6 levels are present in muscle. This deficiency diminishes as the disease progresses to the stable pathology in adult mice.[30] In contrast, the canine golden retriever MD model shows upregulated taurine and SLC6A6 levels at age 8 months.[31] Thus, differing results have been obtained in different disease models, which fits with the known interspecies variance of dystrophin deficiency characteristics. In patients with active inflammatory myopathy, muscle taurine levels are significantly reduced.[32] Interestingly, urinary taurine levels are increased in DM/PM[33] and in DMD patients[34] compared to healthy controls, suggestive of deregulation of the plasma/tissue taurine balance. For the taurine transporter SLC6A6, different results have also been reported, from lower levels in 3–6-week old mdx compared to control mice,[30] to unchanged levels.[35] SLC6A6 protein expression is induced in muscle biopsies from inflammatory myopathy patients, mostly in the regenerating and atrophic muscle fibres, notably also in the perifascicular atrophic fibres of DM muscle.[20]

BETAINE

N,N,N-trimethylglycine (betaine) is a naturally occurring small amino acid derivative. The two main physiological roles of betaine are as an osmolyte to regulate cellular tonicity, and as a methyl donor participating in the control of cellular activities and differentiation. Betaine can be synthesised by the cell through oxidation of choline-containing compounds, or imported from the extracellular matrix by the betaine-gamma-aminobutyric acid (GABA) transporter termed SLC6A12. Betaine has been shown to promote myotube differentiation and hypertrophy *in vitro*, via insulin growth factor 1-signalling.[36] In addition to a beneficial effect on muscle regeneration, betaine could also counteract inflammation, as has been demonstrated by its inhibitory effect on hypoxia-induced adipokine expression.[37] Based upon these observations, it can be concluded that betaine could potentially enhance exercise performance, reduce fatigue, and improve muscle function.

Urinary betaine[33] and betaine/creatinine ratios[38] are higher in DM/PM patients than in controls. These findings parallel reports of elevated taurine, and further corroborate the possible existence of a general osmolyte plasma/tissue imbalance in these patients. While absent from healthy muscle, our immunofluorescence studies show strong staining of the transporter SLC6A12 on a subset of muscle fibres in DMD, DM (Figure 1), PM, IBM, and IMNM tissues, most of which are small atrophic or regenerating muscle fibres.

MYO-INOSITOL

The essential nutrient cis-1,2,3,5-trans-4,6-hexahydrocyclohexaan (myo-inositol) is a cyclic polyol and is one of the most abundant small organic osmolytes. It regulates different metabolic pathways, in addition to being a key component in preserving the cell's osmotic balance. Myo-inositol can be synthesised by the cell, or accumulated from the extracellular space. Biosynthesis of myo-inositol starts with the conversion of D-glucose-6-phosphate to L-inositol-1-phosphate in a reaction catalysed by myo-inositol phosphate synthase. In addition to synthesis, import from the extracellular matrix is achieved by the sodium myo-inositol co-transporter SLC5A3. Hypertonic stress conditions lead to the upregulation of *SLC5A3* gene expression,[39] as well as to the displacement of the transporter to the plasma membrane.[10]

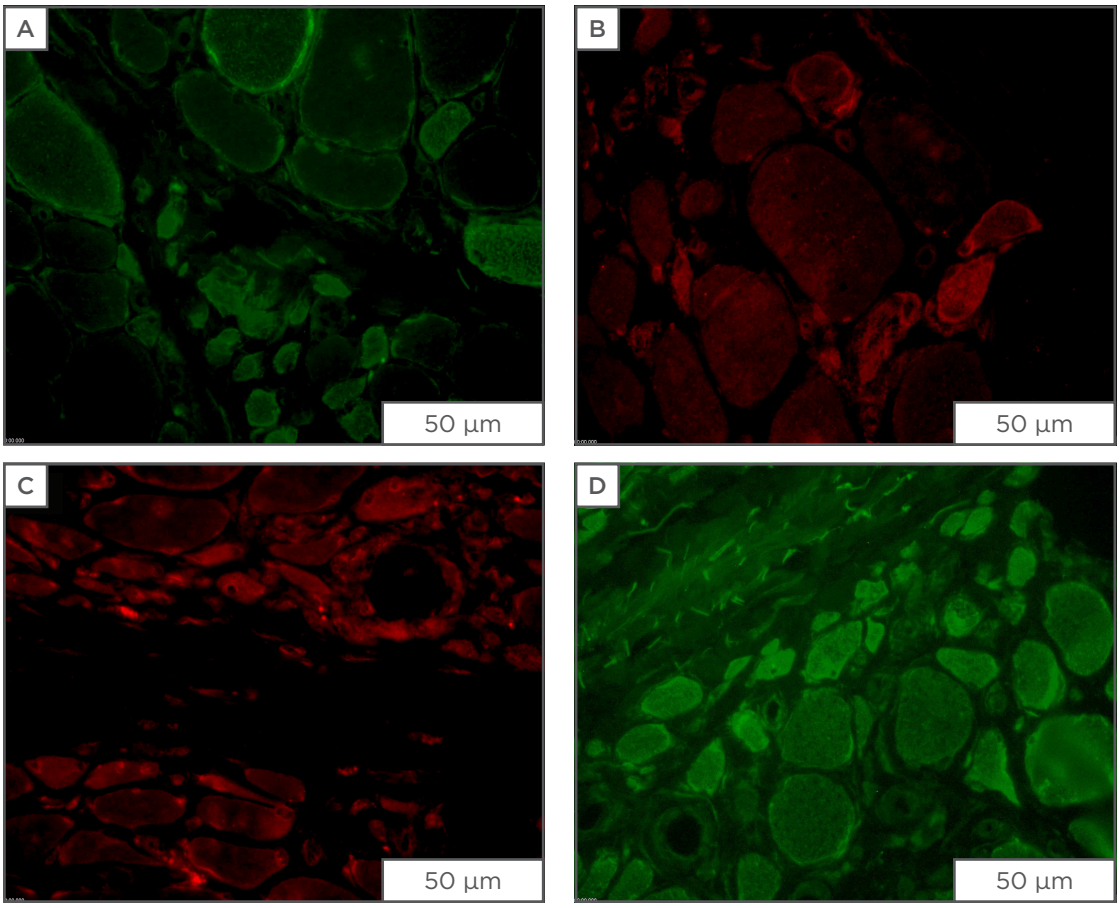

Figure 1: Immunofluorescent staining for osmolyte accumulators in muscle sections from patients diagnosed with dermatomyositis.

Staining for osmolyte accumulators is induced/increased in subsets of muscle fibres of which most are of small-width: A) mouse monoclonal IgG1 anti-SLC6A6 (Santa Cruz Biotechnology, Dallas, Texas, USA; 4 µg/mL); B) mouse monoclonal IgG2b anti-SLC6A12 (Santa Cruz Biotechnology; 4 µg/mL); C) rabbit polyclonal anti-SLC5A3 (Novus, Abingdon, UK; 4 µg/mL); D) goat polyclonal anti-AKR1B1 (Santa Cruz Biotechnology; 1 µg/mL), visualised with secondary antibodies labelled with either AlexaFluor488 (green) or CY3 (red) (Jackson ImmunoResearch Laboratories, Newmarket, UK). Scale bar=50 µm.
Ig: immunoglobulin; AKR1B1: aldo-keto reductase family member B.

The SLC5A3 protein is not present in control muscle material but can readily be detected in the majority of biopsy samples from myositis patients. The transporter is expressed on a subset of muscle fibres but, in addition, SLC5A3 expression has been shown on macrophages and T cells infiltrating DMD, DM, PM, and IBM muscle.[20]

SORBITOL

The sugar alcohol (2R,3S,4S,5S)-hexane-1,2,3,4,5,6-hexol (sorbitol) is synthesised within the cell. The aldo-keto reductase family member B (AKR1B1), also termed aldose reductase, catalyses the reduction of aldehydes and aldehyde forms of glucose. This process makes up the first and rate-limiting step of the polyol pathway of glucose metabolism, reducing glucose to sorbitol. AKR1B1 is expressed in high amounts in normal skeletal muscle, displaying further injury-induced expression.[40]

Sorbitol accumulation is postulated to play a role in skeletal muscle dysfunction associated with diabetes. In rats subjected to streptozotocin-induced diabetes, muscle sorbitol levels are increased, an effect that can be significantly lowered by administering insulin.[41] Insulin decreases intracellular sorbitol by deviating glucose away from the polyol pathway and metabolising it through non-polyol metabolic pathways.[42] When an AKR1B1 inhibitor was given to diabetic rats, skeletal muscle sorbitol levels decreased and muscle contractile properties improved.[43]

Table 1: Osmolytes in patients diagnosed with myositis: summary of the available data.

	DMD	DM	PM	IBM
Taurine	ND	Taurine muscle levels are reduced compared to controls[32]		
	Taurine urinary levels are increased compared to controls[33,34]			ND
	Taurine transporter SLC6A6 protein levels increased in regenerating and atrophic muscle fibres[20]			
Betaine	ND	Betaine urinary levels are increased compared to controls[38]		ND
	Betaine transporter SLC6A12 protein expression induced on regenerating and atrophic muscle fibres			
Myo-inositol	The myo-inositol cotransporter SLC5A3 is induced on regenerating and atrophic muscle fibres and is expressed by a subset of muscle-infiltrating inflammatory cells[20]			
Sorbitol	The sorbitol-producing cellular aldose reductase AKR1B1 is constitutively present in muscle fibres, but is increased in the affected muscle fibres of myositis patients[20]			
	Levels of AKR1B1 protein are increased in patients' muscle tissue[20]			ND

DMD: Duchenne muscular dystrophy; DM: dermatomyositis; PM: polymyositis, IBM: sporadic inclusion body myositis; ND: not determined.

Normal skeletal muscle contains high levels of AKR1B1, and in DMD myotubes *in vitro*, AKR1B1 levels are high and are not further increased by high salt conditions. AKR1B1 levels are increased in the affected muscle fibres of myositis patients, and protein levels are significantly higher in DMD and PM/IBM muscle protein samples compared to control samples.[20]

TREATING MYOSITIS VIA OSMOLYTE PATHWAY INTERVENTION

Evidence is still sparse but clearly points to osmolyte pathway dysregulation in the muscle tissue of patients suffering from myositis (Table 1). An attractive strategy for treating diseases characterised by osmotic disturbances would be to administer positive osmolytes as a nutraceutical supplement. Such supplements are readily available, are relatively cheap, and only have minimal side effects. Based upon the available scientific evidence, two potential compounds come forward for myositis, in particular, taurine and betaine. Intriguingly, glucocorticoids, the most used pharmacological treatment for DM and PM, but also the most common supportive treatment for DMD, have been shown to increase muscle taurine content.[44] In the animal model, the positive effect of glucocorticoids on mdx muscle function can be even further enhanced by administering taurine, with the two drugs exhibiting synergistic therapeutic actions.[45] A beneficial effect of taurine supplements has been firmly shown in mdx mice, increasing the muscle taurine content, improving muscle function, and reducing muscle inflammation.[35,46] Taurine supplementation represents an attractive approach, as it can conveniently be administered orally and presents no serious adverse effects.[47] However, as amino acid patterns of skeletal muscle and blood are age-dependent, with taurine levels increasing from birth to age 15 years,[48] the patients' age may be expected to influence the therapeutic outcome and optimal dosage. The benefits of betaine supplements on muscle endurance and performance[49,50] have also been reported, but another study reported no effect of supplementation in healthy adults.[51]

Reports on the effects of taurine or betaine supplementation in myositis patients have not become available yet. As the upregulation of osmolyte accumulators in regenerating muscle fibres is a general feature of inflammatory myopathy as part of the re-kindling of myogenetic processes to restore muscle damage,[20] osmolyte supplementation could be an amenable supportive therapeutic approach. It should be noted, however, that in DM, osmolyte accumulators are also induced in the perifascicular atrophic muscle fibres, pointing to a possible additional pathogenic role in this subgroup of patients.

Another reason to stimulate the osmolyte pathway could be based upon their known 'chaperoning' effects, mediating refolding of unstable or aggregated proteins,[52] which makes them promising therapeutics for human protein conformational disorders. This offers perspectives for intervention in IBM, a subgroup of inflammatory myopathy

currently still lacking effective treatment options. In IBM, misfolded proteins form multiple protein aggregates likely reflecting failing autophagy. The inclusions contain amyloid-β and components pointing to failing autophagic clearance, such as sequestosome 1, phosphorylated tau, and the standard autophagic marker LC3B.[53] The inclusions also co-localise with the myo-inositol transporter SLC5A3.[20] Whether the presence of the transporter in the aggregates represents a purely dysfunctional aspect due to trapping of the protein remains to be determined. Possibly, trapping of SLC5A3 inside the aggregates could prevent entry of the proper myo-inositol levels in the muscle fibre, disturbing the cell's osmoregulatory system. The complex regulation displayed by osmolytes on protein folding is an open line of research. Betaine concentration-dependent effects have been reported, with betaine dosage tuning the formation/disruption balance of insoluble protein aggregates.[54]

An opposing therapeutic strategy should, however, also be considered, i.e. targeting the pro-inflammatory aspects of individual osmolytes if specific members of the osmolyte pathway with a pivotal role in chronic inflammation can be identified. In this respect, the expression of SLC5A3 on many muscle-infiltrating inflammatory cells[20] puts this myo-inositol transporter forward as a potential target. Myo-inositol can regulate macrophage volume changes during the process of phagocytosis. The plausibility of an anti-inflammatory approach targeting osmolyte accumulators has already been shown with AKR1B1 inhibitors.[55] The latter can significantly prevent inflammation build-up in the allergic lung.[56] This seems an amenable approach, as the beneficiary effects of the pathway members appear to be based mostly on redundant cytoprotective activities. Reductions in individual osmolytes can be compensated for, when individual partners are being targeted, as cells can rely on a complex scala of compatible osmolytes. In muscle cells exposed to hypertonic conditions, protective increases of cell creatine levels have been observed.[57]

In summary, the osmolyte pathway represents potentially beneficial and adversary effects on myositis, and offers an interesting new avenue for therapeutic intervention. Full exploration of this therapeutic strategy will necessitate, however, further unravelling of osmolyte pathway activities in patient muscle tissues.

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ADVANCES IN EPIGENETICS AND INTEGRATION OF OMICS IN LUPUS

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ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic, multi-organ disease that predominantly affects young women of childbearing age. It is also a disease in which epigenetic modulation is emerging as an important mechanism for understanding how the environment interacts with inherited genes to produce disease. Much of the genetic risk for SLE identified in genome-wide association studies has been shown to lie in the non-coding genome, where epigenetic modifications of DNA and histone proteins regulate and co-ordinate transcription on a genome-wide basis. Novel methodologies, including high-throughput sequencing of open chromatin, RNA sequencing, protein microarrays, and gas chromatography-mass spectrometry, have revealed intriguing insights into the pathogenesis of SLE. We review these recent data and their potential contribution to more accurate diagnoses and the development of new therapeutic agents to improve patient outcomes.

Keywords: Systemic lupus erythematosus (SLE), genomics, proteomics, metabolomics.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-system, complex disease in which the environment interacts with inherited genes to produce a broad spectrum of phenotypes with inter-individual variability. These gene–environment interactions lead to a perturbed immunologic state in which autoantibodies, immune complex deposition, and complement activation contribute to systemic inflammation and target tissue damage. The genetics of SLE have been studied extensively; however, risk loci and single genes identified by genome-wide association studies appear to account for ≤25% of the inherited risk for SLE, suggesting that environment also contributes to risk.[1,2] Moreover, most of the genetic risk for SLE lies within non-coding portions of the human genome,[3] demonstrating that the disease may manifest due to perturbations in the regulation of transcription, rather than changes to protein-coding genes that lead to nucleic acid substitutions.

The importance of the non-coding genome was demonstrated by recent work by our research group.[3] Using standard computational techniques, we queried all the single nucleotide polymorphisms (SNPs) previously shown to convey risk for SLE.[1,2] Of the 46 disease-associated SNPs, 30 (65%) were within non-coding regions of the genome. By querying the Roadmap Epigenomics data,[4] we demonstrated that most of the linkage disequilibrium blocks containing the disease-associated SNPs were within non-coding regions that were highly enriched for epigenetic signatures associated with functional elements, such as enhancers. These epigenetic signatures were most prominent in B cells and neutrophils and less

prominent in CD4+ T cells. These data parallel a recent report by Jiang et al.,[5] which demonstrated enrichment for H3K4me1/H3K27ac marks within the linkage disequilibrium blocks containing SNPs associated with juvenile idiopathic arthritis.

The non-coding genome contains numerous functional elements, often identified by specific epigenetic modifications to histone proteins that regulate and co-ordinate transcription on a genome-wide basis.[6,7] The critical element in co-ordinating transcription is the regulation of chromatin accessibility, which is regulated by DNA methylation, alterations in histone proteins, and three-dimensional (3D) chromatin architecture mediated by DNA interactions with transcriptional regulators, such as the CCCTC-binding factor (also known as CTCF).[8] These processes allow transcription to be fine-tuned to specific physiological circumstances.[9] Functional elements that regulate and co-ordinate transcription are typically found in regions of open chromatin,[6,7] and both the ENCODE and Roadmap Epigenomics projects focussed considerable efforts on defining these regions.[10]

Table 1 summarises the most frequently used methods of high-throughput sequencing and mass spectrometry and the information that can be generated using these methods. These methodologies introduce a new era of 'omics', allowing for profiling of genetics, proteins, and metabolites to shed light on disease aetiology and pathogenesis. The same techniques that can be used to diagnose patients can also be used to investigate responses to therapy and describe disease courses on molecular levels.

Table 1: Selected methodologies for integrative omics data.

Methodology	Information provided
Epigenome	
DNAse I hypersensitivity assays	Defines regions of open chromatin
Formaldehyde-assisted identification of regulatory elements	Defines regions of open chromatin
ChIP-seq	Identifies protein-DNA interactions
ATAC-seq	Defines regions of open chromatin
Chromatin architecture	
3C	Analyses 3D chromatin architecture by identifying any region of long-range chromatin interaction
4C	Similar to 3C but combined with microarray
HiC	Identifies long-range chromatin interactions and physical loci in 3D
ChIA-PET	Identifies long-range chromatin sequencing interactions defined by specific protein-DNA interactions
HiChIP	A modification of the ChIA-PET approach to identify long-range chromatin interactions mediated by specific protein-DNA interactions
Transcriptome	
Gene expression microarrays	-
RNA sequencing	-
Proteome	
Mass spectrometry	-
Protein microarrays	-
Metabolome	
Gas chromatography-mass spectrometry	-
Nuclear magnetic resonance	-

3C: chromosome conformation capture; 3D: three-dimensional; 4C: 3C capture-on-chip; ATAC-seq: assays of transposase-accessible chromatin sequencing; ChiP-seq: chromatin immunoprecipitation sequencing ChIA-PET: chromatin interaction analysis with paired-end tag sequencing; HiC: hydrophobic interaction chromatography; HiChiP: highly integrated chromatin immunoprecipitation.

Table 2: Use of omics in lupus to date.

Methodology	Reference	Results
Epigenome		
ChIP-seq	Shi et al.[34]	Found that regions with more extensive histone modifications were enriched in transcription factor binding sites that may be related to interferon signalling in SLE monocytes
ATAC-seq	Scharer et al.[16]	Found changes in chromatin accessibility at the loci of genes involved in B cell activation and differentiation in treatment-naïve adult SLE patients
Transcriptome		
RNA-seq	Dozmorov et al.[33]	Discovered cell-specific patterns of differentially-expressed immunoglobulin genes in SLE B cells when compared to monocytes
Proteome		
Protein microarray	Li et al.,[36] Fattal et al.[37]	Elevated IgG autoantibodies in patients with active SLE when compared to healthy controls
Protein microarray	Fragoso-Loyo et al.,[42] Hu et al.[43]	Patients with neuropsychiatric lupus have elevated levels of autoantibodies, but these autoantibodies are not specific for lupus
Protein microarray	Nicolaou et al.[46]	11 candidate biomarkers were identified for lupus nephritis or neuropsychiatric lupus that are related to cellular growth, development, and/or apoptosis
Metabolome		
GC-MS	Yan et al.[47]	Proteins important in amino acid turnover or protein biosynthesis and lipid-gut microbial metabolism is important in active SLE and can alter with disease activity
GC-MS	Bengtsson et al.[48]	Metabolites associated with oxidative activity and the urea cycle were increased in SLE patients, while levels of tryptophan were decreased

ATAC-seq: assays of transposase-accessible chromatin sequencing; ChIP-seq: chromatin immunoprecipitation sequencing; GC-MS: gas chromatography-mass spectrometry; NPSLE: neuropsychiatric lupus; SLE: systemic lupus erythematosus; Ig: immunoglobulin.

TECHNOLOGIES AND THEIR USES

Methods used to investigate epigenetic factors that regulate transcription in the context of rheumatic diseases, initially focussed on DNA methylation, are either bisulfate-based (such as MethylC sequencing and reduced representation bisulfite sequencing) or enrichment-based (such as methylated DNA immunoprecipitation sequencing [MeDIP-seq], methylated DNA binding domain sequencing, or methylation-sensitive restriction enzyme digestion followed by sequencing [MRE-seq]) and, as noted, can be combined with sequencing.[11,12] Although grossly effective, these methods are not without limitations. In MeDIP-seq, methylated DNA fragments are non-covalently bound to 5-methylcytosine antibodies. Thus, MeDIP-seq does not cover medium-low 5'—C—phosphate—G—3' (CpG) density regions of the genome well and gives a relatively low resolution, limited by the size of the fragments from immunoprecipitation.[12] Moreover, MeDIP-seq requires large amounts of both DNA and antibodies for each assay. MRE-seq and other restriction enzyme-based methods allow interrogation of unmodified and modified areas of genomic DNA, but their coverage and resolution are limited by the specificity of the available enzymes.

Li et al.[12] recently suggested that combining DNA methylation and sequencing methods may yield more sensitive results. In particular, the investigators combined MeDIP-seq with MRE-seq to improve the accuracy of detection of differentially methylated regions and coverage of the genome. A key advantage to integration of these methods is that DNA methylation analysis may be performed on a whole genome level and is not restricted to promoters or CpG islands. Moreover, they describe computational protocols to analyse the data generated from both methods that allow them to increase the sensitivity and accuracy of their results.

Traditional methods such as DNAse I hypersensitivity and formaldehyde-assisted identification of regulatory elements assays have been used to

identify regions of open chromatin and thus, presumably, regions that are functional and biologically relevant. However, the large number of cells these assays require (often >1x10^8) to achieve adequate sequencing depth and signal-to-noise ratios have made them impractical for use in translational studies in SLE or other rheumatic diseases. More recently, Buenrostro et al.[13] developed a method for broadly surveying open chromatin. This technique, called 'assays of transposase-accessible chromatin sequencing' (ATAC-seq), allows researchers to comprehensively survey open chromatin in pathologically relevant cells. By rapidly surveying open chromatin, we may have a comprehensive view of where regulatory elements may be perturbed.[14] ATAC-seq uses Tn5 transposases linked to sequencing adapters to selectively insert constructs into nucleosome-free regions, and can be performed on as few as 50,000 cells,[15] which makes it highly suitable as a tool to study low-abundance leukocyte subsets. In fact, Scharer et al.[16] have described changes in chromatin accessibility that occur at loci surrounding genes involved in B cell activation and differentiation from treatment-naïve adult SLE patients.

Similar advances are being made in techniques to understand how 3D chromatin architecture regulates gene transcription.[17] These methods can be either untargeted (chromosome conformation capture [3C], 3C capture-on-chip, hydrophobic interaction chromatography [HiC]) or targeted (chromatin interaction analysis with paired-end tag sequencing [ChIA-PET] and highly integrated chromatin immunoprecipitation [HiChIP]).[18-21] Untargeted methods simply map any region of long-range interaction at the chromatin level, but, with the exception of HiC, do so at low resolutions. HiC was developed based on 3C.[20] HiC uses 3C to describe not only the genomic sequence of DNA fragments but also where they are physically located in the 3D genomic structure. HiC is also compatible with high-throughput sequencing; this combination identified many long-range interactions between risk loci involved in autoimmune disease and putative target genes in T and B cells.[21]

Targeted methods investigating chromatin conformation can identify interactions mediated by specific proteins and provide higher resolution maps than untargeted methods. Targeted methods include that described by Li et al.,[18] an adaptation of ChIA-PET. ChIA-PET provides high-resolution mapping of long-range DNA interactions mediated by specific proteins. Another method, which the authors designate HiChIP, allows these high-resolution maps to be generated with as few as 1x10^6 cells, or 1% of the number required for ChIA-PET.[19]

PROGRESS IN UNDERSTANDING THE ROLE OF THE EPIGENOME IN LUPUS

Interest in the role of the epigenome in the pathogenesis of SLE has led to several ground-breaking discoveries looking at DNA methylation.[22-24] Coit et al.[24] demonstrated, for example, that epigenetic changes in interferon (IFN) response genes seen in adult SLE patients are associated with disease severity. Investigation of DNA methylation in CD4+ T cells also revealed susceptibility loci that may contribute to the differential manifestations of SLE in different ethnicities, including Europeans and African-Americans.[22]

It is important to note that the epigenetic machinery that regulates gene expression is specific to distinct cell types and that cell-specific expression is a feature of diseases such as SLE.[22-24] For example, B cells have essential roles in antigen presentation and cytokine secretion, and produce autoantibodies that are the key to the diagnosis and pathogenesis of SLE. B cell activation and differentiation also correlate with SLE disease activity and response to therapy.[25-28] Studies using newer technologies, such as high-throughput sequencing, can also be misleading and/or generate conflicting results. For example, using RNA-seq, Rai et al.[29] described dysregulation of specific cytokine pathways in adult SLE patients when stratified by autoantibody profile. IFN transcripts were predominantly dysregulated in patients with only anti-extractable nuclear antigen (ENA) autoantibodies when compared to patients with anti-double-stranded DNA (dsDNA) autoantibodies. Dysregulation of plasma cell-related transcripts were more pronounced in patients with only anti-dsDNA or anti-ENA autoantibodies, when compared with patients who had both sets of autoantibodies. These results conflict with numerous published studies demonstrating that IFN signatures are associated with both anti-ENA and anti-dsDNA autoantibodies,[30-32] possibly because Rai et al. performed RNA-seq on total peripheral blood leukocytes.

Although both epigenetic signatures and transcriptomes are cell-specific, new computational approaches have allowed investigators to infer cell-specific patterns even from complex samples,

such as whole blood, provided there is information available on the ratios of the different leukocyte subsets. For example, using RNA-seq to examine transcriptomes in whole blood samples, Dozmorov et al.[33] used a de-convolution method that allowed them to identify differential expression of immunoglobulin (Ig) genes in SLE B cells, while a monocyte population from the same patients differentially expressed genes comprising a ribosomal signature (Table 2).

De-convolution methods have not been attempted with histone marks, and cell-specific studies continue to be the standard approach. For example, Shi et al.[34] used chromatin immunoprecipitation sequencing (ChIP-seq) to define histone modifications in monocytes of adult SLE patients. ChIP-seq analyses protein interactions with DNA through the genome-wide DNA binding sites for transcription factors and other proteins (e.g. histones). Compared to healthy controls, regions with more extensive histone modification were significantly enriched in transcription factor binding sites that may be related to IFN signalling in adult SLE monocytes. Taken with the information from B cells as described above, these data could help to direct research efforts toward new avenues of therapy targeting different cell populations and intracellular signalling pathways for specific clinical manifestations.

PROTEOMICS AND METABOLOMICS

The transcriptomes and epigenomes of SLE patients only provide a small window into disease pathogenesis and possible response to therapy. Personalised medicine is expected to benefit from the combination of genomic information with regular monitoring of physiologic states by multiple high-throughput methods that query a broad range of cellular processes. Novel approaches using mass spectrometry enable a closer look at the proteome and metabolome (the composition of all small molecule metabolites in human cells).

Protein, or 'autoantigen', microarrays allowing for detection of autoantibody profiles in SLE were described over 10 years ago.[35] These arrays carry thousands of proteins that can be found in many rheumatic diseases, including SLE. The arrays also allow for detection of antibody isotypes (IgG, IgM, IgE, and IgA). The advantages of microarrays over mass spectrometry for protein profiling are the ability to analyse low abundance proteins and that microarrays are not as time-consuming or labour-intensive to perform. Recently, protein microarrays were used to detect proteomic profiles correlating with specific disease manifestations of SLE. Microarrays were used to distinguish between adults with lupus nephritis, neuropsychiatric lupus (NPSLE), and pulmonary involvement.[36-39] Li et al.[36] found that SLE patients had increased levels of IgG autoantibodies in their sera. Combining these findings with transcriptional profiling using conventional hybridisation-based microarrays revealed a correlation with elevated expression of IFN genes, indicating that IFN may play a role in class switching of IgM to IgG antibodies in SLE. Fattal et al.[37] found increased levels of IgG autoantibodies against dsDNA, single-stranded DNA, Epstein–Barr virus, and hyaluronic acid in the sera of patients with active lupus nephritis when compared to healthy controls. Although Fattal et al. found these levels remained high even after the patients achieved long-term clinical remission, indicating independence from disease activity, much evidence using traditional assays has established that anti-dsDNA antibody levels do fluctuate with disease activity and remission.[38-41]

Fragoso-Loyo et al.[42] found elevated levels of autoantibodies using protein microarrays in the sera of patients with NPSLE. However, these autoantibodies could be seen in other rheumatic diseases; none were specific for NPSLE. Hu et al.[43] used a protein microarray with 17,000 distinct proteins to evaluate NPSLE sera. These experiments identified 137 autoantigens (including auto antibodies) associated with SLE. Two of these proteins, anti-60S acidic ribosomal protein P2 and anti-SSA in cerebral spinal fluid (CSF), were significantly correlated with those in sera of NPSLE patients. The findings suggest CSF proteins are potential biomarkers for NPSLE, but there have been conflicting studies.[44]

There remains a definite challenge in finding biomarkers for pulmonary diseases associated with SLE. Protein microarrays for a broad range of cytokines and chemokines were performed on sera from nine adults with SLE who had known pulmonary involvement. Data were compared from nine adults with SLE without pulmonary involvement.[45] A significant increase in CC chemokine ligand 21 (CCL21) and IFN-gamma induced protein 10 (IP-10) levels were seen in patients with SLE and pulmonary involvement. The changes in CCL21 and IP-10 were associated with changes in diffusion capacity of those same

patients, indicating that these chemokines may serve as biomarkers for pulmonary disease in patients with SLE.

A systematic review of the published reports on proteomic biomarkers discovered by mass spectrometry-based methods in adult SLE patients found that ≤28 candidate biomarkers had been validated in the laboratory. Eleven candidate biomarkers were identified in more than one study.[46] Many of these biomarkers are thought to be significant in the diagnosis of lupus nephritis or NPSLE. The functions of the biomarkers appear to be related to maintenance of cellular functions such as growth, division, and apoptosis. However, to date, these biomarkers require further study to assess their clinical utility and significance in clinical practice.

Metabolomes have been profiled from the sera of a cohort of 80 Chinese adult SLE patients using gas chromatography-mass spectrometry.[47] This analysis revealed that proteins associated with changes in amino acid turnover or protein biosynthesis, and lipid and gut microbial metabolism, might act as a 'metabolic signature' in SLE patients. This study also demonstrated that metabolomes varied with differences in disease activity. These alterations predominantly involved metabolites such as glutamate, citrate, linoleic acid, and prophylparaben.

Another group[48] compared adult SLE patient metabolomes with those from patients with primary Sjögren's syndrome and systemic sclerosis. These investigators found an increase in the circulating abundance of metabolites associated with oxidative activity and the urea cycle in SLE patients.[41] SLE patients also had decreased levels of tryptophan compared to those with Sjögren's syndrome or systemic sclerosis. These findings suggest that SLE changes the enzyme activity of a decarboxylase and/or activation of the kynurenine pathway, which may be a novel metabolic checkpoint in the pathogenesis of SLE.[49] These data suggest new ways of treatment by targeting small molecule metabolites and biosynthesis pathways, in addition to the more traditional methods of targeting immunologic pathways.

Nuclear magnetic resonance has been touted as a new and emerging technique for investigating the metabolome; it is faster, less labour-intensive, and does not require as many separations to obtain data as gas chromatography-mass spectrometry. Nuclear magnetic resonance can also measure up to hundreds of metabolites at once. However, there are no studies to date using this technique in SLE.

AUTHORS' PERSPECTIVE

The emergence of this new era of omics and personalised medicine in rheumatic disease is exciting. The newer methodologies to examine the epigenome, transcriptome, proteome, and metabolome will generate previously unimagined amounts of data about health and disease states. These new methodologies also allow for more innovative and comprehensive approaches to pathobiology, prognostication, and therapy. The development of the era of personalised medicine, where the wealth of information available from omics data may be applied to the treatment of individual patients, has the potential to dramatically improve patient outcomes.

Over the past decade, information from the genome and epigenome has allowed us to more accurately diagnose and treat cancer patients in a manner uniquely suited to each individual.[50] Similar advances may soon be applied to rheumatic disease using results generated from high-throughput sequencing methodologies. When combined with clinical disease correlations, this approach may facilitate monitoring of disease phenotypes. In particular, data generated from the epigenomes and transcriptomes of patients with SLE, combined with proteomics and metabolomics, may allow us to predict how time and treatment alter the natural history of the disorder. This combination may also enhance diagnosis and treatment while improving epidemiologic data on SLE and other human diseases.[51] A long-term goal will be to tailor therapies based on individual patient characteristics and more accurately monitor individual responses to individualised therapies, thus improving individual patient outcomes.

However, enthusiasm for personalised medicine and the future of omics in rheumatic disease must be tempered with a word on costs. Currently, these methods are for laboratory and research purposes only, and are not available for clinical use. Each array or sequencing assay is for single use and typically costs thousands of dollars. Data generated from these arrays and assays may result in files that are hundreds of megabytes; data from several patients would require terabytes (10^{12}) of storage on a computer with at least a 2.7 GHz microprocessor. Not only are the datasets large, they can be quite time-consuming to analyse. They require

interpretation first by experts in bioinformatics and then careful clinical correlation to specific diseases by subspecialists with a keen understanding of the underlying mechanisms of disease pathogenesis and progression. In addition, there is the added challenge of integration of data from different sources and platforms that requires the development of more sophisticated, robust bioinformatics tools.

Moreover, there is rising concern over privacy issues with the deposition of genomic data into public cloud computing settings.[52-54] While accessing and integrating genomic data with clinical phenotypes are important for research, these processes must be handled carefully to avoid inadvertent leakage of sensitive information to unauthorised persons and the improper use of available data. When data are shared between multiple institutions, there is additional concern about data being used beyond agreed-upon research scope and potential processing in unsafe computational environments. Establishment of rules and regulations in this field to protect the donor as well as the user of readily available genomic data will greatly support and enhance the use of these technologies in the future.

Combinatorial omics data from SLE patients may give us new ways to subset SLE patients. This will allow for better efficacy in clinical trials requiring a smaller number of patients. Again, we must emphasise that the power of these high-throughput sequencing techniques in SLE appears to be in the provision of ways to advance therapeutics through analysis of earlier responses to therapy in distinct SLE clinical phenotypes and subsets of patients. As most studies are currently, and will continue to be, conducted on patients with long-standing SLE, the use of omics may reflect their disease course. Additionally, some patients may have received heavily immunosuppressive or ablative therapies that will affect the results from omics methodologies. Care must be taken to analyse the correct tissue types and cellular populations. SLE patients may experience a constant low-level of inflammation; omics data may lead us to discover new therapies that could return the genome to 'normal' in specific disease remission states and thus improve patient outcomes by reducing the burden of disease.

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NANOMEDICINES FOR INCREASED SPECIFICITY AND THERAPEUTIC EFFICACY OF RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA), the most frequent chronic inflammatory autoimmune disease, can lead to pain, bone and articular destruction, and limb deformity and impairment, with great impact on the activities of daily life. Several drug modifiers of the inflammatory process have been used in the treatment of the disease, all with specific patient targets and indications. However, the side effects are a frequent cause of undertreatment and non-adherence. To promote better compliance with the therapy, drug researchers have been trying to develop a new carrier of the immunomodulated molecules to increase their concentration in the target cell (mostly synovial), avoiding side effects for organs that are not targeted, as well as providing an easier manner of administration. The research results from animal models are promising and the clinical applications will show if these results are similarly impressive. This paper aims to explain the major applications of nanomedicine in RA treatment and diagnosis. The use of nanocarriers able to act as a diagnostic imaging agent and targeted drug delivery system, simultaneously, also known as nanotheranostics, can allow an improved efficacy and safety pharmacological profile, earlier detection, and thither monitoring of the disease.[1] Commercial successes of RA active targeting of nanomedicine and products under development will be revised.

Keywords: Intracellular delivery, nanomedicine, rheumatoid arthritis (RA), safety, surface modification, targeted delivery, theranostics.

Rheumatoid arthritis (RA) is one of the most common and severe autoimmune diseases related to the joints.[2,3] Regrettably, the RA inflammatory process remains puzzling, and finding effective therapies for the disease, as well as a new means for its early diagnosis, has been a daunting task. It is characterised by chronic inflammation of the synovial membrane, which often leads to destruction of articular cartilage, periarticular bone erosion, and permanent deformities. Consequently, more macrophages, lymphocytes, and fibroblasts are activated and the RA inflammatory process remains.[3-5] Thus, macrophages play a pivotal role in the features and progress of RA, and effective diagnosis and therapy may include the ability to target these cells.

Currently, the main target of RA therapy is to control the inherent inflammatory response, alleviate pain, and avoid structural bone damage and deformities. Several therapeutic options have been used to manage and slow the progression of the disease, which include the use of sulfasalazine,

hydroxychloroquine, corticosteroid-related drugs, or methotrexate (MTX), a first-line, disease-modifying anti-rheumatic drug.[6,7] MTX is widely used due to its satisfactory safety profile, efficacy, and low cost. It is an analogue of folic acid that disrupts cellular folate metabolism by inhibiting its target enzyme, dihydrofolate reductase.[7-9] Still, there is a lack of specificity for MTX and/or other similar drugs.

More recently, biologic agents were developed to target components of the immune response involved in RA, including proinflammatory cytokines and immune cells. Most of these biologicals include cytokine antagonists (tumour necrosis factor [TNF] blockade, interleukin-1 receptor blockade, and interleukin-6 receptor inhibitors), B-cell depleting agents, T cell co-stimulation modulators, and kinase inhibitors.[10] Despite their efficacy, biologicals are also associated with invasive routes of administration, stability issues, and significant adverse effects, which may limit their use. Recent developments in the understanding of inflammation have led to an increased interest in the use of nanomedicine in the treatment of RA, overcoming pharmacokinetic and pharmacodynamic issues related to the classical intravenous formulation, such as poor solubility of active molecules in physiological fluid or premature degradation of drugs.

Nanotechnology is a multidisciplinary research field, concerning the study of devices usually ranging from 1–100 nm, though in larger systems this is ≤1,000 nm. As a consequence of its vast success on the development of biocompatible colloidal systems, such as nanoparticles, nanocapsules, micellar systems, and conjugates, nanomedicine has thrived and is now providing new possibilities for the use of nanomaterials in medical applications for drug delivery and tissue regeneration.[11-13] In this technology, unique phenomena enable novel applications as nanosystems have new properties, such as large surface-volume ratio, surface charge, and small and controlled size. Nanoparticles built with biodegradable, biocompatible biomaterials have gained attention from the scientific community, which is a result of their controlled and sustained release properties, subcellular size, and feasibility of production.[14] The most commonly used polymeric nanoparticles include poly(lactic acid), poly(lactic-co-glycolic acid) (PLGA), poly-ε-caprolactone, poly(alkylcyanoacrylates), and chitosan, while proteins such as albumin and gelatin have been widely explored. Lipid-based nanocarriers have also been proposed. Colloidal drug delivery systems have been described as reducing systemic side effects and maintaining of appropriate drug concentration in the required place.

Nanomedicine may also offer new opportunities to combine diagnosis and therapy in a single approach. Improved theranostics processes (combining diagnostics and therapy) are being studied to develop new means to diagnose, fight, and follow disease. The release and action of anti-rheumatic drugs may be enhanced and controlled, potentially without injuring healthy tissues and organs, while simultaneously providing a non-invasive and specific imaging tool for RA. A theranostic nanosystem combines non-invasive diagnosis and treatment, with the possibility of monitoring real-time drug release and distribution, thus predicting and validating the effectiveness of the therapy. This optimised algorithm treatment to each patient can be specifically performed, achieving the call for personalised nanomedicine.[15]

Conventional radiographs, magnetic resonance imaging (MRI), and ultrasonography are used to measure joint damage in patients with RA. MRI is especially sensitive in detecting early pathology due to its duality in assessing both inflammatory and structural lesions.[16] In the context of MRI-based diagnosis, superparamagnetic iron oxide nanoparticles (SPION) are most frequently used.[17] Despite their intrinsic diagnostic capabilities, these magnetic nanoparticles can be co-encapsulated with a therapeutic agent into other nanoparticles, such as US Food and Drug Administration (FDA)-approved polymer PLGA.[18] SPION can also be associated with other imaging tools, such as optical/fluorescence or offer additional targeting approaches. For example, they can be guided to target sites using external magnetic fields or heated by an external source to provide hyperthermia.[19]

At present, marketed products based on nanomedicines are useful tools for the diagnosis and treatment of prominent diseases. There are different examples of nanomedicines with potential application in RA therapy based on different kinds of nanoparticulate systems, as recently revised.[10] However, the clinical translation of such nanomedicines into clinical trials or products is still in its infancy. There are no clinical trials enrolling RA and nanoparticles considering the new biologics (e.g. adalimumab) as matrix nanostructures. A significant number of the studies have their proof of concept in disease animal models

resembling the RA condition, expecting a future translation into humans.

Additionally, further surface functionalisation of nanoparticles to impart precise biological functions is considered a promising approach in drug delivery. It holds the potential to play an important role in personalised medicine, namely in addressing clinically unmet needs that are highly variable among patients.[20] The modification of the surface of particles provides physicochemical and biological characteristics that can radically change the properties of a material. With RA, nanomedicines can be designed to remain in blood circulation for a longer time, may be surface-conjugated with ligands to facilitate active targeting, can be tailored to macrophage uptake or targeted to certain receptors, and pass through interendothelial cell gaps in diseased tissue. Several active targeting strategies for nanoparticles in RA have been proposed in recent years. In an active targeting approach, ligand molecules, such as antibodies and specific adhesion molecules, attach to the surface of nanoparticles, which can increase the probability of binding to membrane receptors upregulated in RA key local effector cells. These are mainly macrophages or monocytes and fibroblasts.[21] They are local and systemic amplifiers of RA severity and perpetuation is maintained by means of cytokine and cell contact-mediated activation of neighbouring inflammatory cells.[22] Cellular surface proteins, such as CD11b, CD90, HLA-DR, and CD64 receptor of inflammatory macrophages,[23] have been recognised as highly overexpressed antigens in RA-affected synovial tissue, which may be promising biomarkers for novel targeted drug delivery and diagnosic approaches in RA.[23-26] Other agents involving the folate receptor-β of rheumatoid arthritis synovial macrophages,[27,28] or intercellular cell-adhesion molecule-1[29] have also been described. Functionalised nanomedicines may suffer from intrinsic biological variability due to pathology and physiological variations, and possess a limited landscape as far as scale-up production is concerned. Being more elaborate in terms of production steps, due to the surface conjugation of ligand moieties, means that additional steps in the production chain may be required, which makes the manufacturing process more complex.

In a recent study for the treatment of inflammatory arthritis, using a well-established and clinically relevant rat model of adjuvant-induced arthritis, different nanomedicine formulations of dexamethasone, including liposomes, micelles, slow-releasing, and fast-releasing polymeric prodrugs were evaluated.[30] It was found that after a single intravenous injection, formulations with the slower drug release kinetics maintained longer duration of therapeutic activity than those with relatively faster drug release kinetics, resulting in improved joint protection. This finding will be instructional in the future development and optimisation of nanomedicines for the clinical management of RA.

In order to target nanoparticles in RA therapy, hyaluronic acid-MTX conjugates that can specifically bind to CD44 on the inflammatory cells and release MTX in an inflamed tissue of RA have been developed.[31] Those nanoconjugates were taken up by activated macrophages more efficiently than free MTX through binding CD44 and hyaluronic acid. *In vivo* biodistribution tests confirmed that hyaluronic acid–MTX conjugates were selectively accumulated into the inflammatory joint site of the collagen-induced arthritic mouse, improving clinical arthritis indices and reducing proinflammatory cytokines and pathogenic immunoglobulin G.[31] It was also confirmed that the use of hyaluronic acid as a nanocarrier increases the residence time of MTX in affected joints.[32] Hyaluronic acid-MTX conjugates exerted anti-arthritic effects in two different models of arthritis, with a wider therapeutic window than oral MTX.

The use of gold compounds for the treatment of RA is well-established, although the mechanism of action of chrysotherapeutic agents is not very clearly understood. Clinical protocols have been proposed by injecting patients with regular-sized gold compounds to reduce inflammation. Attempts have been made to expose macrophages to gold nanoparticles and compare them with gold complexes, which have resulted in a significantly greater uptake of gold without significant cytotoxicity towards macrophages.[33] These results support the potential of these colloidal systems as anti-RA agents. When combining the delivery of gold nanoparticles with MTX to the region of inflammation in collagen-induced arthritic mice, the retention of nanoparticles was enhanced under the external magnetic field,[34] resulting in enhanced therapeutic effects with an MTX dosage of just 0.05% compared to free MTX therapy for the treatment of RA.[34]

The use of small interfering RNA (siRNA) has shown therapeutic effects in diverse disease models by silencing the gene responsible for the

defects, including RA.[35] In order to enhance the therapeutic efficacy of siRNA, incorporation of siRNA into nanoparticles has shown improved target ability.[36] Despite the lack of research in this area, the delivery of siRNA entrapped into nanoparticles has been able to downregulate fundamental protein molecules involved in the RA physiologic process, such as Notch1[35] or TNF-α.[37] In the future, particular attention may be given to Notch1, a signalling receptor, overexpressed in synoviocytes that has been shown to contribute to TNF-α-induced proliferation. Such targeting of siRNA delivery systems in siRNA-nanoparticles has been shown to be a superior effective RA treatment compared to free siRNA by suppressing protein signalling pathways without any undesirable severe toxicity.

Insights on multifunctional anti-CD64 mAb-modified nanoparticles for the combined delivery of MTX and iron oxide nanoparticles (SPION) has also been proposed.[38,39] Polymeric nanoparticles have the potential to provide a new theranostic approach for RA management. When considering the potential delivery methods for nanomedicines to target RA, intravenous administration is gaining the most attention, both preclinically and clinically. The increased interest in intravenous delivery is not surprising given that nanoparticles delivered systemically have direct access to nearly all parts of the body, including joints, and thus have the most potential to influence clinical care.[40] However, systemically delivered nanoparticles still face exceptional challenges with regard to delivery, clinical translation and regulation. If delivery aspects are easy to overcome, clinical translation and integration relies on a consistent and reproducible product. On the other hand, the ability of nanomedicines to permeate into and/or be retained in the inflamed joint after intra-articular administration has proven to be beneficial in improving RA therapy while reducing systemic exposure of patients to potentially toxic anti-arthritic drugs.[41] This is an alternative worth exploring, considering the controlled and sustained drug delivery properties that nanoparticles may provide in the synovial fluid.

Nanoparticles used in preclinical studies are almost entirely synthesised in small batches and scaling up production for the synthesis of large quantities is not always possible. There is a constant and increasing need to produce consistent and highly reproducible formulations prior to the clinical trial stage.[40] Depending on the methodology of production, scale-up may require additional modifications that most pharmaceutical companies are not prepared to implement. When compared to free drugs, it should also be recognised that nanoformulations will raise the overall cost of production, due to the above-mentioned additional steps. Still, the cost-benefit ratio is clearly well-balanced.

Lastly, from the regulatory perspective, nanomedicine products are mostly administered within a conventional regulatory framework by regulatory agencies. However, additional expert evaluations are necessary to confirm the quality, safety, and efficacy of nanotherapeutics because of their complexity, which may be established in specific guidelines and methodologies, on a case-by-case basis. For regulatory decision-making, it will be imperative to define critical product attributes predictive of product performance *in vivo*.

In summary, there is increasing interest in nanomedicines for the management of RA, with researchers looking for safer and more efficient treatments compared to current medicines. The implementation of nanomedicines, with the potential to control the release kinetics of drugs due to the tailored degradation properties of nanoparticle matrix, can be explored to adjust the therapeutic concentration of anti-RA drugs. Biocompatible and biodegradable materials can be used for the formulation of active payloads, also incorporating scientific knowledge from other drug development processes. In contrast, the optimisation of targeted systems is less straightforward, as mentioned before, regarding the additional steps in the manufacturing process. In the future, it is foreseen that the development of new ligand moieties attached to the surface of nanoparticles may drive those systems to the active sites. Such innovative ligands may enhance the current knowledge of cells involved in the RA process, and increase understanding of the pathophysiology of RA. Additional advantages may be expected by co-administration of different drugs in synergic treatment protocols, with formulation incompatibilities, as nanoparticles offer physical stability during storage. Also, the possibility of formulating more than one drug, with different physical-chemical properties in the same nanoparticulate system, may offer patients less painful treatment regimens and, in the long term, help to overcome the high cost of biologics.

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VITAMIN D DEFICIENCY AND CHRONIC WIDESPREAD PAIN

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ABSTRACT

Vitamin D acts as a steroid hormone possessing important functions in calcium and phosphorus balance and bone health. The presence of vitamin D receptors (VDRs) in many tissues in the human body shows that this vitamin might have effects other than its role in maintaining bone health. Hence, many studies in the last two decades have reported an association between vitamin D deficiency and many musculoskeletal and extra-skeletal diseases. Despite the presence of clear evidence suggesting a causative relationship between musculoskeletal pain and osteomalacia developing as a result of long-term and severe vitamin D deficiency, a putative relationship between vitamin D deficiency and chronic widespread pain (CWP) has recently been an exciting area of discussion. The hypothetical role of vitamin D in the pathophysiology of pain, the availability of VDRs in the muscle tissue and central nervous system, particularly in the hypothalamus, and the reports on the development of muscle hypersensitivity associated with vitamin D deficiency, have provided a basis for a putative relationship between CWP and vitamin D status. This review will discuss these two problems that commonly occur within the general population, and endeavour to reveal this relationship in light of currently available studies.

Keywords: Chronic widespread pain (CWP), fibromyalgia (FM), vitamin D, vitamin D deficiency.

INTRODUCTION

Vitamin D has been known to have a significant function in calcium (Ca) and phosphorus (P) balance and bone metabolism since the 1920s, when it was first discovered.[1] Although there is not a widely accepted cut-off level, a serum 25-hydroxy vitamin D (25-OH D) level <20–25 ng/mL is considered to indicate vitamin D deficiency.[1-4] It has been established that vitamin D deficiency is common, with a prevalence of 25–50% among the general population.[1,3,5,6] The most widely recognised consequences of vitamin D deficiency are rickets in children and osteomalacia, osteoporosis, and increased risk of fractures and falls in adults.[7-10] The availability of vitamin D receptors (VDRs) in many tissues of the human body shows that vitamin D might have other effects apart from its role in maintaining bone health.[2,10] Indeed, many recent studies have reported a relationship of vitamin D deficiency with musculoskeletal disturbances, neuromuscular function disorders, infections, autoimmune diseases, metabolic syndrome, diabetes mellitus, cardiovascular diseases, respiratory diseases, cognitive function disorders, psychiatric disorders, and an increased risk of many types of cancer.[2,3,6,9-11]

Chronic widespread pain (CWP) is a global musculoskeletal disorder causing physical and psychological symptoms. Both the pathophysiology and effective therapies for CWP have yet to be identified. In the American College of Rheumatology (ACR)-1990 definition,[12] CWP was defined as the pain lasting ≥3 months, both above and below the waist, on the right and left sides of body, and in the axial skeleton (cervical spine, thoracic spine, anterior chest, or lower back). CWP is very common (4.2–13.3%) in the general population.[3] As two health problems that are commonly occurring in the general population, the relationship between vitamin D deficiency and CWP is a significant area of research that needs to be elucidated.

Table 1: Major causes of vitamin D deficiency.

Decreased cutaneous endogenous synthesis with sunlight
Limited sun exposure
Latitude, season, and time of day (the solar zenith angle)
Increased skin pigmentation
Ageing (especially >65–70 years)
Sunscreen
Obesity
Smoking
Pollution severity
Insufficient dietary intake
Disturbed bioavailability and metabolism
Obesity (sequestration of vitamin D)
Malabsorption (e.g. intestinal bowel disease [Crohn's disease, steatorrhoea, ulcerative colitis], short bowel syndrome, pancreatic insufficiency, biliary obstruction, coeliac disease, cystic fibrosis, amyloidosis, bariatric surgery)
Hepatic diseases or failure (decreased 25-hydroxylase activity)
Chronic renal insufficiency (decreased 1-α-hydroxylase activity)
Ageing (decreased 1-α-hydroxylase activity)
Nephrotic syndrome (loss of DBP in the urine)
Primary hyperparathyroidism
Type 1 hereditary vitamin D-dependent rickets
Drugs
Anti-epileptic drugs (e.g. phenobarbital, carbamazepine, phenytoin, valproic acid)
Glucocorticoids
Anti-retroviral therapy
Rifampicin, isoniazid
Anti-fungal drugs (e.g. ketoconazole: inhibition of the 1-α-hydroxylase and 24-hydroxylase)
St. John's wort or its extracts
Target organ resistance
Type 2 hereditary vitamin D-dependent rickets (VDR gene mutation)

DBP: vitamin D-binding protein; VDR: vitamin D receptor.

VITAMIN D DEFICIENCY

Sources, Synthesis, and Metabolism of Vitamin D

Vitamin D, which is one of the most important physiological regulators of Ca, P, and bone metabolism, is considered a steroid hormone as it is synthesised in the body, exerts effects through receptors in remote organs, possesses a biofeedback mechanism, and its structure resembles a cholesterol molecule.[5] There are three main sources of vitamin D: cutaneous synthesis with sunlight, diet (natural or fortified), and supplements (prescription or over-the-counter).[13] Vitamin D (D represents D_2 or D_3) is structurally related to two precursors: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). 7-dehydrocholesterol (provitamin$_3$), which is naturally found in the skin, is converted to previtamin D_3 through exposing the skin to solar ultraviolet B (UVB) radiation, and previtamin D_3 is rapidly converted by heat to vitamin D_3.[5,13] This natural endogenous production is the most important and main source of vitamin D for the body.[13-15]

Exposure of arms and legs or face, arms, and hands to direct sunlight for 5–15 minutes at least three-times a week (suberythemal dose) is recommended for the synthesis of a sufficient amount of vitamin D in the skin.[15] Vitamin D synthesis in the skin varies depending on the angle of the sun (maximum in the zenith angle), hour of the day (maximum at noon), and latitude and seasons. With ageing (especially >65–70 years), vitamin D synthesis decreases by 75% due to a decreased amount of 7-dehydrocholesterol, endogenous precursor in the skin.[2] Dark skin directly decreases vitamin D synthesis as melanin absorbs UVB light. Also, sunscreen, obesity, smoking, and air pollution unfavourably affect skin synthesis to a great extent.[2,16] The fact that many factors play a significant role in the skin synthesis of vitamin D, in contrast to the traditional approach, reveals that restricting the effect of clothing or skin tone alone cannot be placed at the forefront (Table 1).[1,2,6,15,16]

The amount of vitamin D supplied with natural food is extremely low.[2,6] Vitamin D_2, which is produced from ergosterol found in cell membranes of fungi through exposure to UV radiation, is a more limited source than vitamin D_3. Vitamin D_2 can only be supplied through certain foods (e.g. shiitake mushrooms, egg yolk) or supplements.[2,6,15] Vitamin D_3 can only be supplied through food of animal origin (e.g. cod liver oil, oily fish, egg yolk), fortified foods, and supplements.[6] Once synthesised in the skin (vitamin D_3) or taken with the diet (vitamin D_3 or D_2), vitamin D enters circulation in its biologically inactive form, which can be stored in the fat tissue or released upon demand.[17]

Table 2: Conditions and diseases causing chronic widespread pain.

Musculoskeletal diseases	**Inflammatory rheumatic disorders**
CWP (primary/idiopathic/non-specific)	Systemic lupus erythematosus
Fibromyalgia	Connective tissue diseases
Myofascial pain syndrome	Rheumatoid arthritis, other forms of polyarthritis
Osteoarthritis/degenerative joint disease/spondylosis	Sjögren's syndrome
Discogenic and radicular pain	Systemic sclerosis
Chronic overuse syndromes (e.g. tendonitis, bursitis)	Polymyalgia rheumatica
Spinal cord lesion	Idiopathic inflammatory myopathies
Hypermobility syndrome	Seronegative spondyloarthropathy
Muscular pain-fasciculation syndrome	Necrotising vasculitis
Trauma, pain, and cramps after exercise	**Neurological diseases**
Faulty or poor posture	Peripheral neuropathy (e.g. diabetic polyneuropathy, Guillain-Barré, porphyria)
Metabolic diseases	Parkinson's disease
Hypothyroidism, hyperthyroidism	Multiple sclerosis
Hyperparathyroidism	Huntington's disease
Osteomalacia, vitamin D deficiency	Central post-stroke pain
Liver and kidney diseases	Alzheimer's disease
B12 deficiency	Syringomyelia
Anaemia	Traumatic brain injury
Electrolyte disturbances	Amyotrophic lateral sclerosis
Amyloidosis	**Other causes**
Glycogen and lipid storage diseases	Chronic fatigue syndrome
Steroid withdrawal syndrome	Psychiatric conditions (e.g. depression, anxiety)
Infectious diseases	Irritable bowel syndrome
Infectious myopathies	Sleep disturbances
HCV, HBV, HIV	Drug-related myalgia (e.g. statins)
Viral syndrome	Neoplastic diseases
Toxic shock syndrome	Paraneoplastic syndrome
Lyme disease	Sarcoidosis
Trichinosis	Peripheral vascular disease
Toxoplasmosis	Other
Poliomyelitis	

CWP: chronic widespread pain; HBV: hepatitis B virus; HCV: hepatitis C virus.

Vitamin D is transported by vitamin D-binding protein in the circulation and converted to 25-OH D by 25-hydroxylase in the liver and then to the active form 1,25-$(OH)_2$D (calcitriol) by 1-α-hydroxylase enzyme in the kidneys. 25-OH D, although biologically inactive, is the major form of vitamin D in the circulation. Vitamin D synthesis is a self-limiting process and is regulated through a negative feedback mechanism involving serum Ca, P, parathyroid hormone (PTH), and fibroblast growth factor 23.[5,13] Biliary excretion of inactive forms (1,24,25-OH D and 24,25-OH D) generated by 24-hydroxylation of 1,25-$(OH)_2$D and 25-OH D represent the first step of inactivation in vitamin D metabolism.[13]

Biological Effects of Vitamin D

Through its receptor-dependent effects, 1,25-$(OH)_2$D promotes sufficient bone (osteoid) mineralisation by increasing intestinal Ca and P absorption, decreasing Ca excretion from the kidneys, and inducing alkaline phosphatase and osteocalcin

synthesis. PTH increases the level of 1,25-$(OH)_2$D by increasing the activity of 1-α-hydroxylase enzyme.[5] Both 1,25-$(OH)_2$D and 25-OH D suppress PTH synthesis.[6] In vitamin D deficiency, decreased levels of ionised Ca in the serum increase the PTH levels. PTH induces 1,25-$(OH)_2$D synthesis, decreases renal Ca reabsorption, and decreases P reabsorption. As a result, vitamin D deficiency results in secondary hyperparathyroidism with normal or increased 1,25$(OH)_2$D levels. Increased PTH and 1,25-$(OH)_2$D levels cause bone resorption.[5,6] 1,25-$(OH)_2$D exerts its effects not only on the kidney tissue, but through VDRs that are widely distributed throughout the body.[6,10,13] With a multitude of targets in many tissues and ~200 genes (transcriptional regulation), 1,25-$(OH)_2$D is known to regulate cell proliferation and differentiation, induce apoptosis, and inhibit angiogenesis and malignant transformation through VDRs.[6,10]

Vitamin D Deficiency and Insufficiency

Although 1,25-$(OH)_2$D is the functional form of vitamin D, the serum 25-OH D level is the most frequently used parameter in clinical practice to reflect vitamin D status in the body. 25-OH D is the main form of vitamin D in the circulation and has a longer half-life (~3 weeks versus ~2 days) and only a certain portion of 25-OH D in the circulation is converted to 1,25-$(OH)_2$D.[1,6] However, optimal vitamin D levels that have gained wide acceptance have not been established. Although many studies have reported a cut-off level of 20–25 ng/mL, in the current approach, a 25-OH D level of ≥30–32 ng/mL is considered to be the lower limit for sufficient calcium absorption, PTH suppression, and optimal health status (skeletal and extra-skeletal).[1-6,13]

In the classification of vitamin D status, most authors have preferred to classify a 25-OH D level of <20 ng/mL as 'vitamin D deficiency', a level of 20–29 ng/mL as 'vitamin D insufficiency', and a level of >30 ng/mL as 'sufficient' vitamin D status.[2,6,13,17] Vitamin D insufficiency can be regarded as the mild form of vitamin D deficiency, which causes bone loss, secondary to hypocalcaemia and secondary hyperparathyroidism. However, the definition of vitamin D insufficiency in current practice is used to consider the possible benefits of vitamin D, particularly on optimal extra-skeletal health, regardless of PTH level or suppression.

Vitamin D deficiency is a considerably is a common condition and the studies have yielded variable results as they were performed in populations with different characteristics, different age groups, different geographic areas, and using different cut-off levels. The studies have reported a prevalence rate ranging from 23–93% for vitamin D deficiency.[1,6,16] Considering these prevalence studies and risk factors, it is estimated that 25–50% of the world's general population and >1 billion people have vitamin D deficiency or insufficiency.[1,5,6]

CHRONIC WIDESPREAD PAIN

The widely known and accepted reference description of CWP was suggested for the first time by the ACR-1990 in diagnostic criteria of fibromyalgia (FM).[12] In this diagnostic criteria, CWP was defined as pain lasting ≥3 months, both above and below the waist, on the right and left sides of body, and in the axial skeleton (cervical spine, thoracic spine, anterior chest, or lower back). In the latest FM preliminary diagnostic criteria published by the ACR in 2010, the definition of CWP in the ACR-1990 criteria was revised and the generalised nature of pain was included in the FM diagnostic criteria based on the examination of 19 anatomical areas.[18] CWP differs from localised pain not only through its distribution, but also with higher pain intensity, longer pain duration, greater disability, more psychosocial problems, and lower quality of life.[3] While the prevalence of CWP in the general population varies between the studies depending on methodological differences, it is reported to range from 4.2–13.3%.[3] In a recent systematic review and meta-analysis (that included 25 articles) the prevalence of CWP was reported to range from 0–24%, and a level of 10% was reported to be the most accurate estimate.[19]

CWP is a clinical condition that could be caused by or associated with many diseases (Table 2),[20,21] but it primarily originates from the musculoskeletal system, in most cases without an underlying organic pathology. CWP including FM is an important health problem without a known aetiology and definitive treatment. Whether CWP is related to vitamin D deficiency is an active area of research.

RELATIONSHIP BETWEEN VITAMIN D DEFICIENCY AND CHRONIC WIDESPREAD PAIN

The presence of VDRs and 1-α-hydroxylase activity in the central nervous sytem, particularly in the hypothalamus, and functioning of vitamin D

as a neuroactive steroid, points to a relationship between vitamin D and central hypersensitivity in chronic painful states.[9,22,23] Vitamin D has been shown to play a role in the pathophysiology of FM through autoimmune and neural regulation, modulation of neurotransmitters, and upregulation of inflammatory pathways in chronic pain (i.e. transforming growth factor-β-1 [TGF-β-1], interleukin [IL]-4, and inhibition of prostaglandin activity).[22,23] TGF-β-1 upregulated by vitamin D suppresses IL-1 and IL-2 and cytokines that are involved in chronic pain, such as tumour necrosis factor-α and interferon-γ.[22,23] Vitamin D has been found to reduce the levels of nitric oxide that play a role in central sensitisation through inhibition of nitric oxide synthase.[23] Similarly, demonstration of a relationship beteen vitamin D status and myopathy, fatty infiltration, and muscle hypersensitivity, has provided the basis for hypothetical pathophysiological mechanisms of vitamin D-CWP interplay.[22,24,25]

When considering the role that inflammatory cytokines, activated by the infections, play in the aetiopathogenesis of FM by modulating central and peripheral pain perception, the role of vitamin D in the regulation of the immune system is a reasonable theory that could explain musculoskeletal pain associated with vitamin D deficiency.[3,26] Briefly, vitamin D has a potential role in the aetiology and persistence of chronic pain through anatomical, hormonal, neurological, and immunological mechanisms.[23] On the other hand, patients with chronic musculoskeletal pain can develop vitamin D deficiency secondary to spending less time in open environments due to depression, limited mobility, or increased fat tissue.[3,26]

In an uncontrolled cross-sectional study involving patient groups of different ethnic origins, Plotnikoff and Quigley[16] reported vitamin D deficiency in 93% of 150 patients with persistent non-specific musculoskeletal pain, a rate which is approximately two-fold higher than the cited studies conducted in a similar latitude, and they suggested the evaluation of patients with persistent non-specific pain for the presence of vitamin D deficiency. Block[27] inquired into this study and reported a prevalence rate of 38% for vitamin D deficiency in patients with non-specific CWP, including FM, a rate similar to that in the general population. Of 9 patients with 25-OH D level <10 ng/mL, only 1 out of 6 patients who completed an 8-week course of vitamin D 50,000 IU/week therapy benefited from the replacement therapy.

As a major global health concern, treatment of chronic pain with a simple and pleasant means of therapy as vitamin D replacement has excited clinicians and this therapy has become the focus of research during the last two decades. Some of the studies that evaluated the relationship between vitamin D deficiency and various types of chronic painful conditions have reported a relationship,[28-34] whereas other studies did not report any relationship.[4,35-37] The studies addressing this interplay have used different methodologies and terminology, making the results confusing.

Although not a systematic review, a PubMed search was conducted to establish the relationship between vitamin D deficiency and CWP, with the keywords "vitamin D" or "vitamin D deficiency" and "chronic widespread pain" or "fibromyalgia" for the period between 1997 and March 2017. Twenty-four original articles (cross-sectional, observational studies) were returned from this literature search in terms of their methodology evaluating the relationship between CWP or FM and vitamin D status. Of these, only two studies were randomised controlled trials (RCTs)[38,39] and eight studies evaluated vitamin D supplementation. Of these eight studies, two RCTs reported conflicting results, and six uncontrolled studies reported a significant improvement with vitamin D supplementation in patients with CWP.[3,40-44] Out of the 16 remaining studies, 11[24,45-54] reported a relationship between vitamin D deficiency and CWP, and 5 studies[55-59] reported no relationship.

A comprehensive review of eight RCTs concluded there were not sufficient data to suggest the use of vitamin D outside chronic pain states, and that further large-scale RCTs were required in this regard.[60] Muscogiuri et al.[11] reported that currently available evidence does not support the role of vitamin D in other chronic diseases except for bone health; however, they highlighted the need for vitamin D supplementation in vitamin D deficiency to reduce the risk of developing chronic disease. In recent narrative[17,22,23,61] and systematic reviews,[26,62,63] sufficient evidence was not found for the use of vitamin D supplementation in chronic pain conditions or CWP/FM and no causative relationship was suggested; further studies were suggested to prove possible benefits in some specific painful conditions. Even further to that, the essential role of vitamin D in bone health (bone mineral density, fractures) and preventing falls seems to have become controversial.[13,64-67]

In the RCT conducted by Warner and Arnspiger,[38] 184 patients with musculoskeletal pain (primary FM) and 104 patients with osteoarthritis (controls) were compared with respect to mean 25-OH D level (diffuse pain: 29.2 ng/mL, controls: 28.8 ng/mL; p=0.78) and the prevalence of vitamin D deficiency (<20 ng/mL) (diffuse pain: 29%, controls: 20%; p=0.09), and no significant difference was reported between the groups. When 50 patients with FM and vitamin D deficiency (9–20 ng/mL) were randomised to treatment and control groups, no significant improvement in pain was found in patients who received ergocalciferol 50,000 IU/week for 3 months compared to the control group. Additionally, no significant decrease was noted compared to pretreatment state. However, Wepner et al.[39] (second of the two aforementioned RCTs) randomised 30 patients with FM and reported a significant reduction in the pain and positive effect in the perception of pain (on the physical role functioning scale of the Short Form Health Survey 36) in the treatment group compared to the control group.

Hsiao et al.[68] performed a meta-analysis of 12 observational studies conducted to establish the relationship between hypovitaminosis D and CWP including FM. They observed a positive crude association between hypovitaminosis D and CWP and suggested that a level of 8–10 ng/mL could serve as a cut-off level determining this relationship. In contrast, in the systematic review of one RCT and eight cross-sectional studies conducted by Daniel and Pirotta,[26] the association between vitamin D deficiency and FM was found to be inconclusive and, according to the best evidence, vitamin D supplementation had no effect; however, it was highlighted that the treatment of vitamin D deficiency in patients with FM would be beneficial with respect to long-term bone health and muscle strength, rather than pain.

CONCLUSION

Vitamin D deficiency is expected to occur commonly worldwide considering the presence of multiple factors affecting skin synthesis of vitamin D, the infeasibility of consuming a sufficient amount of vitamin D in the diet, and the presence of other risk factors that could affect vitamin D status. However, there is no established standardised minimum 25-OH D level required for optimal musculoskeletal and extra-skeletal health that is widely accepted in the scientific milieu. Uncertainty regarding the cut-off level for vitamin D deficiency/insufficiency leads to the use of different cut-off levels, causing limitations in the synthesis and analysis of different studies. According to current evidence, along with the putative role of vitamin D in extraskeletal health, its better-established role in musculoskeletal health (osteoporosis, fracture, and risk of falls) is also weak and debatable. Under these circumstances, the value/discriminative power of currently used cut-off levels (20–25 ng/mL) yielding >25% prevalence rate for vitamin D deficiency, seems to be questionable in differentiating physiological status from a pathological finding. Therefore, normal serum 25-OH D levels must be clarified with powerful studies.

Considering the hypothetical role of vitamin D in the pathophysiology of pain and the wide distribution of VDRs throughout the body (including muscle tissue and CNS), an association is expected between CWP/FM and vitamin D deficiency. However, studies supporting this putative relationship are scarce and of low quality. Musculoskeletal pain that could be associated with vitamin D deficiency is expected to be a widespread pain rather than regional pain and should respond to replacement therapy. Large-scaled double blind RCTs by clinicians specialised in musculoskeletal pain are required with detailed/clear classification of pain and using specific cut-off levels on a specific and homogeneous group of patients. In light of the current data, vitamin D has no place in routine treatment of patients with CWP. In view of the papers reporting musculoskeletal and extra-skeletal benefits for vitamin D and that it is a cheap, simple, 'pleasant' and non-toxic treatment, vitamin D supplementation can still be considered to have a role for patients with risk factors or deficiency.

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Pain in Psoriatic Arthritis Patients

PAIN is prevalent amongst patients with psoriatic arthritis who are being treated with biologic therapies, according to the results of a recently presented study. The study's lead author, Prof Philip Conaghan, University of Leeds, Leeds, UK, declaimed: "These findings highlight the need for psoriatic arthritis treatments that provide sustained improvement in pain to reduce the impact of the disease on patients' daily life, as well as the resultant costs to society."

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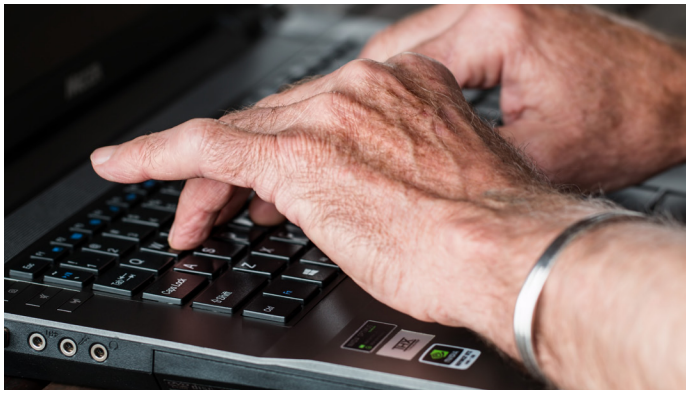
This study focussed on psoriatic arthritis patients who had been receiving traditional biologic treatments for ≥3 months. Patients filled out several questionnaires, which provided a range of information, including health-related quality of life (QoL), work status, impairment in physical function, and impairment in work activity and productivity. Questionnaires included the SF36 and EQ-5D. These questionnaires were completed by 782 patients from 13 countries, who were recruited by rheumatologists and dermatologists.

The questionnaire results showed that a combined total of 66.8% of psoriatic arthritis patients were experiencing pain: 36.8% severe pain and 30% moderate pain. Increased usage of opioids (p=0.0065), prescription non-steroidal anti-inflammatory drugs (p=0.0026), and non-prescription pain medication (p<0.0001) were associated with severe pain.

Pain was found to have a significant impact on health-related QoL. As pain severity increased, QoL was more severely impaired across several domains on the SF36 questionnaire: Social Functioning, General Health, Vitality, Physical, Emotional, and Mental Health, and Physical Functioning. The differences in these scores on the questionnaire were clinically and statistically significant (p<0.0001) at different levels of pain severity. Furthermore, a higher level of pain was found to lead to higher scores for mobility, usual activities, anxiety/depression, and self-care on the EQ-5D questionnaire (p<0.0001). Pain also had an impact on other factors, with more severe pain being associated with greater activity impairment, work time missed, overall work impairment, and greater disability (p<0.0001).

As Prof Conaghan noted, these results indicate that pain needs to be taken into consideration when treating psoriatic arthritis, as it has a variety of negative impacts. Prof Conaghan also added that: "We should also assess whether a given biologic therapy is adequately controlling inflammation and consider non-inflammatory causes of joint pain."



Influence of Comorbidities in Psoriatic Arthritis

FAILURE to adhere, or to respond, to treatment in patients with psoriatic arthritis has been shown to be linked to comorbidities in a population-based study. There are numerous comorbidities associated with psoriatic arthritis, including lymphoma, obesity, diabetes mellitus, hypertension, and other autoimmune diseases; therefore, examining their impact is of importance to those trying to manage the condition.

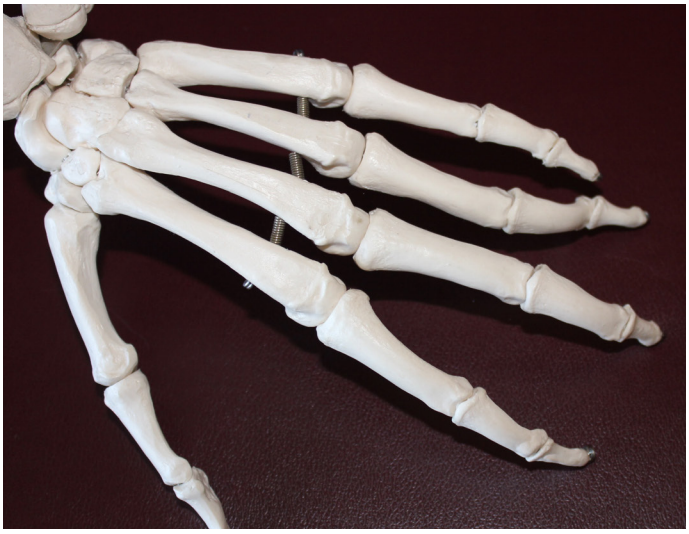
The study population was 1,750 psoriatic arthritis patients from Denmark, who were receiving treatment with their first tumour necrosis factor (TNF) inhibitor. The headline findings were that the level of disease activity was linked to the presence of comorbidities and an increased number of comorbidities resulted in an increasingly negative impact on adherence and response to treatment. Analysing this further, patients with a higher Charlson comorbidity index (CCI) score, when compared to patients with no comorbidities, had statistically higher measures of disease activity when measured at baseline.

Furthermore, the mean time of treatment adherence was 1.3 years, 2.2 years, and 2.6 years for patients with a CCI score of ≥2, 1, and 0, respectively. Additionally, those patients in the ≥2 group had a much greater risk of discontinuing anti-TNF treatment (p=0.001). Finally, it was found that patients in the ≥2 group were less likely to obtain a good clinical response when compared to patients without comorbidities. Indeed, 23% of patients in the ≥2 group achieved a good clinical response as defined by the European League Against Rheumatism (EULAR) criteria, compared with 41% of patients without comorbidities, and the corresponding figures for a good-or-moderate clinical response were 47% and 54%, respectively.

> “To improve the treatment of patients with psoriatic arthritis, it is therefore essential not only to recognise and monitor any co-existing comorbidity, but also to understand their impact on patient management.”

When considering psoriatic arthritis patients with depression and/or anxiety, it was discerned that psoriatic arthritis patients without depression and/or anxiety were more likely to adhere to treatment. The mean treatment adherence times were 2.4 years and 1.7 years, respectively.

Summing up the implications of these findings, lead study author, Dr Lars Erik Kristensen, The Parker Institute, Copenhagen University Hospital, Copenhagen, Denmark, concluded: “To improve the treatment of patients with psoriatic arthritis, it is therefore essential not only to recognise and monitor any co-existing comorbidity, but also to understand their impact on patient management.”



Differences Discerned Between Male and Female Osteoarthritis Patients

SEX-SPECIFIC differences in patients with osteoarthritis have been identified that may account for the difference in osteoarthritis incidence rates between males and females. Although osteoarthritis is more prevalent amongst elderly women than elderly men, the reason for this has hitherto remained unknown. However, findings from a new study focussing on synovial fluid, by researchers from the Medical College of Georgia, Augusta University, Augusta, Georgia, USA, have potentially provided some of the answers to this question, as well providing general insight into the disease. The authors explained that: "...this is the first study to demonstrate gender-specific microRNA (miRNA) profiling in extracellular vesicles of synovial fluid in human osteoarthritis."

Researchers obtained synovial fluid from the knees of male and female osteoarthritis patients, as well as healthy controls. An analysis was conducted on these synovial fluid samples, specifically examining exosomes within the synovial fluid, which are vesicles containing miRNAs. Distinct differences were found in miRNA activity between the two sexes. Indeed, the synovial fluid of women with osteoarthritis contained 91 downregulated and 53 upregulated miRNAs, which were associated with 70 alterations in biological processes. By contrast, the 69 downregulated and 45 upregulated miRNAs in the synovial fluid of men with osteoarthritis were linked to around 50 altered biological processes.

Furthermore, it was discerned that miRNAs that are key in producing collagen and oestrogen signalling were more commonly deactivated or altered in women than men. Previous studies have associated hormone replacement therapy (which increases oestrogen levels) with a reduced risk of osteoarthritis. Additionally, lower oestrogen levels have also been associated with an increased production of bone-destroying cells. With this context in mind, the researchers theorised that their results suggested the influence of oestrogen on the levels of miRNA in the exosomes of synovial fluid. In order to test this theory, they blocked oestrogen availability by utilising aromatase inhibitors, which consequently led to a reduction in miRNA levels. The authors noted: "Synovial fluid-derived exosomes play an important role in the pathophysiology of osteoarthritis. Furthermore, these differentially expressed female miRNAs might be oestrogen-responsive and play a role in toll-like receptor signalling during pathogenesis of osteoarthritis."

However, as well as sex-specific differences, a miRNA known as MiR-504-3p was found to be upregulated in both sexes of osteoarthritis patients. Although the exact function of MiR-504-3p is yet to be elucidated, the researchers suspected it might play a role in the degeneration of cartilage. They intend to conduct further investigations to better understand the impact of MiR-504-3p by inhibiting it. With time, it is hoped that this research will point to future therapeutic options for osteoarthritis.

Measures to Reduce Acute Coronary Syndrome in Early Rheumatoid Arthritis

INCREASED morbidity in rheumatoid arthritis (RA) patients is well known to be predominantly driven by acute coronary syndrome, alongside other coronary diseases. Today, due to new therapeutic strategies and treatment options, the average level of disease activity has decreased. A recent nationwide population-based cohort study by Dr Marie Holmqvist, Department of Medicine, Solna, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm, Sweden, and colleagues set out to consider the efficacy of these treatment strategies in reducing the risk of acute coronary syndrome.

Using the Swedish Rheumatology Quality Register, 15,744 patients with RA that had been diagnosed within 12 months of the onset of symptoms were identified. Alongside these new-onset RA patients, 70,899 controls were included. It was found that 772 RA patients developed an acute coronary syndrome; this represented a 40% higher risk of acute coronary syndrome compared to the control population (hazard ratio: 1.41; 95% confidence interval: 1.29–1.54). It was also noted that the risk of developing acute coronary syndrome was higher in RA patients with seropositive RA and those with a Disease Activity Score 28 (DAS28) >3.2 when first diagnosed.

Although there was a decline in the incidence of acute coronary syndrome during the study period (1997–2014) across patients with RA and the control population, both the excess and relative risks of acute coronary syndrome remained identical. It was also noted that the incidence of acute coronary syndrome was increased during the first year after diagnosis. This led the authors to raise the possibility that increased morbidity during this period was partly a result of the use of COX inhibitors or oral glucocorticoids. Finally, the authors concluded: "Despite improved disease control in new-onset RA, the elevated risk of acute coronary syndrome in RA remains a concern."

> "Despite improved disease control in new-onset RA, the elevated risk of acute coronary syndrome in RA remains a concern."

Survival in Ankylosing Spondylitis Improved by Statins

STATINS have been shown to substantially reduce all-cause mortality in patients with ankylosing spondylitis (AS), according to the results of a recently published cohort study conducted in a general population. Currently, it is known that AS patients have a significantly increased risk of premature mortality as a result of a cardiovascular event compared to the general population. However, previous studies have demonstrated that cardiovascular mortality risk can be reduced by

≤20% in rheumatoid arthritis patients following statin treatment. In this context, a study was conducted to investigate the impact of statin treatment on mortality in patients with AS.

The researchers studied 1,430 patients with AS who had initiated statin treatment and compared them to a group of patients who were not being treated with statins. It was found that 96 patients in the statin treatment group (n=1,108) died, compared with 134 patients in the group who were not prescribed statins (n=1,108). This represented a mortality rate in the statin group of 16.5 per 1,000 person years (PY), which compared favourably with the corresponding rate of 23.8% for those who had not begun statin treatment. This was after accounting for initial characteristics such as the prevalence of cardiovascular disease and cardiovascular risk factors (which were higher in the statin treatment group). The absolute difference in mortality rate was 7.3 deaths per 1,000 PY (95% confidence interval: 2.1–12.5).

Overall, it was found that the initiation of statin treatment was associated with a reduction of 37% in all-cause mortality. This association was made after accounting for age, sex, BMI, socioeconomic status, cardiovascular medication use, total cholesterol levels, healthcare utilisation, and relevant comorbidities. This figure was significantly higher than the corresponding figure from the aforementioned rheumatoid arthritis studies. Future studies need to be conducted to more specifically determine the utility and role of statins in the management of AS, which could ultimately see the inclusion of statin treatment in guidelines for the management of cardiovascular risk in AS patients, potentially changing current practice.

Overall, it was found that the initiation of statin treatment was associated with a reduction of 37% in all-cause mortality.

Impact of Obesity on Axial Spondyloarthritis Treatment

OBESITY has been found to affect responses to tumour necrosis factor (TNF) inhibitors in patients with axial spondyloarthritis (axSpA), according to research performed by investigators from the Swiss Clinical Quality Management (SCQM) programme. Previous research has demonstrated that obesity has an impact on psoriatic arthritis patients, rendering them less likely to reach minimal disease activity on treatment with either conventional or biologic disease-modifying anti-rheumatic drugs or in the absence on systemic immunosuppressive treatment.[1,2] However, in regard to axSpA, infliximab is the only TNF inhibitor that

the impact of BMI has been formally demonstrated for; this was in two retrospective studies.[3,4] Therefore, this study used BMI as a proxy measure for obesity and focussed on its influence on TNF inhibitor responses in a cohort of 624 patients who fulfilled the 2009 Assessment of SpondyloArthritis International Society (ASAS) criteria for axSpA.

These 624 patients, who were starting their first TNF inhibitor, were subdivided into three groups based on their BMI: 332 patients with a normal BMI (18.5 to <25), 204 overweight patients (25–30), and 88 patients who were obese (>30). Patients were followed up for 1 year, with the primary outcome being a 40% improvement in ASAS criteria (ASAS40). A variety of factors were controlled for before multiple adjusted logistic regression analyses were conducted. These factors included age, sex, smoking status, co-medications, axSpA type, physical exercise, and enthesitis. It was found that 44% of normal weight patients (as assessed by BMI) achieved the primary outcome of ASAS40. This was in comparison to 34% of overweight patients and 29% of obese ones (p=0.02). Furthermore, the odds ratio (OR) of reaching ASAS40 was significantly reduced in obese patients when compared to patients with a normal BMI (OR: 0.27; 95% confidence interval: 0.24–1.14).

These results strongly suggest that the management of obesity should be taken into consideration when determining the treatment regime for patients with axSpA.

The researchers noted it would be of utility to conduct studies with smaller axSpA cohorts, as this would make it easier to directly quantify the fat mass of the patients in the studies. This approach would hopefully help to elucidate the reasons why obesity impairs responses to TNF inhibitor in axSpA patients.

These results strongly suggest that the management of obesity should be taken into consideration when determining the treatment regime for patients with axSpA. On a positive note, the authors pointed out that weight loss in psoriatic arthritis patients has been shown to have a positive impact on TNF inhibitor response, which suggests a similarly positive result is likely with axSpA.[5]

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What's New Feature

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UPCOMING EVENTS

45th Congress of the German Society for Rheumatology (DGRh)

6th–9th September 2017

Stuttgart, Germany

Modern rheumatology is becoming increasingly multidisciplinary and this concept of collaboration will take centre stage at this year's DGRh congress. The meeting seeks to a provide a platform for adjacent specialties to discuss rheumatology, exemplifying the rheumatologist's role as a 'conductor' in interdisciplinary networks. New research into diagnostic and therapeutic procedures will also be on display to stimulate discussion.

24th European Paediatric Rheumatology Congress 2017 (PReS 2017)

14th–17th September 2017

Athens, Greece

Set in the heart of the classical, cultural centre of Athens, Greece, this congress offers a unique opportunity to network with leaders in the field of paediatric rheumatology. Featuring presentations on the latest research, including a host of ground-breaking abstracts, this event is sure to inspire a lively debate about the clinical challenges facing the discipline across the globe. Immunometabolomics, autoinflammatory disease, and clinical rehabilitation are just some of the topics covered this year.

British Society for Paediatric and Adolescent Rheumatology (BSPAR) Annual Conference

4th–6th October 2017

Sheffield, UK

This high-quality event, spread over 3 days, presents an excellent platform for medical professionals to network. The event opens with a research day, emphasising the role of young investigators and giving them the opportunity to present their research in a friendly environment. Following that, comes the main body of the conference, where a plethora of medical research will be presented. Finally, the event ends with a gala dinner and drinks, so book your tickets now to be sure not to miss out!

4th International Symposium on Intra-Articular Treatment (ISIAT)

5th–7th October 2017

Prague, Czech Republic

Held every 2 years, the ISIAT symposium gathers a wide range of medical professionals together to discuss intra-articular treatment, including targets, tools, and future developments. This year, the event will feature speeches and lectures by pioneers in the field, as well as hosting a selection of practical courses on ultrasound-guided joint injections. A variety of thought-provoking topics will be included; anybody with an interest in this fascinating sub-speciality is encouraged to attend.

19th Asia Pacific League of Associations for Rheumatology Congress (APLAR 2017)

16th–20th October 2017

Dubai, United Arab Emirates

In collaboration with the Emirates Society of Rheumatology (ESR), this meeting will feature a huge range of rheumatological insights. With a broad scientific programme spanning plenary lectures on the latest research, interactive symposia, and other exhibitions, this event is sure to fully support and influence the future of rheumatology. Specialists from all disciplines are encouraged to attend. Reinforcing this year's theme 'Women Rheumatologists First', female attendees get a tempting 30% registration discount!

American College of Rheumatology/Association of Rheumatology Health Professionals (ACR/ARHP) Annual Meeting

3rd–8th November 2017

San Diego, California, USA

With >450 educational sessions available, the ACR/ARHP Annual Meeting offers boundless opportunities for networking and discussion, providing first-hand access to the cutting-edge of rheumatological research. Workshops and poster sessions feature heavily in this event's diverse programme, so there will be something of interest to everyone over this impressive 6-day schedule. As the world's premier rheumatology meeting, attendance is highly recommended.

54th Congress of the Italian Society of Rheumatology (SIR)

22nd–25th November 2017

Rimini, Italy

Introducing thematic sessions on the discipline's hottest topics for the first time, the SIR congress 2017 promises to be home to enlightening and innovative discourse for 4 days this November. The event strikes a balance between practical and scientific, containing both hands-on demonstrations as well as plenary lectures and abstract presentations. With an emphasis on a multidisciplinary approach, this event is a must for any rheumatological specialist.

European League Against Rheumatism (EULAR) Annual European Congress of Rheumatology 2018

13th–16th June 2018

Amsterdam, Netherlands

Following an incredibly successful 2017 congress in Madrid, Spain, Europe's largest rheumatology gathering moves north in 2018, to Amsterdam, Netherlands. With an enormous scientific programme and tens of thousands of attendees expected, the EULAR congress 2018 is the premier European event for rheumatology research debate. As always, we will be attending, and we hope to see you there!

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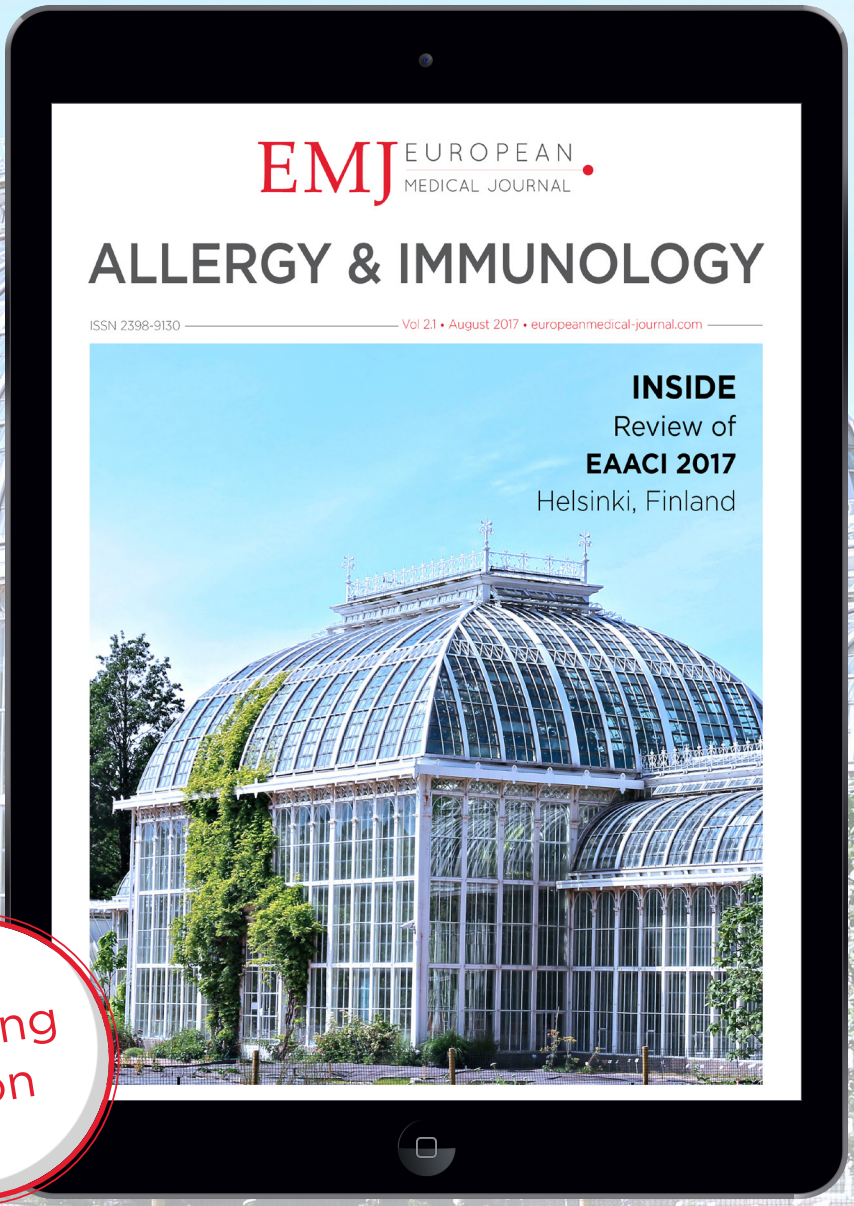

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