EMJ EUROPEAN MEDICAL JOURNAL

# RHEUMATOLOGY

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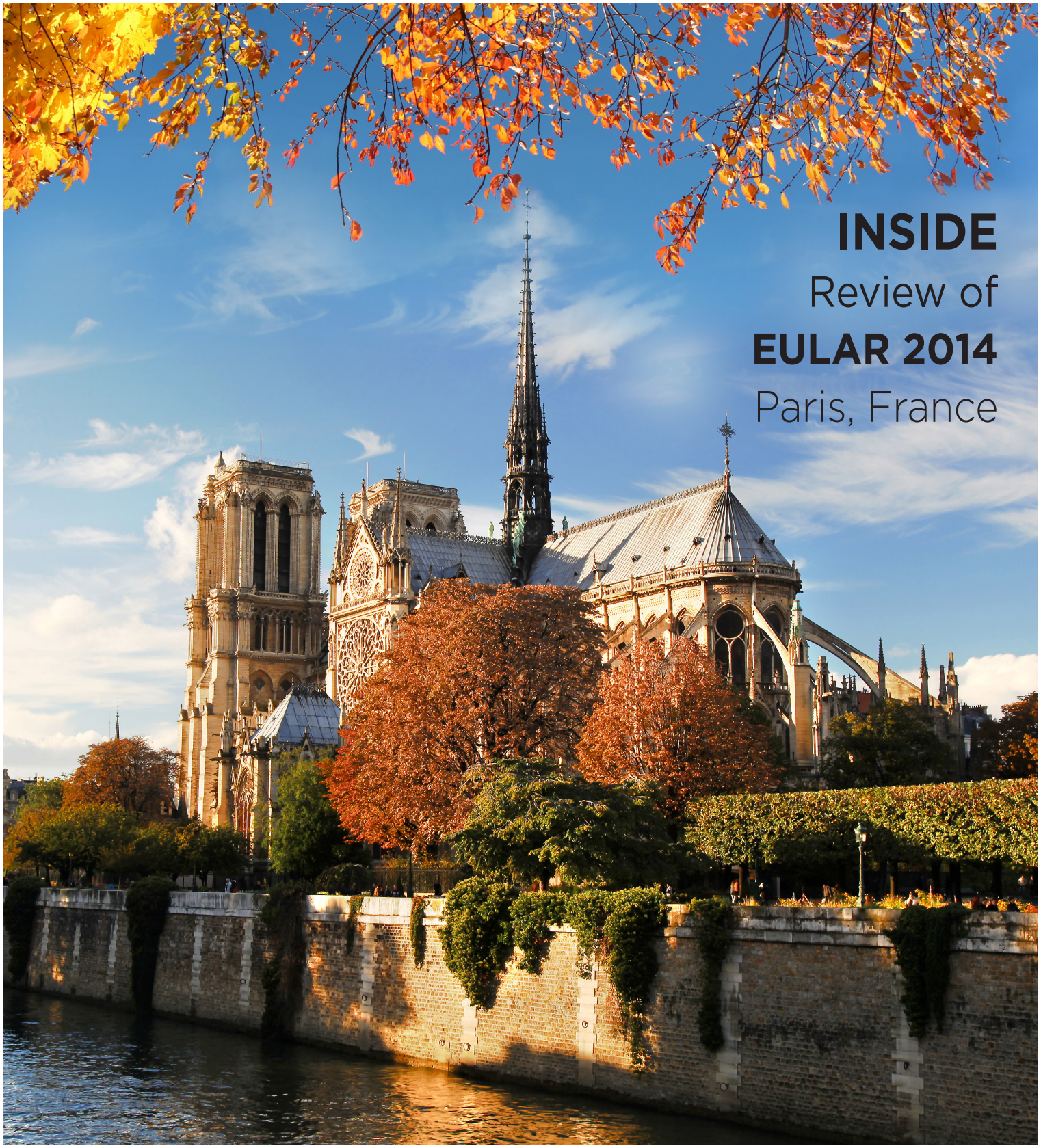

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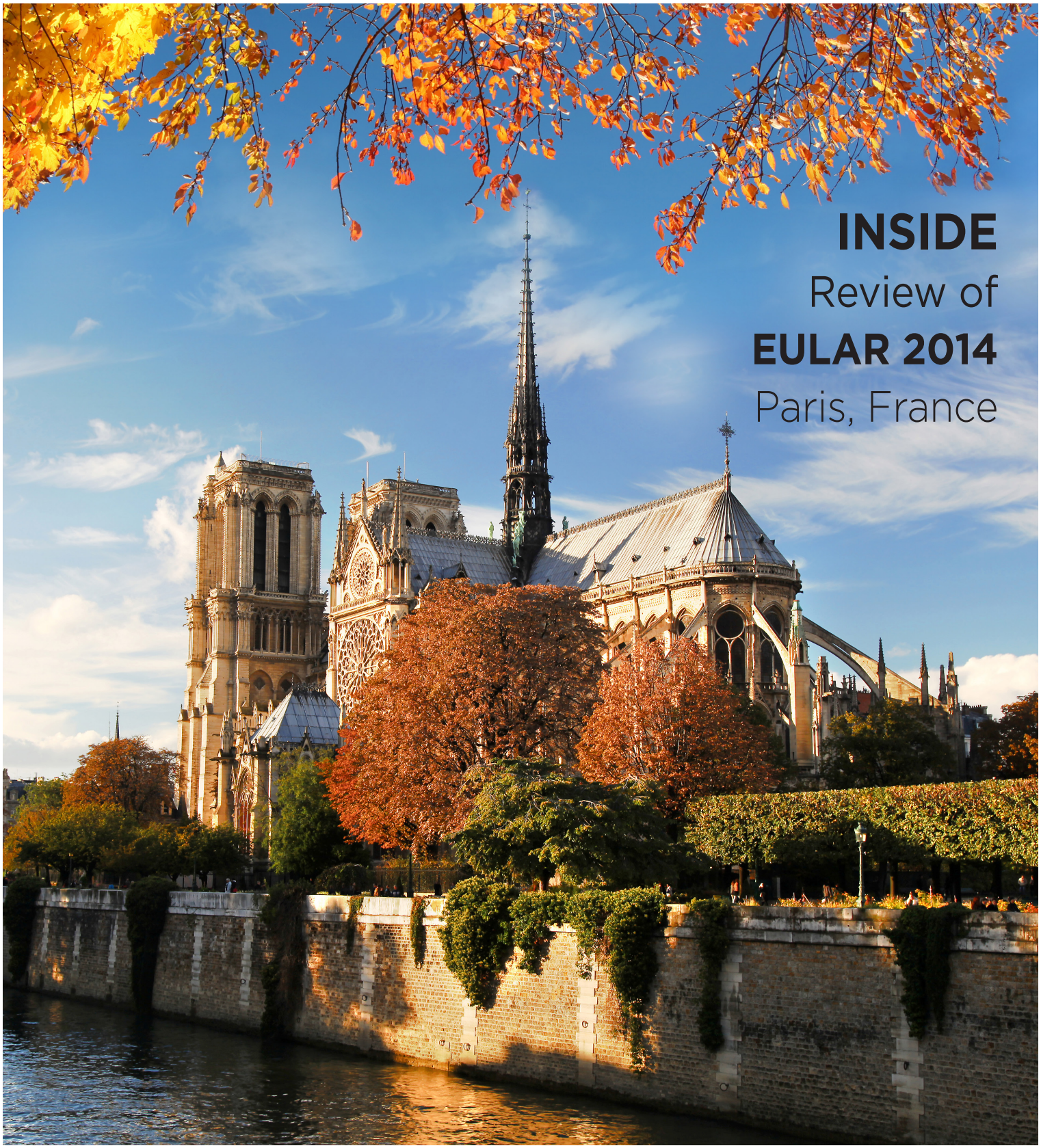

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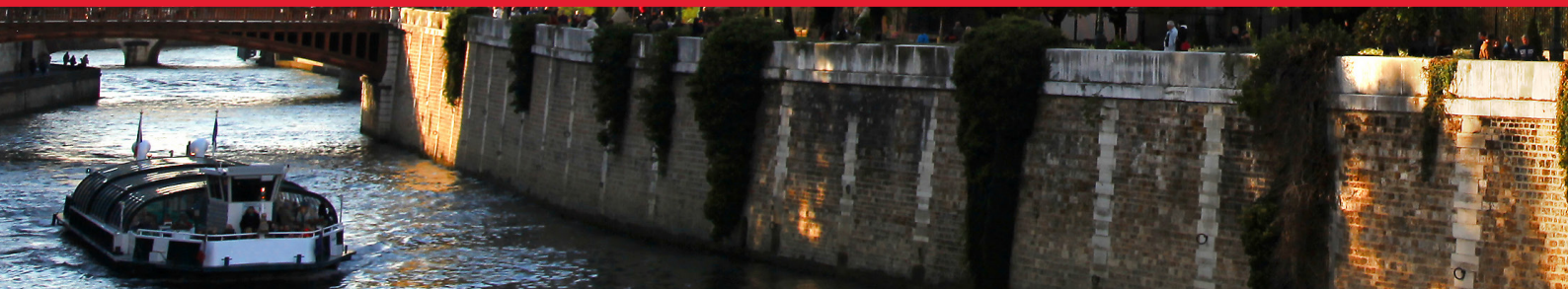

# RHEUMATOLOGY


**ORENCIA® (abatacept) PRESCRIBING INFORMATION.** See Summary of Product Characteristics before prescribing. **PRESENTATION:** 250 mg powder for concentrate for solution for IV infusion containing 250 mg abatacept per vial. Each ml contains 25 mg of abatacept, after reconstitution; 125 mg pre-filled syringe for SC injection. Each pre-filled syringe contains 125 mg of abatacept in 1 ml. **INDICATION:** ***Rheumatoid arthritis (IV infusion and SC pre-filled syringe)***: Treatment of moderate to severe active rheumatoid arthritis (RA), in combination with methotrexate, in adult patients who have responded inadequately to previous therapy with one or more disease-modifying anti-rheumatic drugs (DMARDs) including methotrexate (MTX) or a Tumour Necrosis Factor (TNF) -alpha inhibitor. A reduction in the progression of joint damage and improvement of physical function have been demonstrated during combination treatment with abatacept and methotrexate. See SmPC. ***Polyarticular Juvenile Idiopathic Arthritis (pJIA) (IV infusion only)***: Orencia 250 mg powder for concentrate for solution for infusion is indicated for treatment of moderate to severe active pJIA in paediatric patients 6 years of age and older who have had an insufficient response to other DMARDs including at least one TNF inhibitor. **DOSAGE and ADMINISTRATION:** Treatment should be initiated and supervised by specialist physicians experienced in the diagnosis and treatment of RA. Orencia 250 mg powder for concentrate for solution for IV infusion *Adults and elderly*: Patients weighing < 60 kg: 500 mg (2 vials). Patients weighing ≥ 60 kg to ≤ 100 kg: 750 mg (3 vials). Patients weighing > 100 kg: 1000 mg (4 vials). *Treatment of pJIA*: Paediatric patients, 6 to 17 years of age, weighing less than 75 kg: 10 mg/kg paediatric patients weighing 75 kg or more: to be administered adult dosage, not exceeding a maximum dose of 1,000 mg. See SmPC for details of reconstitution and administration as a 30 minute IV infusion. After initial administration, Orencia should be given at 2 and 4 weeks, then every 4 weeks thereafter. *Children:* Use in children below 6 years of age is not recommended. Orencia 125 mg solution for injection (SC *pre-filled syringe*) *Adults and elderly*: Orencia SC may be initiated with or without an intravenous (IV) loading dose. ORENCIA SC should be administered weekly at a dose of 125 mg by subcutaneous injection regardless of weight. If a single IV infusion is given to initiate treatment (IV loading dose before SC administration), the first 125 mg abatacept SC should be administered within a day of the IV infusion, followed by the weekly 125 mg abatacept SC injections. Patients transitioning from Orencia IV therapy to SC administration should administer the first subcutaneous dose instead of the next scheduled intravenous dose. *Children:* Administration in children below 18 years of age is not recommended. The continuation of treatment with abatacept should be re-assessed if patients do not respond within 6 months. **CONTRAINDICATIONS:** Hypersensitivity to the active substance or excipients. Severe and uncontrolled infections such as sepsis and opportunistic infections. **WARNINGS AND PRECAUTIONS:** *Allergic Reactions*: Caution in patients with a history of allergic reactions. Anaphylaxis or anaphylactoid reactions can occur and can be life threatening. Orencia should be discontinued permanently if a patient develops serious allergic or anaphylactic reaction. *Infections:* Caution should be exercised when considering the use in patients with a history of frequent infections, or underlying conditions which may predispose to infection. Treatment with Orencia should not be initiated with patients with active infections until infections are controlled. Screening for tuberculosis and hepatitis B should be performed prior to therapy. Any patient who develops a new infection should be closely monitored and Orencia should be discontinued if a patient develops a serious infection. Monitor patients for signs of infection when transitioning from TNF-antagonist to Orencia. Co-administration of Orencia with biologic immunosuppressive or immunomodulatory agents could potentiate the effects of abatacept on the immune system. Treatment with immunosuppressive therapy may be associated with progressive multifocal leukoencephalopathy (PML). 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Women should use contraception and not breast-feed during treatment and for up to 14 weeks after last dose treatment. **UNDESIRABLE EFFECTS:** In adult placebo-controlled trials the following adverse drug reactions were reported. *Very Common (≥ 1/10)*: upper respiratory tract infection including tracheitis, nasopharyngitis. *Common (≥ 1/100 to < 1/10)*: Lower respiratory tract infection (including bronchitis), urinary tract infection, herpes infections (including herpes simplex, oral herpes and herpes zoster), rhinitis, pneumonia, influenza, leukopenia, headache, dizziness, paraesthesia, conjunctivitis, hypertension, flushing, blood pressure increased, cough, abdominal pain, diarrhoea, nausea, dyspepsia, mouth ulceration, aphthous stomatitis, vomiting, liver function test abnormal (including transaminases increased), rash (including dermatitis), alopecia, pruritus, pain in extremity, fatigue, asthenia, injection site reactions. *Uncommon (≥ 1/1,000 to < 1/100)*: Tooth infection, onychomycosis, sepsis, musculoskeletal infections, skin abscess, pyelonephritis, pelvic inflammatory disease, basal cell and squamous cell carcinoma, skin papilloma, thrombocytopenia, hypersensitivity, depression, anxiety, sleep disorder (including insomnia), migraine, dry eye, visual acuity reduced, vertigo, palpitations, tachycardia, bradycardia, hypotension, hot flush, vasculitis, blood pressure decreased, bronchospasm, wheezing, dyspnea, gastritis, increased tendency to bruise, dry skin, urticaria, psoriasis, erythema, hyperhidosis, arthralgia, amenorrhea, menorrhagia, influenza like illness, weight increased. *Rare (≥ 1/10,000 to < 1/1,000)*: Tuberculosis, bacteraemia, gastrointestinal infection, lymphoma, lung neoplasm malignant, throat tightness. See SmPC for further details. **LEGAL CATEGORY:** POM **MARKETING AUTHORISATION NUMBER:** Orencia 250 mg concentrate for solution for infusion - EU/1/07/389/001, Orencia 125 mg solution for Injection - EU/1/07/389/008. **MARKETING AUTHORISATION HOLDER:** Bristol-Myers Squibb Pharma EEIG, Uxbridge Business Park, Sanderson Road, Uxbridge, Middlesex UB8 1DH. Tel: 0800-731-1736 **DATE OF PREPARATION:** April 2014. Job No: 427UK14PR03242

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Adverse events should also be reported to Bristol-Myers Squibb Pharmaceuticals Ltd
Medical Information on 0800 731 1736 or medical.information@bms.com.

* RA: Rheumatoid arthritis. References: 1. Summary of Product Characteristics of ORENCIA® 2014. 2. Schiff M, Weinblatt M, Valente R, *et al. Ann Rheum Dis* 2014;73:86-94.

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## Rheumatology

### Editor-in-Chief: Dr Ian Chikanza

Consultant/Senior Lecturer in Adult & Paediatric Rheumatology, Department of Rheumatology, Barts Arthritis Centre, The Royal London Hospital, London, UK.

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Hyaluronic acid fibrous aggregate
produced by bacterial fermentation

# Welcome

It is with great anticipation that I unveil to you our inaugural edition of the *European Medical Journal – Rheumatology*, which includes a review of the Annual European Congress of Rheumatology – the European League Against Rheumatism (EULAR) Congress, high-quality peer-reviewed papers on some of the hottest topics within the field, and the chance to catch up on all other updates in the field in our 'What's New' section.

In our 'Congress Review' section we have a very interesting article on the initiative which has been taken to allow patients to learn more about their condition, embrace the technology which helps them, and assist in the assembly of policy guidelines. It is hoped that this involvement will make patients more aware of the treatments which are beneficial to them.

It is encouraging for patients with these diseases that research is undertaken into new treatments, therapies, and biomarkers, which may help to potentially alleviate this disease. As no disease-modifying drugs for osteoarthritis (OA) have been approved, Dr Geraldine McCarthy has suggested in her paper: '*Why basic calcium phosphate crystals should be targeted in the treatment of osteoarthritis*' that basic calcium phosphate crystals should be explored as a potential target for treating OA. Dr McCarthy argues that it would be of great interest to see the effects of the crystals if they are dissolved or their formation prevented.

Another therapy that could be advantageous to those suffering from OA is the regeneration of old stem cells – this is recycling at its best. In our 'What's New' section we report on a study which discovered that rejuvenating stem cells in older people may help develop therapies to repair worn or damaged cartilage, reducing the effects of pain and improving quality of life.

We have several interesting articles in our 'Congress Review' and 'What's New' sections for those interested in Sjögren's syndrome, personalised therapies for lupus, the identification of biomarkers, and a new RNA-sequence which could be used to treat OA.

Among these interesting reports, we also have an article written by Dr Nelly Ziadé, entitled: '*Osteoporosis-related mortality: time-trends and predictive factors*' which suggests that rheumatologists who manage OA patients should adopt a multivariate approach, based on specific predictive factors.

We hope that you benefit from the content, which we have amalgamated with the help of our esteemed editorial board, and that this journal not only provides you with an insight into the changing world of rheumatology, but will also be of benefit to your patients.

**Spencer Gore**
*Director, European Medical Journal*

*European Medical Journal - Rheumatology* is published annually.
For subscription details please visit www.emjreviews.com



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EMJ GORELY NEW MEDIA

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# Foreword


## Dr Ian Chikanza

*Consultant/Senior Lecturer in Adult & Paediatric Rheumatology, the Royal London Hospital, UK.*


Dear Colleagues,

I would like to welcome you to the first edition of the *European Medical Journal – Rheumatology.*

The past few years have been very exciting for rheumatology patients, especially with regards to the development of target therapies using monoclonal antibodies, which are targeted at specific sites of the inflammatory cascades, and more recently, the use of small chemical molecules that are also targeted, such as the JAK kinase inhibitors.

There have also been revolutionary approaches to managing gout with biotherapies such as anakinra. In systemic lupus erythematosus, targeting B cells has proven to be very promising with a myriad of bio-molecules being developed around this theme and some being tested in clinical trials.

Vitamin D has emerged as the 'God given' molecule being claimed to have preventative and therapeutic potentials for a whole range of autoimmune chronic inflammatory diseases, including effects on infections such as TB; we await viable scientific proof of these observations.

This rapid explosion in scientific research has necessitated the launch of this journal which seeks to review scientific evidence as it is released, to analyse it in a translational context, and assess its merits and potential impact in rheumatology disorders as well as therapeutic utility in the clinic.

At the last international meeting of the European League Against Rheumatism (EULAR), June 2014, Paris, France, we witnessed an unprecedented number of papers on recent scientific discoveries in rheumatology, a number of pivotal clinical trials of new therapeutic entities, and therapy optimisation strategies centred around the 'Treat to Target' approach, whose main aim is aggressive early therapy intervention to induce disease remission and prevent progression and long-term structural damage.

I am very happy for our patients because in the last few years we have witnessed major strides in the management of rheumatic conditions, and the boundaries for disease remission continue to be extended. This has brought direct clinical benefits to patients.

In conclusion, I am very pleased to present to you this inaugural edition of *EMJ – Rheumatology* and invite you to attend the 2015 EULAR meeting in Rome, Italy.

With kind regards,


**Ian Chikanza**

Consultant/Senior Lecturer in Adult & Paediatric Rheumatology, Department of Rheumatology, Barts Arthritis Centre, The Royal London Hospital, London, UK.

# ANNUAL EUROPEAN CONGRESS OF RHEUMATOLOGY 2014

## LE PALAIS DES CONGRÈS DE PARIS
## PARIS, FRANCE
11TH-14TH JUNE 2014

# Welcome to the *European Medical Journal* review of the Annual European Congress of Rheumatology - The European League Against Rheumatism (EULAR) Congress 2014

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**Welcome** to the *European Medical Journal* review of the Annual European Congress of Rheumatology - The European League Against Rheumatism (EULAR) Congress 2014

The European League Against Rheumatism (EULAR) 2014 Congress in Paris, France, set the tone for the future direction of this growing field. A concoction of exciting topics, presented in over 180 sessions by more than 500 experts covering 650 presentations, were displayed in the Palais des Congrès in the city centre, in close proximity to the landmark Arc de Triomphe and the Eiffel Tower.

Attracting a record 4,000 abstracts from over 90 countries, as well as 13,000 participants from around the world, the 15th Annual EULAR Congress was well suited to its luxurious surroundings.

Boasting an outstanding cultural and historical heritage, Paris is a constantly evolving creature in both the arts and sciences; even a walk down the streets of Paris treats you to a kaleidoscope of finery, the river Seine weaving a course through this architectural paradise. This is all befitting of the city's immense scientific accomplishments, its setting for the Curies' radiological legacy being among the most notable.

With over 600,000 students and 17 universities, the Paris Region is a European centre of excellence in training and education, while possessing the highest Research and Development concentration in Europe.

"Unquestionably, research in rheumatic and musculoskeletal diseases (RMDs) in Europe is prospering despite a rather adverse economic environment. It is therefore with great satisfaction that we have seen a progressive availability of EU research funds for RMDs in recent months, following several years of intensive discussion and negotiation by EULAR in the EU Parliament

"Unquestionably, research in rheumatic and musculoskeletal diseases (RMDs) in Europe is prospering despite a rather adverse economic environment."

*Prof Maurizio Cutolo, President of EULAR*

with the Commission in Brussels," said Prof Maurizio Cutolo, President of EULAR.

This section covers the latest wave of breakthroughs in the field of rheumatology, while shining new light on existing therapies for established conditions. A striking story is the emergence of sarilumab, the first fully-human monoclonal antibody targeting the IL-6 receptor, key to joint inflammation. The drug has already delivered impressive results in trials, and has emerged as a potential game-changer for the treatment of rheumatoid arthritis patients who respond inadequately to methotrexate therapy.

Osteoarthritis, one of the top ten most incapacitating diseases in the developed world, is now a less fearful figure with the discovery of revolutionary biomarkers able to detect its most severe state. The potential of the biomarkers to accelerate the early preventative treatment of patients, potentially enhancing quality of life for millions while catalysing crucial savings in economies worldwide, was outlined at EULAR.

Until now, temporal artery biopsy has been perceived as the gold standard for the diagnosis of giant cell arteritis. However, cranial ultrasound was presented at the Congress as a genuine contender to supplant temporal artery biopsy in the doctor's surgery, already saving time and money for physicians and patients by preventing the often unnecessary distribution of hazardous steroids.

Next year's Congress in Rome clearly has a lot to live up to. However, on the basis of Paris there is a golden road leading to a bright future for the global rheumatology community.



# Gout linked to giant killer, coronary artery disease

CORONARY artery disease (CAD), the biggest killer in the USA and UK, may be detected through erectile dysfunction (ED) screening in men with gout. An established symptom of CAD, ED occurs in most men with gout and is often severe.

Gout is a condition that is characterised by recurrent attacks of acute inflammatory arthritis, which causes severe pain in the affected joint, along with swelling and redness. This arthritis is caused by deposits of needle-like monosodium urate crystals in the joints and is associated with abnormal levels of uric acid in the bloodstream.

"Because gout is commonly associated with cardiovascular disease (CVD) risk factors and CAD, and patients who present with ED also have an increased rate of cardiovascular disease risk factors and concomitant silent CAD, all these patients should also be evaluated for possible silent CAD," said Dr Naomi Schlesinger, lead author and Chief, Division for Rheumatology, and Professor of Medicine, Rutgers-Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA.

A sexual health inventory in men (SHIM) survey was conducted on men aged 18-89 with the aim of identifying a correlation between instances of gout and ED. "[This is] the inability to gain and maintain an erection that is sufficient to permit satisfactory sexual intercourse," said Dr Schlesinger.

The survey was carried out on 201 men, 83 of whom suffered from gout and showed a significantly greater proportion of ED (76%) than those men that do not suffer from gout (52%). These data show that gout is potentially a significant risk factor for ED.

Cases of severe ED were also more prevalent among gout sufferers (43%) compared to non-sufferers (30%).

Dr Schlesinger said: "These results strongly support the proposal to screen all men with gout for the presence of ED. Increasing awareness of the presence of ED in gout patients should, in turn, lead to earlier medical attention and treatment for this distressing condition."

It is hoped that these data, which support the hypothesis that gout is indeed a significant risk factor for ED, will lead to the implementation of a screening procedure for men who can then be offered help for ED, but could also save their lives by detecting early signs of CAD.

"These results strongly support the proposal to screen all men with gout for the presence of ED. Increasing awareness of the presence of ED in gout patients should, in turn, lead to earlier medical attention and treatment for this distressing condition."

*Dr Naomi Schlesinger, Rutgers-Robert Wood Johnson Medical School, New Brunswick, USA*

# Could Sjögren's syndrome cause a heart attack?

HEART attack risk is elevated in Sjögren's syndrome (SjS) sufferers, especially in the first year post-diagnosis, signalling the need for active management of patients' cardiovascular risk factors and monitoring for signs of coronary artery disease (CAD).

"Our results support the role of inflammation in cardiovascular disease and the need for increased monitoring for CAD in all patients with this condition, in addition to proper management and modification of their cardiovascular risk factors, to reduce the risk of a future heart attack," said Dr Antonio Aviña-Zubieta, principal investigator and research scientist, Arthritis Research Centre of Canada and Assistant Professor, Department of Medicine, Division of Rheumatology, University of British Columbia, Vancouver, British Columbia, Canada.

SjS is an autoimmune inflammatory disease where the body's immune system attacks glands that secrete fluid, such as the tear and saliva glands. The result of inflammation of these areas is reduced fluid production, causing painful burning in the eyes, and dryness of the mouth, nasal passages, vagina, throat, and skin.

SjS is a relatively common disease, affecting approximately 0.2% of the adult population, highlighting the need to better understand its underlying mechanisms so that treatments can be developed.

There are two types of SjS: primary and secondary. Primary is a somewhat spontaneous type that arises within people that have no history of rheumatological disease, whereas secondary SjS only occurs in those who have another rheumatological disease, such as systemic lupus erythematosus.

The study was conducted on 1,176 SjS patients and 11,983 non-SjS matched controls. Of the 1,176 SjS patients, 28 developed a first-time heart attack (7.7 per 1,000 person years), which was more than double the rate of heart attacks in the control (138 overall; 3.4 per 1,000 person years).

"This is the first general population-based cohort study comparing the relative risk of heart attacks and strokes in patients with new SjS with age, sex, and entry-matched controls; previously we only had limited data on the relative risks in this specific patient group," said Dr Aviña-Zubieta.

Interestingly, the risk of patients developing a heart attack was highest within the first year of being diagnosed with SjS. The study also found an increased risk of stroke for SjS sufferers; however, the aforementioned trend was not identified for stroke.


# Pain relief needles cross swords

> "The injections stimulate the process of tendon repair through an irritation effect, a technique known as prolotherapy."
>
> *Mr Patrick Le Goux, Hôpitaux Universitaires Paris Ile-de-France Ouest, Paris, France*

ULTRASOUND (US)-guided saline injections are just as effective as injections of growth factors containing platelet-rich plasma (PRP) for the treatment of recently developed epicondylitis.

Mr Patrick Le Goux, lead researcher of this latest study, Hôpitaux Universitaires Paris Ile-de-France Ouest, Paris, France, said: "While PRP injections were shown to have no inherent benefit in the treatment of epicondylitis, what is exciting is that pain scores in both treatment groups decreased significantly over the course of the trial."

50 subjects with recent onset epicondylitis (pain ≤3 months), confirmed by magnetic resonance imaging and/or US, were treated with either PRP (ACP®, Arthrex), which aids the tendon repair process, or saline solution through two US-guided injections. These were administered close to but not into the tendon at 4-week intervals, with 25 subjects in each treatment group.

The objective of the study was to prove the superior efficiency of PRP injection on pain over saline injection. However, mean relative improvement in pain (ACP 54.7% versus control 63.6%) was not statistically significantly different between the groups at 6 months or at any other time. After 3-6 months the pain score decreased by 50% in both groups; at 6 months one-third of patients were asymptomatic, which extended to two-thirds at 1 year.

Furthermore, less than one-quarter (23.8%) of patients in both groups experienced persistent pain at the end of the study. No adverse effects were recorded, underlining the safety of the treatments.

"The healing process is stimulated by the echo-guided injection of a substance and/or by the own effect of the needle (needling); the injections stimulate the process of tendon repair through an irritation effect, a technique known as prolotherapy," explained Mr Le Goux.

Further research is required to determine whether the improvement in pain is due to these treatments or the passage of time, as well as a much-needed comparison of injected and non-injected therapies.

# Exercise proved to trample rheumatic disease

LOCAL and systemic inflammations are transiently suppressed by exercise, underlining the significance of regular exercise in achieving maximum efficacy in rheumatic disease control.

"As the inflammatory process in rheumatic diseases is a major cause of disability, we are excited to uncover the process by which exercise works on a molecular level to decrease this inflammation. Our results show the benefits that exercise could have in decreasing the great burden of rheumatic diseases. They also highlight the need for frequent exercise in order to create clinically significant results," said Dr Nicholas Young, lead author, Ohio State University Wexner Medical Center, Columbus, Ohio, USA.

Chronic inflammation is a symptom of every single rheumatic disease, of which over 200 have been identified. Persistent inflammation over time can damage joints and is extremely uncomfortable for the sufferer.

The research focused on the physiological changes created by exercise and their impact on inflammation. In particular, researchers measured the regulation and activation of NF-κB, a protein complex that controls many genes involved in inflammation, and has been found to be chronically active in many inflammatory diseases such as arthritis and inflammatory bowel disease.

To conduct this investigation, an inflammatory response was induced in mice via an injection of lipopolysaccharides (LPS) before exercising some mice and keeping the others relatively calm.

Results showed strong systemic and local inflammatory responses upon injection of LPS, firstly proving that LPS does cause an inflammatory response. NF-κB activation was seen as a result of the LPS injection and was detected in lymphatic tissues throughout the mouse. This contrasts with regularly exercised mice, in which NF-κB activation was significantly inhibited in whole-body systemic analysis.

These findings provide a new method of treatment to enhance the quality of life for sufferers of all rheumatic diseases.

"As the inflammatory process in rheumatic diseases is a major cause of disability, we are excited to uncover the process by which exercise works on a molecular level to decrease this inflammation. Our results show the benefits that exercise could have in decreasing the great burden of rheumatic diseases."

*Dr Nicholas Young,*
*Ohio State University Wexner Medical Center,*
*Columbus, USA*

# An end for immunosuppressant therapy for lupus patients?

ILLUMINATING guidance has been released on ceasing immunosuppressant therapy for most systemic lupus erythematosus (SLE) patients in remission, preventing occurrence of a post-treatment flare of their disease.

"Until now, information on whether and how immunosuppressant therapy might be stopped in lupus patients after achieving low disease activity or remission has been limited," said Dr Zahi Touma, lead author and Assistant Professor of Medicine, Clinician-Scientist, Division of Rheumatology, University of Toronto, Toronto, Ontario, Canada.

The likelihood of a flare is linked to the length of time from the start of tapering to stopping the immunosuppressant; 70% of clinically stable patients experienced successful halt of therapy within 2 years, while half of patients were clear within 3 years and up to 5 years.

Immunosuppressants such as azathioprine or mycophenolate mofetil are designed to specifically target the cells of the immune system and cause a decrease in the overall level of function of the immune system, thereby reducing the effects of SLE. However, this immunosuppression can cause side-effects, including elevated infection risk and cancer.

It was this dangerous side-effect that led Dr Touma to concoct an idea for a study to examine the impacts of halting the use of immunosuppressants in patients who have been in remission for varying lengths of time.

> "The results from our study provide useful guidance on how best to stop the immunosuppressant without triggering a flare. For example, patients who discontinued their immunosuppressant more slowly were less likely to flare within 2 years."
>
> *Dr Zahi Touma, University of Toronto, Toronto, Canada*

The study consisted of 1,678 SLE patients; of the 973 who had been prescribed an immunosuppressant, 99 stopped taking it. Of these 99 people (who were taking a plethora of different immunosuppressant drugs), only 25 flared (had recurring symptoms) within 2 years, and 17 patients experienced a flare after 2 years.

"The results from our study provide useful guidance on how best to stop the immunosuppressant without triggering a flare. For example, patients who discontinued their immunosuppressant more slowly were less likely to flare within 2 years," Dr Touma explained.

# First biomarkers indicative of osteoarthritis have been found

EARLY detection of one of the top ten most disabling diseases in developed countries, osteoarthritis (OA), is looking increasingly attainable following the discovery of the first indicatory biomarkers for the condition.

The presence of three highly promising micro RNA molecules (miRNAs) – miR-454, miR-885-5p, and in particular let-7e – correlated with the development of severe OA of the knee or hip joint, suggesting a potential role for these molecules as biomarkers to predict severe OA in individuals.

"These results indicate that for the first time we will be able to predict the risk of severe OA, before the diseases starts to significantly impact a person's life, allowing us to take preventative action early on. Through the early identification of OA we can decrease both the impact of the disease on individuals and the major socio-economic burden severe disease poses," said Dr Christian Beyer, lead author of the study, Department of Internal Medicine 3 - Rheumatology and Immunology, University of Erlangen-Nuremberg, Bavaria, Germany.

816 subjects, monitored over 15 years, were tested for the presence of the 374 miRNAs and occurrence of OA. Joint replacement (known as arthroplasty) was used as a definitive outcome of severe OA in the knee or hip. 67 of the 816 subjects had one or more total joint replacements for severe knee or hip OA.

A strong correlation between let-7e and joint replacement was found: as let-7e levels decrease, the risk of receiving joint replacement due to severe knee or hip OA increases.

OA, afflicting 10% of the global population, has an average annual cost of between €1,330 and €10,452 per European patient, and will likely continue to bloom through rising life expectancy and obesity incidence.

miRNAs are small molecules that can reflect states of health and disease. Emerging as biomarkers in many fields, they are very stable, can resist harsh conditions, and are easily accessible in the blood.

However, miRNAs remain a long way from establishment in clinical practice. "Our study now opens many new questions that need to be addressed by future studies. We want to know where miRNAs come from: do they come from the disease joint or somewhere else? How do they behave when we follow-up with the patient? Can these miRNAs also reflect disease activity, and can we confirm those data in other cohorts? This needs to be done in future studies," said Dr Beyer.

> "Through the early identification of OA we can decrease both the impact of the disease on individuals and the major socio-economic burden severe disease poses."
>
> *Dr Christian Beyer, University of Erlangen-Nuremberg, Bavaria, Germany*



# Patients winning the fight against rheumatologic conditions

A DYNAMIC initiative has been launched to allow patients to embrace technology, assist in the assembly of policy guidelines, and learn more about their condition. These changes will be welcomed by sufferers of rheumatic diseases.

'New patient empowerment and educational initiatives' were specifically created to address gaps in the ways that patients can take charge of their condition. The initiatives include the partaking of patients in the self-monitoring and initiated follow-up, which reduces the overall cost impact. Patients were trained to interpret their blood test results and monitor side-effects and symptoms, which were then relayed to the nurse.

Another criterion of the initiatives allows patients to undergo training from the European Patient Ambassador Programme to gain skills in healthcare policymaking, which creates awareness of how policies are formulated with regards to the health of people suffering from rheumatologic conditions.

Ms Monica Fletcher, Chair of the European Lung Foundation who developed the programme, explained: "As more people live longer with chronic conditions, such as arthritis and rheumatism, it is essential that they become involved in healthcare to help improve the patient's experience, identify patient priorities, reduce burden on services, and advance research."

The involvement on decision-making for treatment options with the use of a new Patient Decision Aid allows the patient to be more aware of treatments which are conducive to their values.

The use of video-guides allows patients and their families to better understand the condition, and provides tips to help people living with the condition.

Patient involvement in guideline development and political committees has been very active, and supported by the German League Against Rheumatism (the Deutsche Rheuma-Liga).

"Despite the success of this initiative, we still need to find new ways to recruit younger people with rheumatic diseases as patient representatives, who may have less time to become involved in volunteer work," said Dr Cornelia Sander, representing the Deutsche Rheuma-Liga, Bronn, Germany.

"As more people live longer with chronic conditions, such as arthritis and rheumatism, it is essential that they become involved in healthcare to help improve the patient's experience, identify patient priorities, reduce burden on services, and advance research."

*Ms Monica Fletcher, Chair of the European Lung Foundation*

# One-third of psoriatic arthritis patients miss out on optimal therapy

CORRECT doses of psoriatic arthritis (PsA) drugs are not being given to patients, although the resulting efficacy remains satisfactory.

One-third of PsA patients do not receive the dosing of the tumour necrosis factor-alpha (TNFα) inhibitor adalimumab required to achieve optimal clinical benefit, while almost three-quarters were on doses of TNFα inhibitor infliximab lower than recommended in international guidelines; both conclusions were reached in separate studies.

However, a low adalimumab dose still showed reasonable efficacy. Treatment response and adherence were relatively unaffected by a low infliximab dose, which indicates a seemingly efficient strategy when combined with subsequent step-up therapy.

PsA is a condition that affects up to 30% of all psoriasis cases, which occurs in 1-3% of the population. Symptoms can generally be perturbed by subcutaneous delivery of 40 mg of adalimumab every other week.

Mr Erik Volgelzang, lead author of the study on adalimumab, Jan van Breemen Research Institute, Amsterdam, the Netherlands, said: "These results linking serum adalimumab trough concentrations to clinical response in PsA patients confirmed the findings from a previous study in RA patients."

"However, interestingly, of the 103 consecutive patients with PsA prescribed adalimumab, 36 (35%) appeared to be receiving less than optimal dosing, with a trough adalimumab concentration below 5 mg/L (the lowest point of this ideal dose range). A substantial group of PsA patients that use adalimumab are therefore not able to profit from its optimal clinical benefit," Mr Volgelzang added.

"More than 70% of Icelandic and Danish PsA patients treated with infliximab received sustained doses below the recommended 5 mg/kg every 8 weeks," said Dr Bente Glintborg, lead investigator of the study on infliximab, Copenhagen Centre for Arthritis Research, and Centre for Rheumatology and Spine Diseases, Glostrup Hospital, Glostrup, Denmark. "Lower start doses did not appear to affect either drug adherence or response."

Dr Glintborg's study also examined the differences in dosage levels between Denmark and Iceland, which showed that, on average, Danish patients received higher doses than Icelandic patients. "Despite these important differences between Icelandic and Danish patients, they were found to have similar 1-year response rates," said Dr Glintborg.

This study illuminates the inconsistencies of adalimumab and infliximab dosage levels in the clinic, and highlights the need to re-assess the optimum dose for each TNFα inhibitor.

"More than 70% of Icelandic and Danish PsA patients treated with infliximab received sustained doses below the recommended 5 mg/kg every 8 weeks."

*Dr Bente Glintborg, Glostrup Hospital, Glostrup, Denmark*

# Juvenile idiopathic arthritis: possible status detection using biomarkers

PROSPECTIVE biomarkers developed from whole-blood gene expression profiles may predict the status of juvenile idiopathic arthritis (JIA) in children at 12 months of age.

JIA is characterised by inflammation, pain, and swelling in one or more joints for more than 6 weeks. The eyes and lymph nodes are also affected by this condition. It is prevalent mainly in children and adolescents, with an incidence rate of 16-150 children in 100,000. Its aetiology still remains unknown.

"By predicting disease progression in these young children we can better understand the course of the disease and how best to treat the individual," said lead author of the study Prof James Jarvis, Department of Pediatrics, University at Buffalo, The State University of New York, Buffalo, New York, USA.

Blood gene expression profiling has opened many avenues in rheumatology in the past decade, however, it is only possible to predict therapeutic outcomes at 6 months.

The Trial of Early Aggressive Therapy (TREAT) study specifically investigated the whole blood expression profiles in children where methotrexate (MTX) was compared with MTX plus etanercept in newly diagnosed JIA. Emphasis was placed on the genes with expression levels that predicted the outcome (active versus inactive disease) at 12 months.

"The challenge was to test the feasibility of using these prognostic biomarkers from whole blood gene expression profiles in children with newly diagnosed JIA to predict disease status at 1 year," explained Prof Jarvis. "Baseline expression profiles that could predict disease status at 6 months could not predict status at 12 months. However, using 4-month data (the earliest point at which samples were collected from children on treatment) we were able to determine strong predictive properties for disease status at 12 months. Thus, after children had initiated therapy, longer-term outcome was predictable," continued Prof Jarvis.

> "By predicting disease progression in these young children we can better understand the course of the disease and how best to treat the individual."
>
> *Prof James Jarvis, University at Buffalo, The State University of New York, Buffalo, USA*

# Rheumatoid arthritis patients have a friend in valine

RADIOLOGICAL damage in rheumatoid arthritis (RA) faces the music as the amino acid valine at positions 11, 71, and 74 of the HLA-DRB1 gene shine the flashlight on disease outcomes, indicating patients at risk of joint damage and early death.

"This new evidence from our multi-centre cohort studies has shown that positions 11, 71, and 74 on the HLA-DRB1 gene now supercede the classical shared epitope," said Dr Sebastien Viatte, lead author, Arthritis Research UK Centre for Genetics and Genomics, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK.

These findings were the result of a study conducted in the UK that was motivated by the need to find methods of detecting RA early so that doctors have the best possible chance to detect the symptoms.

Although the cause of RA is largely unknown, it was previously thought that a group of alleles on the HLA-DRB1 gene, known as the shared epitope, was thought to have the strongest effect on RA susceptibility.

Genotyping has usurped this assumption and provided three distinct DNA positions, which are all independent genetic determinants of radiological damage in RA. These amino acids are valine at positions 11 (the strongest genetic determinant), 71, and 74.

Three studies were conducted to assess their ability to predict the radiological outcome, anti-tumour necrosis factor (TNF) response, and mortality in patients with RA. These studies were the Norfolk Arthritis Register, the Early Rheumatoid Arthritis Study, and the Biology in Rheumatoid Arthritis Genetics and Genomics Study Syndicate.

The three aforementioned positions together defined 16 haplotypes, which were strongly associated with disease outcome, and most importantly were perfectly correlated with disease susceptibility.

The notion of a strong influence from the genotype of the individual on the epidemiology of RA stems from studies that showed the prevalence of RA on different continents that were all relatively similar (between 0.5-1.0%). However, the data showed a very low occurrence in China and Japan, lending credence to the assumption that RA does indeed have some degree of genetic causality.

"This major advance in genetics might allow stratification of RA patients at the onset of their disease to identify those at risk of joint damage and early death, and also those who are more likely to respond to anti-TNF biological therapy," concluded Dr Viatte.

# Sarilumab eases pain in rheumatoid arthritis sufferers

RHEUMATOID arthritis (RA) patients who respond inadequately to methotrexate (MTX) therapy have been given hope by the impressive Phase III trial performances of an investigational drug called sarilumab.

The drug bolstered major clinical response rates by triggering an upturn in signs and symptoms of RA at 24 weeks - according to the American College of Rheumatology score of 20% improvement (ACR20) - in a major study. This was successfully maintained beyond 52 weeks, securing the secondary endpoints of the trial in the process.

Permanent damage to the joints and extensive cartilage loss are possible in RA, resulting from the host immune system attacking and inflaming the lining of joints. This is catalysed by overly-abundant cytokines, such as interleukin-6 (IL-6), which have been targeted by numerous therapies.

This approach has not been sufficient; the most common class of biologic therapy, tumour necrosis factor inhibitors, have yielded an inadequate response in 20-40% of RA patients. "Despite notable advances, many RA patients continue to struggle with debilitating signs and symptoms, underscoring a clear need for additional options," said Dr Mark Genovese, lead investigator and Professor, Stanford University Medical Center, Stanford, California, USA.

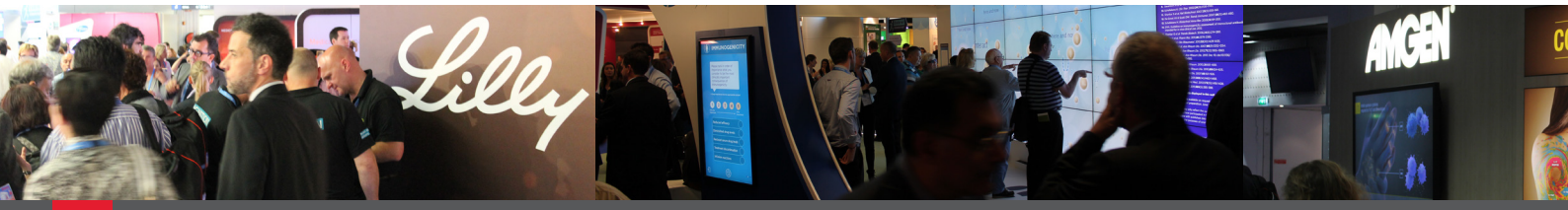
Sarilumab - an every other week, self-administered, subcutaneous injection – is the first fully-human monoclonal antibody which targets the IL-6 receptor; the inflammatory effect of IL-6 is dampened through the selective binding of sarilumab to the IL-6 receptor.

Securing all three co-primary endpoints in the SARIL-RA Phase III trial, a branch of the 2,800-subject SARIL-RA clinical development programme, sarilumab exhibited improvement in disease signs and symptoms at 24 weeks, physical function at 16 weeks, and inhibition of joint damage progression at 52 weeks.

1,197 subjects with active moderate-to-severe RA, who were inadequate responders to MTX therapy, took part in the trial. Subjects were randomised to one of three treatment groups, all combined with MTX: sarilumab 150 mg, sarilumab 200 mg, or placebo.

Both doses of sarilumab (58% for 150 mg, 66% for 200 mg) showed superior improvement over placebo (33%) in signs and symptoms of RA at 24 weeks, extending to 52 weeks with 54%, 59%, and 32%, respectively.

Dr Genovese concluded: "Sarilumab showed efficacy in this study at two different doses, both delivered subcutaneously every other week. We look forward to the results of ongoing trials in this comprehensive registration programme."



# The costly burden of musculoskeletal conditions

> "In these economically challenging times, this research highlights a clear area of focus for policymakers where prioritisation of musculoskeletal disorders could result in longer-term cost-efficiencies."
>
> *Dr Anjte Van Der Zee-Neuen, Maastricht University, Maastricht, the Netherlands*

HEALTHCARE costs are 50% higher in people suffering from musculoskeletal conditions (MCs) than any other diseases, it was revealed in a thought-provoking study.

The new study assessing the impact of the number of diseases an individual has, and its burden in terms of total healthcare costs, was carried out using 8,904 participants.

The significant impact was highlighted especially in the co-existence of two conditions where one of the two conditions is musculoskeletal in nature, making the cost 36% higher for those individuals. Statistically, chronic diseases in Europe account for approximately 70-80% of all healthcare costs – a significantly high proportion is attributed to MCs.

It was also noted that as the number of comorbidities in an individual increased, there was a relatively sharp increase in healthcare costs. Data have revealed that MCs, whether alone or as comorbidity, showcase a higher cost impact than any other disease condition.

The study gathered data on a wide spectrum of self-reported physician-diagnosed diseases (such as MCs, diabetes, cardiovascular diseases, cancer, etc.) along with their healthcare usage. Reference prices from the Dutch manual for pharmaco-economic healthcare evaluations 2010, which was adjusted according to inflation, were utilised to calculate the total healthcare cost. Healthy individuals were used as the reference point in the investigation.

MCs comprise over 150 conditions that affect the body's muscles, ligaments, bones, nerves, joints, etc, which cause pain and hinder daily activities. Some commonly known examples include rheumatoid arthritis, osteoarthritis, osteoporosis, and lower back pain.

"It is clear that the cost of delivering care to those patients with musculoskeletal conditions is considerably higher than those with other diseases. In these economically challenging times, this research highlights a clear area of focus for policymakers where prioritisation of musculoskeletal disorders could result in longer-term cost-efficiencies," said Dr Anjte Van Der Zee-Neuen, Division of Rheumatology, Maastricht University, Maastricht, the Netherlands.

# The big cure to rheumatoid arthritis disease activity

EARLY aggressive treatment of rheumatoid arthritis (RA) is gathering momentum as obese patients display higher disease activity scores (DAS) than non-obese patients, regardless of their disease stage.

Severely reducing DAS using a treat to target approach has been shown to be more effective in triggering clinical remission through decreased disease activity than standard care. This diminishing effect is catalysed by obesity in patients, who have boosted DAS, thus, entailing a more drastic attack by treat to target than in non-obese patients.

"Increasing levels of body fat are associated with heightened production of proinflammatory signalling proteins and raised levels of inflammatory markers. This systemic inflammation could inflate standard DAS and mean that obese patients receive more aggressive treatment than their non-obese counterpart," said Dr Christopher Sparks, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK.

Early RA (eRA; disease duration <12 months) and established RA (disease duration ≥12 months) cohorts were identified using clinical data from an international RA database. Patient demographics, DAS28 and body mass index (BMI) were gathered from the first recorded visit on the database.

The cohorts were categorised into five groups according to their BMI: 1) Underweight <18.5; 2) Normal 18.5-24.9; 3) Overweight 25-29.9; 4) Obese 30-34.9; and 5) Obese II ≥35. The primary outcome measure of the study was high disease activity, i.e. DAS28 of >5.1.

3,534 subjects with a mean age of 54.7 were examined, 72.5% of whom were female. The eRA cohort involved 1,553 patients with <1 year of disease duration; median disease duration was 7.2 years in the established RA cohort. Both the eRA and established RA cohorts shared similar distributions of BMI categories with a mean BMI of 27.1 and 26.8, respectively.

An inverse association between increasing BMI and lower levels of radiographic joint damage was indicated, reinforced by a repeat of the analysis in the established RA cohort.

"Not only do these results provide an explanation for the paradoxical relationship between BMI and disease outcome in RA, they clearly support the benefit to all RA patients of early and aggressive treatment," concluded Dr Sparks.

Further research is required to assess if obese patients, for whom a potentially more harmful higher dose could offer greater protection of joints, experience a higher efficacy of treat to target in the long term.

# Giant cell arteritis, a pain in the head

CRANIAL ultrasound (US) may have overtaken temporal artery biopsy (TAB) in the diagnosis of giant cell arteritis (GCA), possessing superior sensitivity and a comparable specificity.

"Although TAB has historically been considered the gold standard diagnostic test for GCA, the exciting results of this new study suggest cranial US may soon replace TAB in the assessment of patients with a suspected diagnosis of GCA in routine clinical practice," said Dr Adam Croft, lead author and Clinical Fellow in Rheumatology, University of Birmingham, Birmingham, UK.

GCA is a condition that involves the inflammation and narrowing of medium and large arteries in the head and neck. This is known to result in severe symptoms such as painful headaches and blindness due to occlusion of the artery supplying the back of the eye.

It is therefore essential that a prompt, accurate diagnosis of GCA is made, and treatment with high dose steroids begins as soon as possible.

The current diagnostic tool is to perform a TAB, which is a minor, invasive procedure involving the removal of arteries in the head. It is extremely inaccurate at diagnosing GCA, diagnosing only 58% of confirmed clinical diagnoses after 3 months.

However, future GCA sufferers have reason to be optimistic; a new approach known as cranial US promises to replace traditional diagnostic tools to become the new gold standard. This new procedure diagnosed an impressive 96% of confirmed clinical diagnoses at 3 months, a figure that shows that this new approach will be able to reduce the number of patients that are given potentially harmful steroids unnecessarily.

"Being able to reliably confirm the diagnosis is important not just to ensure those patients with GCA receive high-dose steroids to help prevent blindness, but also to prevent patients who do not have GCA continuing high-dose steroid therapy unnecessarily. High doses of steroids can cause a variety of unpleasant side-effects including weight gain, infection risk, osteoporosis and fracture risk, high blood pressure, diabetes, cataracts," added Dr Croft.

It has also been shown that prompt treatment of GCA with steroids is essential to prevent permanent loss of vision. In one study, 14% of patients permanently lost their vision because of GCA, and in 94% of these patients the visual deficit developed before steroid therapy had even begun.

# Impact of diet and disease activity on arthritis

INFLUENCE of the relationship between dietary intake of monounsaturated fatty acids (MUFAs) and cholesterol with disease activity in rheumatoid arthritis (RA) and osteoarthritis (OA) respectively, has been investigated in two independent studies.

In the assessment of disease activity in RA, the TOMORROW study comprised of 208 RA participants and 205 healthy volunteers whose daily food and nutrient intake was monitored by means of diet history. Reference results from the control group were calculated using the Mediterranean diet scores.

It was revealed that dietary intake of MUFAs was an independent predictor of remission in RA patients. The proportion of MUFAs to saturated fatty acids was considerably lower in RA patients with higher disease activity than those in remission and those with low disease activity.

"Confirming that daily intake of monounsaturated fatty acids, as a component of the Mediterranean diet, is an independent predictor of remission in patients with RA suggests that MUFAs might actually be suppressing disease activity," Mr Yoshinari Matsumoto, Department of Medical Nutrition, Graduate School of Human Life Science, Osaka City University, Osaka, Japan.

A previous study postulated that the adoption of the Mediterranean diet in RA patients is associated with abundant benefits such as the reduction of inflammatory activity, surge in physical function, and improved vitality.

"Confirming that daily intake of monounsaturated fatty acids, as a component of the Mediterranean diet, is an independent predictor of remission in patients with RA suggests that MUFAs might actually be suppressing disease activity."

*Mr Yoshinari Matsumoto, Osaka City University, Osaka, Japan*

Cholesterol influence on disease activity was evaluated in mice models with OA who were apolipoprotein E (ApoE) deficient (models with very high levels of low-density lipoprotein [LDL] cholesterol), and were compared to their healthy counterparts. The ApoE mice fed on a normal diet had significantly higher LDL levels than the healthy mice. On the cholesterol-rich diet, the ApoE mice had developed increased synovial thickening and ectopic bone formation.

"With one of the characteristics of metabolic syndrome being increased cholesterol levels, research has been ongoing into clarifying the relationship between cholesterol and OA pathology," explained Dr Wouter de Munter, Department of Experimental Rheumatology, Radboud University Medical Centre, Nijmegen, the Netherlands.

# New drug shows significant increase in bone density

ROMOSOZUMAB has shown to significantly increase bone mineral density and bone content when compared with the current standard of care, teriparatide.

Increasing bone mineral density and bone content is a result that will be of great interest to postmenopausal women with low bone mass, and those suffering from osteoporosis (approximately 30% of postmenopausal women), of which 40% go on to sustain one or more fragility fractures in their lifetime.

"The use of teriparatide, an effective anabolic agent which can stimulate bone growth and reduce the risk of fractures, is potentially problematic due to the requirement for daily subcutaneous injection, its relatively high cost, and also a black-box warning about the risk of inducing osteosarcoma in rats," said Prof Harry Genant, Professor Emeritus of Radiology, University of California, and Co-founder, CCBR Synarc, Inc., San Francisco, California, USA.

Another noteworthy advantage that romosozumab has over teriparatide is that it only needs to be administered by injection at monthly intervals over 12 months, whereas teriparatide requires subcutaneous delivery every day.

Experimental results for romosozumab were largely positive. In the lumbar spine, however, treatment with the new drug only managed to produce a similar significant gain in trabecular bone density compared to teriparatide. Conversely, at the hip, romosozumab was able to produce significantly higher bone mineral density gains than that which was seen using teriparatide.

Cortical bone mineral density gains were larger with romosozumab compared with teriparatide at both the lumbar spine and hip. Mineral gains were also larger with romosozumab compared with teriparatide at both the lumbar spine and hip.

Hip, spine, and wrist are commonly subjected to fractures in women with postmenopausal osteoporosis. Intense back pain and deformity are the result of vertebral fractures, while in elderly patients, hip fracture can cause loss of independence and possible death.

"A large Phase III clinical trial programme is underway, evaluating romosozumab against both placebo and an active comparator in more than 10,000 women with postmenopausal osteoporosis to evaluate its potential to prevent osteoporotic fractures, and to confirm its safety for long-term use," said Prof Genant.

"The use of teriparatide...is potentially problematic due to the requirement for daily subcutaneous injection, its relatively high cost, and also a black-box warning about the risk of inducing osteosarcoma in rats."

*Prof Harry Genant, University of California, San Francisco, USA*


# DNA methylation holds the key to faster treatment for rheumatoid arthritis

A POTENTIAL biomarker of response to etanercept and adalimumab in rheumatoid arthritis (RA) patients has been identified in the form of DNA methylation.

Methylation of genomic DNA has long been known to play a role in many different diseases such as Rett syndrome and Prader-Willi syndrome. However, whether DNA methylation, or epigenetics in general, play a role in RA has remained largely unknown and unstudied.

Until now, RA sufferers have been treated with two different anti-tumour necrosis factor (anti-TNF) therapies – etanercept and adalimumab. These have undeniably proved a huge advance for the treatment of RA and have transformed the treatment of inflammatory arthritis for millions of people around the world. However, only 20-40% of patients achieve a good response to the therapy.

"It can take several years to identify the most effective treatment for an RA patient. This is not only costly in terms of the financial burden, but also in terms of patient outcomes and the irreversible joint damage that is being done," said Ms Amy Webster, postgraduate research student, University of Manchester, Manchester, UK.

To examine the degree of correlation between DNA methylation and RA, a study was conducted using patients from the Biologics in Rheumatoid Arthritis Genetics and Genomic Study Syndicate (BRAGGSS) longitudinal cohort, which were judged to have exhibited an extreme response phenotype after 3 months of treatment with either drug.

DNA from each patient was sampled before the therapy was initiated, before an epigenome-wide association study was conducted using HumanMethylation450 (Illumina) to determine methylated/unmethylated cytosines within the DNA.

Regarding the etanercept arm of the study, the most differentially methylated position is mapped onto the LRPAP1 gene. Interestingly, this gene is known to encode a chaperone of low-density lipoprotein receptor-related protein 1, which influences tumour growth factor-beta activity by assisting the nascent glycoprotein during the folding stage.

In the adalimumab patients, the most differentially methylated position maps to the PDZD8 gene. Joint analysis of the two drugs together identified a differentially methylated region overlapping the CRYZ and TYW3 genes, which have previously been associated with inflammation and Type 2 diabetes.

Ms Webster also mentioned: "Because of this, the identification of biomarkers that can predict a patient's response to a treatment is an important area of research, which will allow the most effective treatment for each patient to be identified early in the course of the disease."

# CT-P13 shows unparalleled efficacy for biosimilars

GAME-CHANGING biosimilar monoclonal antibody CT-P13 has shown exquisite similarity to the imitated drug infliximab (IFX). This cost-effective alternative to infliximab for the treatment of ankylosing spondylitis (AS) is capable of demonstrating similar levels of efficacy and safety.

"The challenge for biosimilars is to demonstrate similarity in therapeutic effectiveness, safety, and immunogenicity to their reference product, not just biochemical and pharmacokinetic equivalence," said Dr Won Park, lead investigator of the PLANETAS study, Inha University Hospital, Incheon, South Korea.

AS is a form of chronic arthritis that affects approximately 1.4 million (0.19%) patients in Europe, it causes inflammation in the joints and ligaments which, in turn, causes pain and stiffness in the neck, back, and buttocks.

Unfortunately, the cause of AS remains a mystery; therefore, current treatments are focused on merely alleviating some of the symptoms by keeping the spine as flexible as possible.

To investigate the effectiveness of this new IFX biosimilar, the programme evaluating the autoimmune disease investigational drug (PLANETAS) study was founded. This study captured measures of clinical disease activity using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), captured levels of disability using the Bath Ankylosing Spondylitis Functional Index (BASFI), and captured levels of mobility via the Bath Ankylosing Spondylitis Metrology Index (BASMI).

Results were highly positive, demonstrating significant improvements and similarities when clinical disease activity was measured using BASDAI (from 6.76 to 3.78 for CT-P13, and from 6.57 to 3.70 for IFX), BASFI (from 6.20 to 3.42 for CT-P13, and from 6.24 to 3.46 for IFX), and also BASMI (from 4.0 to 2.8 for CT-P13, and from 4.1 to 3.2 for IFX).

Dr Park seemed optimistic about the results, saying: "By demonstrating comparable efficacy and safety, the results of our clinical trials should give physicians confidence in using CT-P13 as an alternative treatment option to INX in AS patients." He also added: "This is good news for patients who may previously have had limited access to costly antibody biopharmaceuticals."

This investigation not only gives hope to sufferers of AS, but also breeds confidence in biosimilars, which hitherto have been unable to demonstrate appropriate efficacy and/or safety standards.

"By demonstrating comparable efficacy and safety, the results of our clinical trials should give physicians confidence in using CT-P13 as an alternative treatment option to INX in AS patients."

*Dr Won Park,*
*Inha University Hospital,*
*Incheon, South Korea*

# ANNUAL EUROPEAN CONGRESS OF RHEUMATOLOGY 2014

## LE PALAIS DES CONGRÈS DE PARIS
## PARIS, FRANCE
## 11TH-14TH JUNE 2014

# 2014 EULAR ABSTRACT AWARDS

The EULAR Opening Plenary Session honoured the lead authors of 12 outstanding abstracts selected for the 2014 Abstract Awards. These exciting young talents were recognised for their achievements in planting the stepping stones to future knowledge and success in their areas of expertise. The authors of the top three Health Professional abstracts and the best 'People with Arthritis/Rheumatism in Europe (PARE)' abstracts were also awarded.

## CLINICAL RESEARCH ABSTRACT WINNERS

- **Dr Christian Beyer**, Germany

Identified differentially expressed circulating miRNAs in osteoarthritis patients necessitating arthroplasty in a large, population-based cohort.

- **Mr Ahmad Osailan**, UK

Identified predictive factors associated with cardiac parasympathetic activity in patients with rheumatoid arthritis.

- **Dr Hubert de Boysson**, France

Investigated the use of 18F-fluorodeoxyglucose PET scanning to diagnose patients with giant cell arteritis.

- **Dr Luca Quartuccio**, Italy

Reported long-term success with rituximab over a mean follow-up period of ~70 months in ~two-thirds of patients with cryoglobulinaemic vasculitis associated with hepatitis C infection.

- **Dr Pilar Brito-Zerón**, Spain

Identified predictive factors for lymphoproliferative disease in patients with primary Sjögren's syndrome.

- **Dr Marko Yurkovich**, Canada

Led first general population-based study determining risk of myocardial infarction and cerebrovascular accident in Sjögren's syndrome.

## BASIC SCIENCE ABSTRACT WINNERS

- **Dr Stephan Blüml**, Austria

Demonstrated that microRNA 146 has an important anti-inflammatory role, which may be exploited for therapeutic purposes in inflammatory arthritis.

- **Dr Seokchan Hong**, South Korea

Investigated the role of interleukin-32 on proliferative bone formation in ankylosing spondylitis patients via their effect on osteoblast differentiation.

- **Dr Francesco Ciccia**, Italy

Demonstrated that gut-derived innate lymphoid cells are widely found in the peripheral blood, synovial fluid, and inflamed bone marrow of ankylosing spondylitis patients.

- **Ms Meghna Jani**, UK

Identified single nucleotide polymorphisms within several genes of patients with dermatomyositis.

- **Dr Elisa Corsiero**, UK

Showed that B cells from the synovium of rheumatoid arthritis patients who have synovial tertiary lymphoid structures are highly mutated and locally differentiated.

- **Dr Johanne Martel-Pelletier**, Canada

Demonstrated that bone-specific overexpression of the EPhB4 receptor can prophylactically protect subchondral bone during the process of osteoarthritis.

## HEALTH PROFESSIONAL AND PARE AWARDS

**Ms Dawn Johnson**, UK

Led first research into evaluation of effects of the Educational Needs Assessment Tool in educating people with rheumatoid arthritis.

**Mr Paul D. Kirwan**, Ireland

Examined the ability of a physiotherapist with special training in rheumatology to accurately recognise and diagnose inflammatory joint disease.

**Dr Ingrid Larsson**, Sweden

Determined that a nurse-led rheumatology clinic is safe and effective in monitoring biological therapy in stable patients with chronic inflammatory arthritis.

**Mr Rolf Greiff**, Sweden

First ever PARE abstract award winner for a description of the organisation's work in implementing rheumatoid arthritis instructor training programmes.

# OSTEOARTHRITIS: THE CHALLENGE OF ESTABLISHING A PERSONALISED TREATMENT

## Summary of Presentations from the TRB Chemedica-Sponsored Satellite Symposium held at the Annual European Congress of Rheumatology - EULAR, Paris, France, on 11th June 2014


**Chairpersons**

**Johanne Martel-Pelletier,[1] Antonello Pietrangelo[2]**

**Speakers**

**Francis Berenbaum,[3] Jean-Pierre Pelletier,[4] Burkhard Leeb[5]**

*1. Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Montreal, Canada*

*2. Unit of Internal Medicine and Metabolic Diseases, Centre for Hemochromatosis University Hospital, Modena, Italy*

*3. University Pierre & Marie Curie, INSERM UMR-S938, AP-HP Saint-Antoine Hospital, Paris, France*

*4. Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Montreal, Canada*

*5. 2nd Department of Medicine, Centre for Rheumatology, Lower Austria, State Hospital Stockerau, Karl Landsteiner Institute for Clinical Rheumatology, Stockerau, Austria*



**Disclosure:** J. Martel-Pelletier: shareholder of ArthroLab Inc. and consultant for AbbVie, Bioiberica, Merck & Co, Servier, and TRB Chemedica. A. Pietrangelo has not declared any disclosure. F. Berenbaum: consultant for Pfizer, Expanscience, Servier (and research grant), Sanofi, and AbbVie, and symposia for Rottapharm, IBSA, Genevrier, Bioiberica, and TRB Chemedica (and research grant). J-P. Pelletier: shareholder of ArthroLab Inc. and consultant for Bioiberica, Elanco, Endocyte, Ferring, Merck & Co, Pfizer, Servier, and TRB Chemedica. B. Leeb: participated in clinical trials sponsored by IBSA, Servier, Tropon, and received honoraria from IBSA, TRB Chemedica, Servier,CSC, Sanova, Bayer, Lacer, Fidia.

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## MEETING SUMMARY

The symposium, co-chaired by Prof Johanne Martel-Pelletier and Prof Antonello Pietrangelo, opened with a discussion of metabolic syndrome-associated osteoarthritis (OA) and a brief overview of treatment options by Prof Francis Berenbaum. Prof Jean-Pierre Pelletier then discussed the use of symptomatic slow-acting drugs for OA (SYSADOA) for knee OA within the context of the new European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) recommendations. Finally, Dr Burkhard Leeb concluded the session by discussing the indications, contraindications, and side-effect management of diacerein.

# Metabolic Syndrome-Associated OA: A New Phenotype for Targeted Management

Professor Francis Berenbaum

OA is a consequence of the build-up of cartilage degradation products that move to and inflame the synovial membrane.[1] The synovial membrane responds by releasing several mediators including proinflammatory factors such as tumour necrosis factor (TNF) and interleukins ([IL]-1, IL-6, IL-8, etc.) and matrix metalloproteinases (MMPs) (Figure 1). These mediators further degrade the cartilage creating a vicious cycle. Moreover, soluble mediators are also released at the interface between subchondral bone and cartilage leading to degradation in the deep layer of cartilage.

Currently, OA phenotypes are location based, for instance in the knee, hip, hand, and spine. However, there is an emerging paradigm to consider OA phenotypes based on their risk-factors, therefore offering the potential for targeted therapies. These phenotypes include (not exhaustively) post-traumatic OA, metabolic syndrome (MetS)-associated OA, and age-related OA.[2] Typically a post-traumatic OA patient is aged <45 years with OA in the knee, hip, ankle, or shoulder caused by repetitive mechanical stresses or by a unique acute joint trauma (joint fracture, meniscectomy, etc.). Appropriate interventions include joint protection and stabilisation, prevention of falls, and surgery. The typical MetS-OA patient is aged between 45–65 years with generalised OA, overweight or obese, with at least one of the component of the MetS (diabetes mellitus, hypertension, dyslipidemia). Adipokines, insulin resistance, systemic low-grade inflammation, and lipid toxicity are suggested as triggers for initiation of the OA process. Interventions include weight loss, glycaemia, or lipid control, as appropriate. Age-related OA affects patients >65 years in the hip, knee, or hand without any history of trauma or MetS. It could be due to the accumulation of advanced glycation end products (AGEs) or to chondrocyte senescence. There are no specific interventions; however, soluble receptor for AGE (sRAGE)/AGE breakers may be a potential target.

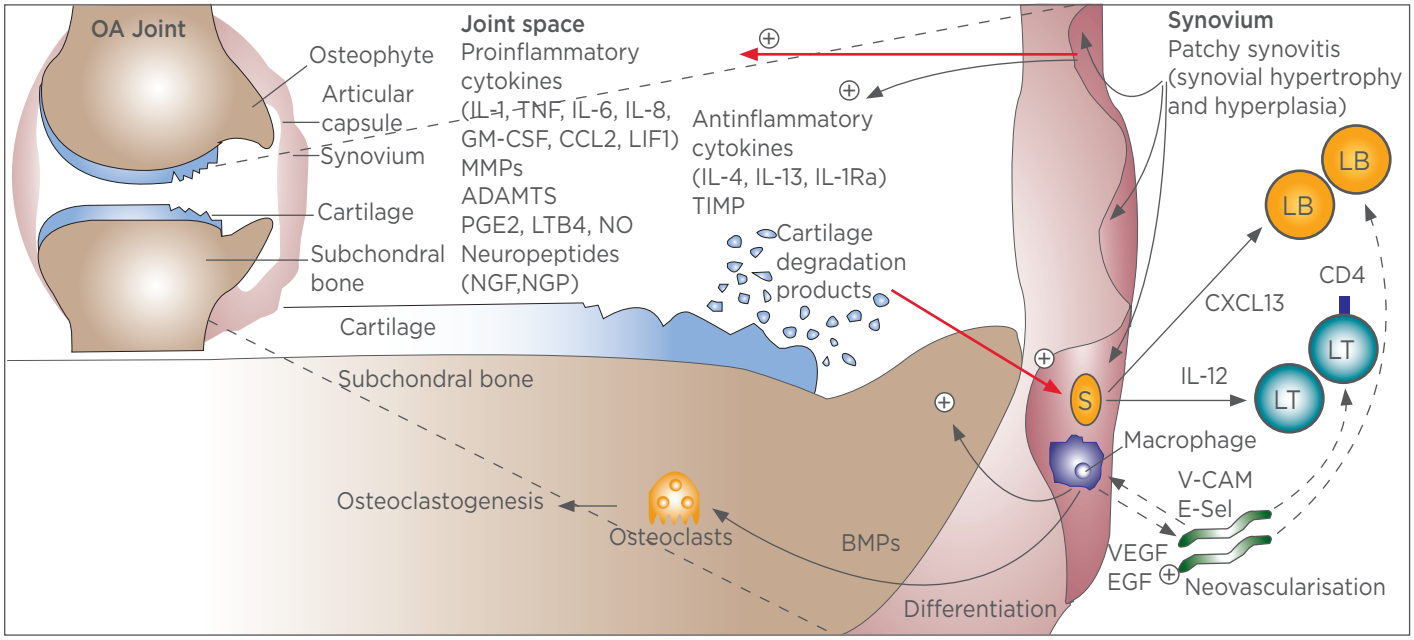


**Figure 1: The pathophysiology of osteoarthritis.**

ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; BMP: bone morphogenetic protein; CCL2: CC-chemokine ligand 2; CXCL13: CXC-chemokine ligand 13; EGF: endothelial growth factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; IL-1Ra: IL-1 receptor antagonist; LIF: leukaemia inhibitory factor; MMP: matrix metalloproteinase; NGF: nerve growth factor; NO: nitrous oxide; OA: osteoarthritis; PGE2: prostaglandin E2; TNF: tumour necrosis factor; vCAM: vascular cell adhesion molecule; vEGF: vascular endothelial growth factor.

*Modified from Sellam J et al.*[1]

Obesity conveys a variable risk of OA depending on the area affected. The relative risk (RR) for the incidence of OA in the knee of a patient with a body mass index (BMI) of 30–35 kg/m$^2$ versus a BMI <25 kg/m$^2$ is 2.4 (95% CI 1.0–5.8), and the RR of progression in a patient with a BMI >25 kg/m$^2$ versus <22 kg/m$^2$ is 2.6 (95% CI 1.0–6.8). The RR for the incidence of OA in the hip of a patient with a BMI >28 kg/m$^2$ versus <24 kg/m$^2$ is 1.9 (95% CI 1.1–3.3).[3-5] OA in the hand is also more frequent in obese patients with a RR of 1.9,[6] despite the mechanical stresses of obesity on the weight-bearing joints, suggesting an alternative mechanism is at play. Adipokines are thought to circulate and accumulate in the joints, causing cartilage degeneration. This phenotype is termed obesity-related or obesity-induced OA. Recent research not only implicates obesity but also metabolic components. MetS is defined as an obese patient having two or more of the following: raised triglycerides (≥150 mg/dL [1.7 mmol/L]); reduced HDL cholesterol (<40 mg/dL [1.03 mmol/L] in males and <50 mg/dL [1.29 mmol/L] in females); raised blood pressure (systolic ≥130 mmHg, diastolic ≥85 mmHg); or raised fasting plasma glucose (≥100 mg/dL [5.6 mmol/L]). The Research on Osteoarthritis Against Disability (ROAD) study followed 1,384 patients for 3 years and showed that the risk of occurrence and progression of knee OA increased in line with the number of MetS components,[7] as did the incidence of knee replacements in 13,753 patients from the Australian Orthopaedic Association National Joint Replacement Registry, even after controlling for weight.[8]

Over 50% of metabolic OA patients suffer from hypertension suggesting that, in addition to insulin resistance, hypertension of the vessels in the subchondral bone, possibly combined with lipid abnormalities of the subchondral bone or the synovial membrane, may increase the risk of OA. Data from the third National Health and Nutrition Examination Survey (NHANES III) show that 28% of OA patients receiving medication for hypertension have diabetes, 12% have angina, 12% have congestive heart failure, 11% had a previous cardiovascular (CV) accident, and 26% have coronary heart disease.[9] Such comorbid CV problems complicate treatment with non-steroidal anti-inflammatory drugs (NSAIDs) as they increase blood pressure by between 3 and 5 mmHg.[10] Since blood pressure is a surrogate marker for ischaemic heart disease,[11] increases such as those caused by NSAIDs increase the risk for CV events. The relationship between myocardial infarction (MI) and NSAID use was investigated in the Taiwanese national health insurance claim database.[12] The risk of an NSAID-induced MI was higher in patients with a prior hypertension diagnosis (n=3,672) than those without (n=4,682). The adjusted odds ratio (OR) was 1.56 (95% CI 1.36–1.79) versus 1.32 (95% CI 1.15–1.51) for oral NSAIDs and 3.43 (95% CI 2.30–5.13) versus 3.18 (95% CI 2.08–4.87) for parenteral NSAIDs. Studies such as these form the basis of the FDA and EMEA special warnings governing NSAID use in patients with, or at risk of, CV disease.

Paracetamol (acetaminophen) is a widely used alternative to NSAIDs and it is now known to raise blood pressure in patients with coronary artery disease after 2 weeks of treatment at a dose of 3 g per day.[13] Opioid analgesics offer another alternative but they are poorly tolerated in older patients with OA as they can cause dizziness, which can, in turn, cause more severe adverse events (AEs). For instance, the risk of fracture is increased by 4.47 (95% CI 3.12–6.41) compared to NSAIDs,[14] which can lead to death in older patients. Therefore, the use of paracetamol and opioids should be considered carefully in OA.

There are many unmet needs in MetS-OA; 63% of patients and 73% of general practitioners are dissatisfied with current treatments.[15] Multiple agents are often required and 53% of OA patients switch to a second NSAID within the first 2 months.[16] Lack of efficacy is the most common reason (33%) with 13% switching due to AEs.[17] There is indeed a critical need for safer treatments in this particular and frequent OA phenotype for which SYSADOA may be an alternative.

Looking to the future, data from the ongoing TRB Chemedica-sponsored DIGItal Cohort Osteoarthritis Design study (DIGICOD) will provide valuable information on risk-based OA phenotypes. DIGICOD will study as many as 500 hand OA patients using radiography and clinical follow-up for 6 years.

## Use of SYSADOA in the Treatment of OA. Who to Treat and How?

**Professor Jean-Pierre Pelletier**

OA is one of the most common chronic diseases, affecting 9.6% of men and 18.0% of women over

the age of 60. The main symptom is pain, with joint tenderness and stiffness being accompanied by limitation of movement. Problems arise due to the large number of patients requiring long-term disease management using a limited number of therapeutic options. Management of OA is complicated by the nature of disease, the severity and number of joints involved, the age of patients, and the fact that pain levels are not always related to structural changes. Furthermore, concomitant disease may influence levels of symptoms. There is also a high variability of both response to treatment and side-effects, and many patients require combined therapy.

OA treatment guidelines have been proposed by the EULAR,[18] the American College of Rheumatology (ACR),[19] the Osteoarthritis Research Society International (OARSI),[20] and the National Institute for Health and Care Excellence (NICE);[21] however, most do not prioritise intervention sequence, are not 'user-friendly', and offer no provision for combined treatment. The ACR guidelines do not make 'strong recommendations' for any particular pharmacological intervention as many of the drugs listed in Europe are not available in the US.[19] The OARSI 2014 guidelines are considered restrictive compared with previous versions and most interventions are classified as uncertain.[22] The recommendations for patients with comorbidities are limited to topical NSAIDs and intra-articular steroids combined with biomechanical interventions, but these are suboptimal for most patients.

Recently, ESCEO formed a working group to propose new guidelines for the treatment of knee OA.[23] The main objective of these new guidelines was the development of a comprehensive, user-friendly OA treatment algorithm allowing prioritisation of interventions using a stepwise approach. The working group consisted of ten rheumatologists, two clinical epidemiologists, and a clinical scientist experienced in OA randomised controlled trial (RCT) design, analysis, and interpretation. The group performed a comprehensive literature search of all interventions considered by current guidelines until February 2014. A draft algorithm recommendation was made in a one-day meeting and a final consensus was reached after three rounds of electronic consultation. The basic principles consist of the need for a combined pharmacological and non-pharmacological treatment, with a core set of initial measures, including patient information access and education, weight loss if necessary, and an appropriate exercise programme.

Four multimodal steps are then established. Step 1 consists of either non-pharmacological or pharmacological background therapy. Non-pharmacological approaches include referral to a physical therapist for realignment treatment if needed and sequential introduction of further physical interventions initially, and at any time thereafter. Pharmacological interventions consist of paracetamol on a regular basis or chronic SYSADOA (e.g. prescription glucosamine sulphate and/or chondroitin sulphate) with paracetamol as needed; topical NSAIDs or capsaicin are added in the still symptomatic patient. Step 2 consists of advanced pharmacological management in the persistent symptomatic patient and centres on the use of cycles of oral COX-2 selective or non-selective NSAIDs, chosen based on concomitant CV, gastrointestinal (GI) or renal risk factors,[20] with intra-articular corticosteroids or hyaluronate used for further symptom relief, if required. There are no age restrictions on the use of NSAIDs as many patients over the age of 75 still benefit from their use in a controlled environment. In Step 3, the last pharmacological attempts before surgery are represented by weak opioids and other central analgesics such as duloxetine. Finally, if symptoms are severely impacting on quality of life, Step 4 consists of end-stage disease management and surgery, with classical opioids as an alternative when surgery is contraindicated. However, the risks of CV events, falls, and fractures leading to increased mortality,[14] discussed above, mean that opioids and narcotics should only be used in knee OA if all other therapeutic options have been exhausted.

The SYSADOA diacerein is recommended by both the EULAR[18] and OARSI[20] guidelines. The OARSI guidelines conclude that diacerein provides a small, but statistically significant, benefit on pain versus placebo (effect size [ES =0.24, 95% CI 0.08–0.39), which compares favourably to the ES versus placebo for paracetamol and NSAIDs of 0.14 (95% CI 0.05–0.22) and 0.29 (95% CI 0.22–0.35), respectively.[20] However, despite a significant risk of diarrhoea (RR=3.5), the guidelines conclude that diacerein is a safer alternative to NSAIDs.[20] Furthermore, the efficacy of diacerein for the treatment of OA symptoms and improvement of function has been demonstrated in four major

meta-analyses that also noted a therapeutic carry-over effect for up to 2-3 months after the cessation of medication.[24-27]

A study of 168 knee OA patients clearly demonstrated the clinical relevance of diacerein using OMERACT-OARSI 2004 responder criteria,[28] which are particularly meaningful for patients as they require ≥20% improvement and absolute change ≥10% in at least two of the three following areas: pain, function, and patient's global assessment. A post-hoc analysis of five major clinical trials[28-32] has assessed the clinical relevance of diacerein using the Minimum Clinically Important Improvement (MCII) and Patient Acceptable Symptom State (PASS) scales.[33] All trials demonstrated an increase in mean pain improvement (MCII) and all but one, a dose-finding study,[32] demonstrated a reduction in mean pain level (PASS).

The carry-over effect was demonstrated in a 16-week study, comparing diacerein with piroxicam 20 mg/day in knee OA patients.[34] Both groups achieved around an 80% reduction in pain by week 16. However, in the 2 months following the cessation of treatment, pain increased in the piroxicam group but was still 30% of baseline levels, whereas, in the diacerein group the pain levels remained lower at about 80% of baseline levels ($p<0.0001$). In the 16-month observational PEGASE study of knee OA, patients treated with NSAIDs plus SYSADOAs experienced a significant reduction in pain 4-8 months after starting treatment (OR 0.72, 95% CI 0.56–0.92) and a significant improvement in function after 8 months of starting treatment (OR 0.77, 95% CI 0.60–0.99).[35]

In summary, diacerein is superior to placebo; it has significant clinical benefits similar to NSAIDs and provides a good alternative to these drugs with a carry-over effect. SYSADOAs improve pain and function and represent the most useful and logical drug treatment for symptomatic OA.

## Diacerein: New Recommendations for Treatment Optimisation

### Doctor Burkhard Leeb

The EMA and the Pharmacovigilance Risk Assessment Committee (PRAC) recently reviewed data on diacerein and confirmed the safety profile established in 1997 by the French Medicines Agency. To improve the benefit/risk ratio of diacerein, PRAC recommended that treatment with diacerein should be initiated by an experienced OA physician. They also recommended that diacerein should be indicated for patients with OA of the hip or knee where the most data have been generated, but it is not recommended for patients with rapidly progressive hip OA as they may respond only weakly to diacerein. According to the EULAR recommendations, a diagnosis of knee OA can be made based on observations of typical symptoms without the need of costly examinations, laboratory tests, or imaging.[36]

As mentioned by the previous speaker, the efficacy and carry-over effect of diacerein treatment was demonstrated in several well-designed controlled clinical studies and meta-analyses.[24,26,28] The Bartels meta-analysis concluded that diacerein could be an alternative therapy for OA patients who cannot take paracetamol or NSAIDs because of AEs such as CV or GI disorders.[26] Soft stools and diarrhoea are the most frequent side-effect of diacerein and usually occur during the first 2-4 weeks of treatment but abate with continued use. To minimise the risk of diarrhoea, PRAC advise starting treatment with a single 50 mg dose daily for the first 2-4 weeks, after which the recommended dose is 50 mg twice daily. Furthermore, PRAC advise that diacerein should no longer be recommended in patients >65 years of age because they are more vulnerable to complications associated with severe diarrhoea. However, if diacerein is prescribed in these patients without problem, no change in the usual recommended dose is necessary. In cases of unusually frequent liquid or watery stools, diacerein should be stopped and alternative treatments discussed with the prescribing physician. Diacerein is contraindicated in patients with inflammatory intestinal disease (ulcerative colitis or Crohn's disease), intestinal obstruction or pseudo-obstruction, and painful abdominal syndromes of undetermined cause. Patients should avoid laxatives and those who need them should be strictly monitored. Care should be exercised with patients taking diuretics and those with low potassium levels.

Although very rare, hepatic disorders may occur during treatment with diacerein. During clinical trials these disorders were infrequent (0.5%), mostly mild, and usually were reversible transaminase increases. Of note, drug-induced liver injury was rare (0.03%). Since the launch of diacerein in

1994, there has been one documented case of acute hepatitis in a 65-year-old woman who recovered after diacerein treatment was stopped and one case of fatal hepatitis in a 68-year-old man also taking NSAIDs and other undocumented drugs.[37,38] The incidence of liver disorders is approximately 1.68 liver disorders per 100,000 patient-years of treatment (PY-T), which compares favourably with data for NSAIDs and paracetamol. Data for NSAIDs show incidences of 10.0 clinically apparent liver injuries and 23.4 liver injuries resulting in hospitalisation per 100,000 PY-T.[39,40] The rate of transplantation due to paracetamol-induced acute liver failure is 0.33 per 100,000 PY-T.[41] Nevertheless, patients should limit their alcohol intake when taking diacerein. Another recommendation is that diacerein is contraindicated in people with past or present liver disease and patients should be screened for major causes of active hepatic disease. Care should be taken when prescribing other drugs known to cause hepatotoxicity. To manage the risks of hepatotoxicity, patients should be taught to recognise the symptoms and contact their physician immediately if they occur. Signs of hepatic injury should be monitored and treatment stopped if elevated hepatic enzymes or suspected signs or symptoms of liver damage are detected.

Diacerein has been marketed in Austria since 2004 where between 2,500-3,000 patients are treated each year with an average treatment duration of 4-5 months. In this period, three unexpected AEs were reported: one case of severe cramps in the calf muscle and foot pain without electrolyte imbalance, one case of vaginal discharge, and one case of loss of consciousness which was not related to diacerein or any drug.

In conclusion, diacerein is indicated for the symptomatic treatment of OA of the hip or knee. Its onset of efficacy is between 2-4 weeks after treatment start. During this time, paracetamol or NSAIDs may be co-prescribed to provide more immediate pain relief. Diacerein is contraindicated in liver disease and monitoring of liver function is recommended. Treatment should be started with a single daily dose of 50 mg, increasing to a twice-daily dose (100 mg daily). Due to the risks of dehydration associated with severe diarrhoea in elderly people, diacerein is no longer recommended in patients over 65 years. Risks and benefits should be considered for each patient and therapy should be individualised wherever possible. Patients with symptomatic hip or knee OA, who are refractory to paracetamol and for whom NSAIDs are contraindicated or ineffective, are ideal candidates for treatment with SYSADOAs, particularly diacerein.

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# INNATE IMMUNITY IN SYSTEMIC SCLEROSIS - ROLE OF TOLL-LIKE RECEPTORS, INTERFERON, AND THE POTENTIAL IMPACT OF VITAMIN D

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## ABSTRACT

Systemic sclerosis (SSc) is an autoimmune disease in which vascular damage and immune activation leads to excessive accumulation of extracellular matrix in the skin and internal organs. Although the focus has been on adaptive immunity in SSc, recent data suggest that innate immunity is critically important. The innate immune system, the first-line barrier against pathogens, modulates mechanisms which activate adaptive immunity. Dysregulation of the innate immune system and toll-like receptors (TLRs) may link immune abnormalities and fibrosis in SSc. TLR signalling pathways might induce production of Type I interferon (IFN) and other cytokines, and represent one of the mechanisms that initiate and develop autoimmunity and subsequent fibrosis. Vitamin D displays many immunomodulatory effects on both innate and adaptive immune responses. Active vitamin D will produce signals via vitamin D receptor and influences TLR stimulation, IFN response, and antimicrobial peptide production. Vitamin D deficiency has been associated with many autoimmune disorders, and can influence clinical phenotype and immune disorders in SSc patients.

Keywords: Systemic sclerosis, innate immunity, toll-like receptor, interferon, vitamin D.

## INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease in which vascular injury and inflammation are followed by an excessive accumulation of extracellular matrix (ECM) in the skin and internal organs, leading to organ dysfunction and premature death. The cause of SSc is due to an interplay between genetic and environmental factors that lead to breach of immune tolerance.[1-4] Immune activation is common in SSc and, although the focus has been on adaptive immunity,[5,6] recent evidence suggests that innate immunity is critically important.[1-3]

## AN OVERVIEW ON INNATE IMMUNE SYSTEM

The innate immune system constitutes the first-line barrier against pathogens that are present from birth, and protect the host between pathogen exposure and initiation of adaptive responses. Components of innate immune system include: physical barriers, enzymes, inflammation-related serum proteins (complement, C-reactive protein), antimicrobial peptides (AMPs: defensins, cathelicidins), pattern recognition receptors (PRRs: e.g. toll-like receptors [TLRs]), and cells (macrophages, mast cells, natural killer [NK] cells, neutrophils, dendritic cells [DCs]).[7]

The innate immune system recognises pathogens through PRRs, which are capable of distinguishing

**Table 1: Toll-like receptor localisation and ligands.**

| PRRs | Localisation | Ligand |
|---|---|---|
| TLR1 | Plasma membrane | Triacyl lipoprotein |
| TLR2 | Plasma membrane | Lipoprotein |
| TLR3 | Endolysosome | dsRNA |
| TLR4 | Plasma membrane | lipopolysaccharide |
| TLR5 | Plasma membrane | Flagellin |
| TLR6 | Plasma membrane | Diacyl lipoprotein |
| TLR7/8 | Endolysosome | ssRNA |
| TLR9 | Endolysosome | CpG-DNA |
| TLR10 | Endolysosome | Unknown |

PRR: pattern recognition receptor; TLR: toll-like receptor; dsRNA: double-stranded RNA; ssRNA: single-stranded RNA; CpG: cytosine guanine dinucleotide.

between self-tissues and pathogens by recognising pathogen-associated molecular patterns (PAMPs) such as components of membrane bacteria, unmethylated microbial DNA, and double-stranded RNA of viral origin.[8,9] When PRRs on cell surface bind PAMPs, they initiate phagocytosis, release toxic oxidants, and in macrophages, pathogen-derived proteins are processed into peptides and presented to major histocompatibility complex (MHC) molecules to engage and instruct antigen-specific T lymphocytes.[8] PRRs can be circulating proteins and receptors.

Circulating proteins include AMPs (e.g. defensins, cathelicidin LL-37), collectins, lectins, and pentraxins.[10] AMPs are important for skin and mucosal membrane protection and for killing phagocytosed organisms. Cathelicidins are released from neutrophils and epithelial cells, and exhibit antimicrobial activities. In both keratinocytes and macrophages, stimulation of CYP27B1, a member of cytochrome P450 superfamily of enzymes, converts vitamin D to its active form 1,25-dihydroxyvitamin D3 (1,25[OH]2D3), which induces the expression of the AMP cathelicidin LL-37.[11]

Receptors include TLRs, C-type lectin receptors, nucleotide-binding oligomerisation domain receptors (NOD-like receptors), and RIG-1-like receptors (RLRs).[10] Ten human TLRs (Table 1) have been identified as membrane proteins or expressed in endocytic vesicles. The ligands for these TLRs are represented by a wide variety of PAMPs, including microbial cell wall components, proteins, and nucleic acids. The final pathway for TLR signalling involves transcription factors that regulate a multitude of genes.[8,10] TLRs are expressed by many immune or non-immune cells such as fibroblasts, epithelial or endothelial cells, and can trigger the secretion of potent pro-inflammatory cytokines including TNF-α, interleukin-6 (IL-6), and pro-IL-1β. TLRs 3,4,7,8, and 9 can trigger production of Type 1 interferons (IFN).[12]

Cells of the innate immune system are phagocytes (neutrophils, monocytes, macrophages, DCs) and also other cells such as epithelial cells, mast cells, and platelets.[7] DCs migrate into lymphoid organs and peripheral sites, internalise microbial products, and molecules released from damaged tissue (called danger signals) present antigen to naïve T lymphocytes and induce their proliferation and activation.[13] PAMPs are potent inducers of DC production of IL-12 and IFN-α and β, and are key regulators of DC development and the Type 1 T helper (Th1) cell immune response. The effector T lymphocyte secretes IFN-γ that further primes DCs to produce great amounts of IL-12 (Figure 1).[14]

## VITAMIN D AND INNATE IMMUNITY

Vitamin D receptors (VDRs) are present on antigen-presenting cells, NK cells, as well as B and T lymphocytes. Vitamin D displays many immunomodulatory effects on both innate and adaptive immune responses.[15-18] Vitamin D has been shown to have a plethora of actions on immune system cells. Active vitamin D negatively

regulates DC differentiation, maturation, and immunostimulatory capacity; decreased MHC-II expression and downregulation of co-stimulatory molecule expression (CD40, CD80, CD86) lead to suppression of antigen presentation to T cells.[19] Moreover, acting on DCs, vitamin D reduces other proinflammatory cytokines such as IL-6 and IL-17, reduces nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation,[20] impairs TNF, IL-1, and IFN-γ secretion,[21] and upregulates anti-inflammatory mediators such as IL-4 and IL-10,[15-18] inducing tolerogenic DCs. Vitamin D enhances monocyte phagocytosis but induces hyporesponsivness to PAMPs, and induces defensins and cathelicidins.[17]

The active form of vitamin D induces synthesis of cathelicidin AMP (hCAP, LL-37) in monocytes/macrophages, as well as other cells such as keratinocytes.[22] Cathelicidins have direct chemoattractant properties for neutrophils, monocytes, T cells, mast cells,[23,24] or can promote chemotaxis by inducing production

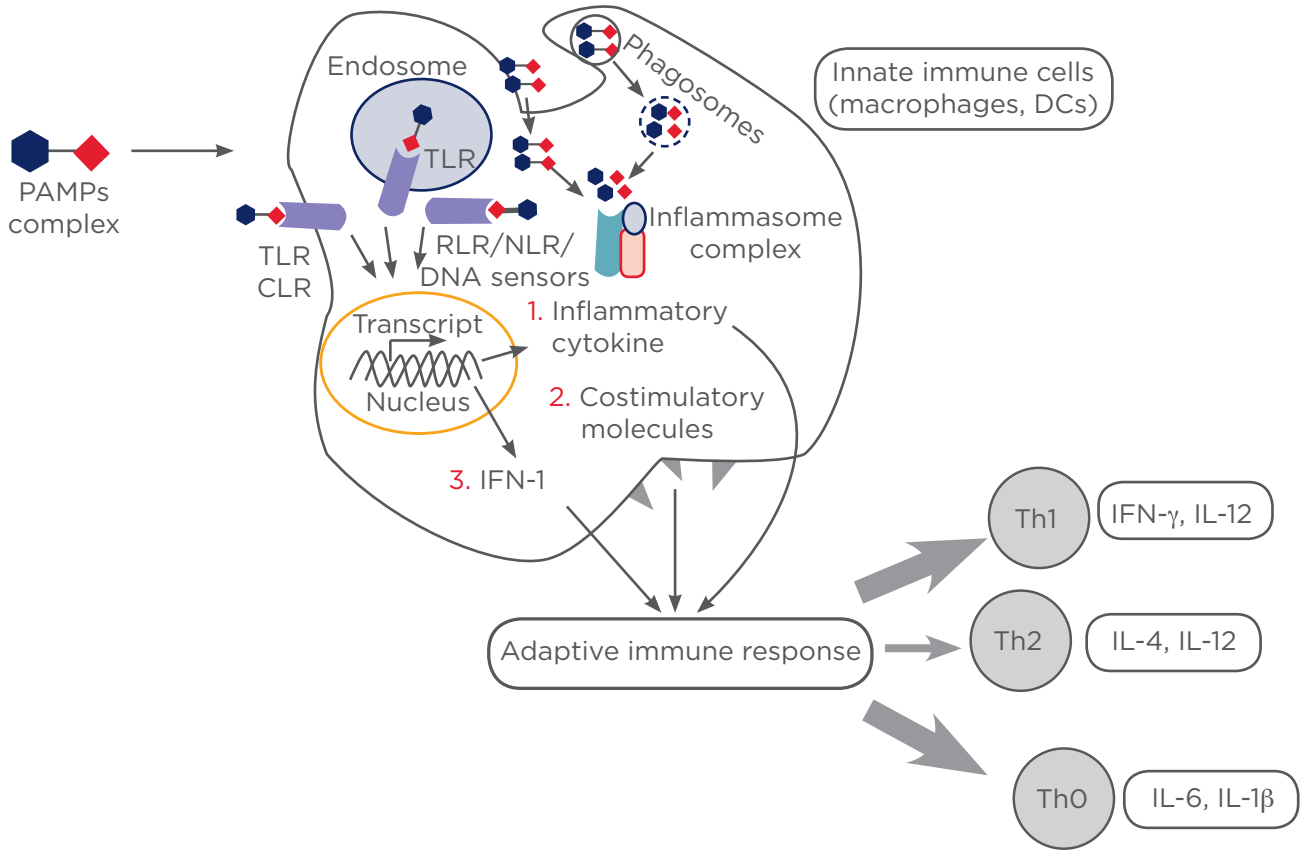


**Figure 1: Link between innate and adaptive immune response.**
Innate immune system responses are mediated by 'pattern recognition receptors' (PRRs). PRRs are capable of distinguishing between self-tissues and microbes by recognising highly conserved PAMPs. PRRs are either membrane and intracellular signal transducing (TLR, RLR, nucleotide-binding domain [NLRs], CLR) or secreted and circulating proteins (e.g. antimicrobial peptides, collectins, lectins). Activated TLRs initiate a signalling cascade that results in pro-inflammatory cytokines being produced along with chemokines and Type 1 IFNs. Innate immune cells internalise PAMPS binded to PRRs and this 'innate' step induces DC maturation, which is accompanied by upregulation of cytokine receptors, the major histocompatibility complex Class 2, and the co-stimulatory molecules CD80 and CD86. Once matured, DCs function as the most potent cells that present antigen to naïve T lymphocytes and induce their proliferation, activities that are central to the antigen-specific adaptive immune response. These effector T lymphocytes actively secrete IFN-γ that further primes the DCs to produce greater amounts of IL-12 in response to stimulation.
PAMPs: pathogen-associated molecular patterns; TLR: toll-like receptor; CLR: C-type lectin receptor; RLR: RIG-1 like receptor; NLR: NOD-like receptors; IFN: interferon; IL: interleukin; DC: dendritic cells; Th1: Type 1 T helper cell.

of chemokines.[25] Cathelicidins appear to be a link between the innate and adaptive immuneresponses by influencing DC activation and polarisation of T lymphocytes.[26,27] LL-37 upregulates the endocytic capacity of DCs and enhances the secretion of cytokines, leading to a Th1-driven immune response.[27]

On the other hand, it was shown that cathelicidins also exhibit anti-inflammatory properties, thus playing a significant role in balancing inflammation and maintaining homeostasis.[22] LL-37 can inhibit cellular immune responses triggered by IFN-γ, which is a key cytokine for polarisation of Th1 responses.[28] Cathelicidins alter the TLR-to-NF-κB pathway in the presence of exogenous inflammatory stimuli and selectively suppress specific proinflammatory cellular responses such as TNF-α, IL-1β, and NFκB.[25,29] The immunomodulatory effects of cathelicidin on macrophage TLR response may vary both on the exogenous/endogenous origin of peptide, upon the cell type and activation status, timing of exposure, and other immune mediators present.[22]

The intracellular TLRs are differentially regulated by vitamin D; TLR-2, 4, and 9 being downregulated, whereas TLR-3 was unaffected. This may have significant biological relevance and may lead us to speculate that the vitamin D deficiency observed in autoimmune disease may further potentiate autoreactivity to self-nucleic acids recognised by TLR.[30]

Actions of vitamin D of innate immunity have indirect effects on adaptive immunity suppressing effector T cell activation. Vitamin D also has direct actions on T cells: it decreases Th1 proliferation, promotes shift from Th1 to Th2, increases Th2 cell function, inhibits production of IL-2, IFN-γ, TNF-α, and IL-5, decreases levels of IL-2 in CD4+ T cells, enhances transforming growth factor beta (TGF-β)-1 and IL-4 transcripts, regulates Th17 cells and decreases IL-17 expression, and promotes regulatory T cell (Treg) development, differentiation, and functions.[17]

## EVIDENCE FOR INNATE IMMUNE ACTIVATION IN SSC

Cells of the innate immune system are detected at the sites of tissue injury in SSc. Macrophages are present in the infiltrates of the dermis, especially in the perivascular region,[31] and their number is increased in both involved and uninvolved skin.[32] DCs from SSc patients show an important TLR response especially in the early phase of the disease.[33,34] The increased production of Type I IFN and of other cytokines such as IL-6, TNF-α, and IL-10 is specifically found in response to TLR-2, TLR-3, and TLR-4 ligands in DCs.[35] The observations that circulating endogenous TLR-4 agonists are present in SSc patients,[36] combined with high circulating levels of inflammatory mediators often secreted by TLR stimulated DCs/macrophages (TNF-α, IL-6, and IL-12p70),[37,38] stress the potential role of TLRs in this condition.

Interplay between altered endothelial cells, immune cells, and their soluble mediators are responsible for the alteration of fibroblast functional phenotype and excessive accumulation of ECM.

The fibrosing phenotype of scleroderma is associated with the production of Th2 cytokines such as IL-4, IL-13, and profibrotic TGF-β.[4] Innate immune cells are responsible for the secretion of many cytokines mentioned above. IL-13 is produced by DCs and mast cells via TLR-2 activation; mast cells can be another source of TGF-β.[39]

### Role of Different TLRs in SSc

Several studies suggested that TLRs can be important in disease initiation and progression, especially intracellular TLR-3, 7, 9, as well as plasma membrane TLR-2 and 4. Increased expression of Siglec-1, an IFN-regulated gene, in circulating SSc monocytes and tissue macrophages suggests that Type I IFN-mediated activation of monocytes occurs in SSc, possibly through TLR activation of IFN secretion. Stimulation with TLR-3, 7, or 9 agonists dramatically increased Siglec-1 expression on peripheral blood mononuclear cells.[40]

*In vitro* stimulation of DC subsets from patients with early SSc with TLR-2, 3, and 4 ligands produced increased secretion of IL-6 and TNF-α by plasmaytoid DCs (pDCs) compared with controls.[33] A rare polymorphism in the TLR-2 gene (Pro631His) is highly associated with the diffuse form of SSc, high levels of antitopoisomerase-I antibodies, and pulmonary hypertension.[41] SSc monocytes carrying this polymorphism also secreted higher levels of proinflammatory and profibrotic cytokines.[41]

Van Lieshout et al.[42] demonstrated that the level of the chemokine ligand CCL18, which was a T cell chemoattractant and profibrotic factor, is higher in the serum from SSc patients compared with

healthy controls. CCL18 and IL-10 were secreted by CD14+ circulating monocytes and DCs after TLR-4 stimulation. Monocytes derived from SSc patients with interstitial lung disease have an enhanced pro-fibrotic phenotype and could differentiate into fibrocytes and secrete higher collagen after exposure to a TLR-4 agonist.[43] A recent study[44] demonstrated that TLR-4 was overexpressed in the skin and lung tissue from patients with SSc.

Farina et al.[45] showed that TLR-3 ligand (dsRNA) strongly upregulated ET-1 mRNA expression in dermal fibroblasts isolated from SSc patients, whereas selective blocking of TLR-3 with bafilomycin attenuated ET-1 expression. Agarwal et al.[46] also demonstrated that dermal fibroblasts from patients with SSc had an increased expression of TLR-3 in response to Type I IFN, resulting in an enhanced secretion of IL-6 and monocyte chemotactic protein 1 (MCP-1). The increased IL-6 secretion could contribute to dermal fibrosis through increases in fibroblast survival and proliferation, ECM deposition, and myofibroblast differentiation.

In nephrogenic systemic fibrosis, gadolinium-based contrasting agents bind TLR-4 and 7 in differentiated macrophages and induce activation of NF-κβ and expression of profibrotic IL-4, IL-6, and TGF-β.[47] HSP70 (heat shock protein 70) and HMGB-1 (high mobility group box 1) are PAMPs - danger signals released from damaged cells that can bind TLRs and induce gene expression of proinflammatory mediators.[48,49] Serum HSP70 has been demonstrated to be elevated in SSc compared with controls and associated with pulmonary fibrosis, skin sclerosis, renal vascular damage, oxidative stress, and inflammation.[48] HMGB-1 is elevated in tissue and serum from SSc patients and correlates with the skin score.[49]

### Interaction between TLR and Type I IFN in SSc

Type I IFNs-α/β are a family of cytokines induced rapidly by viral and bacterial infections, and are well recognised for their crucial role in innate defence. Moreover, IFNs-α/β enhance immune responses through the stimulation of DCs, demonstrating that IFN-α/β serves as a signal linking innate and adaptive immunity. In the last years a lot of research focused on the role of Type I IFN in the pathogenesis and severity of SSc. TLR activation stimulates production of IFNs and other cytokines, and IFNs regulate the behaviour of key cells involved in the development of SSc, including DCs, T cells, and dermal fibroblasts.[50]

In SSc, approximately half of patients have an increased expression of IFN-stimulated genes (ISGs), termed the IFN signature, and pDCs were the main source of IFN production upon TLR-7 or 9 activation.[51,52] Probably the strongest evidence implicating IFN in the disease pathogenesis is detection of IFN and ISGs in affected tissues, especially skin.[53-55]

Kim et al.[56] showed that autoantibody subsets in SSc sera differentially induce IFN-α: anti-topoisomerase 1 induced significantly higher levels of IFN-α as compared with anticentromere or antinucleolar antibodies. IFN inducing activity was significantly higher in patients with diffuse SSc than in those with limited forms, and correlated with lung fibrosis, digital ulcers, pulmonary hypertension, or cardiac involvement.[51] Interferon regulatory factors (IRFs) coordinate the expression of Type I IFNs and IFN–inducible genes, and several polymorphisms have been associated with susceptibility for development of SSc.[53,57]

The development of SSc was reported in patients undergoing IFN treatment.[58] A randomised placebo-controlled trial evaluating effects of subcutaneous IFN-α injection on the severity of skin involvement in patients with early SSc showed that treatment with IFN-α resulted in worsening lung function and a smaller degree of improvement in skin thickening scores.[59]

A combined score of the plasma IFN-inducible chemokines, IFN-inducible protein 10, and IFN-inducible T cell chemoattractant highly correlated with the IFN gene expression signature in SSc patients in the Genetics versus Environment in Scleroderma Outcome Study.[60] The chemokine score correlated positively with Medsger Severity Index for muscle, skin, and lung involvement, as well as creatine kinase levels in SSc. There was also a negative correlation with forced vital capacity and diffusing capacity for carbon monoxide. The fact that the IFN chemokine score did not show a consistent trend of change in time suggested that the IFN signature was a stable marker for the more severe subtype of disease rather than a time-dependent immune dysregulation that improved after the initial phase of SSc.[60]

## VITAMIN D IN SSC

The impact of vitamin D on innate immune activation in SSc is not fully understood. Vitamin D could be beneficial by its capacity to inhibit maturation

of DCs, to decrease important inflammatory cytokines such as IL-1, 6, TNF-α, and IFN-γ, to induce hyporesponsiveness to PAMPs, and to decrease TLR-2, 4, and 9 expression that indirectly inhibits T cell activation. Some of the effects of vitamin D could have the potency to worsen SSc, such as Treg cell activation and cathelicidins. Vitamin D activates Tregs, which have tolerogenic properties but can also increase TGF-β production - a key fibrotic cytokine.[15-18] On the other hand, *in vitro* studies showed that impaired VDR signalling with reduced expression of VDR, and decreased levels of its ligand, may thus contribute to hyperactive TGF-β signalling and aberrant fibroblast activation in SSc.[61] Little is known about the role of cathelicidins in the pathogenesis of SSc; whether their chemoattractant properties and Th1 driven immune response would lead to worsening of the disease remains to be studied.

Yet, vitamin D deficiency has been documented in a high proportion of SSc patients (about 80%),[62] but low vitamin D levels were universal and independent of geographic origin or vitamin D supplementation.[63,64] Vitamin D deficiency in SSc is potentially related to several factors: dermal fibrous thickening with reduced synthesis of provitamin-D3, gastrointestinal involvement, and malabsorption. Moreover, patients with SSc experience impairment in physical functioning, and are prone to a sedentary lifestyle and diminished sunlight exposure.[66-68] Vitamin D is still an undiscovered field in SSc; data reported so far are not homogeneous. Different studies correlated levels of vitamin D with higher levels of parathyroid hormone,[68] higher incidence of acro-osteolysis and calcinosis,[68] systolic pulmonary artery pressure,[65,67] inflammatory syndrome,[62,65,67] Rodnan score,[65,69] activity and severity score,[65,67] low bone mass density,[63,68] disease duration, and pulmonary fibrosis.[62,65,67]

Despite these data, evidence linking vitamin D supplementation with reduced disease activity is still lacking. Nevertheless, given the immunomodulatory properties of vitamin D, we advocate vitamin D supplementation to SSc patients in order to raise vitamin D serum concentrations to desired levels, achieving also as a secondary outcome a potentially mitigating effect of an overactive autoimmune response in such patients.

## CONCLUSION

Although great progress has been made in recent years in elucidating the pathogenesis of SSc, the exact molecular mechanisms are still unclear and it still remains a challenging disease to treat. Components of innate immunity-like cells, AMPs, and PRRs, serve to link innate and adaptive immunity. TLR intracellular signalling pathways might be one of the mechanisms that initiate and drive autoimmunity and subsequent fibrosis. Activation of the immune system results in IFN sensitive gene transcription. TLRs may represent the link between immune activation and tissue fibrosis; several TLR agonists are under investigation. Vitamin D modulates key elements of innate immunity like TLRs, IFNs, cathelicidins, DC function, and T cell activation. Whether vitamin D could have a role in the complex pathogenesis of SSc still remains unclear but low levels of vitamin D may represent a marker of aggressive disease.

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# IDIOPATHIC SCOLIOSIS

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## ABSTRACT

Idiopathic scoliosis (IS) is a lifetime condition and is defined as a structural, lateral rotated curvature of the spine of >10° on standing coronal plane radiographs. It should be distinguished from other causes of scoliosis. It can be classified as infantile, juvenile, and adolescent according to age. As a rule of thumb, about 80% of all curves are idiopathic, right convex thoracic, and present in otherwise healthy girls at the beginning of puberty. A family member most commonly detects scoliosis. The structural asymmetry of the spine is best observed by asking the patient to bend forward. IS is often seen in more than one member of a family, but the aetiology remains unknown. Multiple genes are likely to be involved with incomplete penetrance and variable expressivity. Early detection by screening allows for monitoring curve progression and timely initiation of bracing, but school screening is controversial and practises vary worldwide. Most patients have minor scoliosis and treatment is generally not recommended for patients with curves <20°, but in many European countries clinicians recommend physiotherapy (scoliosis specific exercises) for smaller curves. The indication for bracing is a progressive curve of 25-45° in a growing child. Its effectiveness has been debated, but in a large recent randomised study, the number of teenagers with high-risk curves who progressed to the threshold of surgery was significantly reduced. Surgery is recommended for patients with curves >45°. Scoliosis surgery was not successful until the introduction of Harrington's instrumentation in the 1960s. Modern instrumentation has evolved from the Cotrel-Dubousset system in the 1980s, and a variety of methods are available today. Although scoliosis may be a burden, long-term studies suggest that a good quality of life is maintained in most patients.

Keywords: School screening, bracing, surgery, genetics, quality of life, physiotherapy.

## INTRODUCTION

Idiopathic scoliosis (IS) affects 2-3% of children and usually presents in adolescence. It is a life-long condition and is defined as a structural, lateral rotated curvature (Cobb angle) of the spine of >10° on standing coronal plane radiographs. Adolescent IS usually arises in otherwise healthy children in the growth spurt at the beginning of puberty. The spinal curve is most commonly detected by the family and not by healthcare providers and it is difficult to determine the exact age of onset.[1,2] Age at presentation is therefore more accurate.

Most patients with IS have minor curves and are not recommended any treatment.[3,4] About 90% of those treated are girls.[5,6] IS can be classified as infantile (<age 4 years), juvenile (age 5-9 years), and adolescent (10 years or older).[7] Comprehensive reviews on adolescent IS have been published.[2,8] The aim of the present review is to briefly describe the current knowledge about aetiology, natural course, screening and diagnosis, and outline the evidence of physiotherapy, brace treatment, and surgery.

## AETIOLOGY AND DIAGNOSIS

IS is often seen in multiple members of a family. One identical twin may have a large curve while the other has a small curve, which suggests that genetic, epigenetic, and environmental factors are involved. Studies of twins[9] have reported a concordance in 73% of monozygotic twins and

36% in dizygotic twins. Despite a number of genetic studies with different study designs, the aetiology remains unknown. Different methods of inheritance have been reported, but no single locus has been identified.[10] Multiple genes are likely to be involved with incomplete penetrance and variable expressivity. Candidate gene analyses[11-14] have not found associations for connective tissue genes, but for vitamin D and oestrogen receptor genes. Polymorphism of the oestrogen gene has shown association both for the probability of having scoliosis and for curve progression.[13,14] The hypothesis that an abnormality of the paravertebral muscles contributes to the development of IS has not been confirmed.[8] Magnetic resonance imaging (MRI) studies[15] indicate that the growth of the vertebral bodies is disproportionate compared to age matched controls, but the mechanisms involved are poorly understood.

Scoliosis has been a recognised condition for centuries. Structural scoliosis must be discriminated from functional scoliosis that may be caused by leg length discrepancy or back pain in a patient with disc herniation. A structural scoliosis is clinically suspected if it appears as a keel of a boat when examining the patient bending forward (Figure 1). The keel or gibbus is an expression of the rotation of the spine as structural scoliosis, and is of a three-dimensional (3D) deformity.

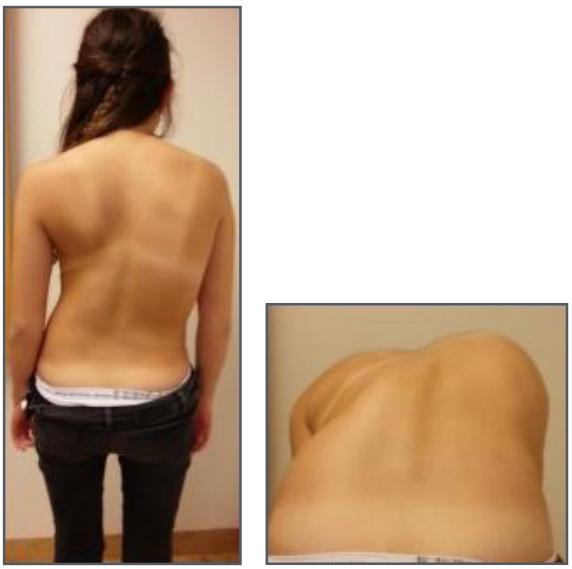

**Figure 1: A structural right convex thoracic scoliosis. The lateral deviation is shown in the left image while the rotation of the spine is shown as a keel of a boat (gibbus) by bending forward in the right image.**

Other rare causes of scoliosis should be excluded, such as vertebral malformation, neuromuscular disorders, and syndrome scoliosis. The diagnosis is confirmed if a standing coronal radiograph shows a curve >10°. Several classification systems have been used for the description and development of curves. Most primary curves are right sided thoracic, but primary curves may, by example, be double shaped, thoraco-lumbar, or lumbar. The IS is usually s-shaped with one major and two compensatory curves, while neuromuscular scoliosis is c-shaped. Patients with rare conditions such as Rett syndrome may have either c-shaped or s-shaped curves.[16]

## SCHOOL SCREENING

Early detection by screening allows for monitoring curve progression and timely initiation of bracing but school screening is controversial and practices vary worldwide.[17,18] Some studies have supported screening whilst others have discouraged routine screening. Currently most international scoliosis societies support and recommend screening.[19,20] While opponents of screening mainly cite the increased costs and lack of effectiveness of the programmes,[21] discontinuation has led to late detection and more surgeries in various countries.[1,22]

School screening is recommended at the onset of puberty, usually twice, at the ages of 11 and 13 years. Since girls are considered to be at higher risk compared to boys, screening in girls only may be more preferable. Screening can be performed by community nurses or physical therapists and provided in connection with other routinely contacts with the school's healthcare system. The examination includes the forward bending test with the use of a scoliometer to measure spinal rotation. School screening is easy to perform by trained examiners and takes an average of 9 minutes per child.[23] Children with rotation >7° are recommended a standing conventional X-ray examination, and those with a major curve >20° are referred for specialist examination.

The most comprehensive longitudinal school screening was performed in 115,190 children in Hong Kong.[24] Of these, 3,158 received X-rays, 264 were braced, 10 had surgery, and 29 had brace and surgery. Comparatively, in Norway which currently has no screening and has age cohorts of about 60,000, in 2012 there were 51 children who had brace treatment and 71 had surgery. Experiences from a clinical trial[5] on the effectiveness of bracing

suggest that patients and their parents prefer bracing to no treatment. The best way to allow for timely bracing may be to reintroduce screening. The procedure itself is cheap but it may not be cost-effective unless implemented in countries which have high surgical rates.

## NATURAL HISTORY

The history of completely untreated IS is unknown.[2] Studies[25,26] that are cited when the natural history is described, may have included braced patients or have follow-up rates <50%. One study[26] that followed patients for 50 years reported that the level of work and disability did not appear different from controls. Although back pain was experienced more often, this was not clearly associated with curve size. Self-image was lower than in controls.

## TREATMENT GUIDELINES

Treatment is generally not recommended for patients with curves <20°, but in many European countries clinicians recommend physiotherapy for smaller and moderate curves in addition to brace treatment.[27]

As scoliosis does not increase in a number of patients, progression to a curve size >25° in a growing child is usually required before bracing is recommended. Skeletal maturity is estimated from images indicating the epiphyseal growth plate at the iliac crest, and is classified as Risser Grade 0-5. Indications for bracing include Stages 0-2 and in some cases Stage 3 if other signs of puberty are not present. Bracing is usually maintained until Risser Stage 4-5 or about 2 years after menarche in girls, and until Risser Grade 5 in boys. Risk factors for curve progression are debated, but young onset age, flexible curves, or thoracic or thoracolumbar primary curves tend to develop faster. Surgery is recommended in the growing child with curve size >45-50°. In addition, other factors such as vertebral rotation and curve localisation are considered in the evaluation for surgery.

### Physiotherapy

Physiotherapy is favoured in many European countries as first-line treatment of small curves and for those with a low risk of progression. Different methods are available and scoliosis-specific exercises are described as different from physiotherapy in general.[28] A recent Cochrane review[29] reported that there is a low quality of evidence that scoliosis-specific exercises may be more effective than electro stimulation, traction, and postural training, but the authors suggest that more research is warranted. A previous systematic review[30] included more studies of low quality and concluded that scoliosis-specific exercises reduced progression rate and brace prescription in patients in early puberty. At present there is little knowledge about the advantages of scoliosis-specific exercises as compared to participation in regular sports or as an adjunct to bracing. Physiotherapy is commonly used in all patients with scoliosis, both in a long-term follow-up of middle-aged previously braced patients, and of younger operated patients; around 30% reported to have undertaken physiotherapy in the last year.[31-33] The use of physiotherapy is also much debated, and scoliosis surgeons often claim that it is not indicated. High-quality studies examining indications and effectiveness in terms of curve reduction and health-related quality of life (HRQoL) are warranted.

### Bracing

Bracing has been used for centuries, the aim is to stop curve progression to avoid surgery. Many different braces are available for the treatment of scoliosis in the growing child or teenager.[34] Principally these can be divided into three types: rigid day and night braces, rigid night braces, and dynamic braces. Advocates of the two latter types argue that these are as effective as rigid day and night braces and more user friendly, but this is not documented in controlled studies. The rigid brace is custom-made from a pre-shaped model and fitted by orthopaedic engineers. A Cochrane review[35] found only one controlled study examining the efficacy of bracing. This study[36] reported that about 25% progressed with brace treatment and 55% without.

The effectiveness of bracing was documented in a large recent randomised study.[5] Bracing significantly decreased the progression of high-risk curves to the threshold of surgery in patients with adolescent IS. Longer hours of brace wear were associated with greater benefit. For the average patient the wearing of a rigid brace did not reduce HRQoL. Patients were recommended to wear the brace for 20 hours daily. The curve reduction in a compliant girl is shown in Figure 2.

Compliance was examined by thermosensors and indicates that patients with brace wear >13 hours

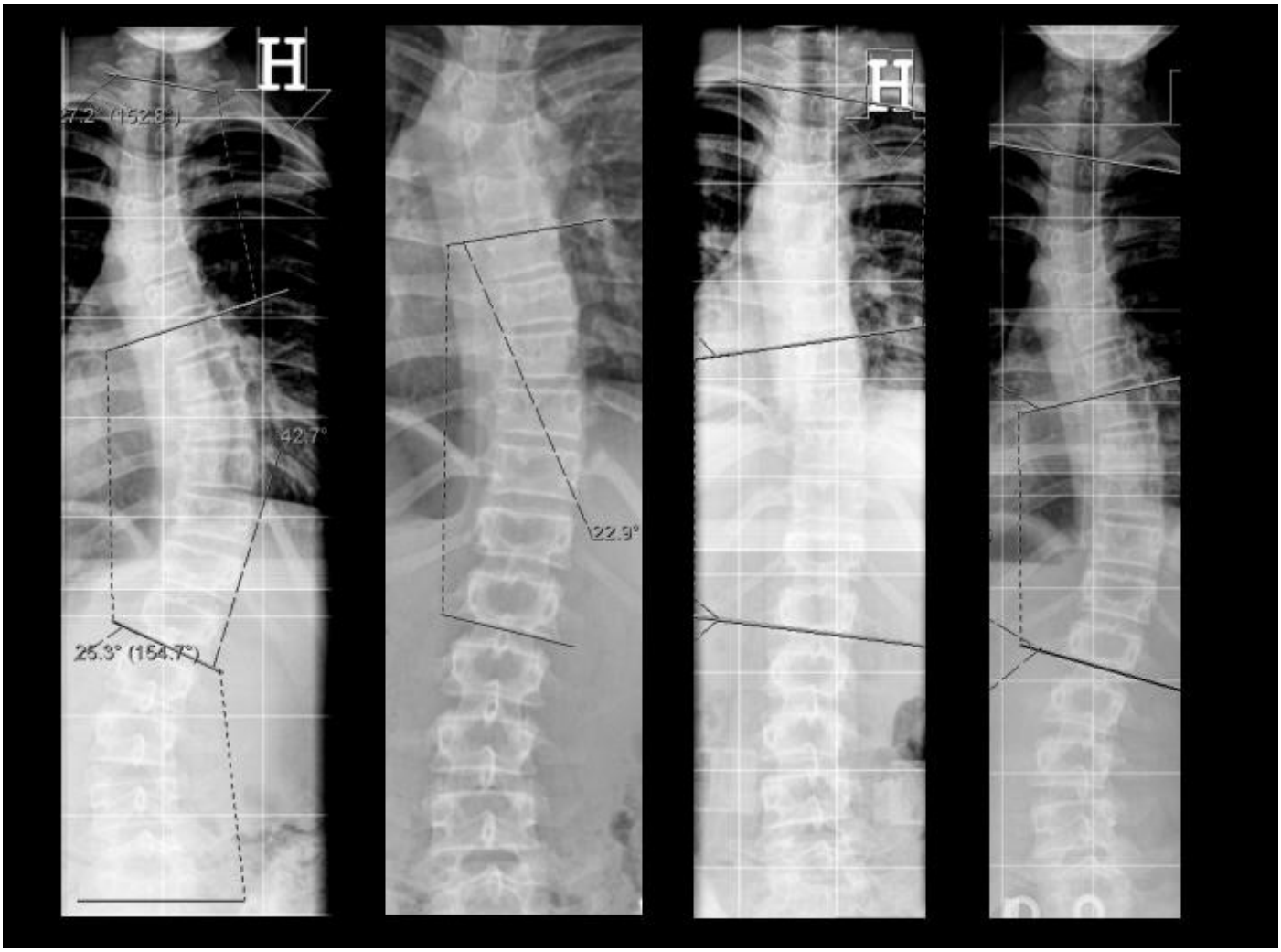


**Figure 2: Radiographs showing a right convex primary curve of 42.7° in a girl with adolescent idiopathic scoliosis who started bracing at age 12 years. She had no signs of growth maturity, Risser was 0, and she had not had menarche. She was highly compliant and used the brace about 20 hours daily. The curve was flexible before bracing and was reduced to 22.9° lying prone, to 15.6° in a rigid brace (indicating good brace fit), and the primary curve was 32.0° at brace weaning. She participated in sports 1-2 hours daily.**

had a success rate of >90%, while those with brace wear <6 hours had a success rate of 42%. This is in agreement with the results of a large longitudinal cohort study with >20 years follow-up.[6] In this study there were 284 compliers and 71 non-compliers. 68 of the compliers and 41 of the non-compliers had progressed >6° at long-term (OR: 5.8 [95% confidence interval 3.3-10.2]) and 17 versus 10 had surgery (OR: 8.6 [3.7 to 19.9]). The curve progression in a girl who initially used the brace as prescribed but later ended treatment is shown in Figure 3.

## Surgery

The aims of surgery are 3D curve correction and improved appearance by balancing the trunk. Scoliosis surgery is major surgery in an otherwise healthy child and it is an overall goal to keep short-term and long-term complications to a minimum. The mortality rate is 1.3 in 1,000 operations and spinal cord injury is reported in about 0.5% of operations.[37,38] Improved preoperative and intra-operative neurophysiologic monitoring and blood salvage procedures contribute to safer surgery. Late infections are usually caused by skin (acne) bacteria and are suspected by onset of pain or signs of a fistula or skin abscess and usually indicate removal of the inserted instruments. Other causes of reoperations are instrument failure or pain. Reoperations are conducted in 5-23% of the patients.[31,39,40] Scoliosis surgery is expensive and total costs are number two of all surgeries in children and adolescents in the USA, next to appendicitis.[5]

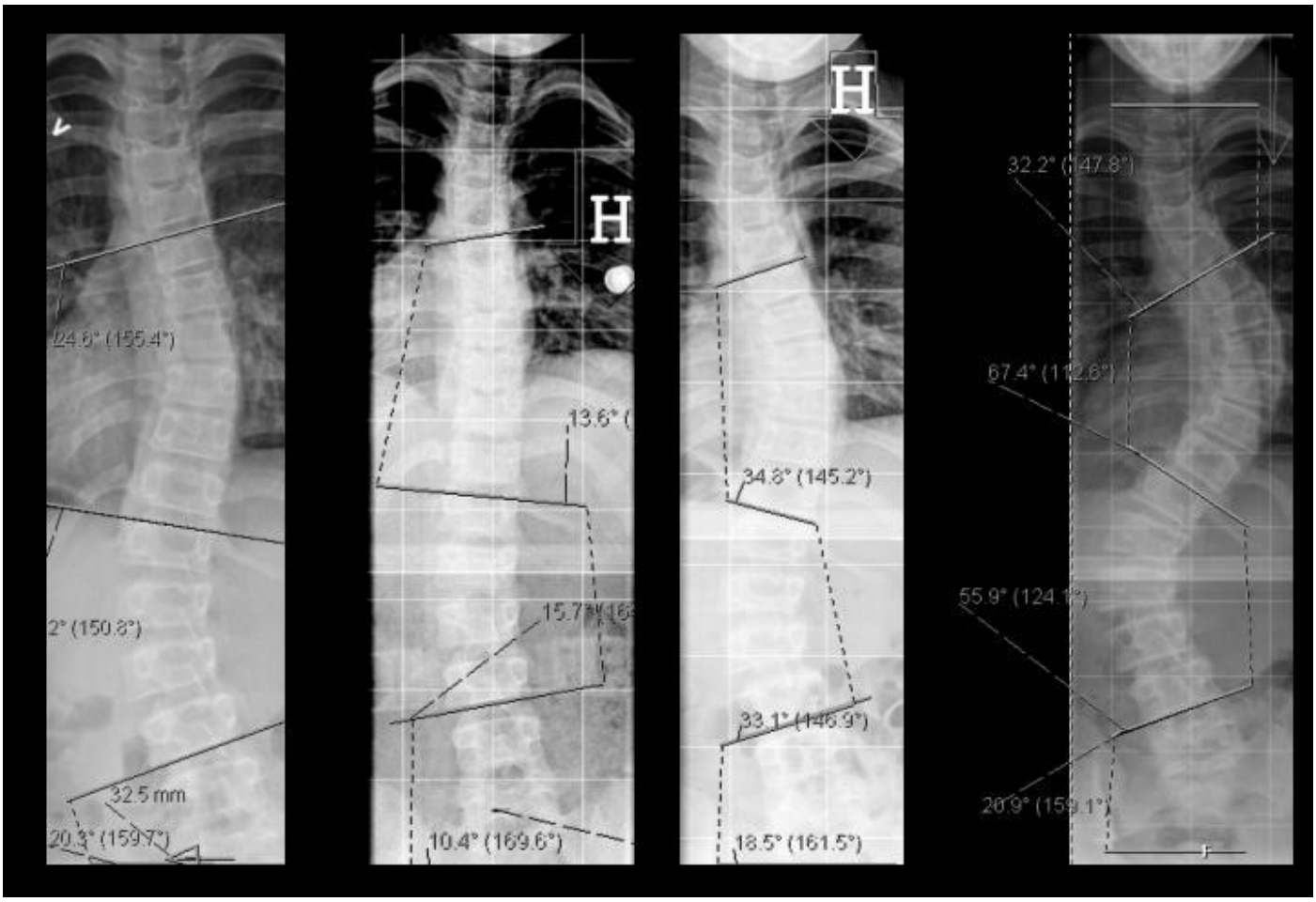


**Figure 3: Radiographs show juvenile idiopathic scoliosis in a girl. She started bracing at age 9 years, the double shaped curve was 24.6° and 29.2°, respectively. Curves were flexible and were reduced to 13.6° and 15.7° in a rigid brace. She started to become non-compliant at age 11 years, curves increased to 34.8° and 33.1°. She stopped to use the brace at 12 years of age and did not attend any appointment for 2 years and her scoliosis had increased to 67.4° and 55.9° at 14 years of age, respectively, and she was referred for surgery.**

Scoliosis surgery was revolutionised by Harrington's instrumentation in the 1960s.[41] Modern instrumentation has evolved from the Cotrel-Dubousset system in the 1980s.[42] Combinations of wires, hooks, pedicle screws, and long rods are used to conduct modern 3D correction. On average, the major curve is corrected by about 60% of its original size and the instrumentation includes about ten segments. The use of segmental pedicle screws in the thoracic spine has been introduced to improve better fixation.[43] No conclusive evidence exists about advantages in outcome, such as improved HRQoL, including better self-image. Interestingly, a previous study[44] reported no difference in long-term outcome after Harrington's instrumentation compared with Cotrel-Dubousset posterior instrumentation at 10-years follow-up. We examined 86 patients at 10-years follow-up after operative treatment with Cotrel-Dubousset.[31] The average primary curve was reduced from 56° to 19° (Figure 4), five patients had implants removed, and 79% of the patients considered their back function as excellent or good. Despite this, 45% reported to have consulted a physician or received physiotherapy the last year before the 10-year follow-up.

Anterior instrumentation is used mainly for isolated thoracolumbar and lumbar curves. The main advantage is the reduced number of fusion levels. A small study[45] with 17-years follow-up reported good correction, no infection, <10% reoperations, and good scoliosis specific HRQoL.

Casts are rarely used post-operatively today. Patients are usually hospitalised for about 10 days,

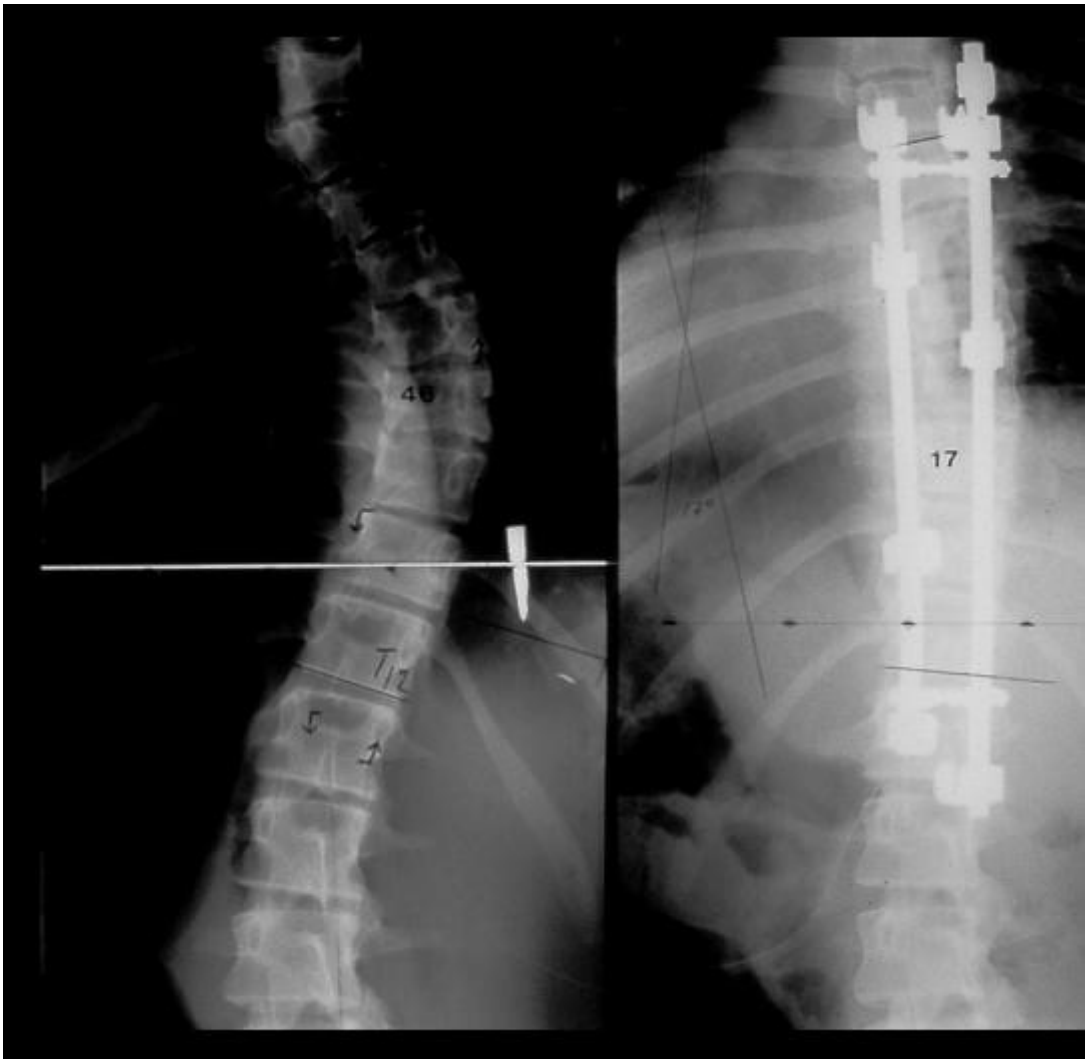


**Figure 4: Radiographs that show idiopathic scoliosis in a girl aged 14 years before and after surgical treatment using Cotrel-Dubousset instrumentation.**

and thereafter stays at home for another 10 days before the general condition is acceptable for attendance of regular school classes.

## HEALTH AND HRQOL

Dyspnoea is associated with curves >80°.[2] Pulmonary function was improved after brace and surgery at 25 years follow-up.[46] Pregnancy, childbearing, and delivery experience of braced and operated patients are comparable to controls.[32,33,47] Spinal mobility is decreased after bracing and surgery, but less after modern instrumentation compared with Harrington's instrumentation.[44,48] Muscle endurance was reduced in one study after bracing and surgery, but muscular strength tests were comparable to controls in another study.[44,48]

Pain is reported more often by operated scoliosis patients than controls without scoliosis.[40,49] Also, a considerable number of patients consulted a physician or had physiotherapy the year before long-term follow-up both after bracing and surgery.[31-33] Average scores of self-image were slightly decreased in both braced and operated patients at long-term follow-up.[31,31-33] QoL was not reduced after bracing and comparable to controls in patients in a recently published clinical trial.[5]

HRQoL is measured by various scoliosis-specific questionnaires. Different self-report outcomes are available. The most commonly used is the Scoliosis Research Society questionnaire, which assess five domains (physical function, pain, self-image, mental health, and patient satisfaction).[50] This questionnaire is validated for use in many different countries. For scoliosis patients this questionnaire is more accurate and valid compared with a generic questionnaire such as the EQ-5D.[51] Reporting of

scoliosis-specific HRQoL in scoliosis patients is influenced by co-morbidity.[52]

## CONCLUSION

The aim of the present review is to briefly describe the current knowledge about aetiology, natural course, screening and diagnosis, and outline the evidence of physiotherapy, brace treatment, and surgery. Despite a number of genetic studies with different study designs, the aetiology remains unknown. The natural course is difficult to outline in the long-term because many patients have been braced. Available evidence suggests that QoL in most patients is, in the long-term, beneficial. The indication for school screening is to detect patients who benefit from bracing. The cost-benefit of screening is debated. Scoliosis is idiopathic in most patients, but curve patterns and other causes should be carefully evaluated to exclude other causes. The evidence of physiotherapy for small curves is sparse, while a recent milestone trial documented the effectiveness of bracing. Surgical methods for operating on large curves are continuously revised and should preferably be evaluated by high-quality studies before implementation.

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# OSTEOPOROSIS-RELATED MORTALITY: TIME-TRENDS AND PREDICTIVE FACTORS

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## ABSTRACT

Osteoporosis is one of the leading causes of handicap worldwide and a major contributor to the global burden of diseases. In particular, osteoporosis is associated with excess mortality. We reviewed the impact of osteoporosis on mortality in a population by defining three categories: mortality following hip fractures, mortality following other sites of fractures, and mortality associated with low bone mineral density (BMD). Hip fractures, as well as other fractures at major sites are all associated with excess mortality, except at the forearm site. This excess mortality is higher during the first 3-6 months after the fracture and then declines over time, but remains higher than the mortality of the normal population up to 22 years after the fracture. Low BMD is also associated with high mortality, with hazard ratios of around 1.3 for every decrease in 1 standard deviation of bone density at 5 years, independently of fractures, reflecting a more fragile population. Finally predictors of mortality were identified and categorised in demographic known factors (age and male gender) and in factors reflecting a poor general health status such as the number of comorbidities, low mental status, or level of social dependence. Our results indicate that the management of a patient with osteoporosis should include a multivariate approach that could be based on predictive models in the future.

Keywords: Bone mineral density, fracture, mortality, mortality indicators, osteoporosis, predictive model, population, time-trends.

## BACKGROUND AND OBJECTIVES

Osteoporosis is one of the leading causes of handicap worldwide and a major contributor to the global burden of diseases.[1-3] The impact at the population level is constantly increasing due to aging in societies,[4,5] although some studies have reported a stabilisation or even decline in the incidence of age-adjusted osteoporotic fractures in Western societies, possibly due to increased average body weight, improved functional ability among the elderly, and specific measures to prevent bone loss and reduce the risk of falling.[6-9]

Osteoporotic fractures are associated with a high mortality rate.[10-13] This excess mortality is mostly elevated in the first 3-6 months following the fracture then seems to decline during the first 2 years post-fracture, but does not return to the levels of the general population even 10 years after the fracture.[10,14,15]

Some studies showed that the excess mortality seems to be stable during the last few decades[13,16,17] while others suggested it is decreasing with time, probably due to advances in the surgical management of the fractures, or to preventive measures in the postoperative periods, such as antibiotherapy and anticoagulation, and to pharmacological primary and secondary preventive measures.[14,18-24] Moreover, the decline in age-specific rates of osteoporotic fractures could indirectly lead to a decline in the global mortality burden.

The recent literature addressed the evolution of the outcomes of osteoporotic fractures with time and

focused on potential risk factors associated with mortality. Magnitude of time trends in absolute and relative mortality following fractures differs between studies. Furthermore, and beyond the fracture event, there is emerging evidence that bone mineral density (BMD) is associated independently with increased mortality.

The objective of the current review is to investigate time-trends of mortality associated with different aspects of osteoporosis: hip fractures, other sites of fractures, and low BMD. The association with specific predictive factors is also addressed.

## METHODS

A review of the US National Library of Medicine (PubMed database) was performed using the Medical Subject Headings (MeSH) terms: "osteoporosis", "fractures", "bone", "mortality", and "population". A second search using the terms: "bone density", "mortality", and "population" was performed in a subsequent step. The search retrieved 433 articles, 64 of which were retained for the analysis. Studies were retained when relevant to the review objectives and when conducted in a population setting. Studies were excluded when their objective related to the efficacy of anti-osteoporotic treatments, sponsored or not. The studies were divided into three categories: mortality following a hip fracture, mortality following any other fracture, and mortality in low BMD patients regardless of fractures. For each study, the population and study design were identified, as well as the mortality indicator used. For every category, the studies were classified by year of publication.

## RESULTS

### 1. Mortality Following Hip Fractures

Among all osteoporotic fractures, hip fracture is the site most commonly associated with mortality. The highest excess in mortality is during the immediate post-fracture period, especially the first 6 months, but remains high several years after the fracture, as shown in population-based prospective studies.[10,14,25] This could be underestimated in randomised trials where the frail individuals at greatest risk of death are rarely considered suitable.

Literature in the past two decades showed that most deaths are related to associated comorbidities, reflecting a poor underlying health condition rather than the fracture itself.[5,26,27] It is clear nowadays that the excess deaths after a hip fracture can be attributed to serious underlying comorbidities that are unrelated to osteoporosis, suggesting that a certain proportion of deaths could not be prevented by reducing fractures.[28,29]

The mortality indicators are shown in Table 1 by study and by chronological order of publication. Although the studies have different populations, methodologies, and mortality indicators, there is a clear trend of decreased excess mortality with time within the same cohort, even if this excess remains present up to 22 years after the hip fracture. At 1 year, hazard ratios (HRs) vary from around 3-10; they decline to 5 at 5 years, and to 2.5 at 10 years. There is also a trend for a decline in excess mortality at 1 year by date of study publication, suggesting a decreased association with mortality over the last decade (Figure 1).

### 2. Mortality Following Fractures at Sites other than the Hip

Osteoporotic fractures at sites other than the hip also lead to a high number of excess deaths.[5,45,46] Most deaths occur within 1 year of fracture, particularly during the first 6 months.[47] Mortality following fractures is higher in men than in women.[5]

The Dubbo study from Australia reported that a high mortality is associated with any type of major fracture compared to the general population,[10] and that this excess mortality persists up to 5 years after all major types of fracture. Many studies, however, showed that mortality rates after forearm fracture are similar to the general population.[10,34]

The mortality indicators are shown in Table 2 by study, by chronological order of publication. Due to the heterogeneity of the studies, a direct comparison cannot be made, but again, in the same cohort, there is a decline in excess mortality with time and higher mortality in males.

### 3. Predictors of Mortality Following Osteoporotic Fractures

Since the fracture itself cannot explain the excess mortality, many studies addressed the associated risk factors with poor survival. Some factors were associated with the fracture, such as the site of fracture and the timeframe following the fracture. The hip site was associated with the higher mortality rates, but other sites considered as major were also associated to a lesser extent (vertebral,

pelvis, shoulder). Fractures at the forearm were not associated with excess mortality. As for time, the mortality risk was higher in the immediate post-fracture period and declined subsequently, remaining however higher than the normal population up to 10 years after the occurrence of the fracture.

However, most factors were independent from the fracture *per se* and yet predicted excess mortality.[26,37,38,55-57] These can be divided in demographic factors, such as age and male gender, and factors reflecting a poor general health status: the number of co-medications, associated chronic diseases (two or more), Charlson index CI) components and medications, low score on entality status test, not walking outdoors before the fracture, lower handgrip strength, use of walking aids, level of social dependence, and being in an institution. It is noticeable that the CI components encompass several chronic diseases. A comparable mortality with the normal opulations was suggested in the absence of risk factors such as low mentality status, low handgrip strength, and fewer than two associated chronic diseases.[55]

Finally, the non-operative conservative management is also associated with poorer outcome. However, this finding could be due to selection bias; the patients chosen for conservative management may have a lower health status or be at higher mortality risk initially, preventing them from reaching the operative option.

## 4. Mortality Associated with Low BMD

Except for pulmonary deaths in women with severe vertebral deformities and kyphosis, strong associations of osteoporotic fractures with specific causes of death have not been identified, suggesting an indirect association with underlying comorbid conditions that may also lead to osteoporosis.[46] This is consistent with the notion that low BMD *per se* is associated with excess mortality from various causes.[28,31,45,46,48,49]

Low BMD is responsible for a growing global health burden, only partially representative of the real burden of osteoporosis. In fact a recent meta-analysis showed that global deaths attributable to low BMD increased between 1990 and 2010, and that low BMD could be responsible for at least one-third of deaths attributable to falls, which is third in the list of major health burdens after road injuries and self-harm.[58,59]

BMD was shown to be associated with mortality independently of age, weight, body mass index, smoking status, previous fracture, physical activity, drug use, and presence of chronic diseases. Low BMD was defined in the studies by low values measured by Dual Energy X-ray Absorptiometry or calcaneus quantitative ultrasound. HR was calculated by linear reduction of 1 standard deviation (SD) of bone density or broadband ultrasonic attenuation (Table 3). The risk of mortality at 5 years was inversely correlated with BMD with HRs of 1.16-1.44, except for one study that included a clinical endpoint (height loss), where HRs were higher (3.43).[60-65]

Factors associated with excess mortality in osteoporosis cohorts are mainly CI components and Elixhauser index.[66] The Charlson comorbidity index predicts the 10-year mortality for a patient who may have a range of comorbid conditions, such as myocardial infarction, congestive heart failure, peripheral vascular disease, dementia, cerebrovascular disease, chronic lung disease, connective tissue disease, ulcer, chronic liver disease, diabetes, haemiplegia, moderate or severe kidney disease, diabetes with end-organ damage, tumour, leukaemia, lymphoma, moderate or severe liver disease, malignant tumour, metastasis, and AIDS.[67] The Elixhauser comorbidity measure developed a list of 30 comorbidities that are significantly associated with in-hospital mortality and include both acute and chronic conditions, relying on the ICD-9-CM coding manual.[68,69]

## Table 1: Summary of studies addressing mortality after hip fracture.

| Year | Population | Follow-up after hip fracture | Mortality Indicator | Reference |
|---|---|---|---|---|
| 1991 | Medicare hip fractures population, USA | 90 days<br>1 year | CF 12.6%<br>CF 23.7% | Fisher et al.[30] |
| 1996 | 9,704 ambulatory women aged 65 years or older enrolled in the SOF | 5.9 years | RR 2.4 | Browner et al.[31] |
| 1997 | 578 community-dwelling females aged 70 and older, USA | 5 years | Excess death 9% | Magaziner et al.[31] |
| 2000 | 6,459 women aged 55-81 years participating in the FIT, USA | 3.8 years | RR 6.68 | Cauley et al.[32] |
| 2001 | Residents aged 60 years and older | 5 years | Men: Mortality ratio 540%, excess death rate 57<br>Women: Mortality ratio 500%, excess death rate 32 | Iacovino et al.[33] |
| 2004 | Hospital setting, Sweden | 1 year<br><br>5 years | RR 10.2 (men)<br>RR 9.1 (women)<br>RR 5.8 (men)<br>RR 5.4 (women) | Johnell et al.[34] |
| 2004 | Prospective, 7,512 volunteer ambulatory women aged 75 and older, France (EPIDOS study) | 3.9 years | RR 2<br>MR 112.4 per 1,000 woman-years, compared with 27.3 per 1,000 woman-years for the non-fractured | Empana et al.[25] |
| 2007 | 43,165 veterans, USA | 1 year | Mortality odds:<br>12% in women<br>32% in men | Bass et al.[35] |
| 2009 | Prospective cohort from the Dubbo Osteoporosis Epidemiology Study of community-dwelling women and women aged 60 years and older, Australia | 10 years | SMR 2.43 | Bliuc et al.[10] |
| 2009 | 786,717 hip fractures aged 65 and older, Medicare, USA | 1 year | MRs<br>32.5% (men)<br>22% (women) | Brauer et al.[20] |
| 2010 | South Korea<br>NHI claims database<br>9,817 hip fractures | 1 year | SMR 2.85 | Kang et al.[36] |
| 2010 | Prospective cohort hip fractures, Taiwan | 1 year | MR<br>31% men<br>16% women<br>Mortality 8-times higher than age-adjusted general population | Vaseenon et al.[37] |
| 2010 | National Hospital Discharge Register<br>41,000 hip fractures, Denmark | 1 year | CM<br>37.1% men<br>26.4% women<br>SMR in the <75 years<br>7.9 men<br>7.3 women<br>SMR in the >75 years<br>3.3 men<br>2.6 women | Kannegaard et al.[38] |
| 2011 | Cohort 6,782 hip fractures in women, UK | 1 year | MRs<br>13.3% in women <65 years<br>31% in women >65 years | Karantana et al.[39] |
| 2012 | Population-based health survey, women aged 65 years and more, Norway | 3 months<br>2.8 years | HR 6.5<br>HR 1.9 | Gronskag et al.[40] |

**Table 1 continued.**

| Year | Population | Follow-up after hip fracture | Mortality Indicator | Reference |
|---|---|---|---|---|
| 2013 | Population-based cohort of 63,257 middle-aged and elderly Chinese men and women in Singapore | 5 years | HR 1.64 (men)<br>HR 1.58 (women) | Koh et al.[41] |
| 2013 | 5,180 hip fractures, Norway | 1 year | SMR 3.64 (men)<br>SMR 2.78 (women) | Finnes et al.[18] |
| 2013 | Dubbo Osteoporosis Epidemiology Study, Australia | 1 year<br>5 years<br>10 years | RS<br>0.83 in women<br>0.63 in men<br>0.59 in women<br>0.48 in men<br>0.31 in women<br>0.36 in men | Frost et al.[42] |
| 2013 | National Insurance Database<br>143,595 hip fractures, Taiwan | 1 year<br>2 years<br>5 years<br>10 years | SMR 9.67<br>SMR 5.28<br>SMR 3.31<br>SMR 2.89 | Wang et al.[43] |
| 2013 | Population-based cohort study, 2,901 residents, USA | 22 years | SMR 1.2 | Melton et al.[44] |
| 2014 | 81,867 first hip fracture patients (nationwide), Norway | 1 year | SMR 4.6 men<br>SMR 2.8 women | Omsland et al.[14] |

CF: case fatality; SOF: Study of Osteoporotic Fractures; FIT: Fracture Intervention Trial; RR: Relative Risk; EPIDOS: Epidemiology of Osteoporosis Study; MR: mortality rate; NHI: National Health Insurance; SMR: standardised mortality ratio; CM: cumulative mortality; HR: hazard ratio; RS: relative survival.

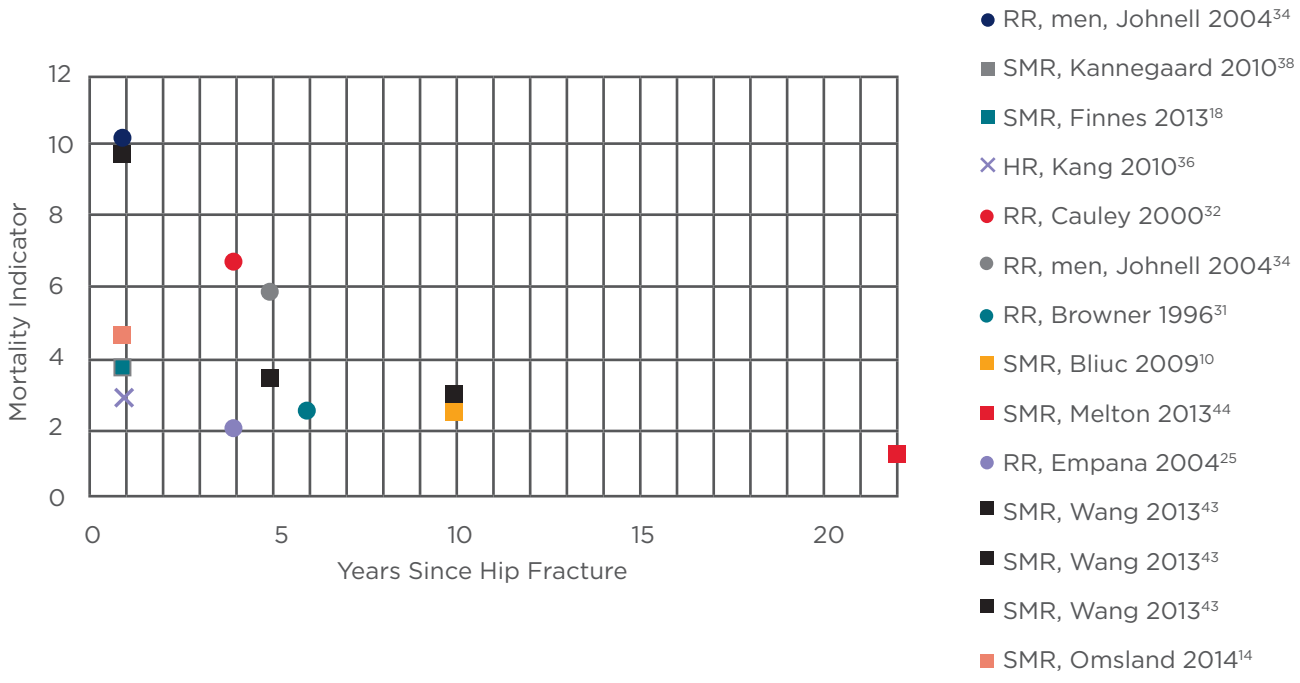


**Figure 1: Mortality indicators after hip fracture by study and by time to fracture.**
Mortality indicators are derived from different studies and different populations. RR (dots): relative risk, defined as the ratio of the probability of an event (mortality) occurring in an exposed group (fracture) to the probability of the event in a comparison non-exposed group (population). SMR (squares): standardised mortality ratio, defined as the ratio of the observed deaths in the study group (fracture) to expected deaths in the general population. HR (cross): hazard ratio, defined in survival analysis as the ratio of probability of death in the fracture arm compared to the non-fracture arm.

**Table 2: Summary of studies addressing mortality after fracture at sites other than the hip.**

| Year of publication | Population | Site of Fracture | Follow-up after fracture | Mortality Indicator | Reference |
|---|---|---|---|---|---|
| 1993 | Rochester residents, USA | Vertebral | 5 years | Survival 61%<br>(76% in normal population)<br>Relative survival 0.81 | Cooper et al.[48] |
| 1998 | Population-based survey, 6,480 subjects, Europe<br>EPOS | Vertebral deformities | 2.3 years | Rate Ratio<br>1.3 in men, 1.9 in women | Ismail et al.[49] |
| 2000 | 6,459 women aged 55-81 years participating in the Fracture Intervention Trial, USA | Clinical Vertebral Fractures | 3.8 years | RR 8.64 | Cauley et al.[32] |
| 2003 | 598 normal population, Sweden (EVOS) | Vertebral deformities | 10 years | HR 2.4 in men<br>HR 2.3 in women | Hasserius et al.[50] |
| 2003 | 677 patients, Finland | Osteoporosis + Vertebral fractures | 3.2 years | HR 4.4 | Jalava et al.[51] |
| 2004 | 2,847 fractures, Sweden | Spine | 1 year<br>5 years | RR 10<br>RR 4.3 | Johnell et al.[34] |
| 2004 | 2,847 fractures, Sweden | Shoulder | 1 year<br><br>5 years | RR 4 (men)<br>RR 2.7 (women)<br>RR 2.1 (men)<br>RR 1.4 (women) | Johnell et al.[34] |
| 2004 | 2,847 fractures, Sweden | Forearm | 1 year<br><br>5 years | RR 1.1 (men)<br>RR 1.8 (women)<br>RR 1.2 (men)<br>RR 1.9 (women) | Johnell et al.[34] |
| 2005 | 2,847 fractures, Sweden | Vertebral deformities | 22 years | 111.7/1000 py in men<br>(73.4/1000 py in normal population)<br>95.1/1,000 py in women<br>(62.0/1,000 py in normal population) | Hasserius et al.[52] |
| 2009 | Prospective cohort from the Dubbo Osteoporosis Epidemiology Study of community-dwelling women and women aged 60 years and older, Australia | Major | 5 years | SMR 1.65 | Bliuc et al.[10] |
| 2010 | 629 patients, Osteoporosis Screening Execise, Japan | Vertebral | 10 years | SR 69% | Ikeda et al.[53] |
| 2010 | National Claim Registry, Korea | Vertebral | 2 years | SMR 2.53 (men), 1.86 (women)<br>Mortality rates 20.61% (men), 10.48% (women) | Lee et al.[54] |
| 2013 | 2,901 fractures, Olmsted county, USA | | 22 years | SMR 1.2<br>But mainly in the first 5 years following the fracture | Melton et al.[44] |

EPOS: European Prospective Osteoporosis Study; RR: relative risk; EVOS: European Vertebral Osteoporosis Study; HR: hazard ratio; SMR: standardised mortality ratio; SR: survival rate.

**Table 3: Summary of studies addressing mortality associated with low BMD.**

| Year of publication | Population | Site of BMD | Follow-up | Mortality Indicator | Reference |
|---|---|---|---|---|---|
| 2002 | 5,816 women, aged 70 years and above, USA | Quantitative Ultrasound of calcaneus | 5 years | HR 1.16 | Bauer et al.[60] |
| 2006 | 275 postmenopausal elderly women, Brazil | Femur dual energy X-ray absorptiometry | 5 years | HR 1.44 (total mortality) HR 1.28 (cardiovascular mortality) | Pinheiro et al.[61] |
| 2010 | 3,145 community dwelling people aged 65 years and above, China | Height Loss >2 cm, correlated with low BMD | 5 years | HR 3.43 | Auyeung et al.[62] |
| 2011 | 1,429 ambulatory postmenopausal female volunteers aged over 50 years, Japan | Lumbar spine BMD | 4.5 years | HR 1.39 | Shiraki et al.[63] |
| 2013 | Prospective population-based observational study on 390 white North European women aged 48 at study start, Sweden | Distal forearm BMD | 3.4 years | RR 1.36 | Svejme et al.[65] |

BMD: bone mineral density; RR: relative risk; HR: hazard ratio.
The mortality risk indicators were calculated by 1 standard deviation reduction of bone density or broadband ultrasonic attenuation.

## DISCUSSION AND CONCLUSION

We reviewed population studies addressing osteoporosis-related mortality, and identified a trend towards decreasing mortality over time. This review has some limitations. The included studies present a high degree of heterogeneity: different populations, different methodologies. The mortality indicators were highly heterogeneous, which also limits the comparisons. This heterogeneity may result in biased interpretation of comparability between populations at the same time point, between different populations at different time points, but also within the same populations at different time points. When separated by decades, confounding factors such as higher life expectancy may interfere with the interpretation of trends of crude mortality rates.

When considering major endpoints such as mortality, the patient should be viewed as a whole and their prognosis cannot be summarised by a single risk factor figure or a single health event. Otherwise healthy and fit patients do not seem to have increased mortality subsequent to the fracture. Male sex, age, site of fracture, the immediate post-fracture period, and poor general health status seem to be universally accepted risk factors for excess mortality.

Some risk factors, when taken individually, may be subject to controversies in different studies. Obesity for example is traditionally associated with lower fracture rates, but recent data from the GLOW study[70] suggest that obesity is associated with higher fracture rates at some specific sites such as the ankle[26] and also with longer hospital stays and poorer functional status.[71]

Due to the important contribution of comorbidities to mortality associated with osteoporosis, a general multivariable approach is suggested to predict mortality, rather than individual indicators such as BMD. Predictive models, similar to models used in cardiovascular diseases, may be developed for more patient-tailored preventive programmes in the future.

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# USE OF ULTRASOUND FOR DIAGNOSIS AND FOLLOW-UP OF PSORIATIC ARTHRITIS

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## ABSTRACT

Musculoskeletal ultrasound (US) is increasingly used as a bedside tool for diagnostic and monitoring purposes in patients with psoriatic arthritis (PsA). The sonographic differentiation between PsA and rheumatoid arthritis (RA) may be challenging because the morphological appearance of synovitis is similar in both conditions. In contrast, perisynovial inflammation is a specific finding of early PsA, and enthesitis is more frequently detected in PsA than in RA. After initiation of effective therapies, a reduction of US signs of synovitis and enthesitis can be seen along with clinical improvement. A numeric US score for regular monitoring of disease activity and damage in PsA patients has not been established yet. While sonographic findings can be discordant from clinical results, their relevance is unclear, although it is a concern that ongoing subclinical inflammation results in worse structural outcomes. Ongoing studies address the value of sonography as a diagnostic and prognostic marker in PsA, and we expect that these results will emphasise the role of diagnostic US for the routine evaluation of PsA patients.

Keywords: Psoriatic arthritis, ultrasonography, disease activity, spondyloarthritis.

## INTRODUCTION

Psoriatic arthritis (PsA) belongs to the group of seronegative spondyloarthropathies and is characterised by inflammation of joints, tendons, and/or entheses associated with psoriatic skin and/or nail lesions.[1] Clinical presentation and clinical course are highly variable, ranging from subtle pain at tendon insertions to mutilating arthritis, from monoarthritis to a 'rheumatoid arthritis (RA)-like' polyarticular phenotype, or from mild spinal inflammation to frank ankylosing spondylitis.[2] As PsA usually arises in patients with preexisting psoriasis, clinicians have the unique opportunity to screen a defined population (namely patients with psoriasis) in order to identify arthritic patients at an early stage.[3]

Unfortunately, there are no specific laboratory markers for the disease, and conventional radiography is of limited value for early diagnosis.[3] Imaging techniques such as magnetic resonance imaging (MRI) or musculoskeletal ultrasound (US) are new attractive tools supporting diagnostic and management decisions in PsA.[2] Sonography has the advantage over MRI of being widely available, having no contraindications, a higher resolution, and causing lower costs. On the other hand, some anatomical locations cannot be judged, and intraosseous lesions, such as bone marrow oedema, cannot be detected by sonography.[4]

### Clinical Diagnosis and Overlap of PsA with Other Diseases

For diagnosis of PsA the classification criteria for psoriatic arthritis (CASPAR) are commonly applied, although these criteria were primarily developed for classification of patients in clinical studies.[5] The diagnostic work-up of PsA patients may further be challenged by a clinical overlap with other diseases. Patients with psoriasis and polyarthritis, for example, may simultaneously fulfil the CASPAR criteria for PsA, and the 2010

American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA.[5,6] The correct diagnosis, however, has fundamental implications on initial and subsequent treatment strategies; EULAR recommendations suggest the use of non-steroidal anti-inflammatory drugs (NSAIDs) as a first-line treatment in PsA, whereas RA patients are initially treated with conventional synthetic disease-modifying anti-rheumatic drugs (DMARDs), such as ethotrexate.[7,8] Besides, there are more biologic agents approved for RA than for PsA.

Similarly, patients with distal interphalangeal (DIP) joint arthritis and bony thickening may be either classified as PsA or osteoarthritis (OA) and again, treatment of these two conditions is fundamentally different.[9] Whether sonography may help to differentiate overlapping conditions in patients with psoriasis will be discussed in the following sections.

## THE ROLE OF US IN PSA

### Detection of Inflammatory and Structural Lesions in PsA

There is now clear evidence that US has a high sensitivity and specificity to detect inflammatory lesions in patients with rheumatic diseases.[4] The new EULAR recommendations on the use of imaging methods in RA emphasised the possibility that sonography may be used to confirm the clinical finding of an 'involved' joint for the purpose of classifying patients with at least one joint with definite clinical synovitis.[10] One study in patients with very early arthritis suggested that US investigations may shift the diagnosis from monoarthritis to oligoarthritis or polyarthritis; the addition of sonographic findings to the 2010 CR/EULAR criteria increased the number of patients also fulfilling the 1987 ACR criteria after an 8-month follow-up period.[11]

In PsA, US studies have focused on the examination of peripheral joints and entheses (see Figure 1 for examples), although there was also some interest in the examination of sacroiliac joints.[2]

### PsA Associated Arthritis

Similar to the reports in RA, US revealed synovial inflammation more commonly than clinical examination; sonography was useful to exclude arthritis particularly in painful large joints.[12-14] Besides, the majority of PsA patients with clinically suspected oligoarthritis were reclassified as having polyarthritis based on the US result.[12,13] For structural lesions in hands, US and MRI were more sensitive than X-ray examination, and when comparing sonography with MRI, the former identified more osteophytes in small joints and erosions in proximal interphalangeal (PIP) joints, whereas the latter performed better for detection of erosions in metacarpophalangeal (MCP) and DIP joints.[14]

### PsA Associated Enthesitis

Several studies evaluated the relevance of US for the diagnosis of enthesitis in patients with established spondylarthritis (including PsA patients) revealing an overall better performance of sonography to identify inflammatory lesions as compared to clinical examination.[15,16] In patients with early or new onset PsA, US was also useful to exclude active inflammation at some tender entheses.[17,18]

### US of Sacroiliac Joints

Sacroiliac joint US was found to have a moderate-to-good sensitivity for diagnosis of spondylarthritis. However, this method is nevertheless of limited value because of the small acoustic window at sacroiliac joints, and because of the fact that bone marrow oedema, the most important sign of spondyloarthritis, cannot be detected by sonography.[19-23]

### US Findings in PsA Associated Dactylitis

US changes in dactylitis have been reported controversially in the literature.[24] The combination of arthritis and tenosynovitis was deemed as the underlying pathology of dactylitis in earlier publications, whereas recent US studies indicate that isolated tenosynovitis is the most common US abnormality, and arthritis occurs in only half of cases.[24,25] Recent MRI studies further suggest that soft-tissue oedema and/or collateral tendon enthesitis are characteristic findings of dactylitis.[26] A project of the Outcome Measures in Rheumatology (OMERACT) is currently underway to agree upon an US definition of dactylitis.[24]

### US of Skin and Nails

US has been used to investigate skin and nail lesions in patients with psoriasis. In B-mode, a psoriatic lesion is characterised by a thickened dermis and epidermis; Power Doppler (PD) may show increased blood flow within the dermis.

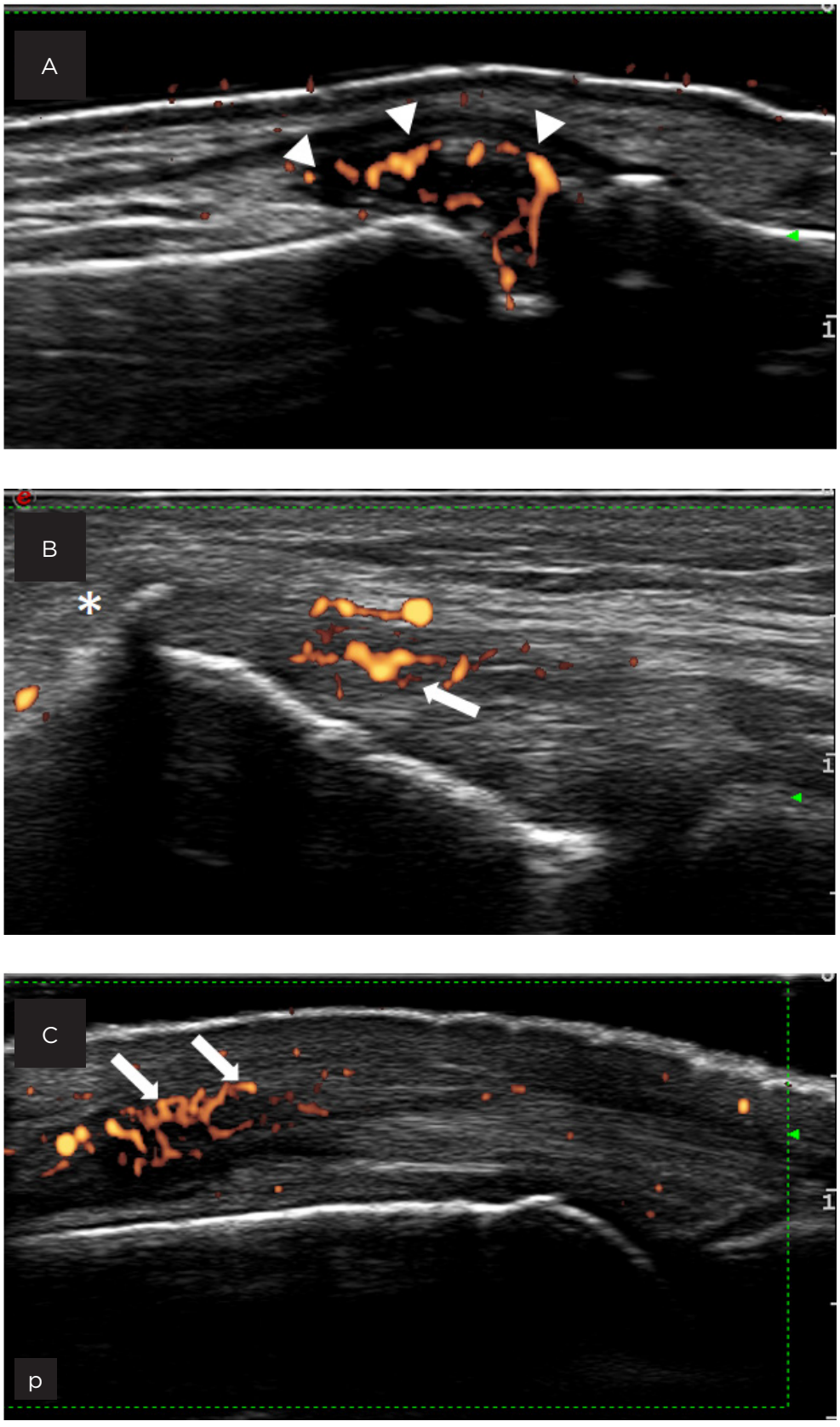


**Figure 1: Examples of ultrasound findings in psoriasis arthritis (PsA).**
A) Longitudinal dorsal scan of a distal interphalangeal joint from a PsA patient. Arrowheads indicate active synovitis with extensive Power Doppler (PD)-signals; joint space is marked with an asterix. B) Longitudinal scan of the lateral epicondyle from a PsA patient with enthesitis. Arrows indicate PD-signals within the enthesis and the open arrow marks an enthesophyte. C) Longitudinal dorsal scan of a metacarpo-phalangeal joint revealing active perisynovitis (arrows). Synovia are indicated by arrowheads and joint space by an asterix. p: proximal.

In patients with psoriatic onychopathy, US reveals hyperechoic parts and/or a loss of definition of nail plates. At later stages, a wavy or thickened appearance of plates with or without increased blood flow in the nail bed may be visible.[27]

## DIFFERENTIATION OF PSA FROM CLINICALLY OVERLAPPING CONDITIONS

### PsA and RA

Despite the fact that synovial tissue samples have suggested a difference in the histopathology of PsA and RA tissue,[28] joint synovitis from PsA and RA patients appears to be indistinguishable by means of MRI or US investigations.[14,29,30] In contrast, extrasynovial inflammatory changes are deemed as characteristic findings of PsA.[13,30] An MRI study of hands, for example, reported periarticular inflammation in small finger joints, particularly in collateral ligaments and periarticular soft tissue in PsA but not RA patients.[31] An Italian US study found that perisynovitis, an extensor peri-tendon inflammation at MCPs, is a specific pathology for patients with early PsA.[32] In patients with shoulder pain, synovitis at the acromioclavicular joint suggested underlying PsA, whereas glenohumeral joint effusion was the most common finding in RA patients.[33]

The presence and extent of US-verified enthesitis was useful to distinguish PsA from RA in one study,[34] whereas in another study clinical but not US scores of enthesitis were greater in PsA compared to RA patients.[35] RA patients from the latter study, however, were older and had a longer disease duration possibly affecting the result toward similar US findings in both groups.

The number and size of US-verified erosions at wrists, MCPs, PIPs, and metatarsophalangeal (MTP) joints may help to differentiate PsA from RA and OA. RA patients had generally more and larger erosions than PsA patients and the number and size of erosions was larger in PsA than in OA.[36] Besides, we know from a micro-computed tomography study that erosions in PsA are mostly Ω-shaped and tubule-shaped, whereas U-shaped lesions are characteristically found in RA.[37] Unfortunately, the morphology of erosions cannot be determined reliably by sonography because the overlying intact bone limits the acoustic window. Osteophytes were generally increased in number, extent, and size in PsA compared to RA, often affecting the entire circumference of the bone (so called 'bony corona').[37]

### PsA and OA

DIP joint involvement belongs to the most characteristic manifestations of PsA.[1] Differentiation of PsA-related DIP arthritis and (activated) OA, however, might be challenging. In an MRI study comparing DIP joints affected by PsA or OA, entheseal and ligament enhancement, extracapsular changes, and diffuse bone oedema were more commonly observed in PsA than in OA.[38] Differentiation of the two conditions by MRI in individual cases, however, was limited because none of the items were specific enough for a reliable diagnosis.

### PsA and Psoriasis

The Madrid Sonographic Enthesitis Index (MASEI) was used to distinguish between patients with PsA and psoriasis (without arthritis), revealing higher inflammatory and damage subscores in the former group compared to the latter. A MASEI ≥20 had a specificity of 90% to correctly classify PsA patients.[39] Another study showed that subclinical enthesitis in PsA is linked with more PD signals than subclinical enthesitis in psoriasis.[40]

Subclinical synovitis and enthesitis was more commonly observed in patients with psoriasis than in healthy individuals, particularly in cases with psoriatic nail disease.[17,41-44] The relevance of this finding for patients' outcome is elusive so far as only a small study suggested that US-verified subclinical enthesitis in patients with psoriasis might predict later onset of PsA.[45]

## CLINICAL AND SONOGRAPHIC MONITORING OF PSA PATIENTS

### Association between Clinical Composite Scores and US Findings

Regular measurement of disease activity and adjustment of therapy targeted at remission are important principles of current EULAR guidelines and 'treat to target' recommendations for PsA.[7,46] In routine practice and clinical trials, PsA disease activity is usually measured with tools 'borrowed' from RA.[46] Recently, new PsA specific composite scores have been proposed; the Disease Activity Index for Psoriatic Arthritis (DAPSA) combines the number of tender (TJ) and swollen joints (SJ),

patients' pain, and global assessment, as well as the C-reactive protein (CRP) level,[47] whereas the Composite Psoriatic Disease Activity Index (CPDAI) corroborates PsA specific domains including joint disease, enthesitis, dactylitis, and skin and axial manifestations, as well as quality of life (QoL).[48] As part of the GRACE (GRAPPA Composite Index Exercise) project, the Psoriatic Arthritis Disease Activity Score (PASDAS) was developed based on the combination of TJ, SJ, global assessments, enthesitis, dactylitis, QoL, and CRP.[49]

None of these new scores, however, has been validated sufficiently so far, and only one study compared the DAPSA and CPDAI with sonography - reporting a considerable disparity between clinical and US results.[50] In this study, the correlation between clinical examination and US was better in joints compared to other PsA manifestations, and the joint-focused DAPSA performed better to identify patients with US-verified active disease than the multifactorial CPDAI.[51] US-verified enthesitis, dactylitis, tenosynovitis, and perisynovitis were not reflected by clinical parameters.

## US as a Tool to Measure Disease Activity in Follow-up Studies

In contrast to the large number of studies investigating the value of sonography for follow-up of RA patients,[4] a few studies have been performed in PsA so far. One study reported that sonography was helpful to monitor the improvement of knee synovitis in PsA and RA patients treated with etanercept;[51] a retrospective study observed a decrement of both US and clinical signs of inflammation in PsA patients treated with adalimumab,[52] and a prospective multicentre Spanish study found improvements in sonographic and clinical scores after infliximab therapy.[53] In a trial aimed at the validation of the Sonography of LArge joints in Rheumatology (SOLAR) score, 126 PsA or AS patients were investigated before and after instigation of conventional synthetic or biologic DMARD therapy.[54] Grey-scale and PD scores of all joint areas exhibited a significant improvement at follow-up.

The responsiveness of US-verified enthesitis to anti-TNF-α therapy was tested in a large Spanish study involving 35 centres. Naredo et al.[55] investigated 197 patients with spondyloarthritis including 34 (17%) PsA patients. B-mode abnormalities and PD signals were reduced after therapy, whereas structural changes such as calcific deposits and cortical abnormalities were not responsive to treatment. Similar observations were made in another study focusing on the Achilles tendon.[56]

Latest US developments also allow for the detection of dermal perfusion changes in patients with psoriatic plaques.[57] In patients receiving TNF-α blocking therapy, a significant correlation between changes of PD, Psoriasis Area and Severity Index (PASI), and the histologically-determined number of blood vessels within psoriatic lesions was observed.[58]

## Remission Assessment in PsA

Although remission is the overarching therapeutic goal in PsA, a definition of clinical remission has not been established so far.[7] Criteria for minimal disease activity (MDA) were recently validated in two prospective PsA cohorts and were useful to distinguish between patients at high and low risk of radiographic progression.[59-61] Complete abrogation of structural damage, however, was not achieved despite MDA, and it is concerning that the presence of subclinical inflammation, as observed in a considerable proportion of patients, may be linked with structural deterioration.[50] A similar concept is currently discussed for RA, but future studies are necessary to test the possible link between US-verified inflammation and radiographic outcomes in both RA and PsA.[4]

## US Composite Scores in PsA

In clinical studies and/or routine practice, US composite scores may be applied for a regular sonographic scoring. In RA, several scoring systems have been proposed, whereas in PsA only two US composite scores have been evaluated so far.[62-66] The Italian 'Five Targets Power Doppler for Psoriatic Disease (5TPD)' US score focuses on 'five targets' (joints, tendons, entheses, skin, and nails) and revealed adequate sensitivity to change in the short-term follow-up of anti-TNF-α therapy. However, as only one anatomical site was investigated for each target, this score is of limited value to determine actual disease activity.

The German US7 score was primarily developed for RA and was only later tested in a small group of PsA patients.[62,65] In RA, the US7 score better reflected the extent of joint inflammation than the DAS28. For PsA, this score is of limited value because of the omission of enthesitis and DIP arthritis, which are important PsA manifestations.

We therefore need a new US composite score that includes all important PsA manifestations, is sensitive, reliable, and feasible in clinical routine.

## PROGNOSTIC VALUE OF US IN PSA

Treatment decisions in rheumatology are usually based on prognostic factors predicting clinical, structural, and functional outcomes as well as treatment success. In RA, high disease activity state, autoantibody positivity (rheumatoid factor and/or antibodies to citrullinated proteins), and the early presence of joint damage are associated with a high risk of rapid radiologic damage.[67,68] Similarly, we know that in PsA a high clinical disease activity, radiographic progression, functional limitations, elevated acute phase reactants, and previous corticosteroid therapy are predictors of a worse outcome.[8,69]

### US for Prediction of Disease Flare and Structural Deterioration

Several studies investigated the prognostic value of sonography in RA, whereas, the number of such studies in PsA is scarce. In RA patients with clinical remission for example, the presence of US-determined synovial hypertrophy and/or enhanced vascularity was associated with an increased risk of developing future clinical flares and experiencing radiographic progression.[70-72] In PsA, only a single study evaluated the value of ultrasonography as a predictor of structural progression in patients with recently diagnosed PsA.[73] The authors reported that a grey-scale score of ≥2, a PD score of ≥2, the presence of enthesitis, and US signs of onychopathy at baseline, as well as persistent synovitis and enthesitis after 6 months, were significant predictors of structural deterioration.[70]

### US for Prediction of Treatment Response

The value of ultrasonography for predicting therapy response in PsA has not been investigated so far. In RA, it was reported that patients with a high number of PD-signals at baseline have a worse response to biological treatments, and the results of a Danish study revealed that a higher grade of US-verified inflammation predicted a better maintenance of anti-TNF-α therapy after 1 year.[74,75]

## SUMMARY

US may be an attractive tool for the diagnosis and monitoring of PsA patients. Perisynovial inflammation as well as enthesitis appear to be the most characteristic US findings in PsA, enabling the differentiation of the disease from overlapping conditions. During follow-up, US findings in joints, enthuses, and skin are responsive to therapy with biological agents. A PsA-specific US composite score is warranted for a standardised sonographic scoring of patients in clinical studies and daily routine. Such a score should include all PsA manifestations and should be sensitive, reliable, and feasible. The relevance of sonography for remission assessment and the value of this tool as a biomarker in PsA have to be clarified by future research.

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# UPDATE ON GENETIC SUSCEPTIBILITY AND PATHOGENESIS IN JUVENILE IDIOPATHIC ARTHRITIS

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## ABSTRACT

Juvenile idiopathic arthritis (JIA) is a multifactorial disease with a pathogenesis which remains inexplicable. However, genome-wide association studies brought forward within recent years have discovered several new susceptibility genes, and accumulating evidence supports genetic variability as playing a key role in JIA development. This review summarises the present knowledge of human leukocyte antigen (HLA) and non-HLA polymorphisms conferring disease susceptibility, and discusses the areas in JIA genetics, which are still to be investigated in order to apply JIA genetics in a clinical setting.

Keywords: Juvenile idiopathic arthritis, arthritis, juvenile, genetics, genetic predisposition to disease, individualised medicine.

## INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children, with a reported prevalence of around 16-150 per 100,000.[1] The term JIA encompasses a diverse group of arthritides characterised by onset of disease before the age of 16, with arthritis lasting >6 weeks, and with an unknown cause. JIA is divided into seven subgroups according to the classification provided by a task force within the International League of Associations for Rheumatology:[2] systemic arthritis (sJIA), oligoarthritis (oJIA; further divided into persistent oJIA [per-oJIA] and extended oJIA [ext-oJIA]), rheumatoid factor-negative polyarthritis (RFneg-pJIA), rheumatoid factor-positive polyarthritis (RFpos-pJIA), enthesitis-related arthritis (ERA), psoriatic arthritis (PsA), and undifferentiated arthritis. Characteristically, an uneven distribution of female and male cases is seen in the different subgroups of JIA. A female majority is seen in oJIA, pJIA, and PsA, in contrast to ERA where male cases predominate.[1]

The aetiology of JIA is believed to be multifactorial with both genetic and environmental factors[3] - such as infections, breastfeeding, and maternal smoking during pregnancy - although knowledge of the latter remains sparse. For many years, genetic studies were limited to a candidate gene approach with both productive and unproductive outcomes.[4] During the last decade, the introduction of genome-wide association studies (GWAS) with a hypothesis-free approach has proven to be a valuable tool for investigating the genetics of JIA, and has discovered several new loci conferring JIA susceptibility. However, despite this new knowledge, a complete understanding of JIA pathogenesis still remains elusive.

## JIA AS A GENETIC DISEASE

JIA is considered a complex genetic disease[5] with a non-Mendelian inheritance pattern. In addition to the knowledge we have today of JIA pathogenesis and multiple genetic loci conferring susceptibility to JIA, the idea of a genetic aetiology has been supported by reports of affected twins and sib pairs with phenotypic concordance in each family

regarding JIA subtypes.[6-9] A study using the Utah Population Database sought to quantify the familial contribution to JIA and found a significant risk in relatives of JIA patients compared to the background population; 11.6 in siblings ($p<2.59x10^{-8}$) and 5.82 in first-degree cousins ($p<6.07x10^{-5}$), respectively. Additionally, the contribution of familial factors in JIA risk was estimated to be 13%.[9]

It has also become evident that other autoimmune disorders, such as Type 1 diabetes, coeliac disease, autoimmune thyroiditis, and other chronic arthritides, are clustering in relatives of JIA patients and in patients as well.[10-14] In a US cohort, the odds ratio (OR) of a JIA relative having at least one autoimmune disorder compared to controls was 3.4 ($p<1x10^{-6}$).[10] This suggests a shared genetic aetiology in an otherwise heterogeneous group of diseases and has to be considered when investigating the pathogenesis and genetic susceptibility of JIA.

## HLA GENES AND JIA SUSCEPTIBILITY

Ever since the discovery of the human leukocyte antigen (HLA) B27 association in ankylosing spondylitis,[15,16] and subsequently juvenile rheumatoid arthritis (JRA),[17] the HLA gene complex has been subject to vast investigations concerning its role in arthritis pathogenesis. The HLA gene complex, located at 6p21.3, remains the single most significant gene region in conferring susceptibility to JIA with particular importance of the HLA-DRB and HLA-DQA/B genes; this was recently confirmed in the largest genetic study on JIA patients (oJIA and RFneg-pJIA) done so far.[18] A single nucleotide polymorphism (SNP) rs7775055 (G>A), tagging the HLA-DRB1*0801-HLA-DQA1*0401-HLA-DQB1*0402 haplotype, showed definite association with oJIA and RFneg-JIA (OR=6.01, $p<3.14x10^{-174}$), and in particular, oJIA (OR=6.78, $p<2.24x10^{-162}$).[18] An important feature regarding HLA genes is the high degree of linkage disequilibrium (LD); thus, it has been a challenge to determine which of the multiple adjacent loci is truly associated with disease risk. This always has to be taken into account when interpreting genotyping results in the HLA complex.

Table 1 summarises the results (only significant results shown) from two of the largest studies[19,20] investigating associations between HLA alleles/haplotypes and JIA in UK/US Caucasians. The best documented allele is the HLA-DRB1*08 which confers susceptibility to JIA (driven by per-oJIA, ext-oJIA, and RFneg-pJIA). Similar results were found with DQA1*04 and DQB1*04, but these alleles are in LD with the DRB1*08 allele and evidence has been reported indicating that DRB1*08 is the true risk allele.[20,21] Other DRB1-alleles associated with disease predisposition are: DRB1*01 (only ERA and PsA; LD with B27 and DQA1*0101), DRB1*11, and DRB1*13 (only per-oJIA). Protective alleles are: DQA1*0102, DQA1*02, DQA1*03, DRB1*04, and DRB1*07, of which DQA1*03 and DRB1*04 are in LD. It should be noted that DRB1*04, which is a rheumatoid arthritis (RA) susceptibility allele, is associated with risk of the juvenile analogue to RA, RFpos-pJIA. Other alleles outside the DRB1 and DRQ genes, such as A*0201, C*0202, and DPB1*0201, are also associated with oJIA risk, whereas A*0101 is protective.

Additionally, Hollenbach et al.[20] showed age-at-onset effects of different haplotypes; 0801-0400-0402 and 1103/04-0500-0301 conferring risk of an early onset of disease (<6 years). Still, the exact mechanisms of how the different alleles are involved in JIA pathogenesis are poorly understood. More recently, studies have tried to identify the actual causal variants in the different DRB1 alleles by focusing on particular amino acid residues in the antigen binding cleft of the DRβ1 protein.[22,23] Thomson et al.[22] studied the amino acid residues (9-86) encoded by exon 2 in the HLA-DRB1 gene and found that variations in the amino acids 13, 37, 57, 67, 74, and 86 are important risk factors in JIA susceptibility. Prahalad et al.[23] investigated the frequency of RA-associated amino acid sequences in residues 70-74 (the so-called shared epitope [SE]) in RFpos-pJIA patients and found a significantly higher frequency of SE alleles in cases compared to healthy controls. Further investigations on the role of key amino acids are important to elucidate the mechanisms of HLA in JIA pathogenesis and autoimmunity in general.

## NON-HLA GENES AND JIA SUSCEPTIBILITY

Prahalad et al.[24] estimated the HLA-DR region to account for 17% of JIA risk, and more recent estimates of the whole HLA complex have been as low as 8-13%.[18,25] This supports the increasing amount of research focusing on finding susceptibility loci outside the HLA gene complex. As mentioned earlier, studies have - for many years - been limited to a candidate gene approach often based on findings from gene expression levels.

But in the past 5 years, with the use of genome-wide genotyping methods and analyses, many new susceptibility loci have been identified.[25-27]

Additionally, the idea of a closely related genetic pathogenesis among several different autoimmune diseases has also proved to be a successful method in finding new JIA susceptibility loci.[18,28-32]

**Table 1: JIA subtypes and associated HLA allele/haplotype variants in US/UK Caucasians.**

| Allele/haplotype | Ref. | JIA | Per-oJIA | Ext-oJIA | RF-pJIA | RF+pJIA | sJIA | ERA | PsA |
|---|---|---|---|---|---|---|---|---|---|
| A*0101 | 20* | | | 0.46 | | | | | |
| A*0201 | 20 | | | 1.96 | | | | | |
| B*27 | | | | | | | | Criteria | |
| C*0202 | 20 | | [a]2.05 | | | | | | |
| DPB1*0201 | 19 | | 2.1 | 2 | | | | 0.1 | |
| DQA1*0101 | 19 | | | | | | | 2.8 | 4.2 |
| DQA1*0102 | 19 | 0.6 | 0.6 | 0.4 | | | | | |
| DQA1*0103 | 19 | 2.7 | 5.7 | | | | | | |
| DQA1*02 | 19 | 0.6 | 0.4 | | | 0.1 | | | |
| DQA1*03 | 19 | 0.6 | 0.4 | 0.2 | | 4 | | | 0.3 |
| DQA1*04 | 19 | 4.4 | 5.1 | 10 | 4.2 | | | | |
| DQA1*05 | 19 | 1.7 | 2.5 | | | | 2.6 | | |
| DQB1*03 | 19 | | | | 0.7 | 6.1 | | | 0.5 |
| DQB1*04 | 19 | 3.5 | 5 | 7.4 | | | | | |
| DQB1*05 | 19 | | | 1.8 | | | | 3.5 | 4.4 |
| DRB1*01 | 19 | | | 2 | | | | 3.6 | 2.7 |
| DRB1*04 | 19 | 0.6 | 0.3 | 0.1 | 0.7 | 3.2 | | | 0.3 |
| DRB1*07 | 19 | 0.6 | 0.3 | | 0.5 | 0.1 | | 0.3 | |
| DRB1*08 | 19 | 3.0 | 3.9 | 6.3 | 3.1 | | | | |
| DRB1*11 | 19 | 2.0 | 2.5 | 2.5 | | | 2.8 | | |
| DRB1*13 | 19 | | 1.8 | | | | | | |
| [b]01-0101-0501 | 19 | | | 1.9 | | | | 4.9 | 3.8 |
| 0401-03-03 | 19 | 0.6 | 0.4 | 0.1 | | 3.9 | | | |
| 0401-0300-0301 | 20 | [c]0.34/NS | 0.21/NS | | | | | | |
| 0701-0201-0201 | 19,20 | 0.42/NS[20] | 0.2[19,20] | | | | | | |
| 0801-0400-0402 | 20 | 7.14/4.08 | 8.70/5.35 | 5.36 | 5.52/3.31 | | | | |
| 0801-0401-0402 | 19 | 4.1 | 6.1 | 10.3 | | | | | |
| 11-05-03 | 19 | 2.1 | 2.2 | 3 | | | 4.3 | | |
| 1103/04-0500-0301 | 20 | 5.99/3.55 | 5.43/NS | 4.65 | 6.90/NS | | | | |
| 13-01-06 | 19 | 3.0 | 6.4 | | | | | | 4.5 |
| 1301-0103-0603 | 20 | 2.04/NS | 2.3 | | | | | | |
| 1501-0102-0602 | 20 | 0.28/0.61 | 0.21/0.54 | 0.48 | 0.28/NS | | | | |

JIA: juvenile idiopathic arthritis; HLA: human leukocyte antigen; Per-oJIA: persistent oligoarticular juvenile idiopathic arthritis; Ext-oJIA: extended oligoarticular juvenile idiopathic arthritis; RF-pJIA: rheumatoid factor-negative polyarticular juvenile idiopathic arthritis; RF+pJIA: rheumatoid arthritis-positive polyarticular juvenile idiopathic arthritis; sJIA: systemic juvenile idiopathic arthritis; ERA: enthesitis-related arthritis; PsA: psoriatic arthritis; NS: not significant.

*Hollenbach et al.[20] investigated a cohort limited to per-oJIA, ext-oJIA, and RF-pJIA cases; [a]OR: odds ratio; [b]Haplotype DRB1-DQA1-DQB1; [c]<6 years/≥6 years.

In 2013, the results from the largest genetic JIA study so far, utilising both of these two approaches, were published.[18] A study which included 2,816 patients (oJIA and RFneg-pJIA) and 13,056 healthy controls using the Immunochip array,[33] analysed a total of 123,003 SNPs. 17 loci reached genome-wide significance ($p<5x10^{-8}$), of which 3 loci (HLA, PTPN22, and PTPN2) previously have shown genome-wide significant associations with JIA; 5 loci have supporting evidence from previous studies (STAT4, ANKRD55, IL2-IL21, IL-2RA, and SH2B3-ATXN2). Furthermore, an additional 11 loci almost reached genome-wide significance ($5x10^{-8}$, $p<1x10^{-6}$).

## The PTPN22 Gene

Table 2 lists the genetic polymorphisms that have shown an association with JIA susceptibility and have been confirmed in ≥2 cohorts (a comprehensive review of both productive and unproductive candidate gene studies has previously been done by Sampath and Glass[4]). The best verified non-HLA gene involved in JIA susceptibility and several other autoimmune diseases, such as RA, Type 1 diabetes, systemic lupus erythematosus, and Graves' disease,[34,35] is PTPN22 (OR ~1.55).[18,28,35-40] PTPN22 encodes the lymphoid protein tyrosine phosphatase (Lyp) involved in modulation of T cell receptor signalling. Of particular interest is the non-synonymous SNP rs2476601 (G>A, also known as c.1858C>T or R620W), a missense mutation proposed to be functional by compromising the ability of Lyp to bind to the C-terminal Src kinase (Csk) and create the Lyp/Csk complex. This complex is responsible for the inactivation of lymphocyte-specific protein tyrosine kinase (Lck, a key mediator of T cell receptor signalling).[34]

## Other Potential Disease-Causing Variants

Many of the SNPs associated with JIA risk are intergenic or intronic, and therefore these SNPs are less likely to be the actual disease-causing variants. Instead, these SNPs suggest an involvement of the adjacent genes in disease pathogenesis. Still, it is important to note that the role of the non-coding SNPs should not be neglected entirely, since a study on the genetic variation in different common diseases (JIA not included) found many of the non-coding SNPs to be located in regulatory DNA.[64] The current knowledge of the mechanistic effects of associated SNPs, however, is generally limited to non-synonymous SNPs located in exons or SNPs in the promoter regions of genes. rs3184504 (also known as c.784T/C or R262W), which is associated with JIA susceptibility (OR 1.20),[18] is located in exon 3 of the SH2B3 gene and is therefore a possible causal variant. This polymorphism has been reported to influence the level of T cell proliferation.[65] The 32bp deletion (Δ32) of CCR5, associated with a lowered JIA risk (OR ~0.80), causes a frame-shift leading to a non-functional chemokine receptor. This is thought to impair the recruitment of T cells in the autoimmune reaction.[55]

Other polymorphisms such as rs755622 (-173G>C, macrophage migration inhibitory factor [MIF]), rs1800629 (-308G>A, tumour necrosis factor [TNF]), and allele 3 of the $(GT)_n$ microsatellite repeat (SLC11A1) are located in the promoter regions of their respective genes. These variants have been associated with a higher expression of their gene products and are therefore likely contributors to JIA pathogenesis.[43,45,66] Conversely, the protective SNP rs1800795 (-174G>C, Interleukin 6 [IL-6]) is associated with low IL-6 levels.[58] The functions of other genes, listed in Table 2, support their relevance as JIA susceptibility genes, but how polymorphisms/mutations in these regions affect gene expression or function is still to be discovered.

Of the loci, whose associations are still to be replicated, those reported in the Immunochip study,[18] of course, also need to be mentioned (TYK2, ERAP2-LNPEP, UBE2L3, C5orf56-IRF1, RUNX1, IL-2R, ATP8B2-IL6R, FAS, and ZFP36L1). Of particular interest is the rs34536443 SNP, located in a coding region (exon 23) of TYK2; thus, it is likely to be a causal variant.

# SYSTEMIC JIA – A SEPARATE ENTITY

Systemic JIA (formerly known as Still's disease) is defined by the presence of arthritis accompanied or preceded by a quotidian fever lasting >2 weeks and including at least one of the following features: evanescent erythematous rash, generalised lymphadenopathy, hepatosplenomegaly, or serositis.[2] The pathogenesis of sJIA is characterised by an activation of the innate immune system leading to an unbalanced secretion of inflammatory cytokines. Due to the distinct clinical features along with a characteristic pathogenesis of autoinflammation rather than T cell-driven autoimmunity, sJIA is regarded a separate disease entity.[67,68]

This idea is also supported by the findings of genes conferring susceptibility to sJIA. First of all, variation in the HLA genes is generally not associated with risk of sJIA, although Thomson et al.[19] did find the HLA-DRB1*11-allele to be associated with sJIA (Table 1). Instead, several genes encoding cytokines have been reported to be associated with sJIA susceptibility. The synonymous SNP, rs1800795 (G>C), in the promoter region of IL-6, has been reported to confer protection in Caucasians in two studies,[57,58] and rs1800896 (G>A) near IL-10 has been associated with sJIA risk in both UK and German cohorts.[51,52] Additionally, studies have found an association between sJIA susceptibility and variants in the IL-1A[69,70] and IL-20[52,71] genes. But due to overlapping cohorts, these findings still need to be replicated. So far, no GWAS has been published investigating patients with sJIA. However, a study is currently underway.[72]

The distinct characteristics of sJIA pathogenesis also seem to influence the efficacy of different disease-modifying anti-rheumatic drugs (DMARDs). Methotrexate (MTX) and corticosteroids (the side-effects considered), as well as biological anti-TNF agents, have been rather unsuccessful. The knowledge of sJIA pathogenesis has instead proven valuable in generating new treatments, and today, anakinra (IL-1 receptor antagonist) and tocilizumab (humanised antibody against the IL-6 receptor) are the most efficacious options in the treatment of sJIA patients.[73]

**Table 2: Non-HLA polymorphisms conferring JIA susceptibility*.**

| Association | Gene region | Chr. | SNP/allele | Gene function | Ref. |
|---|---|---|---|---|---|
| Risk | PTPN22 | 1 | rs2476601 (G>A)<br>rs6679677 (C>A) | Encodes the Lyp – a key modulator of TCR signalling.[34] | 18, 28, 35-40 |
| | STAT4 | 2 | rs7574865 (G>T)<br>rs10174238 (A>G)<br>rs3821236 (G>A) | Stat4 is a transcription factor, particularly involved in IL-12-receptor signalling leading to Th1 cell differentiation and IFN-γ production. | 18, 28,29, 31, 37, 39, 41 |
| | PTPN2 | 18 | rs7234029 (A>G)<br>rs2847293 (T>A) | Modulating several cytokine-signalling pathways such as IL-2, IL-4, IL-6, and IFN-γ. | 18, 28, 32 |
| | SLC11A1 | 2 | $(GT)_n$ allele 3 (MS) | Nramp1 is a membrane protein in macrophages, modulating their activation. Conversely, allele 2 is associated with infections. | 42, 43 |
| | SH2B3-ATXN2 | 12 | rs3184504 (G>A)<br>rs17696736 (A>G) | SH2B3 is a part of the TCR signalling pathway. | 18, 28-30, 37 |
| | TIMMDC1-CD80 | 3 | rs4688011 (C>T)<br>rs4688013 (G>A) | TIMMDC1 is a complex 1 assembly factor in the mitochondria. CD80 is placed on the surface of B cells, providing co-stimulatory signals for T cell activation. | 18, 25, 37 |
| | AFF3-LONFR2 | 2 | rs6740838 (C>A)<br>rs1160542 (A>G) | AFF3 is expressed in lymphoid tissue and is believed to regulate lymphoid development. | 18, 44 |
| | IL2-IL21 | 4 | rs17388568 (G>A) | See below. | 28 |
| | MIF | 22 | rs755622 (G>C) | MIF binds to CD74 and induces secretion of TNF-α, IFN-γ, IL-1β, IL-6, and IL-8 by Th1 cells. | 45, 46 |
| | TRAF1/C5 | 9 | rs2900180 (C>T)<br>rs10818488 (G>A) | TNF-receptor associated factor 1 is involved in T cell survival through 4-1BB (CD137). C5 encodes complement component 5. | 31, 37, 47, 48 |
| | TNF† | 6 | rs1800629(G>A) | TNF-α mediates systemic inflammation and acuse phase response. | 49, 50 |
| | IL10‡ | 1 | rs1800896 (G>A) | Anti-inflammatory cytokine secreted by monocytes and Th2 cells. | 51, 52 |
| | TNFAIP3 | 6 | rs6920220 (G>A) | See below. | 29, 31 |
| | WISP3 | 6 | rs2280153 (G>A) | Regulates cellular functions (adhesion, migration, and differentiation). | 53 |

**Table 2 continued.**

| Association | Gene region | Chr. | SNP/allele | Gene function | Ref. |
|---|---|---|---|---|---|
| Protective | ANKRD55 | 5 | rs71624119 (G>A) rs10040327 (C>A) | Ankyrin repeat domain containing protein 55. Involved in N-glycosylation of immunoglobulin G. | 18, 32 |
| | IL2-IL21 | 4 | rs1479924 (A>G) rs13143866 (G>T) rs6822844 (G>T) | IL-2 is important for clonal expansion of lymphocytes. IL-21 is involved in activation and differentiation of several immune cells (macrophages, NK cells, and B cells). | 18, 28, 32, 44, 54 |
| | IL2RA | 10 | rs7909519 (A>C) rs2104286 (A>C) | IL-2 receptor α subunit, mediates IL-2 signalling. | 18, 27, 28 |
| | COG6 | 13 | rs7993214 (G>A) | Subunit in the oligomeric Golgi complex which ensures the integrity of the Golgi apparatus. | 18, 28 |
| | ANGPT1 | 8 | rs1010824 (C>T) | Angiopoietin-1 activates the TIE2 receptor leading to angiogenesis, neutrophil chemotaxis, and secretion of MMPs. | 28 |
| | VTCN1 | 1 | rs2358820 (G>A) | A B7 costimulatory protein (B7-H4) on the surface of antigen presenting cells interacting with T cells. | 27 |
| | CCR5 | 3 | Δ32 allele | Expressed on T cells and macrophages and serves as receptor for MCP-2, MIP1α/β, and CCL5. | 55, 56 |
| | TNFAIP3 | 6 | rs10499194 (C>T) rs13207033 (G>A) | TNFα-induced protein 3 modulates NF-κB signalling downstream of TNFα and TLRs. | 29, 31, 39 |
| | IL6‡ | 7 | rs1800795 (G>C) | IL-6 is a proinflammatory cytokine secreted by macrophages, mediating fever and acute phase response. | 57, 58 |

*The table shows only susceptibility loci with significant association in ≥2 cohorts. † Studies regarding polymorphisms in the TNF gene region have been rather inconclusive and unreplicated associations of several different polymorphisms have been reported[59-63]; ‡ only associated with sJIA susceptibility. Other loci are associated with JIA (often attributable to oJIA and pJIA).

HLA: human leukocyte antigen; JIA: juvenile idiopathic arthritis; MS: microsatellite; SNP: single nucleotide polymorphism; TCR: T cell receptor; Lyp: lymphoid protein tyrosine phosphatase; IL: interleukin; MIF: macrophage migration inhibitory factor; Th1: Type 1 T helper (Th1); IFN-γ: interferon-gamma; TNF: tumour necrosis factor; NK cells: natural killer cells; MMP: matrix metalloproteinase; TIE2: tyrosine kinase with immunoglobulin-like and EGF-like domains 2; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; CCL5: chemokine ligand 5; TLRs: toll-like receptors.

## FUTURE PERSPECTIVES IN JIA GENETICS

### What is Next in Future JIA GWASs?

The vast majority of genetic studies done so far have focused on finding loci conferring susceptibility to JIA. These studies have been crucial for acquiring an understanding of the pathogenesis of JIA but also in terms of developing new treatment options. However, an increasing number of studies have been focusing on more specific outcomes, often related to either disease course or treatment responses (pharmacogenomics). A list of such findings is shown in Table 3.

As mentioned earlier, the big advantage of a genome-wide approach is that it is hypothesis-free. However, due to the many SNPs genotyped, it is challenging to reach significant results after correction for multiple testing (e.g. genome-wide significance, typically $p<5x10^{-8}$). It requires high demands on the size of the cohorts investigated and, as such, international collaborations such as the International Childhood Arthritis Genetics Consortium are essential to acquire enough patient material. The Immunochip study[18] with 2,816 cases (oJIA and RFneg-JIA), including US, UK, and German samples, is the only study to have reached genome-wide significance ($p<5x10^{-8}$) in multiple loci.

**Table 3: Genetic variations associated with specific outcome in JIA patients.**

| Category | Gene region | Chr. | SNP/allele/GT | Association | Ref. |
|---|---|---|---|---|---|
| Disease course | HLA | 6 | B27 | HLA-B27-pos. children had higher odds of not being in remission after 8 years of disease than HLA-B27-neg. children | 74 |
| | MIF | 22 | rs755622 (G>C) | Higher number of affected joints and higher C-HAQ scores | 75 |
| | NLRP3 | 1 | rs4353135 (T>G) | Increased need for etanercept (anti-TNF) treatment | 76 |
| | IL6 | 7 | rs1800795 (G) | Pain scores (VAS) | 77 |
| | TNF | 6 | rs1800629(G>A) | Higher disease activity, C-HAQ scores (trend), and TNF-α levels | 66 |
| | | | rs1800629(G>A) | Poor outcome | 78 |
| | VTCN1 | 1 | rs10923223 (T>C) | Remitting disease course | 79 |
| | CDK6 | 7 | rs42041 (C>G) | Remitting disease course | 79 |
| | MBL2 | 10 | XA/O or O/O genotype | Remitting disease course | 80 |
| | TGFB1 | 19 | rs1800471 (C>G, GG) | Protective effect against joint space narrowing | 77 |
| MAS susceptibility | IRF5 | 7 | rs2004640 (G>T) | Higher susceptibility to MAS | 81 |
| | PRF1 | 10 | rs35947132 (G>A) | Higher susceptibility to MAS (did not reach statistical significance) | 82 |
| | UNC13D | 17 | 12-SNP haplotype | Higher susceptibility to MAS (9 of 16 sJIA patients with MAS) | 83 |
| MTX response | ABCB1 | 7 | rs1045642(G>A) | Good response to MTX | 84 |
| | ABCC3 | 17 | rs4793665 (C>T) | Good response to MTX | 84 |
| | SLC16A7 | 12 | rs10877333 (T>G) | Good response to MTX | 85 |
| | | | rs3763980 (T>A) | Increased risk of non-response to MTX (validated) | 85 |
| | ATIC | 2 | rs12995526 (T>C) | Increased risk of non-response to MTX | 86 |
| | | | rs4673990 (T>C) | Increased risk of non-response to MTX | 86 |
| | ITPA | 20 | rs2295553 (T>C) | Increased risk of non-response to MTX | 86 |
| | SLC19A1 | 21 | rs1051266 (C>T) | Increased risk of non-response to MTX | 84 |
| MTX toxicity | MTHFR | 1 | rs1801133 (C>T, TT) | High incidence of any adverse effects | 87 |
| | GGH | 8 | rs1800909 (T>C, CC) | Risk factor for liver dysfunction | 88 |
| Glucorticoid response | MIF | 22 | rs755622 (G>C) | Shorter clinical remission after intra-articular glucocorticoid injection (46% reduced time in remission) | 89 |
| | | | rs755622 (G>C) | Longer duration of glucocorticoid treatment (daily regimen) needed | 75 |
| Anti-TNF response | TNF | 6 | rs1800629(G>A, AA) | Low response to etanercept treatment and lower effect in reducing MMP-9 levels | 50 |
| Biologic effects | COL1A1 | 17 | rs1800012 (G>T, GG) | Increased risk of LBMD in pubertal JIA children (Tanner II-III) | 90 |
| | | | rs1107946 (G>T, GG) | Increased risk of LBMD in pubertal JIA children (Tanner IV-V) | 90 |

JIA: juvenile idiopathic arthritis; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; C-HAQ: childhood health assessment questionnaire; VAS: visual analogue scale; TNF: tumour necrosis factor; MAS: macrophage activation syndrome (complication in systemic JIA patients); MTX: methotrexate; MMP: matrix metalloproteinase; LBMD: low bone mineral density.

To utilise future GWASs, investigating more specific outcome measures as well as other JIA subtypes, and the necessity of international collaborations will be even more essential, both in terms of collecting large homogeneous cohorts and acquiring comparable clinical data, e.g. remission rate or treatment response, for association analysis.

## Utilising Novel Sequencing Techniques in JIA Research and Personalised Medicine

As mentioned earlier, most of the common genetic polymorphisms known to confer risk or protection from JIA development are non-coding, and their particular role in the pathogenesis is unknown. Some SNPs might have a regulatory role, but it is also likely that some SNPs tag other more rare and functional variants nearby due to LD. Novel high-throughput sequencing methods (also known as next-generation sequencing [NGS]) have enabled numerous genes (targeted sequencing) or even the entire exome/genome to be sequenced in a single run. The utilisation of such approaches would be interesting to JIA research in order to detect these rare variants with a high impact on JIA pathogenesis, both in genes with known association to JIA susceptibility and new genes not detected in GWASs. This would help researchers to acquire more detailed knowledge on how genetic variation is involved in JIA.

In addition, NGS offers several other applications, such as sequencing of the methylome (bisulfate sequencing), transcriptome (RNA sequencing), transcription-factor binding sites and their interactions with proteins (chromatin immunoprecipitation [ChIP]-sequencing), nucleosome positioning (ChIP-sequencing), and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET). The utilisation of these applications on disease-relevant cell types will enable researchers to investigate the role of the epigenome in JIA pathogenesis. In 2012, Ellis et al.[91] did the first genome-wide methylation study in JIA, finding a lowered methylation level at the gene encoding the proinflammatory cytokine IL-32 in CD4+ T cells. Still, the field of epigenomics in JIA remains undiscovered and it will be interesting to follow the findings of future studies on this matter. Finally, NGS also enables metagenomic analyses of the human microbiota, which is thought to contribute to the development of autoimmune diseases.[92]

In the years to come, genetics are predicted to play an ever-increasing role in the care of JIA. Knowledge of individual genetic variability can support the paediatrician's assessment in determining the right diagnosis, prognosis, and treatment in order to limit patient symptoms, treatment side-effects, and long-term disability. Bulatovic et al.[93] constructed a prediction model for MTX response including information on erythrocyte sedimentation rate and genotyping of four SNPs; it showed a 72% power to predict MTX non-responders. This confirmed the relevance of including genetic variability in determining the right treatment for JIA patients. However, much more knowledge is needed on how results from genome-wide genotyping or sequencing are to be interpreted and used in patient care.

## CONCLUSION

Our knowledge of how genetic variability influences JIA susceptibility has evolved rapidly in recent years, and several new susceptibility loci outside the HLA gene complex have been discovered. These associated polymorphisms, however, are often non-coding and much investigation is still to be done on finding the functional genetic variants and pathways directly involved in the pathogenesis. Genetic variability has also been shown to influence more specific clinical outcomes, such as disease course and treatment response. Expectedly in the years to come, this accumulating knowledge - along with the increasing availability of genome-wide genotyping and sequencing analyses - will assist in diagnosis, prognosis, and more personalised medicine.

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# THE IMPORTANCE OF THE MTOR REGULATORY NETWORK IN CHONDROCYTE BIOLOGY AND OSTEOARTHRITIS

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## ABSTRACT

Osteoarthritis (OA) is a chronic disorder associated mainly with pain, limited range of motion, stiffness, joint inflammation, and articular cartilage (AC) destruction. Recent studies demonstrated the involvement of chondrocyte differentiation (hypertrophy) as one of the mechanisms of cartilage degradation in OA. This indicates the involvement of profound alterations in chondrocyte metabolism in the course of cartilage resorption orchestrated by principal changes in the regulation of cellular function. Mammalian target of rapamycin (mTOR) controls critical cellular processes such as growth, proliferation, and protein synthesis, and integrates extracellular signals from growth factors and hormones with amino acid availability and intracellular energy status. The importance of mTOR activity during AC destruction in OA is supported by considerable alterations in the mTOR regulatory network, involving multiple intracellular (availability of growth factors, adenosine triphosphate [ATP], and oxygen as well as autophagy) and extracellular (glucose, amino acid, lipid, and hexosamine) signals. Moreover, variable mTOR gene expression in the peripheral blood of OA patients is associated with increases in pain or synovitis, and indicates a profound metabolic dissimilarity among patients that might require differential approaches to treatment. These issues are discussed in the present review article.

Keywords: Mammalian target of rapamycin (mTOR), osteoarthritis, articular cartilage, peripheral blood, nutrient signalling pathways.

## INTRODUCTION

Osteoarthritis (OA) is a systemic condition that can affect single or multiple joints, and involves degenerative changes in the articular cartilage (AC), remodelling of the subchondral bone, and limited synovial inflammation.[1-4] The disability in OA is related to pain and reduced mobility due to AC degeneration. Recent evidence has been presented that disease manifestation is preceded by phenotypic modification (hypertrophy) of articular chondrocytes similar to that observed in foetal chondrocytes during their maturation in the epiphyseal growth plate.[1,5-11] These phenotypic changes were associated with upregulation of genes involved in cartilage destruction, altered expression of apoptosis markers, regulatory growth, and transcription factors.[7-14] However, subsequent inhibition of cartilage degradation by genetic abrogation of the local proteolysis of aggrecan and collagen in animal studies reduced pain, experimental disease severity, and subchondral bone changes, whereas osteophyte development was not affected.[15,16] Moreover, clinical trials applying inhibitors of proteinases or inflammatory cytokines were also unsuccessful.[17-21] Therefore, identification of the upstream factors that regulate expression of catabolic molecules and/or chondrocyte hypertrophy in AC is important for a more profound understanding of the regulatory mechanisms that control articular chondrocyte function.[22]

Previous studies have demonstrated that the majority of the identified genes involved in OA encode signal transduction proteins,[23,24] and numerous signalling pathways have been shown to regulate chondrocyte activities.[24-27] These signal transduction pathways are flexible and, therefore, potentially liable to intervention and modification.[25] As AC destruction in OA is associated with chondrocyte hypertrophy, signalling molecules - which regulate chondrocyte activities both in the growth plate and adult AC during OA - could be of particular interest.[28] For example, it has been reported that ERK1/2 phosphorylation and suppression of p38 phosphorylation produce hypertrophic differentiation of AC chondrocytes.[29,30] At the same time, targeting specific signalling pathways in OA might not be easy due to the high variety and crosstalk among pathways.[31] For example, direct targeting of beta-catenin might be risky because of its importance both in the maintenance of articular chondrocyte phenotype stability and cancer development.[32]

With this information in mind, tracking nutrient signalling pathways, which are thought to be linked to seven of the top ten causes of sickness and death including heart disease, obesity, several cancers, diabetes, and others, is more promising.[33] Traditionally, nutrients such as amino acids, carbohydrates, and lipids were considered as substrates for the generation of high-energy molecules and biosynthetic precursors of macromolecules. However, at present, it is obvious that nutrients can function as signalling molecules in nutrient sensing signalling pathways, which regulate various aspects of energy metabolism and control cell growth, proliferation, and survival.[34]

## THE MTOR SIGNALLING PATHWAY

In humans, gene expression is regulated by nutrients interacting with signalling pathways primarily involving mammalian target of rapamycin (mTOR), which integrates contributions from amino acids, growth factors, and molecules involved in the energy status of the cell.[34-37] mTOR is a catalytic subunit of two different complexes including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). These complexes are distinguished through the binding of mTOR to accessory proteins. Raptor is a rapamycin (RAP)-sensitive regulatory protein associated with mTORC1. mTORC1 is regulated through actions on the tuberous sclerosis (TSC) 1/2 tumour suppressor protein complex. TSC1 has no catalytic activity, whereas TSC2 functions as GTPase-activating protein that inhibits Ras homolog enriched in brain (Rheb). Inactivation of the TSC complex results in activation of mTOR[38] (Figure 1). It has been shown recently that mTORC1 could also be activated by RAS-like GTPase RALB.[39] mTORC1 is in charge of the growth factor and nutrient responses, and therefore, critically regulates proliferation, metabolism, and cell survival. Rictor is a RAP-insensitive companion of mTORC2.[40] The activity of mTORC2 is associated with cell migration, glycogen metabolism, and possible regulation of gluconeogenesis.[41]

## MTOR REGULATION

### Chondrocyte Function in Foetal Development

The mTOR signalling pathway is responsible for positive regulation of chondrocyte maturation, proliferation, cartilage matrix production, and cell growth during skeletal development.[42-46] (Table 1). RAP administered to young rats significantly reduced endochondral bone growth as evidenced by enlargement of the hypertrophic zone (due to decreased parathyroid hormone/parathyroid hormone-related peptide [PTH/PTHrP] expression and increased Indian hedgehog [Ihh] expression) and a decrease in chondrocyte proliferation associated with downregulation of mTOR. This was accompanied by a reduced number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated chondro/osteoclasts and decreased expression of receptor activator of nuclear factor kappa-B ligand (RANKL), and vascular endothelial growth factor (VEGF).[44] RAP also reduced insulin-induced growth of foetal rat metatarsal explants due to a selective effect on the hypertrophic zone but not cell proliferation. In the ATDC5 chondrogenic cell line, RAP inhibited proteoglycan (PG) accumulation, Type 10 collagen (COL10A1), and Ihh expression.[47] In the case of nutrient starvation, stress, or reduced availability of growth factors, cellular metabolic adjustments involve inhibition of mTOR activity and induction of autophagy, which serves to promote cell survival. Autophagy was shown to affect foetal chondrocyte differentiation,[48,49] as it developed in terminally differentiated chondrocytes, and permitted these cells to survive in the local microenvironment.[50,51]

### Autophagy

During an autophagic state, the cell cannibalises itself to generate energy and/or to remove

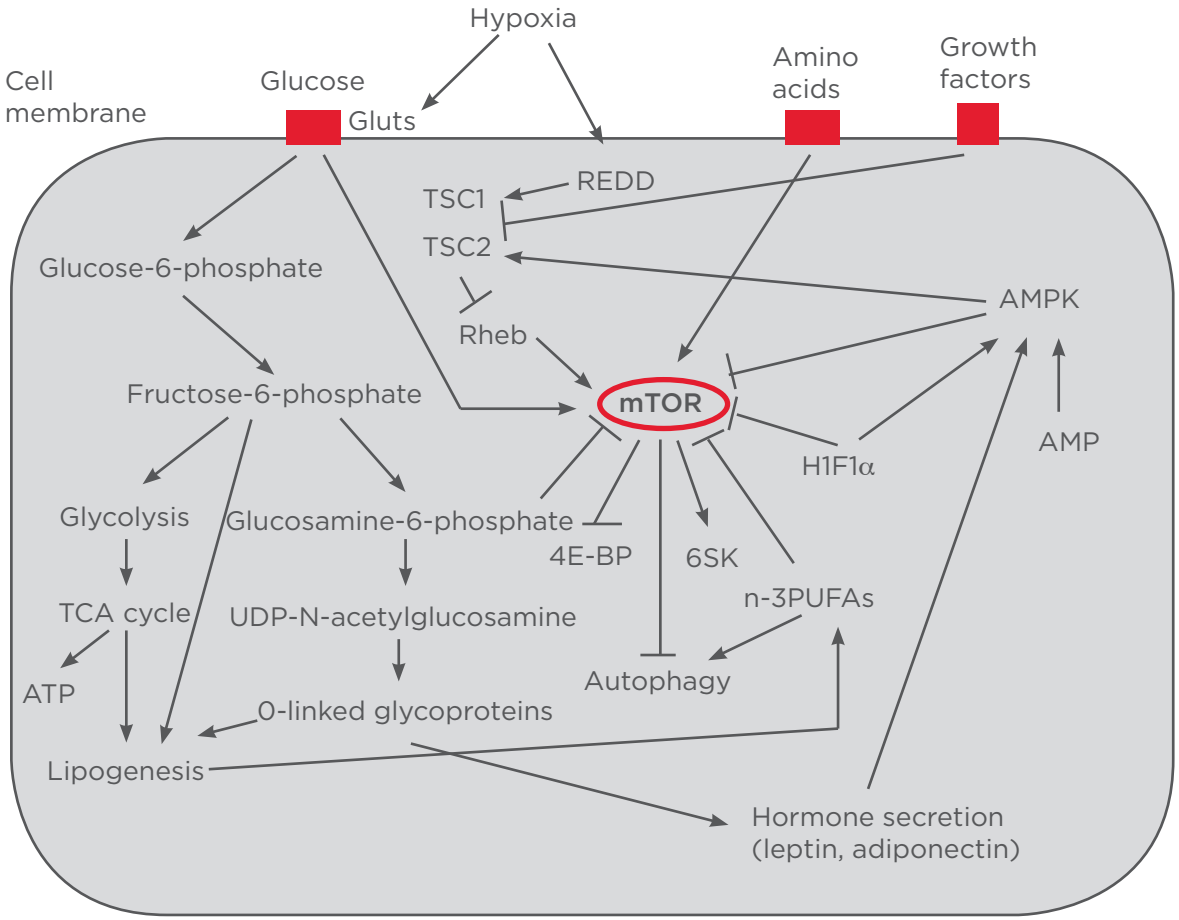


**Figure 1: mTOR regulatory network in chondrocyte.**
mTOR: mammalian target of rapamycin; Gluts: glucose transporters; REDD: regulated in development and DNA damage responses; TSC1/2: tuberous sclerosis 1/2 tumour suppressor protein complex; Rheb: Ras homolog enriched in brain; AMPK: AMP-activated protein kinase; AMP: adenosine monophosphate; ATP: adenosine triphosphate; TCA: tricarbonic acid cycle; HIF1α: hypoxia inducible factor 1α; 4E-BP: eukaryotic translation initiation factor 4E binding protein; 6SK: ribosomal protein S6 kinase; n-3PUFAs: omega 3 polyunsaturated fatty acids.

defective organelles. When autophagy is extended, Type 2 apoptosis can be activated.[52,53] In chondrocytes, autophagy is regulated by adenosine monophosphate (AMP)-activated protein kinase (AMPK) and mTOR activities in a hypoxia-inducible factor (HIF)-dependent manner.[42,54,55] Increased autophagy was associated with mTOR inhibition upon cell growth cessation.[56,57] Autophagy might be protective during cell stress conditions, as it is increased in normal chondrocytes under nutritional (1% foetal bovine serum [FBS]) or catabolic (interleukin-1 beta [IL-1β] or nitric oxide [NO]-generating agent, sodium nitroprusside) stresses.[58]

Autophagy was observed in the superficial and mid-zones of AC in early animal OA,[59] and in human normal and OA articular chondrocytes.[42,60] Increased autophagy was also noted in mild human OA AC versus normal and severely damaged specimens, and in cultured human OA chondrocytes when compared to normal.[58] However, some studies have also described a decreased autophagic response in mild and severe OA cartilage compared to normal cartilage in humans.[60-62]

## Chondrocyte Function in Experimental OA

In mouse experimental OA upregulation of mTOR expression in the knee AC was associated with downregulation of autophagy[61,63] (Table 1). Autophagy has been shown to be capable of ameliorating OA as its activation on mTOR inhibition by RAP or by mTOR deletion reduced disease severity in animal studies.[61,63] This was accompanied by reduced cartilage degradation,

decreased A Disintegrin, and Metalloproteinase with Thrombospondin Motifs (ADAMTS)-5, matrix metalloproteinase (MMP)-13, IL-1β expression, and synovitis.[63] Moreover, activation of autophagy on mTOR gene deletion was associated with a reduction of PG loss, synovial fibrosis, transforming growth factor beta/Mothers against decapentaplegic homolog 3 (TGF-β/SMAD3) signalling, MMP-13, MMP-induced Type 2 collagen degradation, and apoptosis.[61]

## Chondrocyte Function in Human AC

At present, some studies also indicate the importance of mTOR signalling in articular chondrocyte metabolism, extracellular matrix (ECM) maintenance, and OA development, as mTOR expression has been reported in human normal and OA articular cartilage.[61,62,64] mTOR upregulation in end-stage OA articular cartilage was associated with downregulation of autophagy, cyclin-dependent kinase inhibitors, and upregulation of regulators of cell death and apoptosis, increased expression of chondrocyte hypertrophy-related COL10A1, and ECM degrading MMP-9 and MMP-13[61,62] (Table 1). The value of mTOR signalling in chondrocyte biology is further supported by studies on the role of mTOR regulators in AC function, as being a major regulator of various cellular processes it is itself a target of regulation.

## Functions of Positive mTOR Regulators in Chondrocytes

Nutrients such as amino acids and glucose act through mTOR and directly affect chondrocyte differentiation and long bone growth.[43,47]

### Amino acids

Essential amino acids are considered a limiting factor as they are required as substrates for protein synthesis and also act as signalling molecules in several regulatory pathways. Leucine is the most potent regulator of mTOR signalling.[65] The chondroprotective and anti-inflammatory effects of a herbal leucine mix have been demonstrated by a strong inhibition of inducible nitric oxide synthase (iNOS), MMP-9 and MMP-13, NO-production, glycosaminoglycan (GAG) release, and upregulation of COL2A1 expression in human OA chondrocytes and cartilage explants stimulated by IL-1β.[66] In contrast, leucine restriction produced a dose-dependent inhibition of foetal rat metatarsal explant growth. This was accompanied by reduced cell proliferation and hypertrophy, and partial inhibition of mTOR activity. In chondrogenic ATDC5 cells, leucine restriction inhibited cell numbers and PG accumulation as well as COL10A1 and Ihh expression.[43]

### Glucose

Glucose regulates mTOR by several mechanisms including inhibition of mTOR during glucose limitation due to a decreased ATP/AMP ratio and a concomitant AMPK activation,[67] and by a glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-dependent inhibition of Rheb.[68] mTOR might be an important player in chondrocyte glucose metabolism as it is subject to regulation by glucose.

As AC is an avascular non-insulin-sensitive tissue, it utilises glucose as a main energy source, a precursor for GAG synthesis, and a regulator of gene expression.[69] In a hypoxic milieu of AC, anaerobic glycolysis is considered a central element in generating ATP to support ECM synthesis and chondrocyte viability, while mitochondrial oxidative phosphorylation (OXPHOS) serves as a physiologic reserve for ATP production[70] and a source of oxidants generated in mitochondrial electron transport chain (ETC) to maintain cellular redox balance in favour of glycolysis.[71] The importance of glycolysis in AC PG synthesis has been confirmed by enhanced inhibition of this process by a glycolysis inhibitor compared to an OXPHOS uncoupler. Moreover, oxidation of GAPDH by hydrogen peroxide resulted in inhibition of PG core protein synthesis *in vitro* and in an animal model of acute arthritis.[72]

Glycolysis is regulated by glucose transporter (GLUT) expression via cytokines. Both anabolic (TGF-β1) and catabolic (IL-1β) factors have been shown to be equally capable of accelerating glucose transport in normal human cultured chondrocytes. However, TGF-β1-stimulated glucose transport was not associated with increased expression of GLUTs (1, 3, 6, 8, 10), and involved protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) activation. However, in a study of IL-1β-stimulation, glucose transport was accompanied by increased expression of GLUT1 and 6, dependent on PKC and p38 mitogen-activated protein (MAP) kinase, and produced higher levels of lactate indicating glycolysis activation.[69]

**Table 1: Effects of mTOR signalling on chondrocyte function.**

| Condition or treatment | mTOR response | Tissue/cell response | Tissue, animal or cell type | Ref |
|---|---|---|---|---|
| Human OA disease | Upregulation | Decreased expression of autophagy marker ULK1, CDKN1A (p21), increased expression of Type X collagen, MMP-13, and MMP-9 | Human end-stage knee articular cartilage | 62 |
| Human OA disease | Upregulation | Downregulation of 20 autophagy related genes including ULK1, LC3B, Beclin 1, ATG-3, -5, -13, GABARAPL1, and also BNIP3, CDKN1B (p27), FAS, HSP90AA1, and HSPA8. Upregulation of 5 autophagy related genes and cell death/apoptosis regulators APP, CTSB, BCL2, and BAD | Human end-stage knee articular cartilage | 61 |
| Surgical OA in animals | Upregulation | Downregulation of autophagy markers LC3 and ATG5 | Dog and mouse knee articular cartilage | 61 |
| Inducible cartilage-specific mTOR knock-out mice | Downregulation | Increased expression of ULK1, AMPK1, ATG5, BNIP3, and LC3, protection from cartilage degradation, reduction of proteoglycan loss and articular chondrocyte cellularity, reduction of synovial fibrosis, TGF-β/SMAD3 signalling, MMP-13, and MMP-induced Type 2 collagen degradation, and apoptosis | Knee articular cartilage | 61 |
| RAP treated animals | Downregulation | Reduced severity of cartilage degradation, decrease in synovitis, expression of ADAMTS5 and IL-1β, and activation of autophagy marker LC3 | Mouse experimental OA articular cartilage | 63 |
| Increase in the endogenous n-3 PUFAs | Downregulation | Decreased cartilage destruction and osteophytosis, downregulated MMP-13 and ADAMTS5 expression, reduced chondrocyte loss and ECM degradation, and increased autophagy | Fat-1 transgenic mice articular cartilage | 116 |
| Artificially induced temporomandibular condylar cartilage degeneration | Downregulation | Increased expression of autophagy markers Beclin 1 and LC3, and reduced MAPK4K3 activity | Cartilage of rat temporomandibular joint | 57 |
| RAP treated mechanically injured cartilage | Downregulation | Enhanced expression of autophagy markers ULK1, Beclin 1, and LC3, cell viability, and decreased sulfated glycosaminoglycan loss | Human and bovine articular cartilage explants | 125 |
| RAP treated cells | Downregulation | Reduction of proteoglycan accumulation, Type 10 collagen and Ihh expression | ATDC5 chondrogenic cell line | 47 |
| mTOR silenced by siRNA in cells | Downregulation | Increased autophagy marker LC3 expression, less association of BCL2 with Beclin 1 | Mouse chondrocytes | 42 |
| RAP treated cells | Downregulation | Increased autophagy marker LC3 expression | Mouse chondrocytes | 42 |
| RAP treated cells | Downregulation | Decrease of IGF-1-stimulated proteoglycan synthesis | Normal human articular chondrocytes | 77 |
| Glucosamine treated cells | Downregulation | Increased autophagy marker LC3 expression | Normal human articular chondrocytes | 93 |
| Leucine restriction | Partial downregulation | Inhibition of metatarsal bone growth, reduction of proliferation and hypertrophy | Foetal rat metatarsal explants | 43 |
| Leucine restriction | Partial downregulation | Inhibition of cell numbers, proteoglycan accumulation, Type 10 collagen and Ihh expression | ATDC5 chondrogenic cell line | 43 |

**Table 1 continued.**

| Condition or treatment | mTOR response | Tissue/cell response | Tissue, animal or cell type | Ref |
|---|---|---|---|---|
| RAP treated cells stimulated by IL-1β | Downregulation | Enhanced lysosomal activity, increased expression of autophagy markers Beclin 1 and LC3, COL2A1, aggrecan, reduced expression of MMP-13 and ADAMTS5 | Normal human articular chondrocytes | 58 |
| RAP treated cells | Downregulation | Increased autophagy markers LC3 and ULK1; AMPK1, Type 2 collagen and aggrecan expression, decreased MMP-13, CCL5/RANTES, and CCL2/MCP-1 expression | Human OA chondrocytes | 61 |
| IL-1β-treated cells | Upregulation | Increased expression of MMP-13, CCL2, and CCL5; decreased expression of Type 2 collagen | Human OA chondrocytes | 61 |
| Pten-deficient mice | Upregulation | Accelerated hypertrophic differentiation, increased expression of Type 10 collagen, alkaline phosphatase, PDK1, and PI3K signalling | Mouse long bone growth plate | 126 |
| RAP treated animals | Downregulation | Reduction of body and tibia growth, decrease in chondrocyte proliferation, enlargement of growth plate hypertrophic zone, increase in Ihh and reduction in PTH/PTHrP, RANKL, VEGF expression, and decline in TRAP-positive multinucleated cells | Weanling rat growth plate | 44 |
| RAP treated explants | Downregulation | Decreased insulin-induced bone growth stimulation | Foetal rat metatarsal explants | 47 |
| Endochondral ossification: proliferative zone | Upregulation | Inhibition of autophagy | Proliferative growth plate chondrocytes | 48, 50 |
| Endochondral ossification: hypertrophic zone | Downregulation | Increase in autophagy and AMPK activity | Terminally differentiated growth plate chondrocytes | 48, 50 |

mTOR: mammalian target of rapamycin; OA: osteoarthritis; MMP: matrix metalloproteinase; TGF-β: transforming growth factor beta; ADAMTS-5: A Disintegrin and Metalloproteinase with Thrombospondin Motifts-5; IL-1β: interleukin-1 beta; PUFAs: n-3 polyunsaturated fatty acids; ECM: extracellular matrix; RAP: rapamycin; IGF-1: insulin-like growth factor 1; PTH/PTHrP: parathyroid hormone/parathyroid hormone related peptide; RANKL: receptor activator of nuclear factor kappa-B ligand; VEGF: vascular endothelial growth factor; TRAP: tartrate resistant acid phosphatase; AMPK: AMP-activated protein kinase; siRNA: small interfering RNA; PDK1: pyruvate dehydrogenase kinase-1; PI3K: phosphoinositide 3 kinase; MAP4K3: mitogen-activated kinase kinase kinase kinase 3.

Glycolysis inhibition by sodium fluoride (NaF) induced a dose-dependent decrease in ATP production, inhibition of chondrocyte proliferation and differentiation, and cell death promotion in a human chondrocytic cell line. Moreover, chondrocyte treatment by a combination of NaF and lactate upregulated the expression of several genes associated with chondrocyte hypertrophy, including alkaline phosphatase (ALP), VEGF, COL10A1, and MMP-13 and MMP-9.[73] Altered glycolysis function has also been shown to be involved in OA. For example, development of spontaneous OA in guinea pigs was associated with depletion of knee chondrocyte intracellular ATP by 50% despite a lack of mitochondrial ultrastructure abnormalities and the presence of an adaptive augmentation of glycolysis indicated by an increased ratio of lactate to pyruvate.[70]

However, proteomic studies in human OA chondrocytes revealed decreased concentrations of proteins involved in glycolysis (enolase, GAPDH, and fructose bisphosphate aldolase).[74] Moreover, a GAPDH inhibitor, monosodium acetate, caused chondrocyte apoptosis evidenced by upregulation of cytochrome-oxidase C and caspase-3 protein levels and reactive oxygen species (ROS) production.[75] In addition, a significant reduction in GLUT1 mRNA observed in clinical OA cartilage samples resulted in failure of OA cartilage repair.[76]

### Growth factors

Growth factors, primarily insulin-like growth factor 1 (IGF-1), are known to be mTOR positive regulators in many tissues. In AC, growth factor-related ECM maintenance has also been shown to be mediated by mTOR. Accordingly, a decrease in IGF-1-stimulated PG synthesis in cultured normal human articular chondrocytes was observed upon inhibition of mTOR.[77]

## Functions of Negative mTOR Regulators in Chondrocytes

### AMPK

AMPK is a heterotrimeric serine-threonine kinase, which is activated when intracellular energy is limiting. It stimulates ATP catabolism and inhibits its synthetic activity.[78] In mammals, AMPK activates the TSC2-TSC1 complex, thus inhibiting mTOR.[79] AMPK regulates energy homeostasis and cellular metabolism, and also exerts anti-inflammatory effects in multiple tissues.

AMPK activity also supports AC homeostasis, as it is constitutively present in normal articular chondrocytes and cartilage but decreased in OA articular chondrocytes and cartilage as well as in normal chondrocytes treated with IL-1β or tumour necrosis factor alpha (TNFα). Attenuation of AMPK resulted in enhanced catabolic responses to IL-1β and TNFα in human and mouse chondrocytes, and was associated with increased MMP-3 and MMP-13 release. Moreover, AMPK activators suppressed cartilage/chondrocyte procatabolic responses to IL-1β and TNFα, and the capacity of TNFα and IL-8 to induce COL10A1 expression.[80-82]

### Hypoxia

Hypoxia regulates mTOR via REDD (regulated in development and DNA damage response) 1/2 proteins. REDD1 inhibition of mTOR is mediated by the TSC1/2 complex.[83] A hypoxic environment is optimal and protective for AC as chondrocyte exposure to hypoxia inhibited caspase-8 and the generation of ROS, which were induced in primary articular chondrocytes co-treated with the proteasome inhibitor and apoptosis stimulator, TNF-related apoptosis inducing ligand (TRAIL), under normoxic conditions.[84] In the presence of an optimal (5%) oxygen concentration for porcine articular chondrocytes, maximum ATP generation and the highest protection against IL-1β and NO stimulation were observed. However, in the presence of 20% or 1% oxygen, reduced ATP levels and increased AMPK expression were demonstrated.[85,86] Moreover, hypoxia stimulation induced by cobalt chloride (a hypoxia mimetic) increased glucose uptake and lactate production, and upregulated GLUT1 mRNA expression in primary articular chondrocytes.[76,87]

HIF transcription factors represent a central control mechanism of oxygen sensing.[88] HIF activity is important in both foetal and adult AC. For example, HIF-1α is expressed in the central part of the growth plate,[89] and its inactivation in foetal chondrocytes dramatically inhibits anaerobic energy generation and ECM synthesis.[90] In adult AC, HIF-1α was detected both in normal and OA chondrocytes while an increase in HIF-1α expression was associated with disease severity in OA cartilage.[90] Moreover, HIF-1α was suggested to be involved in cartilage repair, as hyaline-like matrix synthesis was increased upon HIF-1α overexpression in the presence of IGF-1 or BMP-2 in the periosteal cells from animal chondral knee lesions.[91]

### Hexosamine pathway

The hexosamine signalling pathway is an additional glucose sensor and is responsible for glucose redistribution either for ATP production or conservation in lipids and/or glycogen. This pathway may be involved in leptin and adiponectin synthesis, which are capable of activating AMPK and inhibiting mTOR function.[34] Another mechanism for the involvement of the hexosamine pathway in mTOR inhibition was observed in normal articular chondrocytes, where glucosamine activated autophagy and inhibited glucose uptake in a manner consistent with the actions of a competitive inhibitor.[92,93]

Glucosamine is an amino sugar widely used to relieve symptoms associated with OA likely because chondrocytes utilise this sugar as a structural component for ECM (glycosaminoglycan) synthesis.[94] Glucosamine has been shown to decrease both foetal and articular chondrocyte proliferation, differentiation, and mineralisation[95,96] due to downregulation of catabolic MMPs, aggrecanases, pro-inflammatory mediators, and the induction of pro-anabolic hyaluronic acid *in vitro*.[97-99]

Some clinical studies reported a decrease in pain and reduction of knee joint space loss after glucosamine treatment.[100] These could be associated with the induction of tissue TGF-β1 and connective tissue growth factor (CTGF) expression, as well as reduction of cartilage oligomeric matrix protein, an AC degradation marker.[101,102] However, the majority of clinical trials have reported numerous non-responders or the absence of this effect when compared with non-pharmacological treatment methods, such as exercise or weight loss.[103-105]

### Lipids

Obesity is one of the main risk factors for OA. Activated white adipose tissue increases synthesis of proinflammatory cytokines while adipokines are capable of promoting synovial inflammation, upregulation of cartilage degrading enzymes, and bone matrix remodelling.[106,107] For example, adiponectin induced an increase in MMPs and collagen degradation activity in OA cartilage or cultured human chondrocytes, which was mediated by an mTOR inhibitor, AMPK.[108-110]

Altered lipid metabolism associated with OA involved increased cellular phospholipid and lipid deposition in the joint[111-113] and distorted cholesterol and fatty acid metabolism in OA chondrocytes.[113-115] It has been shown recently that n-3 polyunsaturated fatty acids (PUFAs) supplement significantly alleviated AC destruction and decreased MMP-13 and ADAMTS5 expression in an animal model of OA. Both exogenous and endogenous n-3 PUFAs downregulated mTORC1 activity and promoted autophagy in articular chondrocytes. Moreover, enhancement in synthesis of endogenous n-3 PUFAs from n-6 PUFAs was shown to be capable of delaying the incidence of OA.[116]

## MTOR AS A MARKER OF SYSTEMIC OA MANIFESTATIONS

The data described above demonstrate the importance of mTOR signalling in chondrocytes both in normal and OA cartilage. However, systemic OA manifestations require additional studies focusing on tissues outside of the AC which play a role in OA.[117] Alterations in non-tissue-specific regulatory protein expression associated with disease manifestation may suggest differential gene expression in tissues other than cartilage. This is supported by the observation of modified expression of genes associated with foetal chondrocyte differentiation, such as bone morphogenetic proteins 2, 4, and 6, as well as runt-related transcription factor 2 (RUNX2), in the peripheral blood of OA patients.[118]

Assessment of gene expression changes measured in the whole blood is an emerging approach in OA research. Blood-based transcriptome and microarray gene expression analyses appeared capable of distinguishing OA patients from control subjects.[119,120] Moreover, upregulation of IL-1β gene expression in the blood was accompanied by increased pain and predicted a higher risk of radiographic progression of the disease,[121] while high expression of TNFα was associated with high mTOR expression and a higher incidence of synovitis.[62]

Upregulation of mTOR gene expression in the PBMCs might occur concomitantly with increased AC destruction as a positive correlation between mTOR gene expression in the blood and AC was noted in end-stage OA patients.[62] In addition, elevated mTOR gene expression was observed in both peripheral blood and AC of end-stage OA patients.[62] At the same time, excessive inhibition of mTOR expression is also deleterious as it might result in significantly more pain upon joint function,[62] which might be associated with the ERK pathway activation in sensory neurons.[122] Considering this information, although treatment of mice by mTOR inhibitors has been shown to be capable of reducing the severity of experimental OA[60,63] and inflammatory arthritis,[64,123] and is suggested for treatment of human OA,[124] mTOR inhibition in OA patients should be considered with caution.

## CONCLUSION

The importance of mTOR regulation in chondrocyte biology and altered activity of positive and negative regulators of mTOR signalling pathway associated with OA suggest its involvement in the disease onset, progression, and outcome. However, the majority of studies on mTOR signalling associated with OA were performed using animal models and cultured chondrocytes. The results gained in these conditions do not necessary imply that exactly the same processes are involved in human OA. Therefore, clinical studies are warranted in order to truly identify the role of mTOR signalling in OA. As mTOR regulation involves both environmental nutrient signalling and is capable of modulating chondrocyte energy turnover, cell growth, proliferation, and survival, further detailed studies of mTOR signalling in OA patients might provide opportunities for the identification of new targets for therapeutic intervention, which could lead to secure and efficient therapies that reduce the symptoms and slow the progression of OA.


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# WHY BASIC CALCIUM PHOSPHATE CRYSTALS SHOULD BE TARGETED IN THE TREATMENT OF OSTEOARTHRITIS

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## ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis and results in significant social, psychological, and economic costs. It is characterised by progressive cartilage loss, bone remodelling, osteophyte formation, and synovial inflammation with resultant joint pain and disability. Since OA affects the entire joint, it is not surprising that there has been difficulty developing an effective targeted treatment. Treatments available for structural disease modification are limited. Current options appear to mostly reduce symptoms. Basic calcium phosphate (BCP) crystals represent a potential therapeutic target in OA; they have been found in 100% of knee and hip cartilages removed at joint replacement. Intra-articular BCP crystals are associated with large joint effusions and dissolution of intra-articular structures, synovial proliferation, and marked degeneration as assessed by diagnostic imaging. While BCP deposition has been considered by many to be simply a consequence of advanced OA, there is substantial evidence to support BCP crystal deposition as an active pathogenic mediator of OA. BCP crystals exhibit a multiplicity of biologic effects *in vitro* including the ability to stimulate mitogenesis and prostaglandin, cytokine, and matrix metalloproteinase (MMP) synthesis in a number of cell types including macrophages, synovial fibroblasts, and chondrocytes. BCP crystals also contribute to inflammation in OA through direct interaction with the innate immune system. Intra-articular BCP crystals can elicit synovial inflammation and cartilage degradation in mice *in vivo*. Although intra-articular BCP crystals are difficult to detect at the bedside, advances in modern technology should allow improved identification and quantitation of BCP crystals. Our article focuses on why basic calcium crystals are important in the pathogenesis of OA. There is ample evidence that BCP crystals should be explored as a therapeutic target in OA.

Keywords: Osteoarthritis, basic calcium phosphate, calcium crystals, degenerative disease.

## INTRODUCTION

Osteoarthritis (OA) is the most common form of joint disease and is one of the leading causes of pain and disability worldwide as it affects up to 13% of the world's population. The lifetime prevalence of symptomatic hip OA is estimated at 25.3%.[1] Knee OA is even higher at 44.7%.[2] OA results in significant social, psychological, and economic costs.[3] Billions of euros are spent on the management of OA especially surgical interventions, mainly joint replacement, which is still the gold standard of treatment for advanced OA. Piscitelli et al.[4] reviewed the socioeconomic burden of total joint arthroplasty for hip and knee OA in the Italian population and showed that hospital costs increased from €741 million to €1 billion over a 5-year period. A US study revealed that OA raised aggregate annual medical care expenditures by $185.5 billion.[5]

OA is a complicated disease as it affects all structures of the joint. Not only does it affect articular cartilage, but it also affects the subchondral bone, synovium, ligaments, tendons, and menisci. The multifaceted nature of the disease poses understandable difficulty in developing targeted therapies.

The multifactorial nature of OA is well-recognised. These factors can work independently or in combination to lead to joint degeneration. While

the clinical and structural characteristics of OA are well-recognised, the aetiopathogenesis remains poorly understood. Non-modifiable risk factors for OA include advanced age and genetics. However, aging appears to be insufficient for the development of OA as bone and cartilage changes in OA are different from those of normal aging. Genetic defects can give rise to premature OA but in the majority of those with OA, no such genetic defects have been identified.[6] Risk factors such as obesity and joint injury are potentially modifiable. But we are aware that OA occurs in those with normal body mass index (BMI) and in those who have never experienced joint trauma.

OA involves dynamic biochemical, biomechanical, and cellular processes. Synovial inflammation is frequently observed and can occasionally mimic RA synovium.[7] Furthermore, inflamed synovium is an important source of pain in OA. Inflammation in OA is now well recognised and this is reflected in ongoing research. Abou-Raya et al.[8] recently demonstrated in a randomised placebo-controlled trial that methotrexate significantly reduced pain and improved synovitis and physical function in patients with OA. They suggested that methotrexate may be a therapeutic option in the treatment of pain and inflammation related to knee OA.[8] A recent systematic review has shown that serum high-sensitivity-C-reactive protein (hs-CRP) levels were modestly but statistically significantly higher in OA than in controls.[9]

OA remains the focus of many academic and, to a lesser extent, industry research programmes. These studies are largely focused on molecular genetics, imaging, biomarkers, and novel pain targets in OA. However, there is paucity in the literature regarding the influence of BCP crystals in the pathogenesis of this disease. This is despite the fact that BCP crystals have been found in 100% of knee and hip cartilages removed during joint replacement, and calcium pyrophosphate dihydrate (CPPD) crystals were found in 20%.[10]

Current treatments for OA include non-pharmacological therapies such as exercise, weight loss, and orthotics to alter joint biomechanics. Pharmacological therapies include analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), viscosupplementation, and intra-articular corticosteroids.

Our article focuses on why basic calcium phosphate (BCP) crystals are important in the pathogenesis of OA, and why these crystals should be further explored as a target for the treatment of OA.

## BCP CRYSTALS

'BCP crystals' is an umbrella term to describe a few types of calcium phosphates. These include partially carbonate-substituted hydroxyapatite (HA), octacalcium phosphate (OCP), tricalcium phosphate, and magnesium whitlockite.[11] HA crystals are the most prevalent. BCP crystals deposit synovium in the cartilage, the joint capsule, tendons, and even in intervertebral discs. BCP crystal deposits increase with age and the crystals frequently co-exist with CPPD crystals. The origin of BCP crystals is not fully understood; however, both CPPD and HA crystals may be generated in matrix vesicles (MV) derived from articular cartilage.[12] There is histologic evidence of MV near BCP crystal deposits in the articular cartilage. Substances within the extracellular matrix (ECM) strongly influence the mineralising activity of MV *in vivo*.[13] Another likely source of BCP crystals in advanced OA is the bony shards embedded in damaged cartilage, and bony debris resulting from the exposure of subchondral bone due to cartilage erosion.[14]

A prime illustration of the potent and destructive nature of BCP crystals is Milwaukee shoulder syndrome wherein abundant intra-articular BCP crystal deposits are found. This syndrome typically occurs in elderly females and is associated with large, and sometimes massive, joint effusions, complete rotator cuff tears, dissolution of the intra-articular portion of the long head of biceps, gross cartilage degeneration, and eburnation of subchondral bone. Rupture of the joint effusion can lead to a massive extravasation of blood and synovial fluid into the surrounding tissues.[15]

### Detection of BCP Crystals

Some progress has been made in the past few years in the detection of BCP crystals. However, unfortunately unlike monosodium urate (MSU) and CPPD crystals that are easily detectable using polarised light microscopy, BCP crystals are too small (20–100 nm) to be identified by conventional techniques. Under a light microscope, clumps of BCP crystals are not birefringent and they can be mistaken for artefacts or debris. BCP deposits are rarely detectable using plain radiography, unlike CPPD that is more often visible on knee and wrist radiographs. Larger BCP aggregates have been detected by Alizarin Red S (ARS) staining, but

this method is difficult to interpret and also stains other calcium containing particulates. Rosenthal et al.[16] showed that BCP crystals could be identified using oxytetracycline staining in conjunction with ultraviolet light. There were fewer false-positive test results than with ARS staining and oxytetracyline did not bind to other particulates in joint fluid. Estimates of the quantities of synthetic BCP crystals were also possible.

More advanced microscopic techniques for detecting BCP crystals include electron microscopy, atomic force microscopy, electron microprobe, Raman spectroscopy, radiograph diffraction, scanning, a binding assay using 14C-labeled ethane-1-hydroxy-1,1-diphosphonate, and bisphosphonate-modified superparamagnetic beads. These techniques are expensive and unfortunately not readily available. BCP crystals must first be isolated from the synovial fluid prior to analysis. Therefore, progress in appreciating the role of BCP crystals in OA has been hampered by difficulties in bedside identification.[17]

## BCP Crystals: Cause or Effect

Deposition of BCP crystals is a common finding in advanced OA. There is controversy in the literature as to whether these crystals cause OA, are a consequence of the degenerative process, or merely exacerbate the disease. Current evidence suggests that calcium crystal deposition contributes directly to joint degeneration and causes inflammation within the joint. Despite this, reviews of OA, written or presented, rarely include BCP crystals as a potential pathogenic factor in OA.[18]

Even if intra-articular BCP crystals are present as a consequence of joint damage, they can still play a role in perpetuating and aggravating the symptoms and signs of OA, especially by their effects on the synovium. Supportive evidence includes the fact that larger joint effusions are seen in knee joints containing BCP crystals when compared to joint fluid from knees without crystals.[19] BCP crystals correlate strongly with the rapid progression of arthritis and the severity of radiographic OA.[20] Furthermore, BCP crystals have been found not only

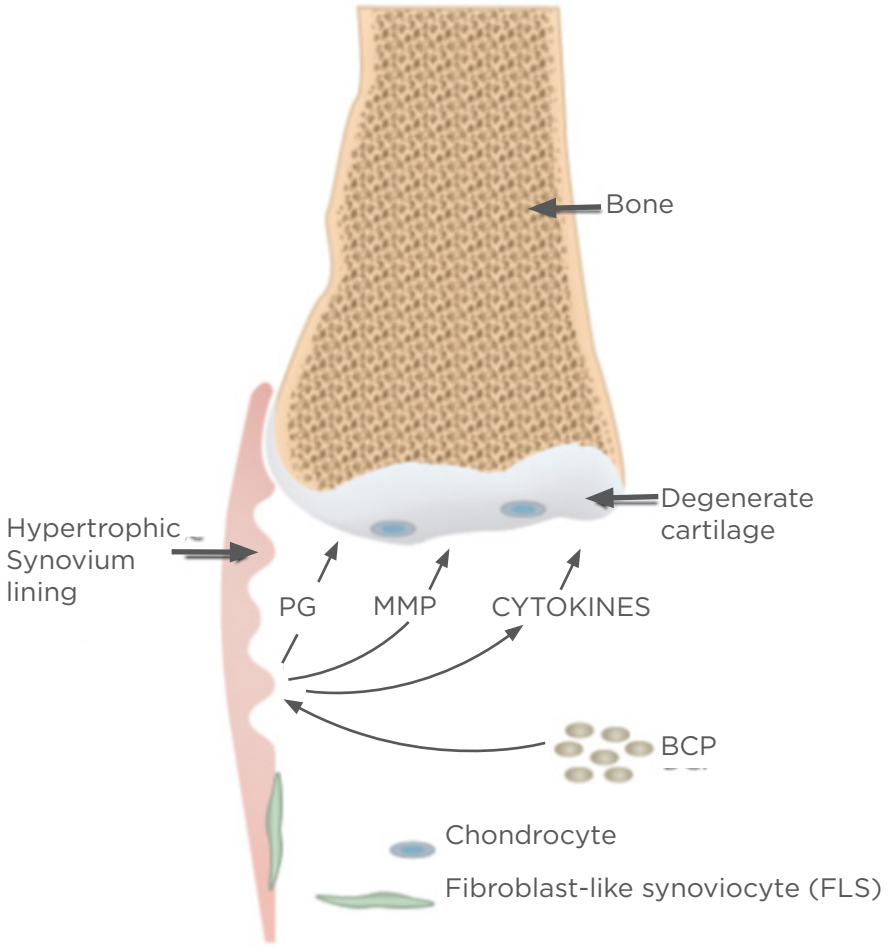


**Figure 1: Proposed pathogenic effects of BCP crystals.**
PG: prostaglandin; MMP: matrix metalloproteinase; BCP: basic calcium phosphate.

in advanced, but also in mild and moderate OA.[21] If BCP deposition was merely a consequence of bone exposure resulting from cartilage wear, how could the relative lack of BCP deposition in inflammatory, potentially destructive arthritis such as rheumatoid arthritis be explained?[22] All evidence points to a unique association between OA and BCP deposition.

## *In Vitro* Findings

An understanding of the molecular mechanisms involved in the pathological effects of BCP crystals, although incomplete, has been significantly advanced in recent years. Recent studies have emphasised the important role of the innate immune system in the pathogenesis of OA. The NALP3 (NACHT, LRR, and PYD domains-containing protein 3) inflammasome complex has been implicated in MSU and CPPD crystal induced inflammatory disease. Activation of Toll-like receptor (TLR) pathways may play an essential role in progression of OA, and BCP crystals appear to be inherently involved in this process. BCP crystals have been shown in numerous studies to have multiple biological effects on articular cells such as chondrocytes and synovial fibroblasts (Figure 1).

*In vitro* BCP crystals induce cellular proliferation and stimulate matrix MMP expression. MMPs accelerate the degradation of cartilage matrix components such as Type 2 collagen, fibronectin, laminin, and proteoglycan. BCP crystals can activate synovial fibroblasts through numerous pathways, including extracellular signal-related kinases (ERK) 1 and 2, nuclear factor κB (NFκB), and protein kinase C (PKC). This in turn leads to upregulation of various inflammatory cytokines including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-1β.[23-25] These cytokines target articular chondrocytes and synovial cells, inducing expression of cartilage degrading enzymes that ultimately lead to joint destruction.

Nitric oxide (NO) is a known central mediator in OA. NO is generated by the oxidation of arginine, catalysed by the nitric oxide synthases (NOSs). BCP crystals increase NO production. BCP crystal deposition in OA cartilage enhances chondrocyte hypertrophy and apoptosis. Cheung et al.[26] demonstrated that treatment of cultured chondrocytes with the NO donor sodium nitroprusside stimulated calcification.

BCP crystals are unusual in that they upregulate both cyclooxygenase-1 and cyclooxygenase-2, followed by increased prostaglandin E2 in human fibroblasts.[27] They induce apoptosis in synovial fibroblasts and articular chondrocytes. These combined processes lead to an imbalance in anabolic versus catabolic mediators of cartilage turnover, which ultimately leads to ECM degradation.

*In vivo* studies have shown that accumulation of crystals in the joint leads to upregulated calcification or friction which activates NALP3 in synovial macrophages, leading to the production of IL-1β and IL-18. Jin et al.[28] showed that HA crystals lead to release of inflammatory cytokines in an NLRP3-dependent manner via reactive oxygen species (ROS) production, potassium efflux, and lysosomal damage.

IL-1β in particular has been identified as a key driver of destructive and inflammatory responses in OA as a result of its ability to upregulate aggrecanases and MMPs while also suppressing the biosynthesis of ECM. In keeping with this, IL-1β has been shown to be increased in the articular cartilage and synovial fluid of patients with OA. BCP crystals initiate IL-1β–mediated inflammatory processes through NALP3 inflammasome-dependent as well as inflammasome-independent pathways. Non–lipopolysaccharide (LPS)-primed murine macrophages incubated with BCP crystals produce high levels of IL-1β as well as IL-18, also an important cytokine in propagating joint damage. More importantly, longer incubation of LPS-primed macrophages with BCP crystals resulted in production of S100A8, a well-described damage-associated molecule that may further activate the macrophages through TLRs leading to production of IL-1β. Therefore, BCP crystals may cause the production of IL-1β both directly and indirectly through the autocrine effect of S100A8.[29] Also, the spleen tyrosine kinase (SyK) and PI3 kinase appear necessary for the induction of IL-1β following macrophage activation by BCP crystals.

The ability of BCP crystals to induce mitogenesis in many cell types including synoviocytes and macrophages may explain the macroscopic synovial proliferation found in OA. For example, BCP crystals activate human OA synovial fibroblasts (HOAS), leading to the induction of mitogenesis and MMP-1 production. Also, BCP crystals can act synergistically with IL1-α and TNF-α to promote MMP production and likely subsequent joint degeneration.[30] BCP crystals also induce the secretion of several other MMPs including MMP-1, 3, 8, and 9, and can also downregulate tissue inhibitor

of metalloproteinases (TIMP).[31-33] BCP crystals can also induce proto-oncogenes, c-fos, and c-myc.[34]

Sun et al.[35] showed that BCP crystals may stimulate the endocytosis of various extracellular molecules, such as DNA fragments, nucleotides, and small peptides that might contribute to the pathogenesis of BCP crystal-associated diseases.

## Animal Studies

Narayan et al.[36] demonstrated that OCP crystals induce inflammation *in vivo* through IL-1-dependent peritoneal inflammation without requiring the NALP3 inflammasome. BCP crystals injected into the peritoneal cavity of mice led to neutrophil recruitment and up-modulation of IL-1α, IL-1β, and myeloid-related protein (MRP)-8-MRP-14 complex, to levels comparable with those induced by MSU crystals. This OCP crystal-induced inflammation was both IL-1α and IL-1β-dependent, as shown by inhibitory effects of anakinra and anti-IL-1β antibody treatment. This study[36] indicated that macrophages, rather than mast cells, are important for initiating and driving OCP crystal-induced inflammation.

Hang-Korng Ea et al.[37] showed that intra-articular BCP crystals have a direct pathogenic role in OA. BCP crystals injected into mouse knees induced synovial inflammation, cartilage degradation, and chondrocyte apoptosis. The effects observed were independent of the inflammasome-IL-1 pathway.

Two studies to date have looked at the effects of preventing BCP crystal deposition using pharmacological agents. Krug et al.[38] evaluated phosphocitrate (PC), a potential therapy for BCP crystal deposition. PC is the only agent to date that blocks the effects of BCP crystals and prevents calcification; the murine progressive anklyosis (MPA) model was used. This is a manifestation of an autosomal recessive mutation that produces an inflammatory joint disorder, associated with BCP crystal deposition, and results in fusion of the joints. Mice with MPA were treated with PC *in vivo* and there was a significant difference in disease progression and severity between the treated and the control group. Unfortunately, this model was somewhat inadequate as it resembled inflammatory arthritis more than OA.[38,39]

Cheung et al.[40] examined a guinea pig OA model with meniscal calcification, consistent with BCP crystal deposition. After weekly treatment of this animal model for 3 months with a new, more potent formulation of PC containing salt and calcium (CaNaPC), the content of calcification in menisci and cartilage degeneration was examined. As a control they evaluated whether similar CaNaPC treatment had a therapeutic effect in a hemi-meniscectomy model with no known crystal involvement. Meniscal calcification correlated with the cartilage degeneration in this animal model. CaNaPC treatment led to significant reduction of calcium deposits and arrested OA disease progression. Similar CaNaPC treatment had no effect in the hemi-meniscectomy model in which articular calcification does not occur. These results support the hypothesis that calcification in the form of BCP crystals plays an important role in OA disease progression, and that CaNaPC is a potential therapeutic agent for CPPD and BCP crystal deposition disease.[40] Unfortunately, no version of PC has been studied in humans nor is any available for clinical use.

## CONCLUSION

No disease modifying osteoarthritis drug (DMOAD) has been approved by a regulatory body for OA as no DMOAD has clearly shown definite efficacy in patients with OA. With OA being the most prevalent rheumatic disease, affecting approximately 40 million patients in Europe, it is essential that we develop an effective treatment.[41] Enhanced efforts should be made to pursue BCP crystals as a potential target for OA. Advances have been made in the understanding of BCP crystals in the pathogenesis of OA.

We unfortunately have no therapy that prevents BCP crystal formation or removes BCP crystals. Drugs that could dissolve BCP crystals or prevent their formation would be of great interest. Modern technology should allow improved identification and quantitation of BCP crystals. Similar BCP crystal deposits are found in atherosclerotic vessels so there is now an increasing interest amongst cardiologists in exploring the biological effects of calcification in vascular disease. We are in an urgent need of an effective safe disease modifier for OA. There is ample evidence in the literature to show that BCP crystals should be explored as a therapeutic target in OA.

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# EXTRA-ARTICULAR MANIFESTATIONS OF RHEUMATOID ARTHRITIS, NOW

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## ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease, characterised by polyarthritis and extra-articular organ disease, including rheumatoid nodules, ophthalmologic manifestations, cardiopulmonary disease, vasculitis, neuropathy, glomerulonephritis, Felty's syndrome, and amyloidosis. Extra-articular manifestations of RA (ExRA) occur in 17.8–40.9% of RA patients, 1.5–21.5% of them presenting as severe forms and usually associated with increased morbidity and mortality. They can develop at any time during the course of the disease, even in the early stages, and are associated with certain predisposing factors, such as the presence of rheumatoid factor, smoking, and long-standing severe disease. Rheumatoid nodules, the most common ExRA, have been found to be associated with the development of severe features, such as vasculitis, rheumatoid lung disease, pericarditis, and pleuritis, especially in those patients who develop them within 2 years from RA diagnosis. There is no uniformity in the definition of the term ExRA, which limits comparability between different studies. Several recent surveys suggest a lower frequency, probably due to a better control of disease activity. Diagnosis of ExRA is a challenge for clinicians, given its variable and complex presentation, and the lack of specific diagnostic tests; it must be based on clinical recognition and exclusion of other causes of the signs and symptoms. Furthermore, management continues to be difficult with a bad prognosis in many conditions. This article reviews the clinical aspects of major ExRA, focusing on incidence, clinical features, and therapeutic approaches, and how modern immunosuppressive therapy can change the outcome.

Keywords: Rheumatoid arthritis, extra-articular manifestation, management, biologic treatment.

## INTRODUCTION

Rheumatoid arthritis (RA) is an immune-mediated chronic inflammatory disease. Despite being characterised by inflammation of the synovial membrane and progressive destruction of the articular cartilage and bone, RA is a systemic disease often associated with other extra-articular manifestations, with a significant impact on mortality and morbidity.[1] A better control of disease activity in the last decades due to the availability of more efficacious drugs has resulted in a lower frequency of extra-articular manifestations of RA (ExRA), as well as better outcomes in many patients.

### Epidemiology

ExRA can develop at any time during the course of the disease, even in the early stages.[2] They occur in 17.8–40.9% of RA patients, 1.5–21.5% of them presenting as severe forms and usually associated with greater comorbidity and premature death.[3] Higher frequencies are seen in Northern European countries, rather than Southern Europe, suggesting a role of environmental and genetic factors in their pathogenesis. Hospital-based studies also show more prevalence, probably due to inclusion of patients with more severe disease.[4] Until now there has been no uniformity in ExRA definition or classification, despite the fact that they have been

studied in numerous RA cohorts. This partly justifies the differences seen in descriptive reports and incidence studies.

### Predisposing Factors

Prete et al.[4] recently reviewed all relevant articles related to ExRA published up to June 2011, and found smoking, early disability, antinuclear antibodies, and rheumatoid factor (RF) positivity as the principal predictor factors of severe ExRA. Gender was not found to have any effect, with the exception of one Italian study, which reported higher risk of developing any ExRA in men than in women (odds ratio, 1.68). Moreover, Turesson et al.[5] described the presence of rheumatoid nodules (RNs) as a predisposing factor for development of severe ExRA, such as vasculitis, rheumatoid lung disease, pericarditis, and pleuritis, especially in those patients who develop them within 2 years from RA diagnosis, and found a particularly high risk in those patients with long-standing, severe disease.

## CLINICAL MANIFESTATIONS

Many different tissues and organs can be involved in RA patients in addition to the characteristic peripheral polyarthritis (Table 1). Several general symptoms can represent a major problem during the course of RA, many of them also being present before its diagnosis. Weight loss, fever, prolonged early morning stiffness, fatigue, generalised muscle weakness, low mood, and depression are often responsible for a significant loss in the quality of life of patients. Fatigue is reported in 40–80% of RA patients as their most disabling symptom.[6] The OMERACT (Outcome Measures in Rheumatology) network of international researchers highlighted fatigue as a main outcome, recommending its measure whenever possible.[7]

As a result of the inflammatory process, RA patients frequently developed normochromic-normocytic anaemia.[8] Other manifestations associated with chronic inflammation include injury of exocrine glands with the development of a secondary Sjögren's syndrome (SS), sarcopaenia, and osteoporosis.

RNs are the most frequent skin manifestation. They occur in about 30% of RA patients, mostly in RF-positive subjects, and are usually located subcutaneously on pressure areas, including the olecranon process and proximal ulna, finger joints, sacral prominences, occiput, and Achilles tendon. Usually painless, they have a variable consistency from a soft, mobile to a hard, rubbery mass attached firmly to the periosteum. Histologically they are characterised by a central necrotic area rimmed by a corona of palisading fibroblasts that is surrounded by a zone of tissue affected by perivascular cellular infiltration enriched with lymphocytes, plasma cells, and histiocytes.[9]

Regression of nodules may occur during treatment with disease modifying anti-rheumatic drugs (DMARD). Paradoxically, in 8–11% of methotrexate-treated RA patients an accelerated rheumatoid nodulosis can occur, with nodules usually located in the fingers or in the metacarpophalangeal and proximal interphalangeal joints. The condition regresses when methotrexate is reduced or withdrawn and if hydroxychloroquine or sulphasalazine treatment is started. Etanercept has also been related with the development of this type of nodulosis.[10] No effective treatment is available.

Ocular involvement occurs in 27% of RA patients.[11] Keratoconjunctivitis sicca (KCS), the most frequent and usually benign ophthalmologic manifestation, occurs in at least 10% of patients together with xerostomia, usually as a part of a secondary SS. Symptoms such as burning or a foreign body sensation can be warning signs. The diagnosis is supported by a positive Schirmer test and a reduced tear break-up time. On the other hand, a reduced salivary flow rate can confirm xerostomia. Some patients develop scleritis, episcleritis, peripheral ulcerative keratitis, or vasculitis involving retinal vessels. A clinical suspicion of such disorders in a patient with RA should lead to immediate referral to an ophthalmologist. Episcleritis, inflammation of the layer superficial to the sclera, usually correlates with the activity of RA. It presents in <1% of patients with RA and is generally a self-limiting condition. Symptoms are usually limited to focal redness and irritation of the eye without altering visual acuity. Scleritis is a more aggressive process, characterised by an intensely painful inflammation of the sclera itself. It is seen in patients with vasculitis and long-standing arthritis. There are three types of anterior scleritis: diffuse, nodular, and necrotising. The latter is also referred to as scleromalacia perforans, the most severe type. It is a degenerative thinning of the sclera that occurs in FR-positive female patients. It has been attributed to a vasculitic process with deposition of immune complexes.

**Table 1: Extra-articular manifestations in rheumatoid arthritis.**

| Affected tissue or organ | Extra-articular manifestation |
|---|---|
| General symptoms | Weight loss<br>Fever<br>Prolonged early morning stiffness<br>Fatigue<br>Generalised muscle weakness<br>Low mood and depression |
| Inflammatory-process associated features | NNA<br>Secondary SS<br>Sarcopenia<br>Osteoporosis |
| Skin | RNs<br>CuV<br>RP |
| Eyes | KCS<br>Scleritis<br>Episcleritis<br>PUK<br>Vasculitis involving retinal vessels |
| PS | PNs<br>PE<br>ILD |
| CVS | PC<br>MC<br>CA<br>CoV<br>Arrhythmia<br>VDs<br>CHF<br>IHD |
| NS | CD<br>CM<br>CNS vasculitis<br>Rheumatoid nodules located within the CNS or meningitis<br>Stroke<br>MM<br>SPN |
| Kidneys | GN<br>IN<br>Secondary amyloidosis |
| Haematological system | FS |

NNA: normochromic-normocytic anaemia; SS: Sjögren's syndrome; RNs: rheumatoid nodules; CuV: cutaneous vasculitis; RP: Raynaud's phenomenon; KCS: keratoconjunctivitis sicca; PUK: peripheral ulcerative keratitis; PS: pulmonary system; PNs: pulmonary nodules; PE: pleural effusion; ILD: interstitial lung disease; CVS: cardiovascular system; PC: pericarditis; MC: myocarditis; CA: cardiac amyloidosis; CoV: coronary vasculitis; VDs: valve diseases; CHF: congestive heart failure; IDH: ischaemic heart disease; NS: nervous system; CD: cognitive dysfunction; CNS: central nervous system; CM: cervical myelopathy; MM: mononeuritis multiplex; SPN: sensory peripheral neuropathy; GN: glomerulonephritis; IN: interstitial nephritis; FS: Felty's syndrome.

This condition is often painless and can evolve to scleral perforation when it goes untreated.[12]

Pulmonary involvement in RA is frequent, although not always clinically recognised, and includes RNs, pleural effusion (PE), interstitial lung disease (ILD), small airway disease, and pulmonary vasculitis. It is responsible for 10-20% of overall mortality,[13,14] and can occur before the development of joint symptoms.[15,16] Parenchymal pulmonary nodules (PNs) are usually asymptomatic, but may cavitate and cause PEs (Figure 1); they also increase the risk of infections and pneumothorax. They are usually found in RF-positive patients with nodules elsewhere. Sometimes differentiation with neoplasms and infections can be difficult. PE, usually an exudate with mixed cell counts and high protein concentration, is common but frequently asymptomatic; autopsy studies reported pleural involvement in 50% of cases, with only 10% clinically detected.[17]

ILD is the most important pulmonary manifestation of RA, being the commonest pulmonary cause of death and a significant contributor to morbidity.[13,14,18,19] The most frequent histopathological patterns of ILD in RA are usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP) (44–56% and 33–44%, respectively),[20] followed by mixed disease (0–12%). Other forms, such as obliterative bronchiolitis, are rare but associated with a high mortality.[21] Although it tends to occur more often in RF positive male patients with long-standing nodular disease,[22] studies in new onset RA have found lung abnormalities in a high percentage of patients.[23,24] Clinical presentation and course are similar to that of idiopathic pulmonary fibrosis, but the response to immunosuppressants is usually better. Diagnosis is based on clinical presentation, blood gases, pulmonary function tests, and high resolution computed tomography (HRCT).[25] As abnormalities can be detected by HRCT in about 50% of RA patients, but only 10% have clinically significant symptoms,[26] diagnosis should be supported not only on clinical signs and symptoms, but also in abnormal pulmonary function tests and either a compatible HRCT or lung biopsy. Physiological abnormalities include a reduction in lung volume, a low diffusing capacity for carbon monoxide (which is the measure best associated with the extent of disease in ILDs and a poorer prognosis in RA-ILD27), and oxygen desaturation during a 6-minute walk test.

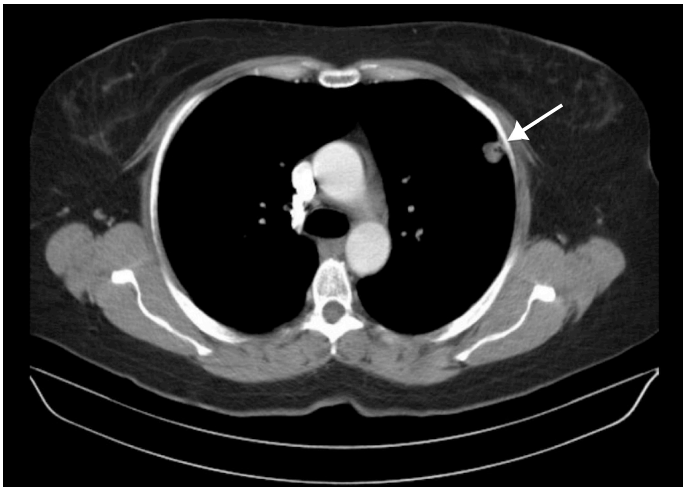

**Figure 1: Pulmonary nodule with a central cavity (arrow).**

Cardiovascular (CV) features in RA are common,[28] including pericarditis, myocarditis, cardiac amyloidosis, coronary vasculitis (CoV), arrhythmia, valve diseases, and, most importantly, congestive heart failure[29] and ischaemic heart disease (IHD). The last two have been associated with an increased morbidity and mortality in RA patients compared with the general population due to an accelerated atherogenesis process that cannot be fully explained by the classic atherosclerosis risk factors;[30,31] the presence of chronic inflammation and a possible genetic component are important contributing agents.[32,33]

Within the classical cardiac manifestations, pericarditis is the most common; both echocardiography and autopsy studies reveal evidence of pericardial inflammation in 50% of patients, although symptoms are relatively uncommon, occurring in about 1–4% of patients. It usually occurs in RF-positive nodular RA, and pericardial fluid analysis reveals features similar to those found in rheumatoid PEs. Conversely, symptomatic myocarditis, endocarditis, and CoV rarely occur, and are almost exclusively demonstrated by autopsy.[34] Cognitive dysfunction is frequently found in RA patients, with prevalence rates ranging from 38-71%.[35,36] Education, income, glucocorticoid use, and cardiovascular disease (CVD) risk factors are independent predictors of its development.[37]

On the contrary, neurological involvement in RA is rare, present in only 1% of patients. Disorders of the central nervous system (CNS) include cervical myelopathy, vasculitis, RNs located within the CNS,

or meningitis. Stroke also occurs with increased frequency.[38] CNS vasculitis is extremely rare. The diagnosis is supported by magnetic resonance imaging (MRI), alone or with magnetic resonance angiography (MRA), showing the segmental vascular stenosis characteristic of vasculitis.[39] Peripheral neuropathy is usually manifested as sensorimotor neuropathy or mononeuritis multiplex. The underlying mechanism is small vessel vasculitis of the vasa vasorum of the nerves with ischaemic neuropathy and demyelinisation as part of the rheumatoid vasculitis (RV) syndrome.

RA and kidney disease (KD) often coincide. There are several potential causes of nephropathy such as drug-related renal disease, secondary renal amyloidosis, and various types of glomerulonephritis (GN). Mesangial proliferative GN is the most frequent histological lesion followed by membranous GN,[40,41] the latter usually being related to gold or D-penicillamine, with both therapies not currently in use. Other infrequent causes of KD can be interstitial nephritis, minimal change glomerulopathy, IgA nephritis, focal proliferative GN, or rapidly progressive GN due to microscopic polyangiitis.[42] Prevalence has been recently established; the MATRIX study[43] found KD in 46.3% of RA patients according to the National Kidney Foundation (NKF) classification[44] with a stage distribution of 11.3% in Stage 1 (normal kidney function with kidney damage), 20.0% in Stage 2 (mild renal insufficiency with kidney damage), 15.0% in Stage 3 (moderate renal insufficiency), and no patients in Stages 4 or 5. The study was not designed to identify the potential causes of KD. A recent retrospective review[45] has shown that RA patients are more likely to develop reduced kidney function over time, with CVD at baseline and elevated erythrocyte sedimentation rate as predisposing factors, and found a relationship between renal impairment and increased morbidity from CVD development.

Secondary (reactive AA) amyloidosis can be seen in long-standing disease and poor response to therapy, and markedly influences these patients' outcomes.[46] Prevalence is around 7%,[47] with clinically symptomatic amyloidosis much lower.[48] Common clinical signs of reactive AA amyloidosis in patients with RA can be found by careful observation for the onset of proteinuria, kidney insufficiency, or gastrointestinal tract symptoms. Biopsy is often necessary to make an accurate diagnosis.

RV is a rare but potentially serious necrotising vasculitis, which can develop in patients with RA sometimes in the absence of active joint disease. RV typically occurs in male patients with long-standing RF-positive erosive nodular RA,[49] in association with a severe disease course and other ExRA features including episcleritis, pleural, and pericardial effusions or pulmonary fibrosis.[50] Smoking is associated with an increased risk of vasculitis among patients with RA[51] and there also appears to be a genetic predisposition, with major histocompatibility complex, Class 2, DR beta 1 (HLA-DRB1)-shared epitope genotypes strongly associated.[52] Any size of blood vessel may be involved, but capillaries, small venules, veins, arterioles, and medium-sized arteries are the most frequently affected. Histopathologically, it is characterised by a necrotising panarteritis showing fibrinoid necrosis of the vessel wall, with an inflammatory cell infiltrate in early lesions. Later on, arterial wall fibrosis with occlusion can appear. It may present with palpable purpura, distal vasculitis (ranging from splinter haemorrhages and fingertips infarction to gangrene), cutaneous ulceration, mononeuritis multiplex, or arteritis of viscera, including heart, lungs, bowel, kidney, liver, spleen, pancreas, lymph nodes, and testis. Sometimes vasculitis is limited to the nail folds, and this has a better prognosis and does not usually herald the onset of systemic disease.[53]

FS is an uncommon ExRA, occurring in <1% of RA patients. It is defined as a combination of RA with neutropaenia and splenomegaly, and occurs mostly among women around the age of 60 with a long history of severe articular disease, RF-positive in association with antibodies to cyclic citrullinated peptides, and who have the HLA-DR4*0401 antigen.[54] Almost 75% of patients with FS will present cutaneous nodules. Other features that are usually present include lymphadenopathy, hepatopathy, vasculitis, leg ulcers, and skin pigmentation. Its poor prognosis is due to a higher incidence of severe infection related to the neutropaenia that normally accompanies it, whose cause lies in both decreased granulopoiesis and increased peripheral destruction of granulocytes.[55] It is important to exclude haematopoietic malignancy when making the diagnosis of FS. The clinical significance of FS resides in the fact that often inactive joint disease distracts the clinician's attention from the severe extra-articular disease and neutropaenia, causing recurrent - sometimes fatal - infections. Furthermore, FS has been

associated with an increased risk of malignant lymphoproliferative disease compared to other patients with RA. This highlights the importance of careful evaluation of these cases.

## MANAGEMENT

The first step in management of RA with or without extra-articular manifestations must be early treatment with DMARD, both to control inflammation with subsequent articular progression and to reduce the risk of further extra-articular complications (Table 2). Progression of scleromalacia perforans can be prevented with this approach, although refractory cases may benefit from the use of biological agents; several papers have shown good results in controlling ocular complications, but clinical trials have not yet been reported.[56]

**Table 2: Management of extra-articular manifestations in rheumatoid arthritis.**

| Extra-articular manifestation | Management |
|---|---|
| General symptoms<br><br>Inflammatory-process associated features | Early treatment with DMARD, and when necessary with biologics, both to control inflammation and to reduce the risk of further extra-articular complications |
| Skin | Hydroxychloroquine or sulphasalazine<br>Avoid methotrexate if accelerated RN occurs |
| Eyes | DMARD and/or biologics to control inflammation<br>Rituximab[56] |
| PS | Cyclophosphamide and high-dose corticosteroids[67]<br>Cyclosporine[68,69] |
| CVS | |
| PC<br><br>MC<br>CoV<br><br>CHF<br>IHD | Non-steroidal anti-inflammatory drugs or steroids<br><br>Immunosuppressive treatment<br><br>Strict control of CVD risk factors[57,58] |
| NS | Cyclophosphamide and high-dose corticosteroids |
| Kidney | Cyclophosphamide and high-dose corticosteroids |
| FS[61] | Methotrexate<br>Granulocyte colony-stimulating factor<br>Rituximab in refractory cases[62]<br>Splenectomy |
| SV | Cyclophosphamide and high-dose corticosteroids[63]<br>TNF-inhibitors[64]<br>Rituximab[65,66] |
| Amyloidosis | Chlorambucil[76]<br>Cyclophosphamide[77]<br>TNF-inhibitors[78,79]<br>Tocilizumab[80,81] |

DMARD: disease modifying anti-rheumatic drugs; RN: rheumatoid nodulosis; PS: pulmonary system; CVS: cardiovascular system; PC: pericarditis; MC: myocarditis; CoV: coronary vasculitis; CHF: congestive heart failure; IHD: ischaemic heart disease; CVD: cardiovascular disease; NS: nervous system; FS: Felty's syndrome; SV: systemic vasculitis; TNF: tumour necrosis factor.

Although there is some evidence that CV risk in RA is reduced by successful suppression of inflammation, it remains important to identify and target traditional CVD risk factors as well. Guidelines emphasising the need for regular screening of patients with RA for CV risks have been recently published.[57,58] Congestive heart failure requires special consideration, since it does not appear to be fully related to traditional CVD risk factors or clinical IHD. Findings from a number of studies have shown that inflammatory cytokines are related to the echocardiographic indices of both systolic and diastolic left ventricular function.[59] In addition, the inhibition of interleukin-1 (IL-1) showed an ability to improve myocardial deformation in these patients.[60]

The majority of traditional cardiac complications in RA are silent and do not require treatment. Symptomatic pericardial disease without haemodynamic compromise can be resolved with non-steroidal anti-inflammatory drugs or steroids, but recurrent forms may need immunosuppressive treatment. Constrictive pericarditis, and rapidly progressive pericarditis, require emergency intervention and can worsen the outcome of patients.

In patients with FS, neutropaenia can be effectively managed with DMARDs, the widest experience being with methotrexate. Splenectomy results in immediate improvement of neutropaenia in 80% of patients, but the rate of infection decreases to a lesser degree. Granulocyte colony-stimulating factor can be useful too. It seems to be logical to suppose that early aggressive treatment of RA may prevent the development of FS, but there are no epidemiological data to support this hypothesis.[61] Some investigators reported a response of FS to rituximab, while others found this treatment questionable. A systematic review evaluating biological treatment in FS concludes that the use of RTX can only be recommended as a second-line therapy in patients with refractory FS; experience with anti-tumour necrosis factor (TNF) agents is very limited with no improvement in neutrophil count.[62] Spontaneous remission of the syndrome can also occur.

For severe ExRA, such as systemic vasculitis, treatment with cyclophosphamide and high-dose corticosteroids has been considered as the recommended approach.[63] According to other reports,[64] we have observed a dramatic response of severe cutaneous vasculitic ulcers after anti-TNF treatment in a RF-positive RA patient with long-standing severe disease (data unpublished, Figure 2). On the other hand, rituximab has proved beneficial in RV, being an alternative to cyclophosphamide in selected patients.[65,66]

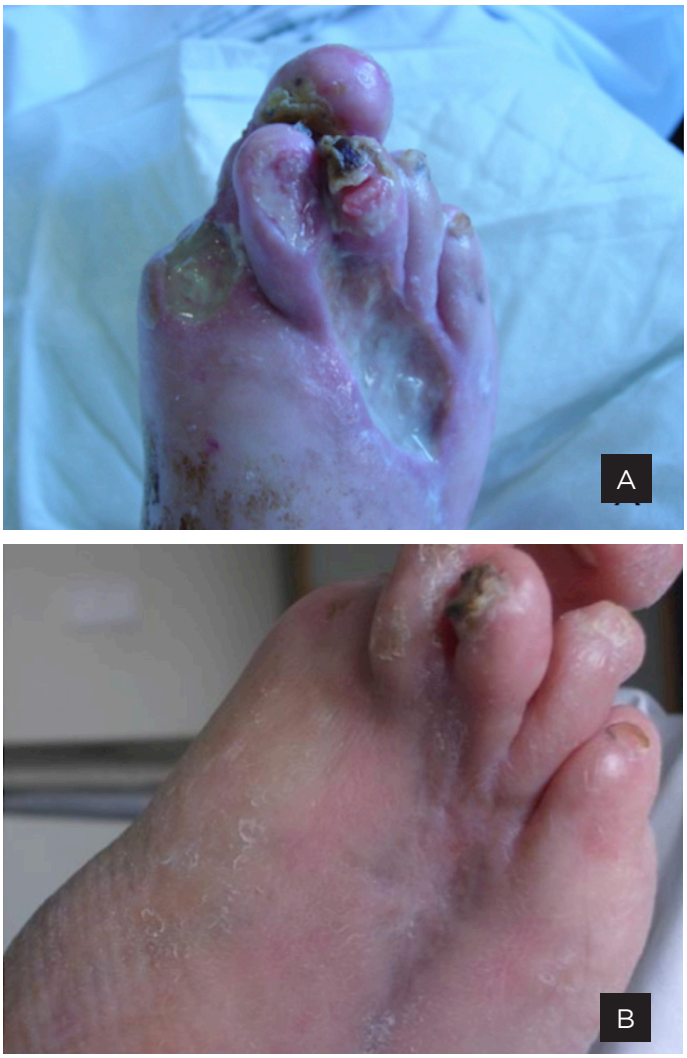


**Figure 2: Complete healing of severe cutaneous vasculitic ulcers after anti-tumour necrosis factor treatment (A: pre-treatment; B: post-treatment).**

RA-associated lung disease treatment is controversial. It is mainly based on systemic steroids and cyclophosphamide,[67] existing also positive data with cyclosporine.[68,69] Although a beneficial effect of anti-CD20 therapy has been described in several case reports, physicians should be aware that this drug could trigger or worsen RA-related pulmonary fibrosis.[70] Conflicting results have been published with TNF-inhibitors, with reports on excellent response in refractory patients,[71] as well as worsening of ILD in others.[72-74] Regression of parenchymal PNs after tocilizumab treatment has been observed.[75]

Standard treatment for AA amyloidosis has been based for a long time on colchicine, chlorambucil,[76] or cyclophosphamide,[77] but TNF inhibitors have proved effective in controlling the progression of renal amyloidosis in patients with RA.[78,79] Since the activation of the serum amyloid A gene depends more on the presence of IL-6 than on the presence of TNF alpha, tocilizumab will probably be a first-line treatment in the future.[80,81]

## EXTRA-ARTICULAR MANIFESTATIONS OF RA, NOW

A better control of disease activity during the last decades has improved the outcome of RA.[82] ExRA incidence seems to be reduced too, although it is not equal for all manifestations: a decline in RV incidence has been found in several studies,[83,84] even though clinical manifestations remain similar and its prognosis remains poor despite modern immunosuppressive therapy.[85] Secondary amyloidosis with clinically apparent organ manifestations is not present in the most recent series of RA patients. On the contrary, it has not been a significant change in the incidence of other severe ExRA manifestations, like RA-associated lung disease. Moreover, milder ExRA manifestations, such as KCS, has been diagnosed more frequently among patients with a more recent onset of RA, possibly because of improved clinical surveillance.[86] The clinician must be familiar with ExRA diagnosis, which is often complicated and difficult, and its management. New immunosuppressive drugs may offer interesting possibilities, although until now quality studies are lacking and there is a need to act with care. Undoubtedly, the future for RA patients is encouraging.

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# ANTIPHOSPHOLIPID SYNDROME NOVEL THERAPIES

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## ABSTRACT

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by arterial and/or venous thrombosis, recurrent pregnancy loss, and persistently positive antiphospholipid antibodies (aPLs). It could be life-threatening as in the case of catastrophic APS where multi-organ failure is observed. APS morbidities are thought to be the result of a combination of thrombotic and inflammatory processes. Over the past decades, the mainstay of therapy of APS has been anticoagulation. As new mechanisms of pathogenesis are being unravelled with time, novel targeted immunomodulatory therapies are being proposed as promising agents in the treatment of APS. In this article, we present an overview of new pathogenetic mechanisms in APS as well as novel antithrombotic and immunomodulatory therapies.

Keywords: Antiphospholipid syndrome, thrombosis, antiphospholipid antibodies, seronegative antiphospholipid syndrome, thromboprophylaxis, immunomodulatory, new oral anticoagulants.

## INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by clinical thrombotic events associated with the presence of antiphospholipid antibodies (aPLs) in patient plasma.[1-3] It was first described in 1983 by Graham R.V. Hughes.[4] APS is recognised as one of the common causes of acquired thrombophilia and can be classified as primary or secondary depending on its association with other autoimmune diseases.[5] Up to 40% of patients with systemic lupus erythematosus (SLE) test positive for aPLs, but only half of these patients go on to develop overt thrombosis or miscarriages.[6] Over the past 30 years, the mainstay of treatment was antithrombotic medications. As we continue to unravel the pathophysiology of the disease, some promising novel immunomodulatory therapies are being introduced. In this article, we will review the pathogenesis of the disease, its clinical manifestations, diagnostic criteria, and advances made in therapy.

## PATHOGENESIS

Although several mechanisms were described as the cause of thrombosis in APS, the ultimate means by which clinical manifestations occur is still not fully understood. aPLs are autoantibodies directed against phospholipid-bound proteins, particularly the $\beta_2$-glycoprotein I ($\beta_2$GPI). It is believed that thrombosis in APS follows a 'two-hit' hypothesis where the first hit disrupts the endothelium and the second hit potentiates thrombus formation.[7-13] One of the many proposed mechanisms that constitute the first hit is increased oxidative stress. Studies in APS patients showed increased lipid peroxidation by-products as well as an increase in intracellular reactive oxygen species (ROS). These were reflected in animal models where ROS contributed to the pathogenesis of murine thrombosis.[14] Another proposed mechanism is impaired endothelial nitric oxide synthase (eNOS) function; some APS patients were found to have decreased plasma nitrite levels and diminished NO-dependent vascular relaxation accordingly, thus, enhancing thrombosis.[15] Disruption of the annexin A5 (AnxA5) shield is also believed to play a role in promoting thrombosis. AnxA5 forms a shield when binding

to phosphatidylserine surfaces; this shield inhibits the formation of procoagulant complexes.[16] Domain I anti-$\beta_2$GPI autoantibodies disrupts this shield when combined to $\beta_2$ glycoprotein 1 ($\beta_2$GPI), thus exposing procoagulant phosphatidylserine and predisposing to thrombosis.[17] An increased expression and activation of tissue factor (TF) was also seen in APS patients where aPLs caused upregulation of the TF in monocytes, neutrophils, and on endothelial cells. TF was thought to play a role in APS-associated thrombotic microangiopathy.[18,19] The second hit is thought to be any triggering event that can cause thrombosis, i.e. local endothelial damage or infection.[7]

Obstetric APS and recurrent pregnancy losses were believed to be the result of different mechanisms acting on placental cells and endometrial tissues.[20] Thrombosis, inflammation, and immunomodulations are thought to affect placental cells. Histological analysis of placenta collected from APS patients showed more thrombotic characteristics than those collected from controls.[20] Complement system activation is also thought to play a role where biopsies from the placental tissue of mice treated with aPLs showed greater deposition of complement components 3 (C3) and 4 (C4) accompanied with a reduction in membrane attack complex (MAC).[21] Recently, immunomodulation was introduced as a possible mechanism in APS-related pregnancy loss. Toll-like receptors (TLRs) have been implicated in the pathological activation of endothelial cells, monocytes, and platelets, thus leading to uncontrolled inflammation and apoptosis.[22] Pathologic mechanisms may also occur at the level of endometrial tissue where some studies showed that aPLs may inhibit endometrial angiogenesis, decrease vascular endothelial growth factor secretion, and inhibit NFκB activation.[23,24] Catastrophic antiphospholipid syndrome (CAPS) was believed to be the result of combined pathogenic mechanisms that involve cellular activation, inhibition of anticoagulants, including the protein C pathway, inhibition of fibrinolysis, and complement activation.[25]

## CLINICAL MANIFESTATIONS

APS usually manifests itself as a thrombotic disorder where patients experience vascular events or pregnancy morbidities. The most common presentation is venous thromboembolism (VTE) where up to 70% of patients can acquire deep vein thrombosis, pulmonary emboli, or develop clots anywhere in the axillary, retinal, or hepatic vascular networks.[26,27] Although arterial bed thrombosis is less common, it is more serious and life-threatening as it affects most generally the central nervous system (CNS) and presents as strokes or transient ischaemic attacks. Obstetric APS appears clinically as recurrent pregnancy losses or premature births due to eclampsia, preeclampsia, or placental insufficiency. Other less common manifestations are listed in Table 1.[28,29]

## DIAGNOSIS

APS is diagnosed by the presence of at least one clinical criterion in addition to one laboratory criterion. Classification criteria were updated in 2006 where some laboratory criteria were modified. Clinical criteria remained unchanged. The revised classification criteria for APS are listed in Table 2.[30]

### Seronegative APS

Hughes and Khamashta[31] were among the first to introduce the term 'Seronegative Antiphospholipid Syndrome' (SNAPS) for patients with clinical manifestations highly suggestive of APS but with persistently negative serologies (lupus anticoagulant [LAC], anticardiolipin antibody [aCL], and anti-$\beta_2$GPI).[23] Although still not widely accepted, some studies showed that

**Table 1: Other less common clinical manifestations.**

| | |
|---|---|
| Thrombocytopaenia | Transverse myelitis |
| Haemolytic anaemia | Leg ulcers |
| Livedo reticularis | Adrenal haemorrhage |
| Cardiac valvular vegetations | Antiphospholipid syndrome nephropathy |
| Myocardial ischaemia/coronary artery disease | Budd-Chiari syndrome |
| Amaurosis fugax | |

SNAPS involve several other antigens than those mentioned in the revised criteria, and new non-criteria antibodies were described that can be utilised in the future as potential diagnostic laboratory markers.[32,33] A list of the non-criteria aPLs is found in Table 3.[34]

## Catastrophic APS

<1% of APS patients tend to develop a severe life-threatening entity called CAPS, which has a 30% mortality rate in the absence of treatment.[35] CAPS was first introduced by Asherson et al.[36] after reporting several patients with accelerated thrombosis and acute organ failure. In 2003, diagnostic criteria for CAPS were proposed and published.[37] A diagnosis of definite CAPS is met when there is evidence of multisystem (≥3) organ involvement over 7 days associated with small vessel occlusion evidence on histopathology and the presence of aPLs in the serum.[37]

**Table 2: Revised classification criteria for APS.**

| Clinical criteria | Laboratory criteria |
|---|---|
| Vascular thrombosis<br>• One or more clinical episodes of arterial, venous, or small vessel thrombosis. It has to be supported by objective validated criteria i.e. unequivocal findings of appropriate imaging studies or histopathology. In histopathology, no evidence of inflammation in the vessel wall shall be present. | • LAC present in plasma, on two or more occasions at least 12 weeks apart. LAC is detected according to the guidelines of the International Society on Thrombosis and Haemostasis.<br>• IgG &/or IgM isotypes of aCL present in serum or plasma, in medium or high titres (i.e. >40 GPL or MPL, or greater than the 99$^{th}$ percentile) on two or more occasions, at least 12 weeks apart. aCL is measured by a standardised ELISA.<br>• IgG &/or IgM isotypes of anti-$\beta_2$GPI present in serum or plasma (in titres greater than the 99$^{th}$ percentile) on two or more occasions at least 12 weeks apart. anti-$\beta_2$GPI is measured by a standardised ELISA according to recommended procedures. |
| Obstetric morbidity<br>• One or more unexplained deaths of a morphologically normal foetus at or beyond the 10$^{th}$ week of gestation. Healthy foetal morphology has to be documented by ultrasound or by direct examination of the foetus. OR<br>• One or more premature births of a morphologically normal newborn baby before the 34$^{th}$ week of gestation due to eclampsia, severe preeclampsia or placental insufficiency. OR<br>• Three or more unexplained consecutive spontaneous abortions before the 10$^{th}$ week of gestation. Maternal anatomical or hormonal abnormalities and paternal and maternal chromosomal causes must be excluded. | |

APS: antiphospholipid syndrome; LAC: lupus anticoagulant; IgG &/or IgM: immunoglobulin G/M; aCL: anticardiolipin antibody; GPL: units for IgG [1 GPL unit = 1 μg of affinity-purified IgG]; MPL: units for IgM [1 MPL unit =1 μg of affinity-purified IgM]; ELISA: enzyme-linked immunosorbent assay; anti-$\beta_2$GPI: anti-$\beta_2$glycoprotein 1 antibody.

**Table 3: Non-criteria antiphospholipid antibodies.**

| |
|---|
| aPE antibodies |
| Antibodies to negatively charged phospholipids other than cardiolipin: PA, PS, and PI |
| Anti-domain I antibodies of $\beta_2$GPI |
| Antibodies to vimentin/cardiolipin complex |
| Anti-PT: aPT-A and aPS-PT |
| IgA, aCL, and IgA anti-$\beta_2$GPI antibodies |

aPE: anti-phosphatidylethanolamine; PA: phosphatidic acid; PS: phosphatidylserine; PI: phosphatidylinositol; $\beta_2$GPI: $\beta_2$-glycoprotein I; aPT: anti-prothrombin; aPS/PT: anti-phosphatidylserine/prothrombin.

## MANAGEMENT

The management of APS thrombosis constitutes either primary thromboprophylaxis or secondary thromboprophylaxis. Primary thromboprophylaxis represents treating aPL-positive patients with no previous thrombosis, while secondary thromboprophylaxis represents treating APS patients with previous thrombotic events. The mainstay of treatment is currently anticoagulation, though multiple novel immunomodulatory therapies are on the rise.

Before initiating any primary prevention, one must exclude the co-existence of autoimmune diseases (such as SLE) and target any other thrombotic risk factors. If any is present, it has to be addressed according to the standards of care. Once the patient is labelled as asymptomatic, testing positive for aPLs, it is not recommended to initiate thromboprophylaxis as per The Antiphospholipid Antibody Acetylsalicylic Acid (APLASA) study which showed no advantage between placebo and low-dose aspirin.[38] If the patient has a high-risk profile (triple positivity - positive LAC, aCL, and anti-$\beta_2$GP1 antibodies), therapy with aspirin should be considered to prevent further vascular events as triple positivity showed to increase the risk of thrombosis.[39]

Secondary thromboprophylaxis depends on the first presentation of the thrombotic event, whether venous or arterial in nature. Randomised controlled trials (RCTs) in patients with previous venous thrombosis indicated the use of warfarin with target international normalised ratio (INR) of 2.0–3.0 as an ideal anticoagulant therapy.[40] If a patient has transient risk factors and a low-risk profile, anticoagulation could be stopped after 3-6 months, otherwise lifelong anticoagulation is recommended.[41-43] Arterial thrombosis presenting mostly as strokes shows a high incidence of mortality and thus, must be managed aggressively. Studies showed that 70% of patients with APS had arterial events with INR <2.5.[44,45] This observation necessitated high intensity anticoagulation to a target INR >3.0 as per Khamashta et al.[46] In the light of increased bleeding complications associated with high intensity anticoagulation, Okuma et al.[47] demonstrated that combination therapy (aspirin and warfarin) had significantly lower stroke recurrence rates with lower bleeding complications than warfarin alone. Anticoagulation is lifelong in the case of arterial events, with no data suggesting that stopping the medications is ever possible.[48]

Rodríguez Garcia et al.[49] concluded that long-term treatment with low molecular weight heparin (LMWH) at anticoagulant dosages could be an option in refractory APS patients, or in those who are contraindicated for oral anticoagulants. This conclusion was based on the results of two studies that observed a total of 47 APS patients treated with LMWH and followed them up for an average of 24 months.[50,51] Both studies showed high rates of clinical improvement with very low incidence of rethrombosis. Keeping in mind that LMWH can cause haemorrhage, osteoporosis, and thrombocytopaenia as a complication of treatment,[52] its use may be an effective and safe alternative for subjects who cannot tolerate oral anticoagulants. Future clinical trials are needed to assess its efficacy and safety when used to treat APS patients.

Warfarin is considered category X in pregnancy; it is associated with several birth defects when given during the first trimester[53] and may cause CNS disorders and eye defects if used in late pregnancy.[54] As warfarin and vitamin K antagonists are harmful during pregnancy, it was concluded by Derksen et al.[55] that combined therapy of heparin and aspirin is the recommended therapy for obstetric APS. This recommendation was based on a meta-analysis which concluded that aspirin alone is ineffective and that low live-birth rate was observed in patients treated with aspirin alone compared to combined therapy.[56] Aspirin should be initiated before conception or at the time of positive pregnancy test, while warfarin must be shifted to heparin or LMWH which has a more predictable dose and has the advantage of easy administration once daily.[57,58] Warfarin can be resumed postpartum after therapeutic INR has been reached.[57]

As CAPS is associated with high mortality rate, recommendations are to treat it aggressively with therapeutic doses of anticoagulation, corticosteroids, plasma exchange, intravenous immunoglobulins (IVIG), and rituximab (anti CD20 monoclonal antibody).[59,60] The 14th International Congress on Antiphospholipid Antibodies Task Force Report on CAPS concluded that anticoagulation and corticosteroids should be the backbone of therapy with Grade B recommendation.[61] Adding plasma exchange to the aforementioned regimen is also recommended, with IVIG added in the case of ongoing infection.[61]

Patients with concomitant autoimmune diseases such as SLE may benefit from extra immunosuppression (i.e. cyclophosphamide).[61] Rituximab may have a role as an initial adjuvant therapy or may be used as a second-line therapy when standard triple therapy (anticoagulation + glucocorticoids + plasma exchange) fails.[61]

## NOVEL THERAPIES

Over the past decade, intensive research in APS field unleashed new pathogenic mechanisms that gave a hope for future targeted therapies. Below we discuss new oral anticoagulants as well as novel immunomodulatory regimens.

### New Oral Anticoagulants

Long-term anticoagulation with oral vitamin K antagonists such as warfarin has been associated with certain limitations as well as undesirable side-effects. It is limited by a narrow therapeutic range, requires frequent laboratory monitoring, has slow onset/offset of action, and interacts with food, drugs, and alcohol. Thus, new agents are being tested currently. These agents include direct anti-Xanthium inhibitors (rivaroxaban, apixaban) and direct thrombin inhibitors (dabigatran etexilate). These agents demonstrated a better safety profile with fewer dietary and drug interactions along with a predictable anticoagulant effect omitting the need of frequent monitoring.[62,63] They are currently FDA approved to be used in the treatment of different conditions based on Phase III prospective RCTs (Table 4).[62,64-69] Although these trials showed superiority of new oral anticoagulants over warfarin when dealing with VTE, its role in managing APS patients is still unknown as aPL status was not documented in any of these trials.[64,65,68] Prospective studies on APS patients are needed as the use of these agents would result in a major improvement in quality of life if proven efficacious.

One trial comparing warfarin versus rivaroxaban in APS patients (Rivaroxaban in Antiphospholipid Syndrome – RAPS) is currently undertaken in the UK.[70]

### Immunomodulatory Regimens

As we continue to understand the different mechanisms underlying APS, new targeted therapies are being explored.

#### Statins

Statins are lipid lowering agents that function by inhibiting the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA), and are used widely as a measure to prevent cardiovascular disease in high-risk patients. Along with its lipid lowering role, it was shown that statins have antithrombotic and anti-inflammatory characteristics due to its ability to modify endothelial functions, inflammatory responses, plaque stability, and thrombus formation.[71] Experiments on different statins revealed that fluvastatin and simvastatin[72,73] were able to inhibit aPL-induced endothelial cell activation and TF upregulation *in vitro*, while others showed that fluvastatin and pravastatin[74-76] prevented aPL-mediated thrombosis and inflammation and pregnancy loss *in vivo*. Data are limited in human subjects although its dual action on tumour necrosis factor-alpha (TNF-α) and TF makes it beneficial for use against the inflammatory and thrombotic features present in APS.[77]

#### Hydroxychloroquine (HCQ)

HCQ is an antimalarial agent used in SLE to prevent thromboembolic events. HCQ has different immunologic effects and acts by inhibiting inflammatory cytokines (Interleukin [IL]-1,2,6, TNF-α), T cell antigen receptor (TCR) and B cell antigen receptor (BCR) induced calcium signalling, and TLR activation.[78] HCQ was also shown to inhibit

**Table 4: FDA approved treatments of various conditions.**

| Medication | Indication |
|---|---|
| Rivaroxaban | VTE prevention after orthopaedic surgery, stroke prevention in non-valvular AF, VTE treatment |
| Apixaban | VTE prevention after orthopaedic surgery, stroke prevention in non-valvular AF |
| Dabigatran | VTE prevention after orthopaedic surgery, stroke prevention in non-valvular AF |

VTE: venous thromboembolism; AF: atrial fibrillation.

aPL-mediated thrombosis in mice by restoring the anticoagulant action of AnxA5 and reducing the binding of anti-$\beta_2$GPI antibodies to the phospholipid bilayer.[79,80] It is currently recommended to combine HCQ with LMWH when attempting to treat recurrent APS.[81] No consensus regarding the use of HCQ for primary thromboprophylaxis in APS patients is currently present. We are currently undertaking a clinical trial to study the efficacy of HCQ as primary thromboprophylaxis in asymptomatic aPL-positive patients, as part of the Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION).

### B cell directed therapies

B cells play a pivotal role in the pathogenesis of APS. Targeting B cells is believed to be beneficial in both treating APS manifestations and preventing the onset of thrombotic events. Rituximab (an anti-CD20 chimeric monoclonal antibody) has proven to be efficacious in treating refractory APS mainly when dealing with haematological manifestations such as persistent thrombocytopaenia and autoimmune haemolytic anaemia.[60] Certain reports also mentioned that rituximab is beneficial in treating diffuse alveolar haemorrhage, skin ulcers, and cognitive dysfunction.[82,83] Rituximab was also tested in CAPS patients where improvement was noticed in six out of seven cases.[84-86] Another promising agent is belimumab (BlyS-specific inhibitor) which acts by inhibiting B cell activating factor (BAFF). Belimumab is currently approved for the treatment of SLE patients.[87] It was tested on murine models with APS where it was able to prevent the onset of the disease and showed an increase in survival rates.[88] Current data on humans are lacking.

### Miscellaneous

Defibrotide, an adenosine receptor agonist, was shown to be successful when used to treat refractory CAPS.[89,90] It acts by blocking monocyte TF expression. Eculizumab (anti-C5) is another agent that demonstrated efficacy in improving the manifestations of APS and preventing aPL-induced thrombosis.[91] It is a humanised monoclonal antibody that acts by inhibiting the cleavage of C5a and C5b and thus, preventing the formation of MAC. It is the first therapy approved for the treatment of paroxysmal nocturnal haemoglobinuria (PNH). Abciximab and dilazep are two antiplatelet agents that exert their action by inhibiting GPIIb/IIIa receptor and blocking TF expression in endothelial cells and monocytes, respectively. Some data support that these agents are effective when used for secondary arterial thromboprophylaxis in APS patients.[92,93] While abatacept (CTLA4-Ig) is currently approved for the treatment of refractory rheumatoid arthritis, it is suggested that by selectively blocking the co-stimulation of T cells, it can prevent B cell activation and aPL production.[88] Thus, it is believed that abatacept may play a role in preventing disease onset although efficacy in APS patients is not yet reported.[94]

As APS is known to be associated with an increase in proinflammatory cytokines, TNF-$\alpha$ blockers were proven to be advantageous when used in patients with recurrent pregnancy loss but not in secondary APS cases (i.e. SLE-related).[95] Several proteins and intracellular pathways are involved in aPL-induced thrombotic mechanisms. By selectively inhibiting these pathways, one can reduce monocyte and endothelial cell activation as well as TF upregulation. Proteins that need to be blocked in order to address the underlying thrombotic state include p38 MAP kinase, NF$\kappa$B, and apolipoprotein E receptor cell-surface receptor among others.[96-98] Some of the previously mentioned new therapies were discussed briefly as studies assessing their efficacy in APS are still lacking.

## CONCLUSION

APS is a systemic autoimmune disease characterised mainly by thromboembolic events. It can affect multiple organs and tissues and may lead to a life-threatening form called CAPS. New molecular mechanisms of the disease are being revealed as research advances; this will open the door for exploring novel targeted therapies. Although immunomodulatory approach is gaining more importance in the treatment of APS patients, anticoagulation remains the mainstay of therapy. More studies addressing the use of immunomodulatory therapies in humans are needed as most of the data we currently have are extracted from experiments done on murine models.

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# A new lease of life from old cells

"This study offers hope [for] improved therapies in osteoarthritis through the replication and rejuvenation of stem cells."

*Prof Alan Silman, Arthritis Research UK, Chesterfield, UK*

REJUVENATING old stem cells in older people could be the answer to developing therapies which will be able to repair worn or damaged cartilages.

"This is pioneering research, which has the potential to help reduce pain and disability... improving quality of life of those with osteoarthritis," highlighted Prof Alan Silman, Medical Director, Arthritis Research UK, Chesterfield, Derbyshire, UK.

Around one-third of people aged 45 years and older suffer from osteoarthritis, a condition which causes both pain and stiffness in the joints as the cartilage at the end of the bones wears away.

As the current treatments for this condition are ineffective, this study is very promising in the eyes of both the researchers and the patients.

A valuable source of potential treatment is the patient's own bone marrow stem cells; these cells can generate joint tissue which will not be rejected when they are re-implanted into the patient. While this is beneficial, it is somewhat troublesome in an older population as the number of stem cells decreases as we age, and the ones which remain are increasingly less able to grow and repair tissue.

Throughout the 3-year study, the researchers, funded by the charity Arthritis Research UK, will first compare the effects of both rejuvenated and non-rejuvenated stem cells, assessing if rejuvenation really does improve cartilage repair.

Secondly, the researchers will investigate the potential to develop new drugs which will be able to rejuvenate stem cells.

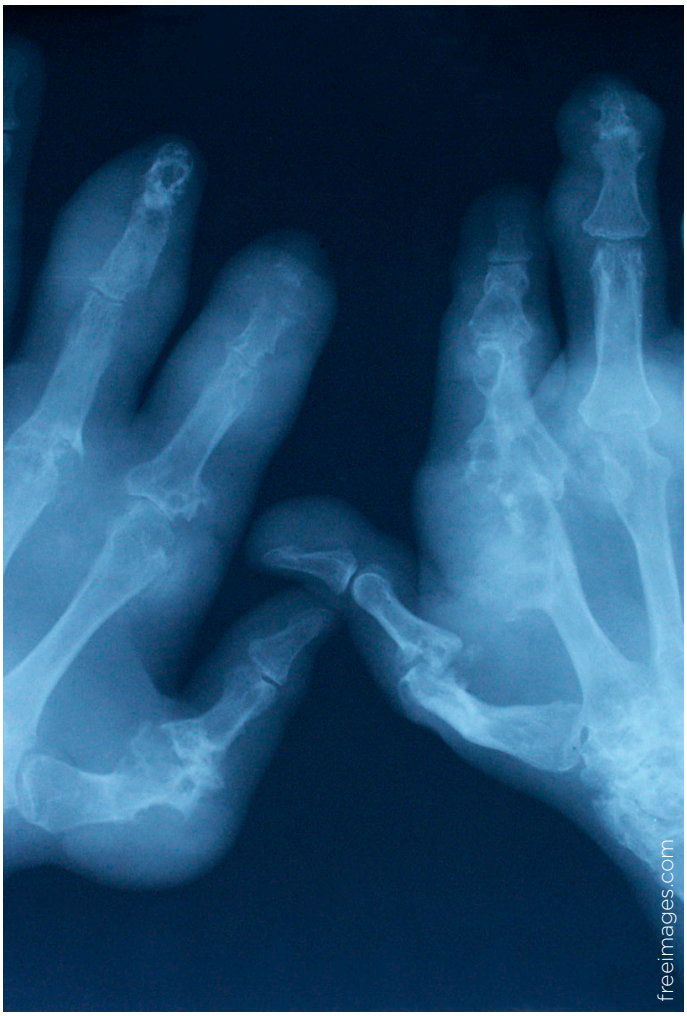
While an answer is not imminent, the researchers remain positive that this discovery will benefit those suffering from osteoarthritis. "This study offers hope [for] improved therapies in osteoarthritis through the replication and rejuvenation of stem cells," said Prof Silman.

# Genetics of secret subtype of rheumatoid arthritis revealed

PATHOGENS and other environmental factors have long been known as triggers of rheumatoid arthritis (RA) in some patients, but not all. Research now defines the genetic basis of another subtype: seronegative RA.

30% of RA sufferers do not test positive for auto-antibodies; they are known as seronegative patients. This subtype has been revealed to be a result of a genetic variant of the protein that causes the better known seropositive variety of RA. This difference might explain why triggers of RA, such as environmental factors or pathogens, can fail to initiate the disease in some patients.

RA is a complex disease that causes swelling, stiffness, and pain in over 400,000 Britons - the effect of their body's own immune cells attacking cells which line the joints in an autoimmune reaction. The ailment presents itself variably and has different outcomes for different sufferers; however, it most commonly occurs in the hands, wrists, and feet where it can leave patients largely disabled. Over time RA can result in further damage to the joint, cartilage, and surrounding bone.




"Now that we have established a genetic basis for these two types of RA, we hope it will lead to patients receiving a swifter, accurate diagnosis and more appropriate, targeted treatment. These findings have opened the door to a better understanding of seronegative RA."

*Prof Jane Worthington, University of Manchester, Manchester, UK*

At present, seronegative RA is widely misdiagnosed; though there is still no cure, this research has implications for leading the way to more acute clinical tests for RA. It also has implications for research into how the body's immune system fights infection.

Prof Jane Worthington, Centre Director, Arthritis Research UK Centre for Genetics and Genomics, University of Manchester, Manchester, UK, commented: "Now that we have established a genetic basis for these two types of RA, we hope it will lead to patients receiving a swifter, accurate diagnosis and more appropriate, targeted treatment. These findings have opened the door to a better understanding of seronegative RA."

# Genes linked to autoimmune terror

"The ultimate goal is to use this knowledge to find new ways to diagnose the disease and develop targeted medicines to treat it."

*Dr Kathy Sivils, University of Oklahoma HSC, Oklahoma City, USA*

GENES linked to the autoimmune disease Sjögren's syndrome have been unveiled following an analysis of the genomes of thousands of people.

Sjögren's syndrome, for which there is currently no cure, is a highly prevalent autoimmune disorder where the immune system attacks and damages the glands that produce tears and saliva, producing symptoms such as dry, itchy eyes, dry mouth with difficulty swallowing and speaking, blurry vision, and a susceptibility to tooth decay.

The causes of Sjögren's syndrome are currently unknown, but it has been proposed that it arises as a result of a complex combination of genetic and environmental factors.

To gain insight into the syndrome's genetic underpinnings, Dr Kathy Sivils, Adjunct Associate Professor, Department of Pathology, University of Oklahoma Health Sciences Center (HSC), Oklahoma City, Oklahoma, USA, set out to identify genes associated with the syndrome.

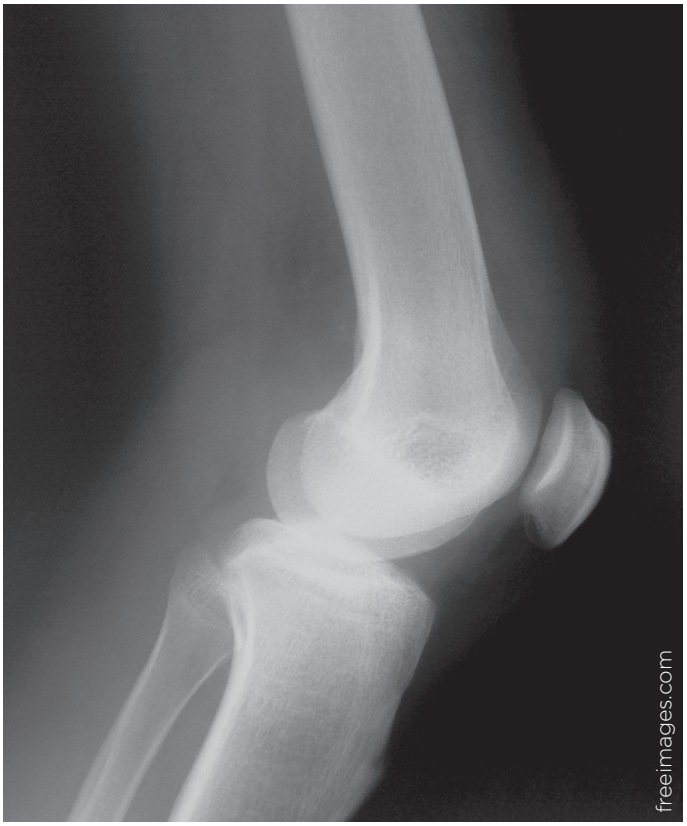
Dr Sivils and colleagues accumulated 2,000 Sjögren's patient DNA samples and compared them to 7,000 DNA samples from healthy volunteers. This allowed the researchers to identify six genes associated with the condition.

The genes that were identified were IRF5 and STAT4 (gene activators that stimulate the immune system), CXCR5 (a cell-surface protein that helps guide B cells to lymph nodes), TNIP1 (binding partner with TNFAIP3 that plays a role in limiting inflammation), IL12A (part of a protein that activates T cells and natural killer cells), and BLK (activates B cells).

Interestingly, some of these genes cause other disorders, such as IRF5 and IL12A being linked with biliary cirrhosis, suggesting a broader role for these genes.

"The identification of these genetic susceptibility factors opens up new avenues for understanding how the immune system goes awry in Sjögren's syndrome," said Dr Sivils. "The ultimate goal is to use this knowledge to find new ways to diagnose the disease and develop targeted medicines to treat it."

# Hope on the horizon for osteoarthritis patients




EFFECTIVE new treatments are on the horizon for osteoarthritis patients; a new gene-sequencing technique, RNA-seq, could be the solution to the problem.

"Most research in recent times has approached osteoarthritis as one disease that will respond to one treatment, and as a result there has been little progress in developing new treatments. We're proposing a new approach to tackle its complexity by identifying different patient groups that will provide targets for developing new treatments," said Prof Ray Boot-Handford, Faculty of Life Sciences, The University of Manchester, Manchester, UK.

For the more than 8 million sufferers of osteoarthritis in the UK, there are very few effective treatment options other than painkillers and joint replacement. Osteoarthritis – a complicated disease - is a condition which affects the joints; it is often used as an umbrella term to describe a disease which affects the cartilage in many ways.

"Not everyone with osteoarthritis has exactly the same pattern of disease, and because not all people's disease is the same it makes sense that not everyone would respond to the same treatment," Prof Boot-Handford explained.

Currently, there are no diagnostic tests which can identify subsets of osteoarthritis patients to help guide treatment. It is the aim of this group therefore to identify patients - and the gene patterns which are active in their joints - who may respond well to treatment.

The RNA-seq will enable the team to analyse gene activity and cell functions in the cartilage of people with osteoarthritis and compare these to a normal cartilage. It is hoped that the distinctive process which drives cartilage damage in different patients can be discovered, which will help guide treatment in the future.

"We're proposing a new approach to tackle its complexity by identifying different patient groups that will provide targets for developing new treatments."

*Prof Ray Boot-Handford,*
*The University of Manchester,*
*Manchester, UK*

# Parental addiction breeds arthritis in kids

CHILDREN growing up with a parent who is addicted to drugs and/or alcohol are more likely to develop arthritis in adulthood.

Those with a history of parental addiction have been shown to have a 30% higher chance of suffering from arthritis, a debilitating condition that results in the inflammation of joints within the body, following the results of a 13,000-adult study.

"We believe that early adversities may change the way the child reacts to stress throughout their lives," said Dr Esme Fuller-Thomson, Factor-Inwentash Faculty of Social Work, University of Toronto, Toronto, Canada.

"Children growing up with addicted parents are often in very difficult home environments. Chronic stress in childhood can create long-term dysfunctions in the hypothalamus-pituitary-adrenal [HPA] axis, which is a key part of the body's stress response system. In turn, abnormal HPA axis functioning has been associated with arthritic symptoms," Dr Fuller-Thomson added.

This provided the foundation and motivation to pursue an observational investigation involving over 13,000 adults with the aim of examining the correlation between parental addiction and arthritis in offspring.

Interestingly, the association between parental addictions and arthritis was shown to be substantially reduced when adverse childhood experiences and all four groups of risk factors (demographics, socioeconomic status, adult health behaviours, and mental health conditions) were included, although researchers maintained that the relationship remained statistically significant.

"Chronic stress in childhood can create long-term dysfunctions in the hypothalamus-pituitary-adrenal [HPA] axis, which is a key part of the body's stress response system."

*Dr Esme Fuller-Thomson, University of Toronto, Toronto, Canada*

Importantly, the researchers caution the fact that the data derives from a survey, making it impossible to determine whether the relationship between parental addictions and arthritis is causal.

"That said, the relationships between childhood psychosocial adversities and risks of chronic disease onset in adulthood deserve increased attention in prospective and records-based retrospective risk factor studies for conditions such as diabetes, heart disease, arthritis, and for chronic pain in general," said Dr Michael Von Korff, Group Health Research Institute, Seattle, Washington, USA.

# Fc receptor holding potential for lupus screening

> "This new finding could play a significant role in the way companies design treatments for autoimmune diseases, in a more targeted approach."
>
> *Dr Robert Kimberly, University of Alabama at Birmingham, Birmingham, USA*

PERSONALISED therapies for patients with autoimmune diseases, such as lupus, are on the horizon; the key being an immune protein, an Fc receptor.

"This new finding could play a significant role in the way companies design treatments for autoimmune diseases, in a more targeted approach.

"Now efforts can be made to target the individuals who will benefit from the treatments, based on gene mutation," said Dr Robert Kimberly, Director, Center for Clinical and Translational Science, University of Alabama at Birmingham, Birmingham, Alabama, USA.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Rather than just eliminating germs in the body, in diseases such as lupus, which can affect lots of different parts of the body, the immune system overreacts and attacks the healthy tissue.

Effective treatments must be based on genes which 'fine-tune' an individual's immune system; one-third of patients do not respond to traditional antibody-based treatments.

Newer therapies aim to decrease the activity of B cells, cells that create antibodies, but these treatments may not be as effective in patients with the Fc receptor variant.

The Fc receptor regulates the assembly of antibodies which attack bacteria in our bodies. It was thought that Fc receptors shut down antibody production, but as 15% of the world's population have the protein it may also activate antibody production. In lupus patients, the Fc receptor would create too many antibodies resulting in an attack on healthy cells.

Identifying this protein in patients offers early warning signs of the disease, and may also allow doctors to tailor specific treatments to patients, saving time and money.

Dr Kimberly suggested: "Future research into the impact of expression of this Fc receptor in B cells on antibody production in both health and disease will likely lead to advances in our understanding of both autoimmunity and natural responses to infectious disease challenges."

# More support needed for arthritis patients

"There is clearly a huge unmet need for people with inflammatory arthritis, and more psychological support would improve the quality of their lives."

*Dr Chris Deighton, President of the British Society for Rheumatology*

AN OVERWHELMING number of patients have emphasised that they want help and support to deal with the pain caused by inflammatory arthritis.

The guidelines from the British Society for Rheumatology (BSR) on the management of rheumatoid arthritis (RA) state: "Effective care, treatment, and support should be available for all patients with RA. Psychological and social support is an important aspect of management. Real and potential psychological distress should be addressed through the multidisciplinary team and appropriate agencies."

Researchers surveyed 1,200 people discussing currently-available support and the type of services they would like in the future.

Only 23% of patients had been asked about social and emotional issues by a rheumatology professional. 97% of patients wanted psychological support from diagnosis onwards, and 96% of these patients said that they would use psychological support services. Moreover, only 6% of patients felt that social and emotional issues were irrelevant to their condition.

"It can be physically and emotionally distressing for patients who develop an inflammatory arthritis. There is clearly a huge unmet need for people with inflammatory arthritis, and more psychological support would improve the quality of their lives. All rheumatology services should provide access to professional psychological support," said President of the BSR, Dr Chris Deighton.

The patients suggested that they would benefit from face-to-face support, self-management or coping clinics, peer support groups, pain management, and patient education.

"Patients have told us that psychological and emotional support is important and they would like help from rheumatology specialists to cope with the impact of living with arthritis. Therefore our research will now focus on supporting teams to acquire the skills and resources necessary to provide psychological and emotional support to improve quality of life for patients," said lead researcher Dr Emma Dures, senior research fellow, University of the West of England, Bristol, UK.

# Featured Suppliers

## Rheumatology

Pursuing a goal of delivering ground-breaking, life-altering therapies to patients in over 50 countries worldwide, Celgene seeks to establish itself as a major player in the global pharmaceuticals industry. A plethora of immune-inflammatory related conditions, including cancer, constitute the targets set by Celgene for the finding, development, and marketing of patented products. These are tested rigorously at key medical centres in over 300 clinical trials. Multiple myeloma, myelodysplastic syndromes, and chronic lymphocytic leukaemia are among the incurable haematological and solid tumour cancers currently being targeted with the development of investigational treatments.

Institut Biochimique SA (IBSA) is a privately owned pharmaceuticals company based in Lugano, Switzerland, marketing its products in over 70 countries, including the USA. IBSA has developed a series of proprietary technologies for improvement of already-available and widely-used molecules and therapeutic solutions. IBSA has become established on a global scale through partnerships and local branches in Italy, France, Hungary, Slovak Republic, Poland, Turkey, and China. Their successes are due to an in-depth competence in basic research, pre-clinical and clinical development, high manufacturing quality, regulatory expertise, and direct marketing of proprietary products.

Sandoz is an established worldwide leader in the field of generics. A subdivision of Novartis, Sandoz currently employs more than 26,500 globally, with its products accessible to 90% of the world's population, spanning 160 countries. This astonishing success is built on the company's mission to supply high-quality, affordable medicines to the world, having secured a web of patients, doctors, healthcare providers, and business partners. With a 1,100-stong portfolio ranging from oral solids, gels, and patch technologies, to complex injectables, inhalers, and state-of-the-art biosimilars, Sandoz continues to pioneer and master affordable, high-quality generics of all complexities.

Dedicated to providing a range of medicines, vaccines, and innovative therapeutic solutions to patients worldwide, Sanofi is forever pursuing the goal of providing healthcare and hope to everyone on Earth. The company, which employs 45,000 across 41 countries, focuses its time and resources on the strategic growth platforms of: diabetes, vaccines, consumer healthcare, rare diseases and multiple sclerosis, other innovative products, animal health, and emerging markets. Central to ambitions is the research and development branch of Sanofi, fuelled by funds from continued growth, which is built on improved global access to quality healthcare.

Based in Geneva, Switzerland, the TRB Chemedica Group is a medium-sized pharmaceutical firm that discovers, develops, and markets patented, innovative medical solutions to patients in over 60 countries worldwide. Founded in 1980, TRB produces innovative therapies for a range of key therapeutic areas including rheumatology, ophthalmology, and neurology. Still regarded as a developing company, TRB currently employs over 700 people globally and has numerous bases across regions such as Europe, South America, and the Far East, with plans for further global expansion, through initiatives including increasing in-house manufacturing capacity, well underway.

# Buyer's Guide

## Exhibitors

- ABBVIE
- ACTELION PHARMACEUTICALS LTD.
- AMGEN INC.
- ANALOGIC ULTRASOUND
- ASTRAZENECA
- ATCC-RA RESEARCH GROUP
- BE THE CURE
- BIOIBERICA, S.A.
- BIOMARIN PHARMACEUTICAL INC.
- BLUE BIO PHARMACEUTICALS LTD.
- BRISTOL-MYERS SQUIBB
- CELGENE CO., LTD.
- CELLTRION HEALTHCARE CO., LTD.
- CRESCENDO BIOSCIENCE, INC.
- CROMA-PHARMA GMBH
- ESAOTE
- FIDIA FARMACEUTICI S.P.A.
- GE HEALTHCARE LTD.
- GLAXOSMITHKLINE
- GUNA S.P.A. ITALY
- HEMICS B.V.
- HOLOGIC INC.
- HOSPIRA INC.
- IBSA INSTITUT BIOCHIMIQUE S.A.
- IMAGE ANALYSIS LTD.
- JANSSEN PHARMACEUTICA N.V.
- LABORATOIRE PIERRE FABRE SANTE
- LABORATOIRES EXPANSCIENCE
- LCA PHARMACEUTICAL
- LILLY
- LUPUS EUROPE
- MDT INTERNATIONAL S.A.
- MEDAC GMBH
- MEDI GMBH & CO. KG
- MENARINI
- MERCK SERONO KGaA
- METEOR
- MIVENION GMBH
- MSD
- NOVARTIS PHARMACEUTICAL LTD.
- PFIZER ITALY S.R.L.
- REGENERON PHARMACEUTICALS, INC.
- ROCHE
- SANDOZ
- SANOFI
- SWEDISH ORPHAN BIOVITRUM A.B.
- TRB CHEMEDICA INTERNATIONAL S.A.
- UCB PHARMA S.A.

## 33rd Annual Meeting of the European Bone and Joint Infection Society

*11th-13th September 2014*

*Utrecht, the Netherlands*

This annual meeting will host a number of scientific presentations which will focus on post-traumatic infections, and also therapy in prosthetic infections. The overall goal of the meeting is to promote prevention and treatment of these diseases. All of those with an interest in infections in the musculoskeletal system - including orthopaedic surgeons, trauma surgeons, infectious disease specialists, and microbiologists - will be attending.

## Comorbidities in Rheumatology

*15th September 2014*

*London, United Kingdom*

Co-badged with the British Society of Rheumatology, this event will explore comorbidities in rheumatic diseases and their consequences. It will also cover how comorbidities can affect disease progression, and how comorbidities can influence the choice of treatment and its efficacy. The event will aim to offer participants updates and an extensive amount of knowledge within the field, with a cutting-edge clinical education programme.

## 21st European Paediatric Rheumatology Congress (PReS) 2014

*17th-21st September 2014*

*Belgrade, Serbia*

This Congress will aim to present the latest clinical developments and cutting-edge innovations in the treatment of rheumatic disease in children. Leading experts and scientists from all over the world will attend, with the event focussing in particular on juvenile idiopathic arthritis. The meeting will provide a forum for attendees to share new developments, foster academic collaborations, and provide continued education in this developing field.

## American College of Rheumatology (ACR) 2014

*14th-19th November 2014*

*Boston, USA*

This annual meeting is seen as the premier scientific meeting devoted to the study of rheumatic diseases. The mission of the ACR is to advance rheumatology and the health of people with rheumatic diseases. This is done through training programmes, education, research, and practice support. All of this is provided to thousands of rheumatologists, rheumatology healthcare professionals, and non-physician healthcare professionals.

## 3rd International Congress on Controversies in Rheumatology and Autoimmunity

*12th-14th March 2015*

*Sorrento, Italy*

This 2-day Congress will present the latest cutting-edge research and analysis concerning rheumatology, autoimmunity, and clinical immunology. As well as acting as a networking venue for all healthcare professionals, there will be also be a number of debates concerning some of the more controversial topics within this field. These will include treatment options for rheumatoid arthritis and systemic lupus erythematosus.

## World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases 2015

*26th-29th March 2015*

*Milan, Italy*

This event aims to be the most informative Congress in the field of rheumatology, with over 4,000 participants from over 100 countries attending. The sessions will focus on treatment, challenges, solutions, and physical rehabilitation, as well as assessing these conditions within other disciplines. There will also be a number of company-sponsored symposia.

## The British Society for Rheumatology: Rheumatology 2015

*28th April–1st May 2015*

*Manchester, United Kingdom*

Throughout this multidisciplinary event there will be a number of innovative sessions focusing on key areas within rheumatology. The sessions, delivered by renowned experts, are all designed to engage the audience through lectures, workshops, and networking seminars. Participants will be able to learn about new technologies and advancements within the field, as well as being able to identify and share best practice.

## 16th European Federation of National Associations of Orthopaedics and Traumatology (EFORT) Congress

*27th-30th May 2015*

*Prague, Czech Republic*

This Congress has a strong history in both education and research in the field of trauma and musculoskeletal surgery. It will provide a programme covering a range of topics, from basic research to areas within daily practice, in order to improve the knowledge of healthcare professions and therefore the quality of life for patients. Sessions at the event will include symposia, instructional lectures, complex case discussions, and debate forums.