EMJ

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+ REPRODUCTIVE HEALTH

The IVF Shopping List: To Tick or Not to Tick

+ HEPATOLOGY

Cholestasis in the Baby and Infant

+ RESPIRATORY

Exercise and Rhinitis in Athletes

THE EUROPEAN MEDICAL JOURNAL

+ EDITOR'S PICK

Systemic Sclerosis: The Role of YAP/TAZ in Disease Pathogenesis

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Monday 30 September

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At the Orion/Menarini co-sponsored evening symposium, we'll focus on the patient – how clinicians can improve clinical outcomes by understanding, and then acting on, their needs and preferences.

Agenda

Co-Chairs: Prof. Eric Bateman & Prof. Piotr Kuna

17:30–17:35 • Prof. Eric Bateman, Cape Town, South Africa
Setting the scene

17:35–17:50 • Prof. José Luis Izquierdo, Guadalajara, Spain
What really matters to patients with asthma and COPD

17:50–18:10 • Prof. Federico Lavorini, Florence, Italy
Harnessing inhaler technology

18:10–18:30 • Dr Mark Levy, London, United Kingdom
Patient factors in successful asthma and COPD therapy

18:30–18:45 • Prof. Eric Bateman, Cape Town, South Africa
Empowering patients with MART

18:45–19:00 • Prof. Piotr Kuna, Lodz, Poland
Panel discussion: the impact of patient preference and inhaler satisfaction on clinical outcomes

Webcast recording will be available to view after the symposium on the website wehale.life/events/ers2019

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EMJ 4.3

European Medical Journal 4.2 2019

By including articles from across the therapeutic spectrum, we hope to create fertile ground for the flowering of fresh ideas that will advance scientific knowledge and patient care.

VIEW JOURNAL ←

Contents

"In the following pages, we present a range of articles in therapeutic areas such as rheumatology, hepatology, dermatology, allergy, and more."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

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EMJ is supported by various levels of expertise:

- Guidance from an Editorial Board consisting of leading authorities from a wide variety of disciplines.
- Invited contributors are recognised authorities from their respective fields.
- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
- An experienced team of editors and technical editors.

Peer Review

On submission, all articles are assessed by the editorial team to determine their suitability for the journal and appropriateness for peer review.

Editorial staff, following consultation with either a member of the Editorial Board or the author(s) if necessary, identify three appropriate reviewers, who are selected based on their specialist knowledge in the relevant area.

All peer review is double blind.

Following review, papers are either accepted without modification, returned to the author(s) to incorporate required changes, or rejected.

Editorial staff have final discretion over any proposed amendments.

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We welcome contributions from professionals, consultants, academics, and industry leaders on relevant and topical subjects.

We seek papers with the most current, interesting, and relevant information in each therapeutic area and accept original research, review articles, case reports, and features.

We are always keen to hear from healthcare professionals wishing to discuss potential submissions, please email: editorial.assistant@emjreviews.com

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1. Fulkerson P, *et al. Nat Rev Drug Discov.* 2013;12(2):1–23. **2.** Caruso M, *et al. Curr Opin Allergy Clin Immunol.* 2013;13(6):677–85. **3.** Hammad H, *et al. Nat Rev Immunol.* 2008;8:193–204.

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SANOFI GENZYME

Welcome

Valued readers, I proudly welcome you to the newest edition of our multidisciplinary flagship journal. The increasing number of interdisciplinary conferences and disciplines today showcases the importance of bringing together expertise from diverse disciplines to solve the current global challenges in healthcare. Consequently, here at EMJ we strive to provide an assortment of articles from various therapeutic areas to facilitate our readers to achieve a broader view on the current trends in research, ultimately sparking creativity and leading to high-impact research. With this in mind, no matter your field of expertise, *EMJ 4.3* will prove a stimulating read.

In the following pages, we present a range of articles in therapeutic areas such as rheumatology, hepatology, dermatology, allergy, and more. In the field of reproductive health, *EMJ 4.3* includes two fascinating contributions: Tesarik provides a review of the causes, consequences, and potential solutions of genetic and epigenetic modifications in spermatozoa, which can negatively affect embryonic development and be the root of various health problems for the potential offspring. Further in the field of reproductive health, the feature by Alteri et al. describes the application of the Provoost model to the 12 *in vitro* fertilisation add-ons reported by the Human Fertilisation and Embryology Authority (HFEA), including key techniques such as assisted hatching, physiological intracytoplasmic sperm injection, and elective freeze all cycle.

For those interested in hepatology, to help the prevention of cholestasis-related chronic liver dysfunction and resultant liver transplantation, Gunaydin and Bozkurter Cil examine the aetiology, diagnosis, and treatment of cholestasis in babies and infants. Furthermore, Sundaram and Jearth provide a paper on a related topic in which they analysed primary sclerosing cholangitis, a rare hepatic cholestatic disorder, and emphasised the risk of malignancies and the management of the disease.

Complementary to these papers, we have our fantastic what's new stories that include the newest developments in haematology, nephrology, and urology. Written by our talented editorial team, these stories cover topics such as detection of anaemia in pregnancy, fluoride and its impact on kidney and liver health, and the use of gold nanoparticles for the treatment of prostate cancer. These will surely enlighten anyone wanting to broaden their knowledge and edge forward in the interdisciplinary field.

Finally, I would like to extend my appreciation to everyone who contributed to *EMJ 4.3*: peer-reviewers, authors, the editorial board, and the diligent EMJ team. I hope everyone enjoys the engaging content that lies ahead because, as they say, today a reader, tomorrow a leader!

Spencer Gore

Chief Executive Officer, European Medical Group

THE ART OF RESPIRATORY DIAGNOSTICS

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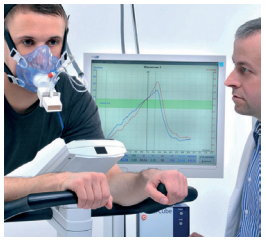

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Foreword

Dear colleagues,

It is with great pleasure that I welcome you to *EMJ 4.3.* This journal contains a host of articles presenting new and exciting topics in the world of scientific research today. I would like to take the opportunity to introduce some of the outstanding reads included in this publication.

Albhaisi reviews the potential of noninvasive procedures in direct comparison with invasive biopsies conventionally carried out in patients with nonalcoholic fatty liver disease. Invasive techniques are costly and may be associated with morbidity and even mortality. It is imperative to explore the possibilities in this field which may replace biopsy and offer more suitable screening modalities for those at risk. The discussion surrounding hepatology continues as Sundaram et al. provide a comprehensive insight into the rare cholestatic disorder, primary sclerosing cholangitis. The review addresses the risk of malignancies associated with the disorder and its management.

Genetic and epigenetic modifications to the human DNA sequence prevail throughout life and remain a fundamental therapeutic area of immense interest. The difference between these modifications in DNA of human spermatozoa and their effects on embryonic development, the incidence of miscarriage, and health of future offspring are unravelled in a paper by Tesarik who dissects the causes and consequences of each.

Food avoidance and emergency medication are currently used to manage food allergies. In addition to increased global prevalence, the severe and life-threatening nature of some allergies directs research towards specific allergen immunotherapy (AIT). Chiera et al. focus on the administration of AIT and the importance of long-term post-desensitisation to reduce the occurrence of allergic reactions.

My Editor's pick is by Walsh, who considers the significant role of the transcriptional coactivator Yes-Associated Protein/Tafazzin (YAP/TAZ) in the pathogenesis of the autoimmune condition, systemic sclerosis. The author compares this to the role of YAP/TAZ in the condition nephrogenic systemic fibrosis when investigating the role of YAP/TAZ cytoplasmic sequestration in scleroderma.

Other topics covered in this publication include the investigation of rhinitis during exercise and the effect on athletes during sports performance and an interesting case report on the diagnosis of scalp sarcoidosis, amongst many more.

Proving to be an enthralling read from beginning to end, this edition of *EMJ 4.3* is wide in its scope and encourages discussion across multiple disciplines.

Professor Mátyás Benyó MD PhD

Fellow of the European Board of Urology; European Academy of Andrology – clinical andrologist; assistant professor, Department of Urology, University of Debrecen, Debrecen, Hungary; senior consultant, National Public Health Center; member of the executive committee of Hungarian Association of Andrology; member of the audit committee of Hungarian Urology Society.

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ACTIVE INGREDIENT: apalutamide. Please refer to Summary of Product Characteristics (SmPC) before prescribing. **INDICATION:** ERLEADA is indicated in adult men for the treatment of non-metastatic castration-resistant prostate cancer (NM-CRPC) who are at high risk of developing metastatic disease. **DOSAGE & ADMINISTRATION:** Treatment with apalutamide should be initiated and supervised by specialist physicians experienced in the medical treatment of prostate cancer.ERLEADA is for oral use. The tablets should be swallowed whole and can be taken with or without food. **Adults:** The recommended dose is 240 mg (four 60 mg tablets) as an oral single daily dose. Medical castration with gonadotropin releasing hormone analogue (GnRHa) should be continued during treatment in patients not surgically castrated. If a dose is missed, it should be taken as soon as possible on the same day with a return to the normal schedule the following day. 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If treatment is started, patients should be monitored for the adverse reactions and dose reduced as per section Dosage and administration. **Hepatic impairment:** No dose adjustment is necessary for patients with baseline mild or moderate hepatic impairment (Child-Pugh Class A and B, respectively). ERLEADA is not recommended in patients with severe hepatic impairment. **CONTRAINDICATIONS:** Pregnant women or women with potential to be pregnant. Hypersensitivity to the active substance or to any of the excipients. **SPECIAL WARNINGS & PRECAUTIONS:** ***Seizure***: ERLEADA is not recommended in patients with a history of seizures or other predisposing factors including, but not limited to underlying brain injury, recent stroke (within one year), primary brain tumours or brain metastases. Treatment should be discontinued permanently, if a seizure develops during treatment. The risk of seizure may increase with concomitant medication that lowers the seizure threshold. ***Falls & Fractures***: Before initiating treatment with ERLEADA, patients should be evaluated for fractures and fall risk and should be monitored and managed continuously for fractures and use of bone-targeted agents should be considered. ***Concomitant use with other medicinal products:*** A review of concomitant medicinal products should be conducted when apalutamide treatment is initiated. Concomitant use of apalutamide with medicinal products that are sensitive substrates of many metabolising enzymes or transporters should generally be avoided if their therapeutic effect is of large importance to the patient, and if dose adjustments cannot easily be performed based on monitoring of efficacy or plasma concentrations. Co-administration with warfarin and coumarin-like anticoagulants should be avoided, if not avoided International Normalised Ratio (INR) monitoring should be conducted. ***Recent cardiovascular disease:*** If ERLEADA is prescribed, patients with clinically significant cardiovascular disease should be monitored for risk factors such as hypercholesterolaemia, hypertriglyceridaemia, or other cardio-metabolic disorders. Patients should be treated, if appropriate after initiating ERLEADA for these conditions according to established treatment guidelines. ***Androgen deprivation therapy may prolong the QT interval:*** In patients with a history of or risk factors for QT prolongation and in patients receiving concomitant medicinal products that might prolong the QT interval, physicians should assess the benefit-risk ratio including the potential for Torsade de pointes prior to initiating ERLEADA. ***Effects on ability to drive and use machines:*** ERLEADA has no or negligible influence on the ability to drive and use machines. Patients on medication with ERLEADA should be advised about risk of seizures with regard to driving or operating machines. **SIDE EFFECTS:** ***Very Common***: skin rash (Skin rash associated with ERLEADA was most commonly described as macular or maculo-papular. Skin rash included rash, rash maculo-papular, rash generalised, urticaria, rash pruritic, rash macular, conjunctivitis, erythema multiforme, rash papular, skin exfoliation, genital rash, rash erythematous, stomatitis, drug eruption, mouth ulceration, rash pustular, blister, papule, pemphigoid, skin erosion, and rash vesicular), fracture (includes rib fracture, lumbar vertebral fracture, spinal compression fracture, spinal fracture, foot fracture, hip fracture, humerus fracture, thoracic vertebral fracture, upper limb fracture, fractured sacrum, hand fracture, pubis fracture, acetabulum fracture, ankle fracture, compression fracture, costal cartilage fracture, facial bones fracture, lower limb fracture, osteoporotic fracture, wrist fracture, avulsion fracture, fibula fracture, fractured coccyx, pelvic fracture, radius fracture, sternal fracture, stress fracture, traumatic fracture, cervical vertebral fracture, femoral neck fracture, tibia fracture), arthralgia, fatigue, weight decreased, fall. ***Common***: hypothyroidism (includes hypothyroidism, blood thyroid stimulating hormone increased, thyroxine decreased, autoimmune thyroiditis, thyroxine free decreased, tri-iodothyronine decreased), hypercholesterolaemia, hypertriglyceridaemia, pruritus. ***Uncommon***: seizure. ***Not known***: QT prolongation. **Refer to the SmPC for other side effects. FERTILITY/PREGNANCY/ LACTATION:** ERLEADA

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ADT=androgen deprivation therapy; nmCRPC=non-metastatic castration-resistant prostate cancer; PSA=prostate-specific antigen.
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is contraindicated in women who are or may become pregnant. ERLEADA may cause foetal harm when administered during pregnancy. There are no data available from the use of ERLEADA in pregnant women. Animal reproductive studies have not been conducted with ERLEADA. It is unknown whether apalutamide/metabolites are excreted in human milk. ERLEADA should not be used during breast-feeding. Based on animal studies, ERLEADA may decrease fertility in males of reproductive potential. It is not known whether apalutamide or its metabolites are present in semen. For patients having sex with female partners of reproductive potential, a condom should be used along with another highly effective contraceptive method during treatment and for 3 months after the last dose of ERLEADA. **INTERACTIONS:** The elimination of apalutamide and formation of its active metabolite, N desmethyl apalutamide, is mediated by both CYP2C8 and CYP3A4. **Potential for other medicinal products to affect apalutamide exposures:** ***Medicinal products that inhibit CYP2C8:*** No initial dose adjustment is necessary when ERLEADA is co-administered with a strong inhibitor of CYP2C8 (e.g., gemfibrozil, clopidogrel) however, a reduction of the ERLEADA dose based on tolerability should be considered. Mild or moderate inhibitors of CYP2C8 are not expected to affect the exposure of apalutamide. ***Medicinal products that inhibit CYP3A4:*** No initial dose adjustment is necessary when ERLEADA is co-administered with a strong inhibitor of CYP3A4 (e.g., itraconazole, ketoconazole, ritonavir, clarithromycin) however, a reduction of the ERLEADA dose based on tolerability should be considered. Mild or moderate inhibitors of CYP3A4 are not expected to affect the exposure of apalutamide. ***Medicinal products that induce CYP3A4 or CYP2C8:*** No dose adjustment is necessary when ERLEADA is co-administered with inducers of CYP3A4 or CYP2C8. **Potential for apalutamide to affect exposures to other medicinal products:** Apalutamide is a potent enzyme inducer and increases the synthesis of many enzymes and transporters; therefore, interaction with many common medicinal products that are substrates of enzymes or transporters is expected. The reduction in plasma concentrations can be substantial, and lead to lost or reduced clinical effect. There is also a risk of increased formation of active metabolites. ***Drug metabolising enzymes:*** *In vitro* studies showed that apalutamide and N desmethyl apalutamide are moderate to strong CYP3A4 and CYP2B6 inducers, are moderate inhibitors of CYP2B6 and CYP2C8, and weak inhibitors of CYP2C9, CYP2C19, and CYP3A4. When substrates of CYP2B6 (e.g., efavirenz) are administered with ERLEADA, monitoring for an adverse reaction and evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. In humans, ERLEADA is a strong inducer of CYP3A4 and CYP2C19, and a weak inducer of CYP2C9. Concomitant use of ERLEADA with medicinal products that are primarily metabolised by CYP3A4 (e.g., darunavir, felodipine, midazolam, simvastatin), CYP2C19 (e.g., diazepam, omeprazole), or CYP2C9 (e.g., warfarin, phenytoin) can result in lower exposure to these medicinal products. Substitution for these medicinal products is recommended when possible or evaluation for loss of efficacy should be performed if the medicinal product is continued. If given with warfarin, INR should be monitored during ERLEADA treatment. When substrates of UDP glucuronosyl transferase (e.g., levothyroxine, valproic acid) are co-administered with ERLEADA, evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. ***Drug transporters:*** Apalutamide was shown to be a weak inducer of P glycoprotein (P gp), breast cancer resistance protein (BCRP), and organic anion transporting polypeptide 1B1 (OATP1B1) clinically. When substrates of P-gp (e.g., fexofenadine, colchicine, dabigatran etexilate, digoxin), BCRP/OATP1B1 (e.g., lapatinib, methotrexate, rosuvastatin, repaglinide) are co-administered with ERLEADA, evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. Based on *in vitro* data, inhibition of organic cation transporter 2 (OCT2), organic anion transporter 3 (OAT3) and multidrug and toxin extrusions (MATEs) by apalutamide and its N-desmethyl metabolite cannot be excluded. No *in vitro* inhibition of organic anion transporter 1 (OAT1) was observed. **Medicinal products which prolong the QT interval:** Since androgen deprivation treatment may prolong the QT interval, the concomitant use of ERLEADA with medicinal products known to prolong the QT interval or medicinal products able to induce Torsade de pointes such as class IA (e.g., quinidine, disopyramide) or classIII (e.g., amiodarone, sotalol, dofetilide, ibutilide) antiarrhythmic medicinal products, methadone, moxifloxacin, antipsychotics (e.g. haloperidol), etc. should be carefully evaluated. **Paediatric population:** Interaction studies have only been performed in adults. **LEGAL CLASSIFICATION:** Medicinal product subject to medical prescription. **MARKETING AUTHORISATION NUMBER(S):** EU/1/18/1342/001, EU/1/18/1342/002, EU/1/18/1342/003. **MARKETING AUTHORISATION HOLDER:** Janssen-Cilag International NV. **PACKS & PRICE:** *Country specific.* Products mentioned in this document may not be registered in all countries. Prescribing Information may vary per country. Health Care Providers must refer to their country prescribing information. Prescribing information generation date or last revised: April 2019. Based on 14 January 2019 EU Summary of Product Characteristics.

References: 1. Smith MR, *et al. N Engl J Med.* 2018;378:1408–18. **2.** ERLEADA® (apalutamide) summary of product characteristics. Janssen-Cilag International NV, Beerse, Belgium, March 2019.

Erleada® (apalutamide) tablets

Date of preparation: June 2019 CP-95128

The IVF Shopping List: To Tick or Not to Tick

Authors: *Alessandra Alteri,[1] Valerio Pisaturo,[2] Kelly Tilleman,[3] Arianna D'Angelo[4]

1. Obstetrics and Gynecology Department, IRCCS San Raffaele Scientific Institute, Milan, Italy
2. Reproductive Medicine Department, International Evangelical Hospital, Genoa, Italy
3. Department of Reproductive Medicine, Ghent Fertility and Stem Cell Team (G-Fast), Ghent University Hospital, Ghent, Belgium
4. Wales Fertility Institute, University Hospital of Wales, Cardiff University, Cardiff, UK

*Correspondence to alteri.alessandra@hsr.it

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Keywords: Add-ons, assisted hatching (AH), calcium ionophore, embryo glue, freeze all cycle, genetic testing, *in vitro* fertilisation (IVF), intracytoplasmic morphologic sperm injection (IMSI), intracytoplasmic sperm injection (ICSI), intrauterine culture.

Citation: EMJ. 2019;4[3]:14-21.

INTRODUCTION

The field of reproductive medicine is recognised for its rapid innovations and advanced technologies, but also for its connection with lucrative medical industry and marketing. *In vitro* fertilisation (IVF) patients deal with multiple challenges when facing fertility treatment which have a limited success and come at a huge cost. In this context, IVF centres offer, and patients ask for, a spectrum of unproven add-on interventions, called 'add-ons', to increase the chances of a having a live birth.[1]

The Human Fertilisation and Embryology Authority (HFEA) recently produced a statement analysing 12 add-ons to disseminate clear patient information.[2] For this purpose, patient-friendly communication strategies have been adopted using a traffic-light rating system that represents how effective add-ons are. Thus, based on the level of evidence, the following system has been used: i) colour green when more than one good-quality randomised controlled trial (RCT) is present in the literature, indicating that the procedure is effective and safe; ii) colour amber when further studies are required due to limited body of evidence, showing that the procedure cannot be considered as standard therapy; and iii) colour red when no evidence of safety and efficacy exists. Although the HFEA tried to guide both professionals and patients on the principles that should be followed when choosing whether or not to use an add-on therapy with IVF treatment, the HFEA statement did not consider aspects other than efficacy and did not discriminate between short-term and long-term data regarding children's safety.

For this aim, the authors used a model framework proposed by the European Society of Human Reproduction and Embryology (ESHRE) in 2014 to distinguish when specific add-ons fall in the 'experimental treatment' category and when they can be moved into the 'innovative treatment' or 'established treatment' categories.[3] This tool, proposed by the Special Interest Group 'Safety and Quality in Assisted Reproductive Technology (ART)' and 'Ethics and Law' and described by Provoost et al.,[3] is based on a scoring system assessing four criteria: efficacy, safety, procedural reliability, and effectiveness. The choice for using this tool to categorise the IVF add-ons and compare the classification with the traffic light system used by the HFEA is based on the fact that

the Provoost model is a consensus-based model. A group of experts has prepared the framework based on the problematic dichotomy between experimental and established technologies. This dichotomy does not include the innovative treatments in clinical practice. The tool gives clarity on the notion of experimental and innovative treatments without deciding to restrict these treatments. The latter is not the purpose of the model proposed by Provoost et al.[3] Moreover, this paper gives guidance on how to implement the classified treatments and to strictly follow-up patients. The classification of the treatments is based on four criteria; the efficacy of the procedure (criterion 1) is scored with 0 when no proof of principle has been demonstrated or with 1 when it is.[3] While the efficacy is a categorical criterion (pass or fail), the other three criteria are ordinal. Safety (criterion 2) is referred to patients as well as the future children, considering safe data in animals (score 1), reassuring preclinical, short, mid, and long-term data (scores 2, 3, 4, and 5, respectively).[3] The different degrees of procedure variability (criterion 3) in different laboratories are taken into account by the procedural reliability, characterised by procedures enormously or highly variable between laboratories (score 1–2) to procedures considered as routine techniques (score 5).[3] The effectiveness (criterion 4) is referred to as the likelihood of producing the desired outcome compared with outcome of established ART techniques (from unknown/low likelihood with score 1–2 to high or higher than conventional ART techniques with score 5).[3] To define a treatment from experimental through innovative to an established one, it is important to assess these four criteria considering evidence-based medicine literature.[3] For each of the criteria a threshold exists, and if a treatment scores below the threshold, even for just one of the criteria, it cannot be moved to the subsequent definition, even though it would score higher for other criteria (Figure 1).[3] Only treatments scored above the thresholds for all the four criteria could be considered innovative treatments (efficacy score 1, safety score 3, procedure reliability score 2, and effectiveness score 2). When treatments showed a score of ≥4 on the last three criteria, they could be considered established treatments (Figure 1).[3]

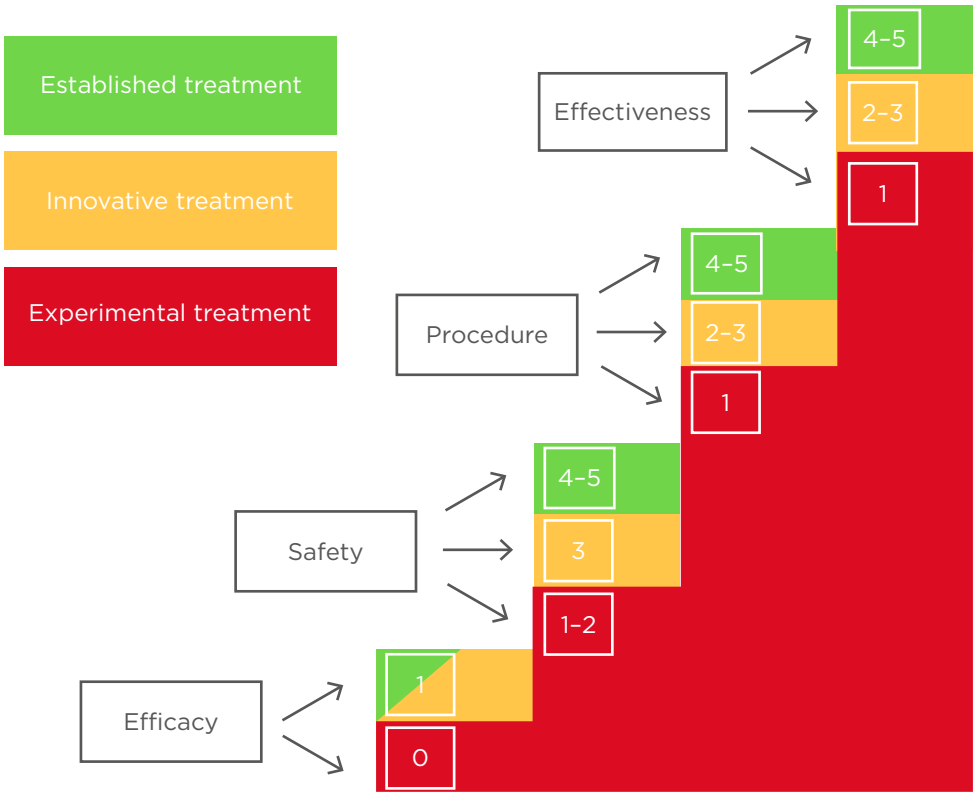

Figure 1: Sequential four-criterion assessment tool to consider the transition of a treatment from experimental (colour red) through innovative (colour amber) to established one (colour green) with the related scores.

This paper describes the application of the four-criterion assessment tool to the 12 add-ons reported by the HFEA, analysing the add-ons with the Provoost model and comparing them to the traffic light representation of the HFEA.

ASSISTED HATCHING

The artificial rupture of the zona pellucida to improve the chances of implantation and clinical pregnancy is known as assisted hatching (AH) and it is characterised by multiple different manipulations, i.e., making holes, thinning, or breaching of different sizes, or using laser, mechanical, or chemical methods on fresh or frozen/thawed embryos at different developmental stages.[4] Although a significant number of RCT is available, these studies are heterogeneous and do not support the efficacy of the technique. As a matter of fact, some meta-analyses were performed to evaluate the effect of embryo AH on clinical outcomes, reporting a borderline significant improvement in clinical pregnancy while the live birth rate has still not proven to be increased by AH.[5,6] Although very few RCT reported an absence of embryo damage and baby's congenital anomalies, a retrospective cohort study with a large sample size also suggested that AH alone does not increase the risk of major congenital anomalies when looking at 36,033 births after AH.[5,7]

The National Institute for Clinical Excellence (NICE) guidelines do not recommend the use of AH to improve pregnancy rates.[8]

Since only neonatal outcomes related to AH have been reported, the AH procedure should be considered as an experimental treatment based on the Provoost model. To move from experimental to innovative treatment on the continuum, the safety threshold should be characterised by reassuring short-term data on children up to at least 3 months post-delivery (score 3).[3]

Likewise, the HFEA states that there is no evidence available supporting the effectiveness of this add-on, and hence defined it as a red-coloured traffic light (Table 1).

PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES

To identify aneuploid embryos that are unsuitable for embryo transfer, 11 RCT based on Day 3 embryos analysed by fluorescence *in situ* hybridisation (FISH) showed no increase in live birth rate and, in some cases, a decrease in outcome.[9] As such, this first version of preimplantation genetic testing for aneuploidies (PGT-A 1.0) using FISH has been neglected. Nowadays, the PGT-A implies the comprehensive chromosome screening of biopsied trophectoderm cells of blastocysts (PGT-A 2.0). Regardless of these improvements, the real clinical value of PGT-A 2.0 is still controversial. In fact, PGT-A 2.0 has increased the implantation rate, reduced the miscarriage rate, and decreased the time to have a baby.[10] Unfortunately, the level of evidence seems to be low, attributable to the paucity of RCT (three RCT in good prognosis patients and one RCT in advanced maternal age women).[10] Moreover, no studies regarding the long-term follow-up of children born after PGT-A 2.0 at the blastocyst stage are currently available.[10] While PGT-A 1.0 was categorised red by HFEA and could be considered an experimental treatment because of lack of evidence on its effectiveness (score 1), the PGT-A 2.0 has been assigned the amber colour by the HFEA. PGT 2.0 could be defined as an experimental treatment in the Provoost model. In fact, there are reports showing some effectiveness (score 2), reassuring preclinical data on children (score 2), and that there is still variability in the procedures (score 2).[10] (Table 1).

PHYSIOLOGICAL INTRACYTOPLASMIC SPERM INJECTION

Hyaluronan-based selection of sperm, so-called physiological intracytoplasmic sperm injection (PICSI), is a strategy to select the best sperm for ICSI through the binding between hyaluronic acid and the sperm plasma membrane. The Cochrane review based on two RCT concluded that evidence was insufficient to show any difference in clinical pregnancy and live birth rates between PICSI and standard ICSI and no clear data on adverse effects are available.[11] Recently, a robust multicentre RCT demonstrated that PICSI did not significantly increase the live birth rate compared to ICSI.[12] Although this study was not powered to

investigate miscarriage, a significant reduction in miscarriage rate in the PICSI group compared with ICSI group had been observed. Nevertheless, the authors concluded that the wider use of advanced sperm selection techniques for assisted reproduction was not recommended.[12] The level of evidence is hence too low to demonstrate that this strategy is effective. Thus, HFEA associated the red colour to this strategy. Although PICSI represents a comparable procedure among laboratories (score 3), there is no short-term follow-up data (score 2), the effectiveness is low (score 2), and thereby could be considered an experimental approach when the Provoost model is applied (Table 1).

ELECTIVE FREEZE ALL CYCLE

With advances in cryopreservation of human embryos, the number of frozen–thawed embryo transfer (FET) cycles has increased steadily, with success rates after FET similar to those of fresh embryo transfer. This led to the development of the so-called 'freeze-all strategies' in IVF, in which the entire cohort of embryos is electively cryopreserved, and the transfer is delayed. This approach is preferred in patients at risk of developing ovarian hyperstimulation syndrome[13] or undergoing PGT cycles. Recently, the elective freeze-all strategy has become more common and it is often offered to patients in order to perform the transfer of a cryopreserved embryo into a more physiologic environment, without the effect of ovarian stimulation on endometrial receptivity. The Cochrane review based on four RCT concluded that elective FET strategy (eFET) is not superior to fresh transfer in terms of cumulative live birth rates with a moderate-quality evidence, but it is associated with lower rates of ovarian hyperstimulation syndrome and a higher rate of pregnancy complications.[14] Moreover, other RCT have been performed but with conflicting results. Recently, a robust RCT showed that frozen single blastocyst transfer resulted in a higher singleton live birth rate than fresh single blastocyst transfer did in ovulatory women with good prognosis.[15] Furthermore, some studies have shown that frozen single blastocyst transfer led to a higher singleton birthweight, which was accompanied by a higher risk of pre-eclampsia.[15] A register-based study on 4,758 children showed that health indicators were similar among FET and fresh ET singletons during a 3-year follow-up.[16] Scoring safety, procedure, and effectiveness with score 3, it is possible to consider the eFET as an innovative treatment, and similarly HFEA assigned the amber colour (Table 1).

INTRAUTERINE CULTURE

There has been an attempt at recreating the *in vivo* embryo development conditions and transpose these to the *in vitro* embryo culture. The *in utero* encapsulation technology introduces microinjected human oocytes into a retrievable and permeable tubing system that is inserted in the uterus. This allows the optimal exchange between the uterine maternal environment and the developing embryo. Because no RCT exist, HFEA considered this strategy as red. Moreover, very little evidence regarding safety and no reassuring preclinical data are available using the device.[17] Currently, the intrauterine culture could be considered as an experimental treatment (Table 1).

ARTIFICIAL EGG ACTIVATION CALCIUM IONOPHORE

Calcium ionophores are used as artificial oocyte activators to improve fertilisation rate (e.g., in a cycle subsequent to a total fertilisation failure or lower fertilisation rate). Two meta-analyses have been published.[18,19] In the latest meta-analysis, supported by the meta-regression analysis, artificial oocyte activation (AOA) appeared to improve the overall pregnancy rate and live-birth rates after ET.[18] Nevertheless, it is important to note that in the same study, no evidence supporting the use of AOA in IVF treatments was observed in a subgroup analysis based on only four RCT. Reassuring mid-term data have been reported in children, aged 3–10 years, born after AOA (score 4 for safety). The neonatal, neurodevelopmental, and behavioural outcomes were within expected ranges.[20] The insufficient evidence to support the overall use of AOA is related to the heterogeneity in the AOA protocols and the indications (score 2 for procedure reliability and score 2 for the effectiveness). HFEA assigned the amber colour to the AOA treatment due to scanty data. Basing on the four-criterion assessment tool it can be considered an innovative treatment (Table 1).

EMBRYO GLUE

The use of hyaluronan-rich (HA) supplemented medium is proposed to increase the chance of the embryo adhering to the endometrium and is stated to improve live birth rate. Benefits in terms of outcomes using HA-enriched transfer media still remain controversial.[1] The latest Cochrane review reported the overall quality of evidence supporting an improvement in clinical pregnancy and live birth rates using HA-supplemented medium as moderate, while a subsequent RCT did not find a beneficial effect on clinical pregnancy, implantation, and delivery rates. The birth weight however was significantly higher in the HA group than in the conventional ET medium group.[21,22] When four criteria are used to categorise this add-on, the use of the HA-supplemented media could be defined as an experimental treatment because only short-term neonatal outcome is available (score 2 for safety). Since further RCT studies are needed to obtain robust conclusions concerning the effectiveness of HA supplemented media, HFEA assigned the amber colour (Table 1).

ENDOMETRIAL SCRATCHING

The induction of endometrial injury to stimulate an inflammatory response and increase angiogenesis has been proposed as a strategy to improve embryo implantation. It is important to underline that the exact biological mechanisms behind the possible beneficial effect on implantation remain unclear. Risks related to this procedure are very rare and include infection and uterine perforation. Multiple RCT and meta-analyses have been performed measuring different effects.[23,24] Studies are of moderate quality at best and the evidence is compromised by the high clinical heterogeneity.[23] A very recent RCT showed that the endometrial scratching did not result in a higher rate of live birth compared to no intervention among women undergoing IVF.[24]

Table 1: Evaluation of 12 add-ons using European Society of Human Reproduction and Embryology (ESHRE) sequential four-criterion assessment versus Human Fertilisation and Embryology Authority (HFEA) grading.

	Efficacy	Safety	Procedure	Effectiveness	Type of treatment	HFEA grading	Concordance
Assisted hatching	1	2	3	1	Experimental		✓
PGT-A 1.0	1	4	3	1	Experimental		✓
PGT-A 2.0	1	2	2	2	Experimental		✗
Physiological ICSI	1	2	3	2	Experimental		✓
Elective freezing all cycle	1	3	3	3	Innovative		✓
Intrauterine culture	1	1	n/a	n/a	Experimental		✓
Artificial egg activation calcium ionophore	1	4	2	2	Innovative		✓
Embryo glue	1	2	3	2	Experimental		✗
Endometrial scratching	1	2	2	2	Experimental		✗
Reproductive immunology tests and treatment	1	1–2	1	1–2	Experimental		✓
Time-lapse imaging	1	2	3	2	Experimental		✗
Intracytoplasmic morphologic sperm injection	1	3	2	2	Innovative		✗
Sperm DNA damage	n/a	n/a	n/a	n/a	Not assigned	Not assigned	Not assigned

HFEA: Human Fertilisation and Embryology Authority; ICSI: intracytoplasmic sperm injection; PGT: preimplantation genetic testing; n/a: not applicable.

The inconclusive and sometimes contradictory results indicate that no solid conclusions can be drawn. No short or long-term data are available regarding children born after IVF cycle with endometrial-scratch procedure. It is because of these reasons and the score 2 for safety that endometrial scratching is categorised in the Provoost model as part of the group of experimental treatments although HFEA assigned the amber colour (Table 1).

REPRODUCTIVE IMMUNOLOGY TESTS AND TREATMENT

Failure of immune-mediated processes required to establish a maternal immune tolerance has been proposed as a potential cause of infertility. Based on this theory, different tests and therapies have been proposed for recurrent implantation failure and recurrent pregnancy loss patients such as the use of corticosteroids, intravenous immunoglobulins, TNFα antagonists (e.g., adalimumab, infliximab), calcineurin inhibitors (e.g., tacrolimus), and intralipid infusions.[25] Conflicting results have been reported concerning the improvement of clinical outcomes via these immunomodulating and immunosuppressive therapies.[25] A paucity of safety data indicates that the use of these tests and treatments requires a careful 'benefit to risk' evaluation.[25] To date, the lack of large RCT, no protocol standardisation related to reproductive immunology, and no reassuring data on safety to support the clinical benefit leaves this topic under investigation. Thus, reproductive immunology tests and treatments can be considered experimental, in agreement with the HFEA opinion for which the red colour was assigned (Table 1).

TIME-LAPSE IMAGING

Emerging as a new technology to guarantee a continuous embryo monitoring, the time-lapse system (TLS) represents one of the potential tools of the IVF laboratory. The possibility of an undisturbed culture associated with the evaluation of morphokinetic parameters linked with an algorithm for embryo selection has the potential to improve live birth rate. Nowadays, RCT and meta-analyses have been performed. The last Cochrane Review reported the quality of evidence ranging from very low to moderate.[26] In fact, there are inconclusive data in terms of live birth, miscarriage, stillbirth, or clinical pregnancy to choose between TLS, with or without embryo selection software, and conventional incubation.[26] No detrimental effects were observed using TLS on obstetric or perinatal outcomes.[27] TLS represents an important innovation in the IVF laboratory with reassuring preclinical outcomes, but today this technology is not more effective than conventional methods for embryo incubation. In conclusion, a lack of published short-term follow-up data on children born after embryo culture with TLS limits the evaluation of this technology. When applying the Provoost model, it can be classified as an experimental treatment because of the score 2 for the safety, although HFEA assign it to the amber category (Table 1).

INTRACYTOPLASMIC MORPHOLOGIC SPERM INJECTION

Sperm morphology assessment using high magnification (over ×6,000) allows identification of sperm abnormalities, such as the presence of nuclear vacuoles. These abnormalities have been suggested to have negative effects on ICSI outcome.[28] The integration of intracytoplasmic morphologic sperm injection (IMSI) technology in the ICSI procedure promised to increase pregnancy and/or birth rates in male factor infertility. No additional risks are reported with respect to ICSI and several RCT and meta-analyses have been published in the last decade. No convincing evidence of any significant difference between IMSI and ICSI in terms of pregnancy rate or live birth rate has been reported however.[29] These results do not suggest a routine use of this technique in IVF treatment, therefore the HFEA assigned it the red colour (Table 1). Reassuring data regarding safety[30] (score 3), relative procedure variability (score 2), and moderate effectiveness (score 2) scoring, categorises IMSI as an innovative treatment by the Provoost model.

ADDITIONAL INFORMATION - SPERM DNA DAMAGE?

Sperm DNA integrity represents an important parameter of male gamete quality. Several assays for sperm DNA fragmentation have been used and the type of damage measured with various

grades of sensitivity are different, generating high heterogeneity among the studies.[31] There is no evidence that endorses the use of one assay over another. An important topic is the management of treatment as a result of this diagnostic test. Different strategies have been proposed such as lifestyle modifications, infection control, or oral antioxidant therapy, but there is not enough evidence of effectiveness for the treatment(s).[1] This culminates in the question of whether sperm DNA testing has a role during andrological examination. In 2013, the Practice Committee of the American Society for Reproductive Medicine (ASRM) concluded that sperm DNA testing cannot be recommended routinely for clinical use based on the insufficient evidence.[32] Because DNA fragmentation is a diagnostic, noninvasive test and not a treatment, no traffic light has been assigned from HFEA to sperm DNA damage, and likewise the Provoost model was not applied (Table 1).

CONCLUSION

Medical assisted reproduction is in continuous progress and development. It is advantageous for patients to have access to the most successful and safest treatments with the highest outcome. However, the emotional race against the fertility clock to conceive and give birth to a healthy child makes patients vulnerable to the application of less effective techniques, sometimes even without reassuring safety data. Various supplementary treatments have found their way into the clinical practice of assisted reproduction without being critically assessed on their effectiveness. The HFEA has categorised 12 'add-ons' according to the traffic-light system to inform patients on the effectiveness of these supplementary treatments. HFEA concludes that rigorous RCT and robust data on safety are often lacking and therefore the use of add-ons should be reconsidered to avoid false hope and unnecessary costs for the patient.

Here, the add-ons were categorised by the Provoost model: a framework using a 3-stage classification of treatments. Most of the add-ons were categorised in agreement with the HFEA classification; the red traffic light corresponded to the class of the experimental treatment, whereas the amber colour aligned with the innovative category. For the PGT-A 2.0 embryo glue, the endometrial scratching, and the time lapse imaging, HFEA assigned an amber light, whereas the application of the Provoost model directed these four to the class of the experimental treatment.

A possible explanation for the lack of concordance between the HFEA traffic light rating system and the Proovost model for some treatments could be that some RCT were not available when the HFEA had released its model; for instance, the RCT on endometrial scratching.[24]

The classification of treatments based on predefined criteria as proposed by Provoost et al.[3] is useful because it gives a structured methodology to evaluate the literature used as input for the model. Although, these evaluations are subject to bias and discussion. Models and frameworks can help; however, every instrument has its pro and cons. The model proposed by Provoost et al. takes into account four criteria to move a treatment onto the continuum.[3] Because many studies report only on perinatal outcome, and not on mid-term safety of the children, this requirement for innovative treatment was not always met, hence the resulting score in the Provoost model.

Aside from the patients' information and these add-on classifications in IVF treatments, it is important that the correct follow-up studies are designed, and that long-term outcome data are published on these treatments. Nowadays, there is a substantial gap in knowledge regarding the long-term health of children born after ART and the effects of the introduction of new ART techniques on offspring must be monitored.[33] Especially for an innovative treatment, IVF centres should make a commitment to closely monitor their practice by conducting follow-up studies. Only when these data are reported can well-informed clinics provide the most effective and safest care for their patients.

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Variability of Biologics and its Impact on Biosimilar Development

This symposium took place on 19th June 2019, as part of the International Conference on Malignant Lymphoma in Lugano, Switzerland

Chairperson: Paul Cornes[1]

Speakers: Huub Schellekens,[2] Martin Schiestl,[3] Wojciech Jurczak[4]

1. Consultant Clinical Oncologist, Comparative Clinical Outcomes Group, Bristol, UK and International Cochrane Gynae Oncology Group, Bath, UK
2. Professor of Pharmaceutical Biotechnology, Utrecht University, the Netherlands
3. Head Regulatory Affairs Policy & Intelligence, Sandoz Biopharmaceuticals, Sandoz GmbH, Kundl, Austria
4. Professor of Hematology, Jagiellonian University, Kraków, Poland

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Meeting Summary

This symposium took place during the International Conference on Malignant Lymphoma (ICML) in Lugano, Switzerland, June 2019, and focussed on scientific aspects associated with development and approval of rituximab biosimilars in lymphoma. The symposium began with an overview presented by Dr Cornes detailing the urgent economic need for biosimilars to improve access to these biologic treatments in oncology and other therapy areas. Prof Schellekens, author of the first paper on biosimilars in 2002, discussed how regulatory strategies for biosimilars were shaped, and how these have evolved in the intervening years. Today, the emphasis of biosimilar development is placed on extensive analytical testing to demonstrate a match with the reference medicine at a fundamental level. Clinical testing plays a confirmatory role, removing any residual uncertainty regarding potential clinical differences between biosimilar and reference medicine. Dr Schiestl presented further detail on analytical perspectives on biosimilars. Development of biosimilars is complicated by the inherent variability of biological synthesis techniques employed in the manufacture of biologics. This variability is further increased by ongoing changes to manufacturing processes, which can result in changes in biological activity. Consistent quality is therefore a cornerstone of biosimilar development. Prof Jurczak provided a comprehensive overview of the

factors that must be considered during clinical development of a biosimilar. Clinical trials for biosimilars have a confirmatory role in the development process, rather than the pivotal role played by clinical trials for reference medicines. Therefore, these trials have markedly different objectives compared with reference clinical trials, resulting in differences in the chosen endpoints. In biosimilar trials, response endpoints, which provide rapid and sensitive assessments of equivalence, are preferred to survival endpoints, which require large and lengthy trials for adequate evaluation. Prof Jurczak illustrated this using data from the Phase III clinical trials of the Sandoz rituximab biosimilar. In this trial, Sandoz rituximab demonstrated an equivalent response rate to reference rituximab. Increasing economic pressure on healthcare systems means that biosimilars are likely to play an increasing role in the treatment of cancer in coming years, requiring clinicians to increase their familiarity with these important medicines.

Introduction to Biologics and Biosimilars

Doctor Paul Cornes

The World Health Organization (WHO) states that access to essential medicines is part of the right to health.[1] However, patterns of disease are changing, with noncommunicable diseases (NCD) becoming more common, now being responsible for almost 70% of all deaths worldwide.[2,3] To adapt to these changes in patterns of disease and control NCD, multiple innovations are required across domains of prevention, early diagnosis, and treatment. In particular, innovation in medicines is required – better treatment means more effective medicines for more diseases.[3] Of all NCD, cancer is a key threat, and this is universal to all countries globally.[3,4] Substantial innovation in cancer medicines has been achieved in recent years, and at the current rate >100 new cancer drugs could be available by 2020, giving a total of 200 cancer medicines.[5-8] Furthermore, >700 molecules were in late-stage development in 2017, an increase of >60% from a decade ago; almost 90% of these drugs are targeted treatments, including biologics.[9]

Biologic drugs are transforming treatment of other hard-to-treat diseases beyond cancer, including multiple sclerosis, heart disease, asthma, and inflammatory bowel disease,[10] because these targeted therapies offer improved effectiveness compared with past generations of small-molecule medicines.[11] However, biologics attract high costs, and therefore represent a good target for cost-control initiatives. Speciality medicines, including biologics, represent a large proportion of the overall medicines budget, ranging from 18–35% in South Korea, Japan, Canada, and Australia, to 35% in the USA and 35–45% in the five largest European markets (2016).[12]

Issues of medicine affordability are becoming evident in many therapy areas, including but not limited to oncology.[9] The median annual cost per life-year gained of a new cancer drug launched in 2014 exceeded $200,000,[13] and novel checkpoint inhibitors all carry list prices of more than $12,000 a month.[14] The best-selling anti-inflammatory biologic Humira (adalimumab) costs $38,000/year in the USA,[15] which equates to more than half the median income of an American household. Overall, total global prescription medicine sales are expected to be $1.2 trillion in 2024, and are increasing by 6–7% year-on-year.[16]

These high costs inevitably result in an impact on access to drugs. Evidence suggests that there are serious gaps in the availability of basic chemotherapeutic and biologic medicines in many Central and Eastern European countries.[17] In addition, a strong correlation exists between the wealth of a country and the number of patients receiving biologics,[18] with only patients in the USA, Germany, and the UK having access to >40 of the 54 oncology medicines initially launched between 2013 and 2017.[19] This exemplifies the stark reality; globally, cancer care is not affordable for most patients and many governments,[20] but the rise in cancer and other NCD cannot be solved without innovation in medical treatments. Therefore, healthcare budgets need to change to bear the costs of innovation; however, this can only be achieved by making savings elsewhere, which in turn must not compromise care. One potential route to such savings is the use of generic and biosimilar medicines to replace originator medicines. Between 2018 and 2024, it is predicted that $251 billion of currently patent-

protected sales could be replaced by more affordable follow-on versions.[16]

Seven of the top ten selling medicines in 2017 were biologics, three for treatment of inflammatory disease and four for treatment of cancers.[21] All of these biologics have, or will soon have, biosimilar brand competition. In 2017, sales of these seven biologics totalled more than $63 billion.[21] With an estimated 33% price saving offered by biosimilars, >$20 billion a year could be saved and used to sustain global healthcare. Previously, experts have discussed how the demand for biologics is putting pressure on healthcare budgets, suggesting that the introduction of biosimilars could potentially greatly reduce costs and increase access to treatment.[22] However, the manufacturing and development of biologics and biosimilars is complex. This was the focus of the symposium and will be discussed in subsequent sections of this report.

Biologics and Biosimilars: The Regulatory Perspective

Professor Huub Schellekens

The first article describing the development of 'off-patent biotech products', which would come to be known as biosimilars, was published in 2002 by Schellekens and Ryff.[23] The publication outlined the need for a new regulatory pathway for this new class of products to augment the existing pathway used for generic medicines. While generics are copies of small molecules that are easy to synthesise chemically, biosimilars are new versions of biologics, which are relatively large and complex proteins manufactured in living systems. The enormous complexity of the cell machinery used in the manufacturing process means that biologic manufacture cannot be rigidly controlled, and a certain degree of variability is present in all biologics, both within and between batches.[24] This variability results in challenges for biosimilar manufacturers, in that they must manufacture a product to a moving target. A further complication is that the proprietary technology used to develop the original molecule is unknown to the biosimilar manufacturer. Therefore, an iterative process is required to gradually guide the fingerprint of the biosimilar towards that of the reference medicine.[25]

The 2002 publication by Schellekens and Ryff[23] also suggested that extensive clinical data would be needed to show the efficacy and safety of a proposed biosimilar, to an extent similar to the reference biologic. In 1998, a formulation change for an epoetin molecule had resulted in the development of cross-reacting anti-epoetin antibodies leading to pure red cell aplasia.[26] This in turn led to an assumption that the clinical performance of biologics could be significantly altered by even small changes in the manufacturing process.

The approach to biosimilars has advanced considerably since 2002. Precision analytical tools are used to provide an extensive physicochemical and biological characterisation of the biosimilar, conclusively demonstrating high similarity to the reference medicine (Figure 1).[27] Once these analytical comparisons are complete, the regulatory pathway recommends clinical comparison of pharmacokinetics (PK) and pharmacodynamics (PD), followed by a single Phase III clinical study, which is considered confirmatory to resolve any potential uncertainty regarding the similarity of the biosimilar candidate and the reference medicine.[27]

Since 2006, the date of the first biosimilar approval in Europe, we have also gained knowledge on the safety of biologics, and the idea that 'even minor differences may affect the safety and/or efficacy of biologics' is no longer a consensus opinion. Manufacturing changes for biologics occur at an average rate of 1.8 per year.[28] For example, to date, 29 high-risk or moderate-risk process changes have been observed for reference rituximab.[29] These changes have not resulted in withdrawal of the products or alterations in their labelling, indicating that the observed changes were predicted by health authorities to not result in an altered clinical profile.[30] Furthermore, no unexpected safety issue has been reported for currently available biosimilars on the European market, including more than 58 medicines and over 700 million patient-days' exposure.[31] As noted by Lamanna et al.,[32] "biopharmaceutical variability resulting in clinical differences is extremely rare with only a single verifiable case resulting in adverse events in over 35 years and over 260 products."

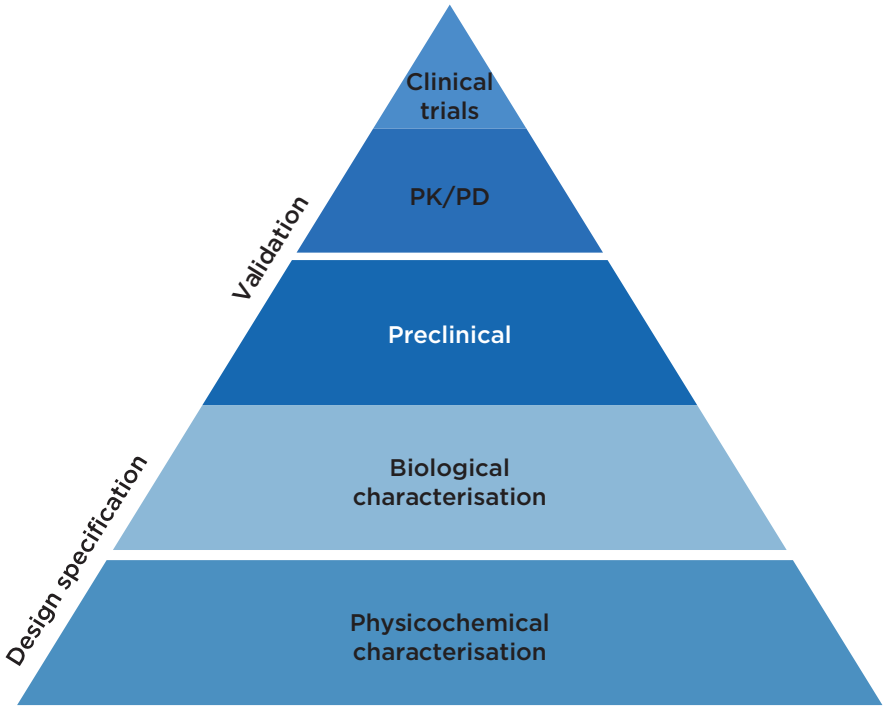

Figure 1: The current approach to biosimilar development.[27]

PD: pharmacodynamics; PK: pharmacokinetics.

Biologics and Biosimilars: The Analytical Perspective

Doctor Martin Schiestl

Biosimilars are precisely engineered to match an existing reference biologic medicine in all relevant attributes. Based on guidance published by regional and national health authorities, key requirements for biosimilarity are matching structure and function; similar PK/PD, clinical efficacy and safety; and the same presentation, dose (strength), and method of administration. Biosimilars must have certain relationships with their reference medicines in terms of structure. The amino acid sequence must be identical, while secondary, tertiary, and quaternary folding patterns must be indistinguishable across multiple redundant methods of analysis. Owing to biosynthesis in living cells, post-translational modifications such as glycosylation and sialylation demonstrate a certain degree of variability for biologic medicines, and each batch of a given biologic can be differentiated from other batches using sensitive analytical methods.[30]

Manufacturing changes may result in more profound shifts in attributes, which in some cases may be associated with changes in biological function. For example, the antibody-dependent cellular cytotoxicity activity of reference rituximab was noted to undergo a noticeable shift following a change in the manufacturing process in batches expiring after mid-2010.[30] However, this variability is tightly controlled within acceptable limits to ensure that such shifts have no relevant clinical impact. Moderate and major manufacturing changes also require pre-approval by the health authorities who grant such a change only if the risk for any clinical impact is sufficiently low. To exclude relevant clinical differences of a biosimilar to its reference medicine, the biosimilar must match the reference medicine as closely as possible in terms of physicochemical structure, with particular focus on attributes known to have the largest potential impact on clinical performance.[25] This close similarity at a molecular level can then be confirmed using *in vivo* biological testing. For example, Sandoz rituximab was shown to have biological activity that fell within the range of reference rituximab

across the key effector functionalities associated with the central mechanism of action.[33]

Consistent manufacturing of biosimilars is ensured through control of the manufacturing process, including control of raw material, process design, in-process testing, and control of process parameters, release testing of harvest, drug substance, and final dosage form. The quality system is governed by Quality Assurance functions, compliance with Good Manufacturing Practices (GMP) and regular inspections by health authorities. Dr Schiestl concluded that consistent quality is a key regulatory requirement for biosimilars, and analytical capabilities and quality systems are continuously improving, allowing for robustness in ensuring consistent quality.[32,34] A biosimilar medicine and its reference medicine are expected to have the same safety and efficacy profile,[10,35] as confirmed by 13 years of clinical experience with biosimilars in the European Union (EU) as of 2019.[36]

Biologics and Biosimilars: The Clinician's Perspective

Doctor Paul Cornes and Professor Wojciech Jurczak

The Biosimilars Forum Survey, conducted in 2015–2016, aimed to assess physicians' knowledge of biosimilars.[37] When asked 'Do you believe biosimilars will be safe and appropriate for use both in naïve and pre-exposed patients?', <50% of respondents were in agreement. Prof Pekka Kurki of the Finnish Medicines Agency has suggested that the root cause of this uncertainty is a discrepancy between the needs of regulators, who assess the totality of evidence, and clinicians, who focus only on clinical testing.[38] Attitudes towards biosimilars are improving however. An analysis of publications mentioning biosimilars between 2004 and 2015 found exclusively sceptical language in 2004, which had changed to be approximately 50% positive and 40% neutral by 2015.[39]

The common desire to see clinical data is learnt from deep familiarity with the development process for innovator drugs, for which clinical trials are indisputably the optimal way to confirm efficacy.[40] In biosimilar development, however, the overall goal of the clinical programme is to demonstrate equivalence of the biosimilar to the reference medicine. The corresponding statement is that the clinical programme aims to detect any difference between the medicines, should it be present. Therefore, the indication chosen for a clinical trial should be sensitive to detect any potential differences, rather than the most frequently prescribed indication for the drug.[41] Similarly, the appropriate endpoints could be very different to 'traditional' Phase III endpoints; PD endpoints are likely to be more sensitive than clinical endpoints.[42] In clinical trials of reference trastuzumab, for example, the PD endpoint pathologic complete response (CR) was shown to be four times more sensitive than overall survival, and achieved in one tenth of the time.[43] Overall, the design features differ in studies for new biologics and for biosimilars, as summarised in Table 1.[44,45]

An equivalence trial is designed to provide statistical evidence that there are no clinically meaningful differences between the biosimilar and its reference medicine.[46] Therefore, the critical first step in biosimilar trial design is to predict the effect of the reference medicine on the chosen endpoint. Then, it is necessary to determine how large a difference could be observed between the effect of the reference medicine and the proposed biosimilar before it would be regarded as non-equivalent; this difference is known as the equivalence margin.[46] Generally, trials are designed to test whether the true difference lies within the equivalence margin with 90% or 95% confidence. If this is the case, then the treatments can be considered equivalent.

Trial designs must also take account of whether to use the risk difference or risk ratio to compare treatments. 'Risk difference' measures the absolute difference between two treatments, where equivalence is denoted by a difference of 0. Conversely, 'risk ratio' measures the relative difference between two treatments, where equivalence is denoted by a difference of 1.[47] Importantly, these points are generally agreed with regulatory authorities before a trial begins.

Survival endpoints (progression-free survival [PFS], overall survival [OS]) are included in almost all trials for originator oncology drugs. However, these endpoints have limitations that make them

less suitable in certain situations, including in the development of biosimilars.[44] For example, survival endpoints require longer follow-up times and larger sample sizes for reliable measurement compared with response-based endpoints.[48] Importantly, survival endpoints do not directly measure the antitumour activity of a drug, and may be influenced by factors not attributable to differences between the biosimilar and reference biologic, including tumour burden, performance status, underlying conditions, and previous and subsequent treatments.[44,49] Therefore, regulatory authorities including the European Medicines Agency (EMA) recommend that endpoints such as response rate may be more sensitive than PFS or OS for detecting differences between a biosimilar and reference medicine, and can be used as surrogates for survival in biosimilar trials.[44]

The clinical development programme for Sandoz rituximab included studies of PK/PD, efficacy and safety from two indications, follicular lymphoma (FL) and rheumatoid arthritis. FL was chosen as an appropriate indication for a Phase III confirmatory clinical trial, as this indication resulted in the largest effect size for rituximab when added to standard chemotherapy, and is the most homogeneous of the approved oncology indications for rituximab.[50] The ASSIST-FL study was a randomised, Phase III trial of efficacy, safety, and PK of Sandoz rituximab versus EU-sourced reference rituximab.[50] Overall response rate (CR or partial response [PR]) was chosen as the most appropriate endpoint for the trial, as to confirm similarity with an overall survival endpoint would have required several thousand patients. Given that large clinical trials (>1,000 patients) have a mean cost of $77 million,[51] this would have been prohibitive.

Table 1: Comparisons between study designs for a new biologic and for a biosimilar.[44,45]

Design features	Pivotal trial for a new cancer biologic	Biosimilar confirmatory clinical trial
Clinical design	Superiority vs standard of care (non-inferiority designs also useful in some situations).	Equivalence study vs reference medicine.
Study endpoints	Clinical outcomes data (OS and PFS) or accepted/established surrogates.	Pharmacokinetic and pharmacodynamic markers; objective RR.
Patient population	Any – homogeneity is desirable.	Sensitive with a high degree of homogeneity.
Safety	Acceptable risk/benefit profile vs standard of care.	Similar safety profile to reference medicine.
Immunogenicity	Acceptable risk/benefit profile vs standard of care.	Similar immunogenicity profile to reference medicine.
Extrapolation to other indications	Not allowed.	Possible if justified.
Statistical inference	Based on p-values.	Based on confidence intervals maintained within pre-defined margins.
Analysis approach	Significance level of 5% for hypothesis testing. Primary analysis on FAS.	90% or 95% confidence intervals. Primary analysis on PPS.
Design type	Superiority or non-inferiority. Powered to show difference for primary endpoint (if one exists).	Equivalence or non-inferiority. Powered to show similarity for primary endpoint.
Error types	Type I: superiority shown but not true Type II (if study not powered): superiority not shown but actually exists.	Type I: equivalence shown but drugs are not similar. Type II (if study not powered): difference shown but drugs are equivalent.

FAS: full analysis set; OS: overall survival; PFS: progression-free survival; PPS: per-protocol set; RR: response rate.

	Response, % (90% CI)	
	SDZ-RTX N=311	EU-RefRTX N=313
Overall response rate	87.1 (83.59, 90.15)	87.5 (84.04, 90.49)
Complete response	14.8 (11.6, 18.5)	13.4 (10.4, 17.0)
Partial response	72.3 (67.9, 76.5)	74.1 (69.7, 78.2)

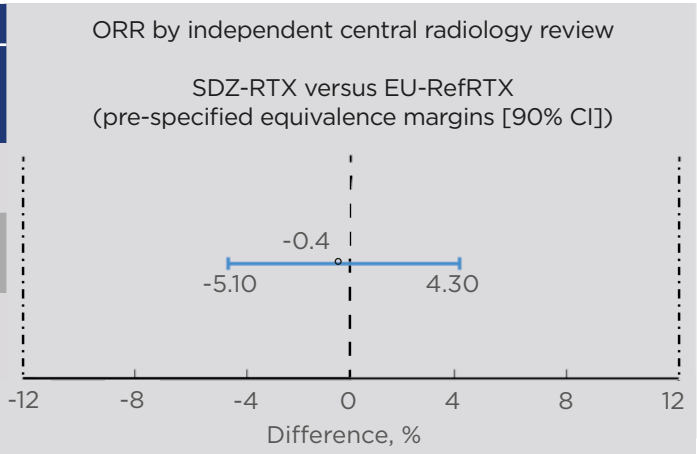

Figure 2: Results on the primary endpoint in the ASSIST-FL trial of Sandoz rituximab.[50,52]

CI: confidence interval; EU-RefRTX: EU-sourced reference rituximab; ORR: overall response rate; SDZ-RTX: Sandoz rituximab.

The study included patients with previously untreated, advanced-stage FL (Ann Arbor stage III/IV, WHO histological grade 1-3a) who were randomised to receive either Sandoz rituximab 375 mg/m^2 + cyclophosphamide, vincristine, and prednisone (CVP) chemotherapy (n=314) or EU-reference rituximab 375 mg/m^2 + CVP (n=315) for eight 3-weekly treatment cycles, known as the induction phase or combination treatment period. Tumour assessment was performed at end of Cycles 4 and 8. During the 2-year maintenance period, responders received Sandoz rituximab (n=254) or EU-sourced reference rituximab (n=252) every 3 months. The primary endpoint was met: equivalence in overall response rate was demonstrated, with a difference between treatments of -0.40% and a 95% CI of -5.94 to 5.14. This was entirely contained within the predefined equivalence interval of -12 to 12%, as was the 90% CI (-5.10 to 4.30%) (Figure 2).[50]

In ASSIST-FL, PFS and OS were unpowered, descriptive secondary endpoints.[52] CR after 30 months (CR30) is considered a surrogate for PFS, as correlation between these two outcome measures has previously been established.[53] CR rates (based on investigator assessment) were similar between treatments at all time points, including 33 months.[52] Safety profiles of Sandoz rituximab and reference rituximab were similar when combined with CVP in the combination phase, or alone in the maintenance phase.[50] Incidences of adverse events (AE), serious AE, and AE leading to discontinuations and deaths were comparable. Most AE were mild or moderate in severity.

Conclusions

Doctor Paul Cornes

The implementation of biosimilars is a central policy imperative for the EU.[54] At a stakeholder event on biosimilar medicines held by the European Commission in Brussels in September 2018,[55] the consensus stated that there are no outstanding serious medical issues regarding biosimilars, that they can be considered as safe and of the same quality as reference biologics, and that they may be associated with significant cost savings.[35,54] Stakeholders also agreed that biosimilars can be used in indications that are approved for the reference medicine but not studied for the biosimilar, enabling pharmacies to stock only one brand,[56] and that brands can be switched safely,[57] possibly as part of the annual tender process. In a decade of use (with >58 biosimilars now approved and >700 million patient-days' exposure) EMA-approved biosimilars have all maintained approval without showing a different risk or benefit profile to the reference medicine. Within 12 months of launch, biosimilar rituximab infusions overtook the reference biologic in the EU5 nations.[58] Ongoing

increases in biosimilar uptake are likely to lead to considerable healthcare savings,[16] broadening access to biologics for patients in wealthy as well as lower income countries, ultimately allowing for increased sustainability of care and an increased budget for development of innovative treatments.

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Moderate Psoriatic Arthritis and Perspectives from Phosphodiesterase-4 Inhibition

This symposium took place on 14th June 2019 as part of the European League Against Rheumatism (EULAR) Congress in Madrid, Spain

Chairpeople: Rubén Queiro-Silva[1]

Speakers: Stefan Siebert,[2] Dafna Gladman,[3] Frank Behrens[4]

1. Hospital Universitario Central de Asturias, Oviedo, Spain
2. University of Glasgow, Glasgow, UK
3. Toronto Western Hospital, Toronto, Canada
4. Goethe-University, Frankfurt, Germany

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Meeting Summary

Although there is no universally approved definition of moderate psoriatic arthritis (PsA), many clinicians see patients who they feel fit into this category: patients with limited joint involvement, but who might also show other manifestations of the disease, as well as a range of comorbidities. In his presentation, Dr Siebert described the challenges faced in treating this group of patients, who are mostly not captured in clinical trials. Recent advances in PsA treatment have focussed towards the severe end of the spectrum, suggesting that more must be learned around treatment options for patients with moderate disease. This represents a large unmet need. Given the heterogeneity of this patient population, a range of effective treatments is needed. Prof Gladman then presented data from longitudinal cohorts to illustrate the high burden of disease in patients with PsA who had a limited number of affected joints. By comparing patients with oligoarticular PsA (i.e., ≤4 affected joints) with those with polyarticular arthritis (≥5 affected joints), Prof Gladman showed that disease burden is not solely driven by the number of affected joints, but also by other PsA manifestations and/or comorbidities. There are clear gaps in our knowledge of PsA; to address these, population studies and trials of potential treatments are needed. Phosphodiesterase-4 (PDE4) inhibition is one potential treatment strategy that is currently being investigated. Dr Behrens described a post-hoc analysis of data pooled from three Phase III clinical trials that suggests the PDE4 inhibitor apremilast may be an effective treatment for patients with moderate PsA. It is hoped that this will be confirmed by the ongoing FOREMOST trial, a Phase IV study of apremilast in patients with oligoarticular PsA.

Introduction: Moderate Psoriatic Arthritis - What Does it Mean?

Doctor Rubén Queiro-Silva

Dr Queiro-Silva opened the session by proposing a definition of the moderate-severity PsA patient: patients associated with a limited number of affected joints, including some with oligoarthritis and some in the lower range of polyarthritis.[1-3] Patients may have other core manifestations of PsA, such as dactylitis (inflammation of fingers and toes), enthesitis (inflammation of the insertion points of tendons and ligaments into bone), spinal disease, and skin and nail involvement.[1-3] They may also have common comorbidities associated with PsA.[4] Patients with moderate PsA therefore have a significant disease burden.

Baseline data from the Spanish Rheumatology Society's (SER) recent-onset PsA registry confirm that patients with a low number of affected joints (i.e., who show an oligoarticular disease pattern) have pain and global disease activity scores that cannot be neglected.[3]

Dr Queiro-Silva illustrated the potential complexity of the moderate-severity PsA patient by describing a case from his clinic. A 24-year-old female presented with a very painful knee. An MRI scan showed that she had enthesitis and physical examination revealed dactylitis, which was not particularly painful. At the time of presentation, there was no joint synovitis.

The challenge for clinicians is therefore not only in defining the patient's disease, but in determining the best therapy based on a number of factors, including disease severity.

Challenges in Psoriatic Arthritis Clinical Practice: Tailoring Treatments to Patients' Profiles

Doctor Stefan Siebert

Increased knowledge regarding the immunopathogenesis of both psoriasis and PsA has led to major advances in our understanding of psoriatic disease. In the skin and enthesis, the immunological pathways are well-defined and relatively straightforward; however, in the joints, the picture is more complicated (Figure 1).[5-7] This should be a consideration when a patient presents in clinic. This increased understanding of psoriatic disease has led to the development of a number of therapies directed at both extracellular and intracellular signalling pathways. The challenge for clinicians is deciding which treatment to choose for individual patients presenting with moderate disease.

Informing Treatment Decisions in Psoriatic Arthritis

To help inform their decision, clinicians can draw on information from several sources, such as clinical trial data, guidelines and recommendations, and real-world data. However, it is important to recognise the limitations of some of these sources. For example, data from randomised clinical trials (RCT) in PsA are not easily translated into clinical practice. These trials are carried out under highly controlled conditions in homogeneous populations, meaning that they ultimately do not provide information on how to treat patients with significant comorbidities, infectious disease, or cancer. The primary endpoint in these studies is the American College of Rheumatology 20% improvement criteria (ACR20). This score has limitations in measuring improvements in patients with a limited number of affected joints, because of the obvious difficulty in showing a 20% improvement in this setting. In addition, ACR20 does not take into account the other manifestations of PsA (i.e., dactylitis, enthesitis, spinal disease, skin involvement) or quality of life (QoL). These are the issues that patients generally want to discuss in clinic. Another limitation of RCT is the over-representation of patients with more severe disease.

The European League Against Rheumatism (EULAR) has published treatment recommendations for patients with PsA.[8] These recommendations move patients through a series of steps depending on how well they respond to treatment. Closer examination of the EULAR recommendations, focussing on the peripheral joints, reveals that there are fewer treatment options (with less supporting evidence) for patients with moderate PsA than for those with severe disease.[8]

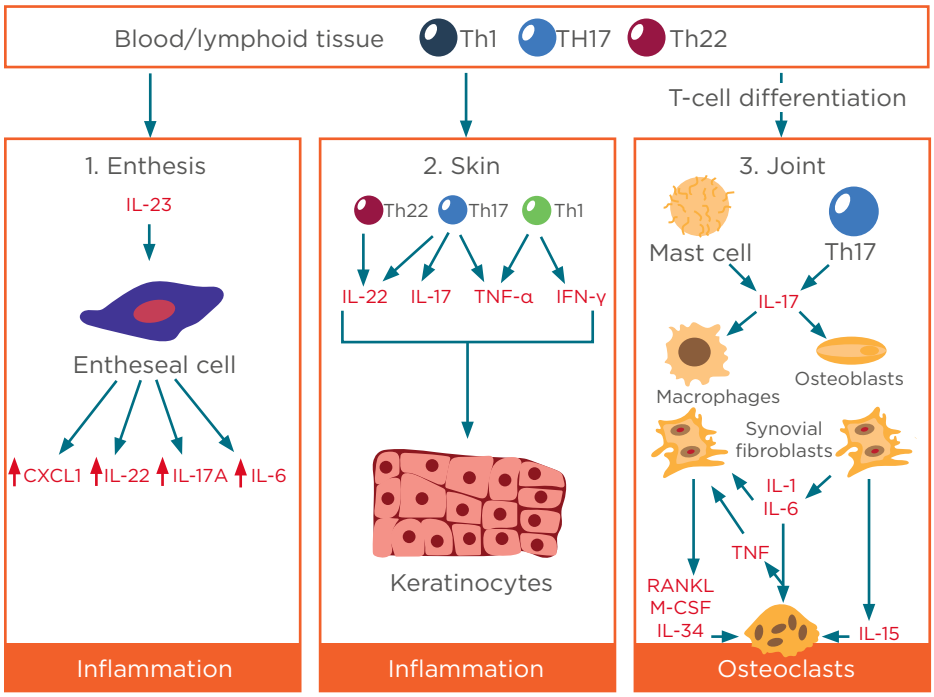

Figure 1: Inflammatory pathways in the enthesis, skin, and joints.

CXCL: chemokine ligand 1; IFN: interferon; IL: interleukin; M-CSF: macrophage colony stimulating factor; RANKL: receptor activator of nuclear factor κB ligand; Th: T helper; TNF: tumour necrosis factor.

Adapted from Lubberts,[5] Merola et al.,[6] and Saxena et al.[7]

As stated by EULAR, "The primary goal of treating patients with PsA is to maximise health-related quality of life." Therefore, when considering which treatment to use, the principle of maximising health-related QoL should be applied.[8] Low disease activity levels are associated with better health-related QoL, patient-reported outcomes, and patient-acceptable symptom state.[9-11] Nevertheless, patients with residual and moderate disease activity remain undertreated: a survey of PsA patients in North America and Europe showed that approximately one-third of those who were not receiving treatment considered themselves to have moderate disease.[12] This undertreatment has an impact on QoL: data from the Dutch early PsA cohort (DEPAR) showed that patients with residual disease activity despite continuous methotrexate monotherapy had worse health-related QoL scores after 6 months than those who had achieved minimal disease activity.[13]

Stratification of patients according to various predictive factors (biomarkers, phenotype, and comorbidities) to inform treatment choice is widely used in other fields, particularly oncology.[14,15] However, in PsA, stratification according to biomarkers is not possible, as none have yet been identified. Stratification by phenotype poses challenges in terms of variation in presentation (which is heterogenous and overlapping), joint distribution (approximately one-third of patients have oligoarthritis[16,17] and are less likely to achieve ACR20 after 48 weeks of treatment than those with polyarthritis),[18] and disease severity (patients with moderate disease activity who do not quite fit into the current treatment guidelines).

Comorbidities are common in PsA, and the concept of multimorbidity (the presence of ≥2 long-term conditions) is an important consideration. Multimorbidity increases with age[19] and is associated with reduced QoL, higher mortality, reduced socioeconomic status, higher treatment burden, higher rates of adverse drug events, and increased use of health services.[19,20] Data from the Australian Rheumatology Association (ARA) database show that 58% of

patients with PsA have at least two conditions other than their PsA.[21] Patients with a higher number of comorbidities have worse disease activity and are less likely to respond to, or remain on, treatment.[22]

The prevalence of metabolic conditions, such as metabolic syndrome, obesity, and diabetes is increased in patients with PsA.[23-28] In addition, patients with PsA are twice as likely to develop nonalcoholic fatty liver disease than patients with psoriasis (who in turn are twice as likely to develop this condition compared with the general population).[29]

Finally, patient preference should also be taken into account. A survey of patients in North America and Europe revealed several burdens that patients associate with their medication, including the need for frequent blood level monitoring, lifestyle compromises, and fear of injections.[30]

Summary

Although there have been major advances in PsA treatment, this has predominantly focussed on the severe end of the disease spectrum. Patients with moderate disease represent a considerable unmet need; their heterogeneity means that several treatment options are needed and tailoring treatment to the individual patient remains a challenge.

Oligoarticular Psoriatic Arthritis: Lessons from Longitudinal Cohorts

Professor Dafna Gladman

A significant barrier to the treatment of oligoarticular PsA is the requirement by some insurance payers that patients have a minimal number of affected joints to be eligible for certain treatments. However, as described below, patients with oligoarticular PsA (≤4 affected joints) have a significant burden of disease that is similar to that in patients with polyarticular disease.

Oligoarthritis is not uncommon: in a series of nine studies carried out between 1976 and 2018, each with >100 PsA patients, 25–50% of patients had oligoarthritis.[31-39] Oligoarthritis occurs earlier in the disease course than polyarthritis;[2] therefore, it is important that these patients are treated effectively before further joint involvement occurs.

The Burden of Oligoarticular Psoriatic Arthritis

Data from several longitudinal cohorts show that patients with oligoarticular PsA have a significant disease burden. Using data from the German Collaborative Arthritis Centres, Huscher et al.[4] compared the burden of disease for patients with oligoarticular PsA (n=287) and polyarticular PsA (n=324).[4] They found that 63.3% of patients with oligoarthritis had osteoproliferations, compared with 43.2% of those with polyarthritis. Comorbid conditions were also more common in oligoarticular patients: present in 82.8% of oligoarticular patients compared with 68.3% of polyarthritic patients. The study also showed that few patients who had had oligoarticular disease for <5 years were given biologics. Patients who had oligoarticular disease for ≥5 years were more likely to receive opioids than those with early oligoarticular disease or those with polyarticular disease.

Baseline data from the SER recent-onset PsA registry show that 81.5% of patients have peripheral arthritis.[3] Oligoarticular PsA has traditionally been thought of as a condition affecting the larger joints. However, data from the GRAPPA Composite Exercise (GRACE) study, in which 266 patients (53.0%) had oligoarthritis, showed that smaller joints are also commonly affected.[40]

In the Dutch early PsA cohort (DEPAR), SF-36 scores recorded at the time of diagnosis were very similar between patients with oligoarticular disease (n=151) and those with polyarticular disease (n=91), indicating a similar burden of disease, regardless of the number of joints affected.[1]

Further data from DEPAR show the value of minimum disease activity (a composite measure of disease activity) in guiding treatment in both oligoarticular and polyarticular PsA.[41] In addition, data from the TIght COntrol of inflammation in early Psoriatic Arthritis (TICOPA) trial show that other composite scores such as the Psoriatic Arthritis Disease Activity Score, GRAPPA composite score, and Composite

Psoriatic Disease Activity Index tools were able to distinguish effectively between patients with oligoarthritis who were aggressively treated and those who received standard of care, and are therefore suitable for use even in patients with few affected joints.[42]

Knowledge Gaps in Oligoarticular Psoriatic Arthritis

There is clearly a large disease burden associated with oligoarticular PsA, but to be able to develop treatment strategies for these patients, a number of gaps in our knowledge must be filled. Firstly, we need to determine how to identify and measure active disease. As explained by Dr Siebert, ACR20 is not appropriate because it is not possible to show a 20% improvement when only four joints are affected. Appropriate treatment goals need to be defined, and we need to determine which treatments have efficacy in this patient group. There is an obvious need for more data in oligoarticular PsA, for example, studies looking at treatment goals that incorporate the patients' perspective of disease burden, population studies looking at phenotype-specific outcomes, and RCT.

The FOREMOST study is one RCT that is currently underway.[43] It is designed to investigate the efficacy, safety, and tolerability of the PDE4 inhibitor apremilast in patients with early, oligoarticular PsA. Patients are eligible if they have had oligoarticular PsA for <2 years and have received nonsteroidal anti-inflammatories and/or ≤1 conventional synthetic disease modifying antirheumatic drug at a stable dose for ≥3 months. Patients are randomised to apremilast or placebo and can continue to take their background therapies. Treatment will last for up to 24 weeks, after which patients can enter an extension phase and continue to receive apremilast for a further 24 weeks. The primary endpoint is minimal disease activity assessed by MDA-joints (i.e., the two articular minimal disease activity criteria plus three of the other five criteria) at Week 24.

Summary

Patients with oligoarticular disease may present with additional PsA core manifestations. In addition, these patients can also suffer from comorbidities. Overall, they can have a significant disease burden. Patients with PsA deserve appropriate treatment regardless of how many joints are affected.

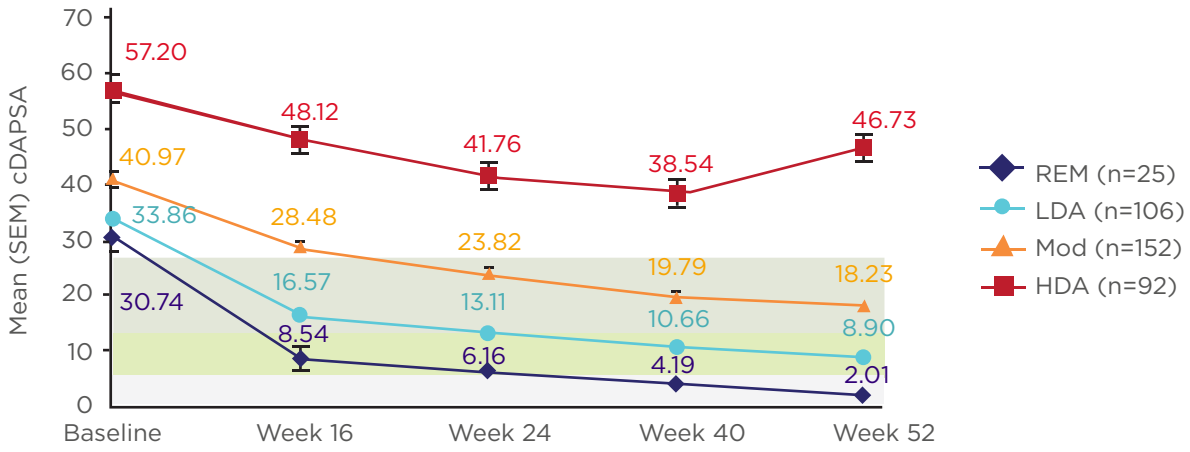

Figure 2: cDAPSA scores over 1 year of treatment with apremilast 30 mg BID, stratified by cDAPSA category at Week 52.[46]

High disease activity (no shading): cDAPSA >27; moderate disease activity (green shading): cDAPSA >13–≤37; low disease activity (blue shading): cDAPSA >4–≤13; remission (grey shading): cDAPSA ≤4.

BID: twice-daily; cDAPSA: clinical Disease Activity for Psoriatic Arthritis; HDA: high disease activity; LDA: low disease activity; Mod: moderate disease activity; REM: remission; SEM: standard error of mean.

Learnings from Phosphodiesterase-4 Inhibition: Optimising Outcomes for Moderate Psoriatic Arthritis

Doctor Frank Behrens

Oligoarthritis is part of the spectrum of moderate disease. Researchers in Switzerland have developed technology with a purely data-driven approach that involves a machine making this decision based on electronic patient records.[44] The technology clusters patients into phenotypes and can differentiate between patients who have oligoarthritis and those who may be closer to a polyarticular phenotype. In the future, this may help clinicians with their treatment decisions. Even with this technology, it is obvious that patients with oligoarticular disease represent a large proportion of the patient population.

As described by Prof Gladman, oligoarticular and polyarticular PsA have a similar impact on patients' QoL (as measured by SF-36). A possible explanation is that this might be driven by enthesitis, rather than joint count,[1] which adds to the argument that in patients with moderate disease, clinicians need to look beyond joint counts.

The goal of treatment in PsA is to optimise long-term health-related QoL and social participation through control of signs and symptoms, prevention of structural damage, and normalisation or preservation of function.[45] Approximately 70% of patients with oligoarticular PsA have non-erosive disease;[38] however, if left untreated they might develop erosive disease. As described by Prof Gladman, composite scores such as clinical Disease Activity for Psoriatic Arthritis (cDAPSA) are valuable tools to measure treatment success.

Evidence for Phosphodiesterase-4 Inhibition as a Treatment Option

The PDE4 inhibitor apremilast is currently licensed for the treatment of active PsA in adult patients who have had an inadequate response or who have been intolerant to a prior disease-modifying antirheumatic drugs (DMARD) therapy. To investigate if patients treated with apremilast could reach treatment targets, an analysis was carried out on pooled data from patients in three RCT who received treatment for 1 year.[46] Patients were grouped according to the cDAPSA category achieved at Week 52, and their mean cDAPSA trajectory was traced over 1 year from baseline. Patients who achieved treatment targets (low disease activity or remission) at Week 52 presented with lower disease activity at baseline. In addition, these patients had a greater and more robust decrease in cDAPSA score over 1 year than those with higher baseline disease activity (Figure 2). Probability estimates of shifting across various cDAPSA score categories from baseline to Week 52 were also presented. The probability of patients with moderate disease activity at baseline achieving treatment target (low disease activity or remission) at Week 52 was 46.9%,[47] while in patients with high disease activity at baseline, the probability of achieving treatment target was 24.9%.[47]

Control of disease activity in patients treated with apremilast also resulted in control of musculoskeletal manifestations of the disease.[46] Patients who achieved treatment target had low mean swollen and tender joint counts (≤1.2 and ≤2.6, respectively) at Week 52. Mean dactylitis count was 0.0 at Week 52 in patients who achieved remission; patients who achieved low disease activity had a mean dactylitis count of 0.5. Similarly, enthesitis was also well controlled: patients who achieved remission had a mean Maastricht Ankylosing Spondylitis Enthesitis Score of 0.4 at Week 52; those with low disease activity had a mean score of 1.2.

It therefore appears that patients with moderate disease who achieve treatment target by Week 52 (based on cDAPSA score) can also achieve control of other PsA manifestations such as dactylitis and enthesitis when treated with apremilast.

In addition to RCT data, Dr Behrens also showed data from routine clinical practice in Germany. He referred to the Lapis PsA study, a prospective observational study carried out in Germany to evaluate the effectiveness of apremilast in 111 patients in a routine care setting.[48] At baseline, no patients had minimal or no symptoms, as assessed using the Physician' Global Assessment. After 4 months of treatment, this had increased to 65% of patients.

Summary

Patients with moderate PsA represent a relevant population in clinical practice. Treatment strategies need to align with individual patient disease profiles. The data presented above suggest that apremilast is a potential treatment option for patients with moderate PsA. Further evidence will become available from the randomised, controlled FOREMOST trial.

Question and Answer Session

Q: In the Swiss artificial intelligence model described by Dr Behrens, how sure can you be that the joint count is accurate?

A: Dr Behrens responded that no clear guarantee could be given, but it is also important to realise that this cannot be guaranteed in clinical and observational studies either. Prof Gladman said that there is research that shows the more highly trained an individual is, the more accurate the joint count. Dr Siebert commented that clinicians often underestimate the number of affected joints, so it is important to do a full 66/68 joint count every time the patient is in clinic.

Q: Regarding comorbidities and extra-articular involvement, which might have the greatest synergy with PsA to increase disease burden?

A: Dr Behrens responded that underestimation and undertreatment of moderate PsA leads to a cumulative disease burden. Inappropriate treatment may increase the risk for other comorbidities. Dr Siebert added that enthesitis seems to have a greater impact than other manifestations on patients' function.

Closing Remarks

Dr Queiro-Silva asked the speakers to sum up how close they think clinicians are to being able to tailor a therapeutic approach to individual patients.

Dr Siebert reiterated that clinicians need a lot of different therapies to choose from for patients with moderate PsA. There are tools available to help clinicians; patients with moderate disease should not be allowed to drift.

Prof Gladman said that clinicians are already trying to use a personalised approach, but that it would be nice to have a biomarker or an algorithm that would help clinicians define that patient's treatment pathway. This could happen in the next 5–10 years.

Dr Behrens stated that there have been many disappointments with biomarkers in other diseases, such as rheumatoid arthritis, and that is it unlikely that researchers will identify just a single biomarker that will give a clear picture. There are a lot of technologies being used, such as genomics and proteomics, but the information obtained has not been integrated. Artificial intelligence may provide a way forward with this. Dr Behrens estimated that we are 5–10 years away from completing the research, but perhaps 20 years away from having something tangible that can help clinicians in daily practice.

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Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: Achieving and Sustaining Remission while Reducing Organ Damage

Interviews with Three Key Opinion Leaders

Interviewees: Annette Bruchfeld,[1] Kirsten de Groot,[2] David Jayne[3]

1. Department of Nephrology, Karolinska University Hospital, Sweden
2. Department of Internal Medicine, Nephrology and Rheumatology, Medical Clinic III, Sana Klinikum Offenbach, Offenbach am Main, Germany
3. Lupus and Vasculitis Unit, Addenbrooke's Hospital, Cambridge, UK

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Due to advances in treatment, antineutrophil cytoplasmic antibody-associated vasculitis (AAV) is no longer a universally fatal condition; however, difficulties remain in managing its chronic relapsing-remitting course. Current standard of care[1] aims to control the multi-system damaging vasculitis but exposes patients to the risk of severe treatment toxicities in the short and long-term, particularly from high-dose or prolonged steroid use. Moreover, a lack of knowledge around disease recognition in real-world clinical practice often impedes patient access to the required specialist care. In this article, three experts in the field of AAV, Prof Annette Bruchfeld, Prof Kirsten de Groot, and Prof David Jayne, offer their views on the current status of disease assessment and management. In a series of interviews conducted by the European Medical Journal in June and July 2019, the experts identified present challenges and future goals, and discussed the impact of remission and relapse on patients with AAV. In particular, they voiced their concerns over the clinical risks of therapy versus sustained disease control and suggested how improvements in healthcare services and communication could transform patient care.

CURRENT CHALLENGES

The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are a group of rare inflammatory diseases including microscopic polyangiitis, granulomatosis with polyangiitis (previously known as Wegener's granulomatosis), and eosinophilic granulomatosis with polyangiitis (previously known as Churg–Strauss syndrome).[2] AAV can cause inflammation and damage to small blood vessels throughout the body, with the kidneys, ears, nose, sinuses, lungs, and skin being most commonly affected. Although treatments are available to induce remission of AAV, the current standard of care, typically involving immunosuppressants and high-dose steroids,[1] is associated with significant clinical risk.

Prof Bruchfeld commented on the ideals of providing effective treatment for AAV, while limiting the patient's exposure to severely toxic side effects. "The goal would be to deliver effective treatment early, with medications that are less toxic than those we have today. We also need to find a way to reduce the number of relapses which, in time, would serve to reduce organ damage."

"Maintenance of remission is the major challenge," agreed Prof de Groot, "Especially for patients who are at high risk of relapse – for those patients, we don't have so many options. Also, we don't currently have treatment-free long-term remission." Identifying patients at greatest risk of relapse was seen as a key area for improvement, with potential stratifying parameters including renal involvement; ANCA target proteins (e.g., myeloperoxidase versus proteinase 3); granulomatosis versus vasculitic disease; persistent microhaematuria; and ANCA conversion at remission.

In Prof Jayne's view, two further main issues at present are access to expert advice, and delays in diagnosis. "Unless they have very characteristic presentations, rare diseases usually result in diagnostic delay. This is compounded by the fact that AAV can present in many different ways, with different parts of the body involved, and it often takes 3–6 months before the diagnosis is made. Also, general practitioners (GP), to whom patients first present, don't tend to do the necessary tests, which is inevitable with a rare disease." Prof Bruchfeld and Prof de Groot concurred, emphasising the importance of physician awareness and education around AAV. Although the situation was said to have improved notably over the last 20 years, educating physicians (especially primary care doctors) to recognise the signs of the disease would further facilitate patient referral to specialist centres for diagnosis and management. "This is very important. The nephrology and rheumatology communities know the disease well now, but it needs someone to send the patient to these specialists, and that person is not so well educated in terms of AAV. We have to transfer our knowledge," said Prof de Groot.

CLINICAL ASSESSMENT

Whilst clinical trial endpoints for assessment of AAV are well described, the means of evaluating disease in the clinic are less clearly defined. The experts highlighted what they felt should be best practice in this area.

Prof Jayne outlined the two typical types of patient presentation: firstly, the patient who is admitted to hospital as an emergency, usually with either kidney or severe lung disease as a presenting feature, and who is diagnosed in hospital; secondly, there is the non-emergency patient who is referred to hospital, typically without a particular diagnosis but with a set of symptoms that are subsequently diagnosed by the hospital as AAV. "The key threshold is suspicion," commented Prof Jayne, and once the physician suspects AAV, the experts agreed that the actual assessment process is fairly standard, involving: X-rays, computed tomography (CT) scans, blood and urine tests, plus other investigations related to the main manifestations of disease, for example renal biopsies. "The ANCA blood test is of particular importance," commented Prof Jayne. "It is positive in around 95% of patients, relatively specific for AAV, and widely available. If the ANCA test is positive and the patient has symptoms suggestive of AAV that essentially confirms the diagnosis," he explained.

Prof de Groot emphasised the importance of an interdisciplinary team in achieving the full clinical assessment. In addition to the treating specialist (nephrologist/rheumatologist), there may be need for a radiologist, neurologist, and likely also an ENT doctor, pulmonologist, and

ophthalmologist. "It is quite a challenge to establish a stable team with experience of the disease," she commented. Prof Bruchfeld added that, in her view, patients should be at the heart of the assessment process. "Often the patient is forgotten. Doctors don't always understand that patients need the best information possible so that they are clear about their disease status and long-term prognosis. For example, if the patient has a lot of chronic renal damage, it is important they understand that the disease can be stabilised but, in the future, they may need to start dialysis," she clarified.

Prof Bruchfeld also pointed out that many of her patients at the University Hospital are enrolled in clinical and surveillance studies and that, in this environment, the use of rating scales is part of the standard patient assessment. She cited the Birmingham Vasculitis Activity Scale (BVAS)[3] as a helpful tool for following disease activity and detecting remission but commented that it is not often used outside clinical research, although she frequently uses BVAS as an educational tool and also advocates its use in smaller centres. While Prof Jayne doesn't believe these scales are suited to routine clinical practice, he agreed that BVAS is a useful tool for education and training, helping physicians to recognise and understand the range of disease manifestations.

Determining Remission

Current European guidelines[1] consider treatment for AAV in terms of remission induction and maintenance therapy, and subsequently, treatment of relapse. This raises the issue of how remission should be defined or recognised, in order to direct ongoing treatment.

"It is important to have a clear picture of when the patient is in remission, so to minimise exposure to the toxicities of initial treatment," said Prof Bruchfeld. Together with Prof de Groot, she advised that a BVAS score of zero is often the predefined target for remission, along with clinical observations that vessel inflammation has ceased in all organ systems. In Prof Jayne's view, assessment most often relies upon pattern recognition and experience. "Each organ system needs to be considered in turn, in order to work out whether persistent symptoms/abnormalities reflect ongoing disease in those areas of the body – and this is part of the skill of managing AAV," he explained. "Tests such as inflammatory markers (CRP [C-reactive protein], ESR [erythrocyte sedimentation rate], etc.) have a certain value, but they also have problems. Can we rely on them? What does it mean if the ANCA test is negative? Then there are patient symptoms that don't necessarily relate to AAV activity. It is a complex picture."

Prof de Groot further articulated these difficulties: "The more precisely you look, the less the patient appears in remission. For example, if you do an MRI of the sinuses every 3 months, you will see that the sinusitis never disappears, so you can never say that the patient is in remission. On the other hand, if you don't do these detailed assessments/radiology exams, then it's clinical judgement alone and the patient is more likely to appear in remission. So we really need to define whether we want to use CT and MRI as remission parameters or just keep to clinical tests and BVAS score." Prof Jayne agreed, pointing out that the division of remission induction and maintenance therapy is slightly artificial. "There's quite a lot of evidence to suggest that the disease is continuing, even though we're labelling people as being in remission. A lot of this has been driven historically by the desire to limit the use of cyclophosphamide (a standard immunosuppressant treatment for AAV) to only 3–6 months because it's a toxic drug – and so the remission induction period has been labelled accordingly. The bottom line is that relatively inexperienced centres may over-treat patients while more experienced centres may be better at determining remission and switching patients to maintenance therapy," he concluded.

Concerning the long-term impact of assessing and achieving remission, Prof de Groot stated, "If a patient is judged as not being completely in remission then they remain on induction treatment for longer. If induction of remission takes >3–6 months, we know that the outcome is worse; so achievement of full remission, rather than partial, within 3–6 months is important." It was also acknowledged that some patients recover more quickly than others, and the experts concurred that the greatest benefits of prompt symptom control are less organ damage and less exposure to the toxicities of treatment (steroids, in particular). Prof Jayne commented, "Steroid doses tend to be tapered along with improved control of the disease, so increased exposure is a

consequence of delayed disease control. Further to this, the organ we are most interested in is the kidney, and we know that we control kidney disease quite slowly, over months. If there was a treatment that could control kidney disease within days, this would have a long-term protective effect on organ function."

RELAPSE – TREATMENT AND IMPACT

Relapse is common in patients following conventional immunosuppressive therapy. After treatment has stopped, the experts estimated that by 2 years, 20–30% of patients will have relapsed, rising to 50–70% of patients within 5–10 years. "Those figures would be higher if you stopped the treatment earlier, and lower if you continued the treatment for longer," said Prof Jayne. It was explained that the rate of relapse also varies according to diagnosis, with granulomatosis with polyangiitis patients more prone to relapse than patients with microscopic polyangiitis, for example. In addition, it is important to distinguish the relapse rates achieved with conventional therapy (as above) from those observed more recently with rituximab, which postpones relapse further. Prof Bruchfeld commented that "we also have to be aware that patients can relapse beyond 10 years. This is why we continue specialist monitoring without fully discharging patients to GP care. It gives a better chance of recognising relapse, and we educate patients to contact us if they have specific symptoms that could indicate relapse." Prof Bruchfeld feels that this monitoring practice is likely to continue for the foreseeable future. "There may well be a group of patients who will have this disease once and never again, but we don't yet know who they are," she said.

The impact of relapse is significant, exposing patients to the risk of further organ damage and greater burden of medication and associated side effects. "About a third of relapses are what we would consider major relapses, which have long-term consequences in terms of mortality risk, and risk of end-stage renal disease," said Prof Jayne. "In contrast, minor relapses don't tend to result in significant organ damage, but they cause patient distress, making them feel unwell and, perhaps most critically, they commit the patient to a lot more treatment and associated toxicity. This is not only due to treating the relapse, but also because the physician is a lot more reluctant to stop the drugs in the future, and patients can remain on treatment for years." Prof Jayne outlined how these more and less severe states of relapse could present in practice: "For example, if you had a patient with known renal involvement, and they had a return of blood and protein in the urine, but with unchanged blood tests, that relapse could be regarded as relatively non-severe because you know it can be controlled and the patient hasn't suffered. In contrast, if your patient with renal disease presents with a relapse in which their renal function has seriously declined, you know that they are going to struggle to recover again, and the consequences from this relapse will be much worse."

Distinguishing between major and minor states of relapse was said to be difficult and could also be seen as an arbitrary divide in what is essentially a continuum of disease. Prof Jayne commented, "The reality is that minor relapses tend to be followed by other minor relapses or major relapses. Any relapse is a bad thing, and minor relapses are often major relapses just picked up early." Prof Bruchfeld concurred, "Deciding how to treat more minor relapses is problematic, as it is uncertain whether a minor relapse will precede a major relapse." However, she also pointed out that in clinical study centres, the major/minor divide is often defined using BVAS scoring, with major relapse being a more severe manifestation of disease that would, in turn, require more aggressive treatment. Prof de Groot added, "It is the difference between whether you reintroduce steroids at a low dose or whether you do a full re-induction treatment with more steroids and probably a more potent immunosuppressant. There is a difference, in terms of severity of disease, in terms of damage accrual, and in terms of need for a more potent immunosuppressant."

The experts confirmed that in patients without contraindicatory comorbidities (e.g., diabetes, osteoporosis) minor relapses are routinely treated by increasing steroid dose: "Even though there are strong data showing that this is not a good approach, and that almost all relapses treated in this way will be followed by further relapses," said Prof Jayne. "Rheumatologists, especially, spend their lives inventing therapies to enable us to avoid the use of steroids, but at the moment we don't have a better concept," added

Prof de Groot. Prof Bruchfeld agreed, "In general, I think most vasculitis doctors would like to find a way out of using so many steroids, because we know that the short and long-term side effects are not good for the patient." As a consequence, said Prof Jayne, there is a move towards the routine use of rituximab as an immunosuppressant treatment for all patients with relapse, whether deemed minor or major. He added, "Steroids are a major driver of long-term damage, so if you look 5–10 years on, about 50% of patients will have an aspect of steroid-induced damage, and that is directly related to relapse driving continuation of steroid dosing."

ADVERSE EVENTS OF THERAPY

Considering the clinical risks of therapy for AAV, infection was said to be the major problem in the short term (first year), strongly correlated to immunosuppression and steroid use. It is a predictor of early death, and the risk of infection is raised in patients who are older, have renal failure, and/or display comorbidities. Although other short-term issues such as steroid-dependent diabetes were noted, the experts were clear that infections dominate in the short term and remain a risk as long as steroid treatment continues. Prof de Groot clarified: "Mortality is high in the first year of treatment. Almost 11% of patients die within the first year, and half of them die from infections – very few die from active disease. This illustrates the issue of (probable) overtreatment within the first year, putting patients at risk of dying from the side effects of treatment."

In terms of more long-term adverse events, Prof Jayne believes that they should be generally viewed as being in one of two groups: either immunosuppressant-related or steroid-related. "Conventional immunosuppressive treatment (cyclophosphamide followed by oral immunosuppressants) is associated with an approximate 3-fold increase in malignancies (particularly skin malignancies) and, in younger patients, fertility problems due to cyclophosphamide," he said. Concerning steroid-related effects, Prof de Groot stated, "We know that every gram of steroids adds to the accrual of damage, and there are a lot of side effects from steroids, like diabetes, osteoporosis, weight gain, cataracts, thinning of the skin; and with every steroid course you add to these side effects."

Another long-term risk of the current standard of care is cardiovascular disease, as outlined by Prof Bruchfeld: "Many of these patients are older and may, due to vasculitis, have hypertension, reduced renal function, or residual proteinuria, all of which increase the risk of cardiovascular disease. So, long-term, we often face a cardiovascular quandary which is important, but difficult to prevent."

Among the many adverse effects, Prof Jayne thinks that the biggest concern for physicians is any event that leads to hospital readmission. This is most likely to be related to an infection, a cardiovascular or thromboembolic event, or symptoms of major relapse/inability to control the disease. Another specific concern is patients having to enter dialysis, said Prof Bruchfeld. However, she believes that this can be an abstract concept to patients, who are themselves likely to be more immediately concerned about steroid-related adverse events and fatigue: that is, aspects which affect their daily life. Prof Jayne agrees that patients simply want to feel better and to survive. "At the outset, patients aren't often made aware of the long-term complications of the disease. Usually, their concerns are functional; they want to be back at work, they want to be looking after their children, and they want relief from symptoms," he explained. In addition, they are concerned about the use of steroids: "Patients hate steroids," acknowledged Prof de Groot. "They know they work well and act very quickly but, longer term, higher dose steroids are disliked not only by doctors, but by patients in particular."

The ability to sustain remission while limiting adverse events is key to the current and future treatment of AAV. The best means of achieving this depends upon the patient profile but there was general agreement that, for a relapsing patient, an immunosuppressant regimen with rituximab dosed just once or twice a year, avoiding (or at very least tapering) steroids and other oral medications, is the best option at present. Prof de Groot added, "Very often we have a steroid-free long-term remission, but there is still continuous use of immunosuppressants. Treatment-free remission, which is what we would wish to have, is not yet available."

LOOKING AHEAD

Considering the future of treatment for AAV, the experts identified several key targets. Prof Jayne listed two main goals: to control the disease more quickly, and to reduce the risk of relapse (essentially curing the disease), giving the patient confidence that there will be no need for further treatment. Furthermore, he believes that better organisation of healthcare systems and resources is another major unmet need. "The reality is that most patients with AAV are treated by generalists, and not vasculitis specialists. So inevitably, there is going to be variability in quality of care. Countries that have made efforts to improve these healthcare services, particularly France, have demonstrated that this directly improves long-term outcomes for patients," he said.

Prof Bruchfeld believes that there should be greater investment in patient concerns; for example, related to treatment toxicities and fatigue. "Fatigue is a continual side effect of the disease and its treatment. If it's from the treatment we should change it, so that the patient can live a more normal life. Taking more interest in patient concerns will give us the incentive to develop their treatment in different ways, and not just focus on inflammation," she explained. Prof Jayne reiterated that patients also need better access to advice, both during the route to diagnosis (which is often long and delayed), and once diagnosis is confirmed. "What I hear about, on almost a daily basis, is patients struggling to access advice in which they feel confident," he noted.

Steroid-free treatment was highlighted by Prof de Groot as another critical need, in addition to immunosuppressant-free long-term remission. "Steroids are something that, in 20 years of vasculitis research, we cannot renounce," she said, "We still can't go without steroids."

CONCLUSION

Prof Bruchfeld, Prof de Groot, and Prof Jayne clearly highlighted the many existing issues in the management of AAV, largely centring on the lack of disease awareness in primary care, the maintenance of remission, and the toxicities of existing treatment (high-dose steroids in particular). Long-term use of steroids and continued immunosuppressant treatment through remission were cited as specific problems. Resulting effects such as infection and organ or tissue damage are strongly associated with a poor prognosis and a raised mortality risk in this complex condition. They represent a key focus for the development of future therapies, alongside a need for improved access to specialist interdisciplinary healthcare services. In addition, the experts felt that greater focus on patient concerns was needed. Indeed, keeping patients central to the management of disease was cited as an overall priority, in order to help manage expectations, deliver trusted advice, and improve daily life for those living with AAV.

Biographies

Prof Annette Bruchfeld

Karolinska University Hospital, Sweden

Prof Bruchfeld received her MD at Karolinska Institutet, Stockholm, Sweden, and is currently a Senior Consultant and Associate Professor in the Renal Medicine Department at Karolinska University Hospital. Her clinical and research interests are mainly in inflammatory kidney diseases, ANCA-associated vasculitis, and hepatitis C in chronic kidney disease and transplantation. She has extensive experience in conducting academic and sponsored clinical trials in these areas. Prof Bruchfeld is a long-standing member of the European Vasculitis Society (EUVAS), and is currently a member of the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) council and a board member of the Immunonephrology Working Group and the ERA-EDTA Scientific Advisory Board.

Prof Kirsten de Groot

Medical Clinic III (Internal Medicine, Nephrology, Rheumatology), Sana Klinikum Offenbach, Germany

Prof de Groot is a trained internist, rheumatologist, and nephrologist, who worked in the Universities of Lübeck and Hannover, Germany, before becoming Head of the Department of Nephrology and Rheumatology at Sana Klinikum Offenbach, the largest teaching hospital of the University of Frankfurt. She is a specialist in systemic autoimmune diseases. The majority of her scientific career has been devoted to clinical research in the field of ANCA-associated vasculitis and clinical therapeutic trials for these diseases. She is a long-standing member of EUVAS.

Prof David Jayne

Lupus and Vasculitis Unit, Addenbrooke's Hospital, UK

Prof Jayne is a clinical scientist with over 30 years of drug development and clinical trials experience in rare autoimmune diseases. He is a professor in clinical autoimmunity at the University of Cambridge, with extensive experience in teaching and in translational research. He has been involved in the evaluation of over 20 therapeutic agents for the care of multi-system autoimmune diseases, as well as the optimisation of standard-of-care regimens, the construction of global collaborative research networks, and the reorganisation of healthcare delivery for complex immunologic disease. Prof Jayne is President and Founder of EUVAS, and acts as an advisor to governments, regulatory and research bodies, industry, and patient-led organisations.

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Systemic Sclerosis: The Role of YAP/TAZ in Disease Pathogenesis

EDITOR'S PICK

Our Editor's Pick for this edition of *EMJ 4.3* is the review paper by Walsh. Providing an original view on the potential contributions of YAP/TAZ to the development of systemic sclerosis, this article delineates whether altered expression of YAP/TAZ is involved in driving the disease pathology of systemic sclerosis or induced as a result of the disease itself. Hopefully, a better understanding of the association between YAP/TAZ and systemic sclerosis will lead to better future treatments.

We hope you enjoy reading this review as much as we did.

Authors: Thomas Walsh

Lancashire Teaching Hospitals NHS Foundation Trust, Preston, UK
Correspondence to tomjwalsh81@gmail.com

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Abstract

Systemic sclerosis (SSc) is a systemic autoimmune condition of unknown cause. Yes-Associated Protein/Tafazzin (YAP/TAZ) are transcriptional coactivators previously demonstrated to be involved in cellular stretch biology, and form the principal effector molecules of the Hippo signalling pathway. The association between YAP/TAZ and stretch is contingent upon their cytoplasmic localisation (with nuclear translocation, the cell adopts a relaxed state). The author weighs the evidence for a central role for YAP/TAZ signalling in scleroderma spanning the major clinical features of the condition. Several of the features unique to SSc are mediated by cytoplasmic localisation of YAP/TAZ, including the stretch phenotype (through binding to NF-2), arterial lumenal obliteration (through their binding to angiomotin), the promotion of hypergammaglobulinaemia (via feedback to the upstream Hippo signalling molecule Mammalian Ste20-like Kinase 1), and the induction of B-Lymphocyte-Induced Maturation Protein-1 leading to the adoption of Th2 lineage, prominent in SSc. One observes that the induction of the fibrotic phenotype of scleroderma is mediated through GLI1/GLI2 (the effector molecules of the Hedgehog pathway). GLI1/GLI2 are induced to reciprocally enter the nucleus when YAP/TAZ is intracytoplasmic. The latter explains the characteristically increased connective tissue growth factor 2 and endothelin-1 expression. In this article, the author references some examples of the role of YAP/TAZ in the biophysically similar condition nephrogenic systemic fibrosis and suggests a role of YAP/TAZ cytoplasmic sequestration in programmed cell death protein 1-ligand antagonist-induced scleroderma.

INTRODUCTION

Systemic sclerosis (SSc) is a multi-system connective tissue disease characterised by excessive fibrosis, microvasculopathy, and autoimmunity. The condition broadly spans two general phenotypes, limited and diffuse, each weighted toward a characteristic but somewhat overlapping antibody and organ involvement profile.

While there has been no shortage of contributory pathogenetic component parts identified to date, a cohesive cell biological explanatory model has been elusive. In this paper, the author makes a case for Yes-Associated Protein/Tafazzin (YAP/TAZ) as the keystone in scleroderma pathogenesis.

YES-ASSOCIATED PROTEIN/TAFAZZIN AND THE CANONICAL HIPPO PATHWAY

The Hippo pathway in mammals is a kinase cascade in which the mammalian Ste20-like kinases 1/2 (MST1/2) phosphorylate and activate large tumour suppressor 1/2 (LATS1/2). This cascade regulates the activity of two transcriptional coactivators: YAP and a transcriptional coactivator with the PDZ-binding motif known as TAZ. When YAP and TAZ are active, they translocate into the nucleus to bind the transcription enhancer factor 1 (TEAD 1) DNA-binding domain and induce expression of a wide range of genes that are involved in cell proliferation and in regulation of organ size. Mammals express four different TEAD (TEAD 1-4). TEAD 1-4 are broadly expressed, but each TEAD has tissue specific expression, which indicates tissue specific roles for each TEAD.[1]

Active MST1/2 phosphorylate SAV1 and MOB1A/B, two scaffold proteins that assist MST1/2 in the recruitment and phosphorylation of LATS1/2. Phosphorylated LATS1/2, in turn, phosphorylate and inactivate YAP/TAZ (thereby confining it to the cytoplasm). Phosphorylation of YAP and TAZ leads to their binding with 14-3-3, causing cytoplasmic sequestration of YAP/TAZ.[2] LATS phosphorylation induces casein kinase 1δ/ε to phosphorylate YAP/TAZ and the recruitment of SCF-ubiquitin ligase which results in the ubiquitination and subsequent degradation of YAP/TAZ.[3]

As transcriptional coactivators, YAP/TAZ do not have DNA binding domains, but, upon nuclear translocation, they regulate gene expression through interaction with TEAD 1–4. TEAD 1–4 can bind to vestigial-like family member 4 (VGLL4) in the nucleus and thus function as transcriptional repressors.

The interaction between YAP/TAZ and TEAD 1-4 dissociates VGLL4 from the latter and thereby activates TEAD-mediated gene transcription to promote tissue growth and inhibit apoptosis (Figure 1).[4]

YES-ASSOCIATED PROTEIN/TAFAZZIN AND THE STRETCH-PHENOTYPE

Upstream of the Hippo pathway are Merlin, KIBRA, and other cell adhesion molecules and polarity-regulating proteins that localise to cell–cell contact points (e.g., adherens junctions). Cell-to-cell junctions are crucial in the regulation of growth dependent control of proliferation.[5,6] Merlin physically interacts with YAP/TAZ and suppresses its nuclear localisation. Cell density normally regulates YAP/TAZ nuclear localisation, thereby controlling cellular proliferation. Mechanical tension suppresses YAP/TAZ nuclear localisation via Merlin-induced nuclear export which occurs when it dissociates from the adherens junction, rendering a highly tense actin belt within the cell. Thus, the cell exhibits a tense phenotype wherein proliferation is inhibited.[7] Cytoplasmic relocalisation of YAP reduces the expression levels of cyclin A, cyclin B, and CDK1 genes, both *in vitro* and *in vivo*.[8] This would be anticipated to prevent progression to completion of mitosis (Figure 2).

TAZ is a component of the Wnt signalling cascade and mediates Wnt biological responses.[9] When Wnt signalling is turned off, YAP/TAZ are sequestered in the β-catenin destruction complex; i.e., preventing nuclear localisation.[10]

RECIPROCAL EXPRESSION OF HEDGEHOG PATHWAY COMPONENTS

Another consequence of non-nuclear YAP/TAZ is enhanced expression of GLI1/GLI2 (effector molecules of the Hedgehog [Hh] pathway).[11] Notably, this phenomenon was demonstrated under hypoxic conditions.

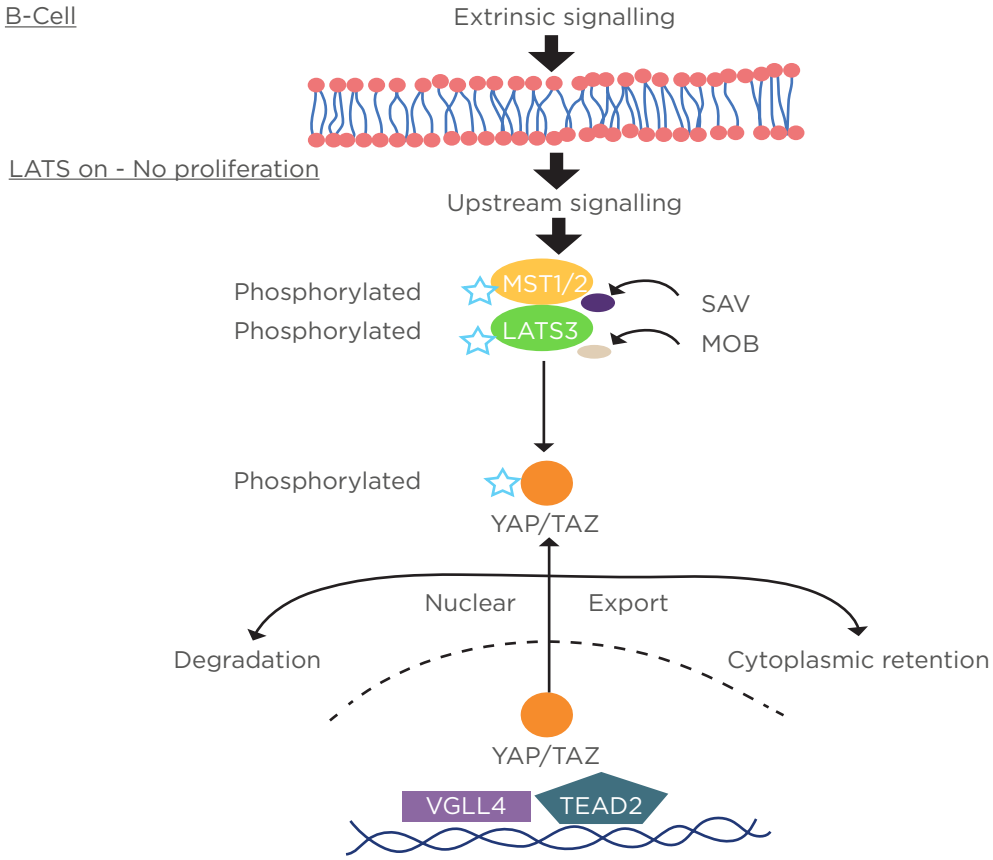


Figure 1: Hippo signalling in the B-cell–YAP/TAZ is intracytoplasmic when large tumour suppressor signalling is on.

LATS: large tumour suppressor 1/2; LATS3: large tumour suppressor 3; MST1/2: mammalian Ste20-like kinases 1/2; TEAD 2: transcription enhancer factor 2; VGLL4: vestigial-like family member 4; YAP/TAZ: yes-associated protein/tafazzin.

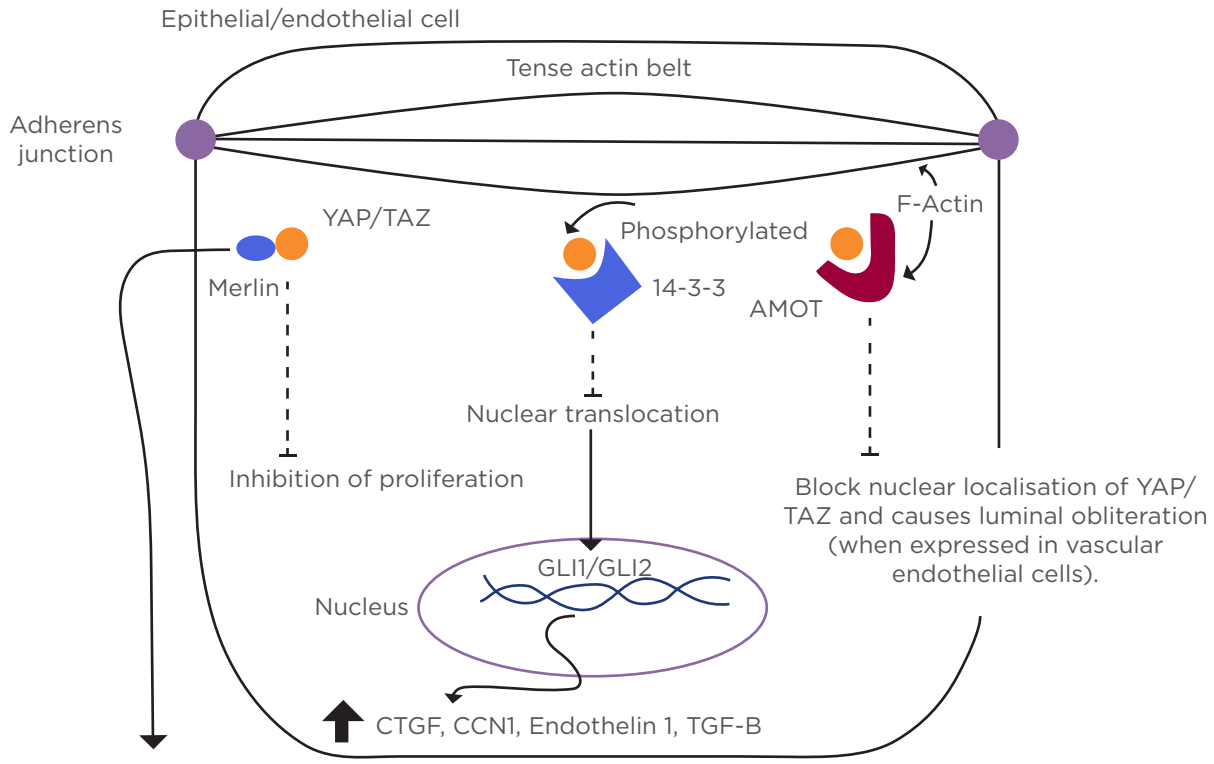
However, there is severely reduced oxygen tension in the skin of SSc patients with resultant over-expression of oxygen-regulated pathways.[12] GLI1/GLI2 expression abrogates activator protein 1 (AP1) activation.[13] Cytoplasmic YAP/TAZ regulates SMAD3/TGFß signalling via induction of SMAD7; however, this mechanism relies upon the AP1 transcription factor.[14] Thus, it can be inferred that this SMAD3/TGFß axis is not necessarily active secondary to cytoplasmic YAP/TAZ per se. Instead, this activity may be mediated through functions of GLI1/GLI2.

Prominent over-expression of sonic hedgehog (SHH) was detected in fibroblasts of endothelial cells and keratinocytes of fibrotic skin from scleroderma patients. Expression of GLI2 was increased in the skin of patients with SSc compared with healthy controls. GLI1 was enhanced to a lesser extent. There was also evidence of prominent accumulation of GLI1/GLI2 in myofibroblasts.[12]

TGFß has been shown to induce transcription of GLI-2 independent of Hh proteins such as SHH, Patched (PTCH), and Smoothened (Smo). TGFß activates Hh signalling in a canonical manner via SMAD3-dependent pathways. In fact, GANT61 (a GLI2 inhibitor) ameliorated fibrosis in SSc through reduction in TGFß levels and Hh target genes.[15] Hh inhibitors appear to be vulnerable to being overcome by copious IL-6 production from M2 macrophages.[16]

CCN MATRICELLULAR PROTEIN EXPRESSION

With reduced nuclear localisation of YAP/TAZ, there is reduced expression of connective tissue growth factor (CTGF or CCN2) and cysteine-rich angiogenic inducer-61 (CYR61, also known as CCN1). The CCN proteins are a family of extracellular matrix-associated proteins involved in intercellular signalling.[17,18] CCN1 regulates



1) Circumferential actin belt suppresses YAP/TAZ nuclear translocation.
2) Merlin physically interacts ith YAP/TAZ to prevent its nuclear translocation by virtue of the fact that merlin nuclear export signal domains are required for YAP/TAZ translocation.

Figure 2: Mechanisms of intracytoplasmic localisation of YAP/TAZ and the resultant cytokine profile.

AMOT: angiomotin; CCN1: cysteine rich angiogenic inducer-61; CTGF: connective tissue growth factor; GLI1/2: glioma-associated oncogene 1/2; TGF-β; transforming growth factor-β; YAP/TAZ: yes-associated protein/tafazzin.

Adapted from Furukawa et al.[7]

keratinocyte growth and survival, as well as promoting angiogenesis, whereas CCN2 likely mediates alterations in the stromal extracellular matrix microenvironment.[19]

CCN1 levels diminish in avascular areas and with vaso-obliteration, whereas CTGF is induced under hypoxic conditions.[20] The dearth of CCN1 expression and over-expression of CTGF/CCN2 in SSc would appear to be not entirely in keeping with YAP/TAZ cytoplasmic localisation given that presumably this results in reduced expression of both secretory proteins.[17]

GLI1/GLI2 are downstream mediators of TGFß (as well as effectors of TGFß transcription) and they are known to enhance CTGF/CCN2 expression,[21] perhaps thereby addressing the reason for the apparently jarring finding that cytoplasmic localisation of YAP/TAZ suppresses both CCN1 (known to be suppressed in scleroderma) and CTGF/CCN2 (which is over-expressed in the condition but suppressed by cytoplasmic localisation of YAP/TAZ per se). Furthermore, overactivity of GLI1/GLI2 in capillary endothelial cells leads to intimal hyperplasia, a characteristic small vessel finding in SSc.[22]

KRUPPEL-LIKE FACTOR 5 AND FRIEND LEUKAEMIA INTEGRATION 1 TRANSCRIPTION FACTOR

Kruppel-like factor 5 (KLF5) and friend leukaemia integration 1 transcription factor (Fli-1) have been demonstrated to be deficient in SSc.[23,24]

KLF5 is a transcriptional activator that binds directly to specific recognition motifs in the promoters of target genes. This protein acts downstream of multiple different signalling pathways and is transcriptionally stabilised by

YAP/TAZ. It may participate in both promoting and suppressing cell proliferation. Cytoplasmic (non-nuclear) YAP/TAZ promotes proteasomal destruction of KLF5.[25]

Fli-1 levels are decreased in sclerodermatous lesional and non-lesional skin compared with healthy controls. There is an inverse correlation between collagen expression and Fli-1 in healthy control skin.[26]

To date, there is no accountable correlation between deficiency of Fli-1 expression[23] and non-nuclear YAP/TAZ. However, the suppression of Fli-1 has been reproducibly demonstrated through a downstream effect of endothelin 1, the release of which is a downstream paracrine phenomenon induced by GLI1/GLI2 expression.[27,28] It should also be noted that Fli-1 deficiency may contribute to depressed CCN1 levels in scleroderma.[29]

As highlighted by Noda et al.,[30] simultaneous repression of both KLF5 and Fli-1 may be a molecular hallmark of SSc, ostensibly functioning synergistically in the pathogenesis of the fibrosis, vascular changes, and over-expression of CD19 that are characteristic of the disease.

FIBROSIS LOOPS

The Autotaxin (ATX)/lysophosphatidic acid (LPA)/IL-6 amplification loop is a driver of fibrosis in SSc, such that ATX mRNA expression was increased 2.6-fold in sclerodermatous skin as compared with healthy skin, and IL-6 expression was raised relative to the skin of controls. Sclerodermatous fibroblasts produced significantly more IL-6 in response to LPA than did control fibroblasts.[31] YAP/TAZ are effector agents for the activity of LPA via LATS kinase inhibition and therefore dephosphorylation promotion, thus localising YAP/TAZ to the nucleus.[32] The latter, in turn, appears to induce Merlin transcription and angiomotin accumulation, both of which will be inclined to sequester YAP/TAZ in the cytoplasm, as least in physiological circumstances.[33]

M2 MACROPHAGE POLARISATION

The signalling protein Wnt5a enhances TGFß1-mediated macrophage polarisation and kidney fibrosis by inducing nuclear expression of YAP/TAZ. Verteporfin-induced inhibition of YAP/TAZ was sufficient to block Wnt5a and TGFß M2 macrophage polarisation. As such, nuclear localisation of YAP/TAZ is crucial for establishing the M2 macrophage phenotype in SSc.[34] Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of IL-6 from M2 macrophages.[35] This effect would appear to be mediated through cAMP signalling. PDE4 inhibitors have all been shown to induce YAP phosphorylation. Thus, once again this would imply that YAP/TAZ are cytoplasmic (or not active) in SSc.[36] These ostensibly contradictory data point to separate simultaneous processes that result in a final common pathway.

NUCLEAR FACTOR ERYTHROID 2 RELATED FACTOR-2

Nuclear factor erythroid 2 related factor-2 (NRF-2) is a key cellular sensor of oxidative stress that can induce transcription of cytoprotective genes (such as glutathione), thus protecting cells from excessive oxidative stress. Given that SSc patients' fibroblasts produce high amounts of reactive oxygen species that contribute to fibroblast activation and proliferation, as well as collagen synthesis,[37-39] and that the pathway is druggable, some experimental trials have been conducted. First, Wei et al.[40] showed that NRF-2 knockout mice displayed an exacerbated phenotype of bleomycin-induced SSc. Then, Toyama et al.[41] addressed therapeutic targeting of YAP/TAZ by dimethyl fumarate (a fumaric acid ester that augments the antioxidant response by enhancing the NFR-2 signalling pathway). They demonstrated damping of TGFß1-induced gene expression through reducing nuclear localisation of YAP/TAZ and PI3K/Akt pathway inhibition.

FIBROSIS IN SCLERODERMA INTERSTITIAL PNEUMONITIS

In idiopathic pulmonary fibrosis, it was shown that mechanosignalling through nuclear localisation of YAP/TAZ is a consistent factor driving fibroblast activation and fibrosis, albeit in a small sample size (n=4 and n=5, respectively).[42,43] In non-specific interstitial pneumonitis (more typical for scleroderma patients), Yeo et al.[44] demonstrated that there was a more dichotomous expression of TAZ, such that there was nuclear over-expression in cellular non-specific interstitial

pneumonitis (n=15) versus non-nuclear expression in fibrotic phenotypes (n=17). This may suggest a changing role for YAP/TAZ in the course of disease progression.

YES-ASSOCIATED PROTEIN/TAFAZZIN AND THE VASCULAR PHENOTYPE OF SYSTEMIC SCLEROSIS

It is notable that, at a microvascular level, the mechanotransductive properties of vascular shear stress on the endothelium results in the nuclear localisation of YAP/TAZ. In fact, the latter appears to be required for maintenance of the vessel lumen. Angiomotin and its family of proteins physically bind YAP and sequester it in the cytoplasm. Cytoplasmic relocalisation of YAP/TAZ (in this context) results in failure of lumen maintenance (i.e., obliteration of the lumen). This is a property that may at least in part be responsible for the microangiopathy component of SSc,[45] perhaps borne out pathologically as capillary dropout.[45-47]

As previously discussed, suppression of CCN1 and KLF5, as well as the indirect effect of endothelin-1[48] (induced by elevated GLI2 expression), contribute to the vascular pathology witnessed in SSc.

YES-ASSOCIATED PROTEIN/TAFAZZIN AND THE METABOLIC PROFILE OF SYSTEMIC SCLEROSIS

The fibrotic state promotes a glycolytic pathway of cellular metabolism. An enzymatically active pool of phosphofructokinase-1 that binds TEAD 1–4 and fosters YAP/TAZ activity (demonstrated in malignant and non-malignant cell lines). Phosphofructokinase-1 stabilises YAP/TAZ interaction with TEAD 1–4.[49]

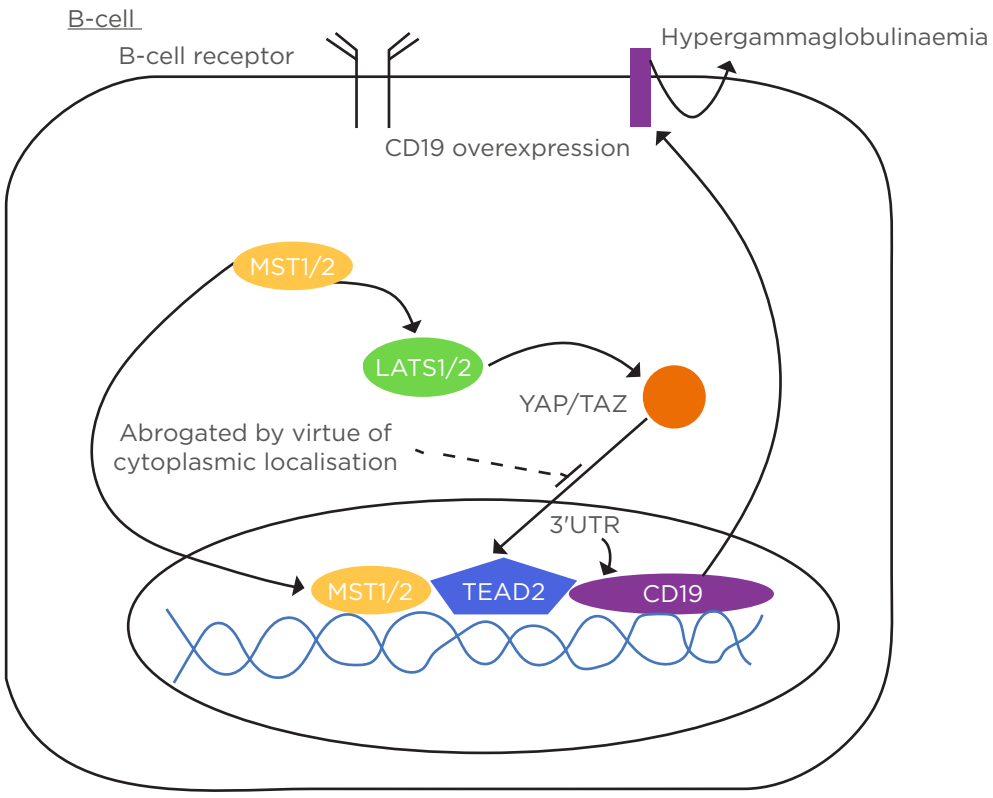


Figure 3: B-cell demonstrating the promotion of CD19 via MST1 occurring in the context of intracytoplasmic YAP/TAZ.

3′UTR: 3′ prime untranslated region; LATS1/2: large tumour suppressor 1/2; MST1/2: mammalian Ste20-like kinases 1/2; TEAD2: transcription enhancer factor 2; YAP/TAZ: yes-associated protein/tafazzin.

TEAD 4 directly targets peroxisome proliferator-activated receptor (PPAR)-γ activity and acts as a regulator in the early stage of adipogenesis. In adipogenesis, TEAD 4 is dramatically increased and binds to promoters of adipogenic genes. TEAD 4, together with its cofactors VGLL4 and C-terminal binding protein-2 (CTBP2), negatively regulate adipogenesis. This ternary complex inhibits adipogenesis and PPAR-γ promoter activity.

VGLL4 is a competitive antagonist of YAP/TAZ at TEAD 4. Both adipogenesis and PPAR-γ expression appear to be curtailed in SSc, once again insinuating cytoplasmic localisation of YAP/TAZ.[50,51] Lats2, which supresses preadipocyte proliferation (characteristic for SSc), enhances the phosphorylation and cytoplasmic accumulation of YAP/TAZ. Notably, PPAR-γ is also induced by KLF5, the expression of which is stimulated by C/EBPß.[52] Korman et al.[53] demonstrated that pharmacological restoration of PPAR-γ signalling may help to control skin fibrosis in SSc.

YES-ASSOCIATED PROTEIN/TAFAZZIN AND THE IMMUNOLOGICAL PROFILE OF SYSTEMIC SCLEROSIS

CD19 is approximately 20% over-expressed in scleroderma. CD19 is a potent positive regulator of B-cell function. It potentiates signals from the B-cell receptor, which results in PI3K activation and subsequent Akt phosphorylation.[54-56] CD19 is a driver of B-cell activating factor, which has been shown to be over-expressed in scleroderma.[57] Furthermore, the expression of proinflammatory adipokines, such as IL-6, MCP-1, COX-2, and haptoglobin, was highly increased in B-cell activating factor-treated 3T3-L1 adipocytes, while the level of adiponectin was grossly reduced.[58]

MST1 (a component of the canonical Hippo signalling pathway) positively regulates B-cell receptor signalling via regulation of CD19 transcriptional levels.[59] YAP/TAZ act downstream from MST1. When MST1 signalling is activated, YAP/TAZ are deactivated and/or located in the cytoplasm. MST1 moves to the nucleus and binds to TEAD 2. TEAD 2 is the specific subtype of TEAD expressed predominantly in B-cells. In binding to TEAD 2, there is interaction with the 3' UTR of CD19 promoting CD19 transcription andconsequent translocation to the B-cell membrane. This, in turn, contributes to hypergammaglobulinaemia and autoantibody expression (Figure 3).

MST-1 is fundamental to T-lymphocyte function from thymocyte migration and antigen recognition, through to shaping the adaptive immune response.[60]

The degradation of YAP contributes to higher expression of B-lymphocyte-induced maturation protein-1 (BLIMP-1), which mediates the terminal differentiation of CD8+ T cells.[61] CTLA-4 activation regulates the expression of BLIMP-1 in CD8+ T-cells by activating (i.e., facilitating nuclear transfer) of YAP/TAZ,[62] a mechanism which may underlie the clinical efficacy of abatacept (a CTLA-4 agonist) in clinical trials of scleroderma treatment.[63] BLIMP-1 expression is mainly in activated T cells and is essential for the production of IL-10 by a subset of Foxp3+ regulatory T cells with effector phenotype.[64] Previous studies have demonstrated an important role for IL-10 in skin and pulmonary involvement in SSc.[65,66]

BLIMP-1 promotes Th2 cell differentiation.[67] Th2 cells secrete IL-4, IL-5, IL-9, and IL-13, and are the predominant T-cell phenotype in scleroderma.[68] Notably, Hh signals have also been shown to promote Th2 differentiation.[69]

In developing plasmablasts, BLIMP-1 is dispensable for IL-10 production (interferon regulatory factor 4 is necessary), and B cells lacking BLIMP-1 fail to fully differentiate into plasma cells.[70] Early expression of BLIMP-1 in B-cells in fact causes autoimmune disease by inducing an increase in self-reactive plasma cells.[71]

To date, the YAP/TAZ cascade does not explain the specific antibody profile of SSc patients (anti-topoisomerase, anti-centromeric antibodies, anti-RNAP-3). The common feature of these antibodies would appear to be the fact that these are both directed against cell-cycle progression elements. As such, these may constitute features of a response to induced cellular senescence.

YES-ASSOCIATED PROTEIN/TAFAZZIN IN SCLERODERMA: PHENOMENON OR EPIPHENOMENON?

Monocytes from African American SSc patients with interstitial lung disease have been shown

to have low levels of caveolin-1 leading to preferential fibrocyte differentiation.[72] Caveolin-1 and YAP are positively regulated (i.e, when YAP is intracytoplasmic, caveolin-1 expression is low).[73]

Nephrogenic systemic fibrosis is a condition that bears some similarity to scleroderma. The condition was first identified in 1997 among individuals on dialysis/renal patients who had exposure to gadolinium-based contrast agents used for MRI. The condition is characterised by tightening of the skin with resultant joint contractures. Pathologically, it differs from scleroderma insofar as there is a proliferation of fibroblasts and deposition of elastic fibres and thickened collagen bundles (closely resembling the pathology of scleromyxedema). The specificity of this finding is due to the relatively prolonged exposure to gadolinium (Gd3+) ions, due to the failure of the kidneys in these individuals to excrete the ions.[74-76]

Gd3+ binds to and inhibits PIEZO-1,[77] a cell-surface-expressed mechanotransductive (stretch activated) calcium channel critical to homeostasis of sheets of epithelial cells *in vivo*. Where stretch of epithelial cells occurs, as in a sheet of epithelial cells, to the extent of 1.6 psi (phi, or the golden ratio), PIEZO-1 transduces a signal that results in cell division.[78] In the absence of this signal, the sheet of epithelial cells enter a 'tense' state wherein they are unable to compensatorily divide sufficient to accommodate 'normal' movements.[77,79]

Specifically, to affect this state, PIEZO-1 activation has been demonstrated to cause decreased nuclear localisation of YAP/TAZ.[80] While the pathology of nephrogenic systemic fibrosis and SSc are not the same, both conditions are undeniably biophysically similar.

It has been demonstrated recently that the immune checkpoint inhibitor and PD1 antagonist, pembrolizumab, may induce a condition indistinguishable from scleroderma.[81] The significance of this is that when leucocyte-associated PD1 binds to the target cell-associated PD1 ligand (PD-L1), cytoplasmic YAP/TAZ acts downstream of the PD-L1 by translocating to the nucleus, binding TEAD, and thereby promoting further PD-L1 proliferation.[82,83] Notably, other pathways also promote PD-L1 proliferation by encouraging nuclear localisation of YAP/TAZ. By blocking the PD1/PD-L1 interaction, no downstream signal is sent to the cytoplasmic YAP/TAZ and thus one may reason that an excess of YAP/TAZ will remain intracytoplasmic. The consequence is that the phenotypic features of scleroderma are expressed. Further study of checkpoint inhibitor-induced scleroderma is necessary to confirm the role of YAP/TAZ in this phenomenon.

CONCLUSION

It remains difficult to parse the exact aetiology of SSc. Part of this difficulty is rendered by the substantial overlap in cytokine and kinase pathways. Fibrosis is a final common pathway of many inflammatory processes and, as such, looking through the prism of established disease distorts underlying causative mechanisms. There are some studies that imply or categorically argue in favour of nuclear localisation of YAP/TAZ in scleroderma. While this may be a consequence of other potent cytokine influences in established disease, this would not make logical sense initially since this would imply a 'relaxed' cellular phenotype with pro-proliferative tendency.

There is conflation too at the level of interpreting the reality of a 'stiff' microenvironment (as seen with a fibrotic extracellular matrix) and a 'stretched' cellular phenotype. The reality is that both coexist *in vivo* in scleroderma. This is a challenging model to recreate per se, let alone to evaluate its functional aberrancies in a diseased state.

There is a weight of evidence supporting a substantive role for hippo pathway mediators in SSc encompassing all the major features of the condition. Most features favour intra-cytoplasmic localisation of YAP/TAZ. There is apparent functional desynchrony of YAP/TAZ, GLI1/GLI2, Wnt pathyway components, TGFß, and intracellular oxidants (Piersma B et al.[84] and Korman B et al.[85] have provided excellent reviews on this subject). This ultimately results in a disease that is grossly refractory to current treatments or cures. Knowing this, careful focus should be applied simultaneously to pathway blockade mechanisms in disease treatment and to further elucidate aetiological mechanisms.

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Noninvasive Imaging Modalities in Nonalcoholic Fatty Liver Disease: Where Do We Stand?

Authors: Somaya Albhaisi

Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA
*Correspondence to somaya.albhaisi@vcuhealth.org

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. Liver biopsy is the gold standard for diagnosis and staging of fibrosis in patients with NAFLD; however, it is invasive, costly, and may be associated with morbidity and even mortality, so is not suitable for screening the large number of individuals who are at risk of, or have, NAFLD. Therefore, there has been tremendous focus on finding noninvasive diagnostic modalities, including imaging. New imaging modalities are emerging and may potentially replace biopsy. This review discusses the different noninvasive imaging modalities for the assessment of NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in developed countries.[1] It is defined as the presence of at least 5% of hepatic steatosis on histology or imaging in absence of significant alcohol use and other secondary causes of steatosis.[2] NAFLD has been clinically associated with metabolic disorders such as obesity, diabetes, and dyslipidaemia. It consists of a wide spectrum of clinico-pathologic presentations ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC).[3-6] The top three leading causes of death in patients with NAFLD, in descending order, are cardiovascular disease, cancer, and liver disease.[3] Therefore, early identification of this disease is paramount.

The gold standard for diagnosis of NASH is liver biopsy; however, this is invasive, costly, and risks complications.[7] Thus, biopsy is not practical for the screening or monitoring of NAFLD.[8,9] Noninvasive diagnostic techniques, such as serum biomarkers and imaging studies, have emerged. Imaging, in particular, has gained importance in the noninvasive diagnosis of hepatic steatosis.

IMAGING IN NAFLD/NASH

Ultrasonography

Ultrasonography is the most commonly used imaging modality for evaluating hepatic steatosis.

Ultrasound (US) is accepted as an initial screening for fatty liver because it is safe, widely available, well tolerated, and inexpensive.[10-13] It also plays

a key role in ruling out focal liver lesions and characterising them.[14] There are numerous sonographic features of steatosis, such as the 'echogenicity' of the liver relative to the adjacent right kidney, hepatomegaly, and blunting of liver structures. Recent studies suggest that fatty infiltration of the liver can change the Doppler waveform of the hepatic veins.[15,16] The degree of steatosis can be subjectively scored as mild, moderate, and severe, or, as reported in some studies, by using ordinal US scores.[17,18]

In a large meta-analysis of patients with suspected or known liver diseases, the reported sensitivity and specificity of US in distinguishing moderate-to-severe fatty liver from the absence of steatosis, was 85% (80–89%) and 93% (87–97%), respectively. Nevertheless, US lacks the sensitivity for detection of liver fat and is considered inaccurate in differentiating fibrosis from steatosis or quantifying the fat accumulation. US can only detect steatosis if the liver fat content is above 12.5–20.0%.[9] Another major weakness of US is its operator dependency. Numerous factors can affect the sonographic features besides hepatic steatosis, such as obesity, renal disease, equipment-related factors, operator dependency, and the qualitative interpretation. Consequently, US has limited accuracy, repeatability, and reproducibility for diagnosis and evaluation of the degree of hepatic steatosis.[20-23] Such limitations may be at least partially overcome by semi-quantitative indices, which are correlated with metabolic derangements and histological features in various liver diseases, notably including NAFLD both in adults and in children.[24,25] Despite its undisputed limitations, US remains a first-line option technique in the investigation of NAFLD.[26]

Computed Tomography

X-ray CT uses the density of liver to spleen ratio to detect hepatic steatosis. NAFLD is typically an incidental finding on CT that are being performed for another indication. CT has fallen out of favour for diagnosis of hepatic steatosis for multiple reasons, including exposure to ionising radiation and lack of accuracy and reliability, especially for the detection of small fractions of fatty infiltration.[27] Moreover, it has been demonstrated that CT attenuation values vary significantly between different manufacturers' scanners and image processing techniques.[28]

Box 1: Relative cost of current available non-invasive techniques for liver steatosis assessment.

Technique	Procedure cost
US	Low
CT	Fair
MRI	High
MRS	High

CT: computed tomography; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; US: ultrasonography.

Magnetic Resonance Imaging

Magnetic resonance (MR) spectroscopy (MRS) is reportedly the most accurate method for the quantification of steatosis,[29,30] but its use is currently limited to research. MRS may be better than histology in assessing longitudinal changes in liver fat content, and is also safe; however, it is expensive and not widely available (Box 1).[31]

Magnetic Resonance Elastography

MR elastography (MRE) is the MR equivalent of transient elastography that is considered among the final options to assess hepatic fibrosis in patients with NAFLD. It uses a modified phase-contrast method to image the propagation of the shear wave in the liver parenchyma. MRE has demonstrated excellent diagnostic accuracy and ability to exclude significant fibrosis. Studies have shown that MRE has a sensitivity and specificity of 98% and 99%, respectively, for detecting all grades of fibrosis.[32,33] When coupled with MRI, MRE can be helpful for the screening of HCC. Another advantage is that MRE accuracy is not affected by obesity or cirrhosis. Since the measured liver area is large on MRE, it can avoid potential sampling errors. On the other hand, MRE may be inaccurate in inflammatory conditions and iron overload. MRE may not be practical for routine screening of NAFLD patients because it is costly, time-consuming, and not readily available. The best indication for MRE may be in morbidly obese patients who fail US-based elastography or need detailed liver imaging.

Magnetic Resonance Spectroscopy

MR spectroscopy (MRS) is the gold standard for quantification of fat in the liver,[34] therefore it can accurately diagnose NAFLD.[35] MRS measures the chemical composition of tissue based on proton signals frequency. Most of the identifiable peaks are derived from water and fat, and the fat signal fraction, also known as proton density fat fraction (PDFF) can be calculated.[34,36] Therefore, MRS is considered the most sensitive and accurate noninvasive method of quantifying liver fat.[30,31,36] MRS has important limitations that preclude its widespread use.[37] MRS is time consuming, not readily available, and requires additional equipment and special expertise.

Vibration-Controlled Transient Elastography

Vibration-controlled transient elastography (VCTE), also known as Fibroscan® (Echosens, Paris, France), is the most commonly used elastography method.[38] VCTE is a noninvasive point-of-care method of assessing liver fibrosis by using an US-based technology for estimation of liver stiffness measurement (LSM).[39,40] VCTE was originally validated for use mainly in the setting of viral hepatitis.[41,42] Studies have shown robust VCTE quality criteria in patients with NAFLD, which include a minimum of 10 measurements that are used to obtain the median LSM and the interquartile range. Two probes are now available: the M-probe and the XL-probe. The latter probe has been introduced due to the high failure rate of VCTE in obese patients.[43,44] XL-probes possess a deeper focal length, increased amplitude, and lower shear wave frequency; therefore, they are more reliable in obese patients.[45] A multicentre prospective study by Siddiqui et al.[46] on NAFLD patients who underwent VCTE found that the diagnostic accuracy of VCTE in differentiating fibrosis stages was lower than previously reported by Tapper et al.[47]

Controlled Attenuation Parameter

The controlled attenuation parameter (CAP) is a novel tool for the assessment of hepatic steatosis available as an adjunct to VCTE.[48] Based on studies, CAP relies on an M-probe of Fibroscan; therefore, it shares the same limitations as VCTE.[43] The first study that assessed its performance in patients with chronic liver diseases has reported that CAP was able to accurately detect steatosis ≥11%, ≥33%, and ≥66% with an area under the receiver operating characteristic (AUROC) of 0.91, 0.95, and 0.89, respectively.[49] Nevertheless, a meta-analysis by Karlas et al.[50] suggested that CAP does not provide accurate reliable quantification of liver fat. Another meta-analysis of studies using the M-probe has suggested optimal cut-offs of 248 (237–261) dB/m, 268 (257–284) dB/m, and 280 (268–294) dB/m, respectively, for detection of steatosis.[51] Others have proposed an optimal cut-off of 288 dB/m.[52] The differences in proposed cut-offs can be explained by the variation in BMI and diabetes prevalence in heterogeneous populations, the use of M-probe, and the small sample size in most studies. A multicentre study in NAFLD patients using the XL-probe reported that CAP had an AUROC of 0.76 for detecting steatosis >5% and a 96% positive predictive value.[53] Only two studies have performed a head-to-head comparison of CAP with US, showing that the performance of CAP for detecting and grading liver steatosis was higher than that of US; however, the rate of overestimation was significantly higher for CAP than for US (30.5% versus 12.4%; $p<0.05$).[54] Overall, CAP is a useful technique for the rapid quantification of steatosis, but it still needs to be better validated with the XL-probe in patients with NAFLD.

Acoustic Resonance Forced Impulse Imaging and Shear Wave Elastography

Acoustic resonance forced impulse imaging (ARFI) is integrated into a conventional US device and relies on elastography to estimate the LSM in shear wave speed. Shear wave elastography (SWE) adapts US imaging to evaluate liver stiffness. SWE can perform measurements over a wide range of frequencies and regions and thereby reduce sampling errors. SWE may be considered a screening test for patients with mild fibrosis stages according to Cassinotto et al.[55] and Leung et al.;[56] however, further studies are needed to confirm its applicability to patients with NAFLD. In general, SWE and ARFI are more reliable compared to VCTE in the assessment of liver fibrosis, but the utility of their use in NAFLD is yet to be confirmed as data are currently limited. The quality criteria for the application of ARFI or SWE

are limited; thus, further studies are needed to establish those criteria and to define the role of ARFI and SWE in NAFLD so their readings can be standardised.

Discussion

US is not sensitive but is highly specific for detection of moderate-to-severe hepatic steatosis. MRI–PDFF/MRE is considered the gold standard to quantify liver fat due to its high diagnostic accuracy; however, it may not be routinely available and is expensive. It may be used when other tests fail and can otherwise be reserved for clinical studies. CAP readings can be highly reliable if the interquartile range is <30 db/m.[57] It becomes less accurate with a dynamic range of liver fat; therefore, it is not reliable in differentiating closely related steatosis stages.[42] CAP, when combined with VCTE, may be helpful in screening obese patients for NAFLD. Elastography has gained wide acceptance. The most validated imaging modality in NAFLD is VCTE, which can be performed as a point-of-care test. It is best used to exclude significant fibrosis; however, VCTE is less accurate for low stages of fibrosis. SWE or ARFI may be useful for risk stratification of patients with NAFLD. Imaging in NAFLD is an area of increasing research focus. Further studies are needed to evaluate and quantify the relationship between imaging modalities and clinical status in NAFLD.

Noninvasive imaging methods, together with serum-based biomarkers, can be used as part of targeted screening strategies for NAFLD in primary care settings to improve specialist referral. There is a need for an integrated management plan for NAFLD between primary and secondary care, with robust pathways for subsequent referrals. The absence of well-defined referral strategies can potentially result in missing a substantial proportion of the population at risk.[58]

CONCLUSION

The noninvasive assessment of NAFLD has progressed significantly. It is important to tailor the choice of noninvasive tests to the setting (primary care, tertiary referral centre, or clinical trial) and clinical needs (screening, staging of fibrosis, or follow-up). Although various imaging techniques are available, US remains the first line technique to be adopted in the evaluation of NAFLD. MRI–PDFF is the most accurate method for detection and grading of steatosis, but it is neither routinely available nor affordable, making it strictly used in research. Until now, there is no imaging modality that can reliably discriminate NASH from simple steatosis. Imaging can help with the identification of advanced fibrosis and, therefore, the appropriate referral for a liver biopsy. The combination of serum markers and liver stiffness, measured using transient elastography, can identify NAFLD patients at a high risk of liver-related complications.

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Scalp Sarcoidosis with Systemic Involvement: A Case Report and Literature Review

Authors: Michela Starace, Nicolò Brandi, Carlotta Baraldi, Bianca Maria Piraccini, *Aurora Alessandrini

Department of Experimental, Diagnostic and Specialty Medicine, Division of Dermatology, University of Bologna, Bologna, Italy
*Correspondence to aurora.alessandrini3@unibo.it

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Abstract

Scalp sarcoidosis is generally uncommon and it may present itself with varying morphologies; therefore, it is important to differentiate this disease from other forms of cicatricial and non-cicatricial causes of alopecia. Trichoscopy and histopathology are essential to rule out other skin diseases and to confirm diagnosis. Treatment options include topical, oral, and intralesional corticosteroids; immunosuppressive agents; and hydroxychloroquine, in order to arrest the progression of alopecia. Here, the authors present a case of scalp sarcoidosis with systemic involvement, in which dermoscopy gives important clues for its diagnosis. The authors have also reviewed the literature and identified 46 cases of sarcoidosis that induced alopecia.

INTRODUCTION

Sarcoidosis is a multisystem disease, of unknown aetiology, which can involve any organ of the body, even if it more frequently affects the lymph nodes, lungs, skin, and eyes. Scalp sarcoidosis is a rare localisation of cutaneous sarcoidosis and is usually associated with other skin lesions of the body, which can result in very different morphological presentations.[1,2] The clinical and dermoscopic findings of alopecia due to sarcoidosis have recently been detailed.[3] Here, the authors report a case of progressive scarring alopecia due to scalp sarcoidosis associated with stable lung involvement, where dermoscopy suggested the diagnosis of the scalp disease.

CASE REPORT

A 50-year-old Caucasian female was referred to the authors' Department of Dermatology with 2-years' history of alopecic patches of the scalp, which had always been small and stable but had gradually increased in size over the previous few months. The patient was affected by pulmonary sarcoidosis for around 20 years but at the time of presentation to the clinic was asymptomatic and had been followed-up without treatment for 10 years. The patient reported a negative QuantiFERON-TB test and a normal spirometry.

At the time of examination, the clinical manifestation of the scalp showed the presence

of several irregularly round and confluent patches of alopecia localised at the vertex that were red-orange in colour. Examination of the whole skin and mucosal surfaces of the patient revealed the presence of a small atrophic red-brown plaque on her back, measured to be 1 cm by 2 cm (Figure 1). Trichoscopy of the alopecic patches showed loss of follicular ostia, a reddish-orange discoloration of the skin, and capillary telangiectasias. Other dermoscopic findings included white cicatricial skin spots, brown dilated follicular ostia with emerging hairs at the periphery of the patches, and a few dystrophic hairs. Dermoscopy of the skin plaque on the back showed a red-orange hue and dilated vessels (Figure 2).

A 4 mm punch biopsy was performed from the margin of an alopecic area of the scalp and showed non-caseating granulomas in the dermis composed of epithelioid histiocytes and a few multinucleated giant cells with surrounding infiltrate of lymphocytes. No follicular structures were evident (Figure 3). No fungal organisms or mycobacterial species were observed after periodic acid–Schiff (PAS) and Ziehl–Neelsen stain.

Taking these dermoscopical and histological results into account, the authors' clinical suspicion of scalp sarcoidosis associated with lung sarcoidosis was confirmed. While waiting for her pneumologist to make a check-up and decide the best treatment, the patient was treated with local therapy based on clobetasol propionate ointment twice a day for 2 months. At the 2 month follow-up, the scalp lesions appeared to have not improved, while the back lesion was evidently reduced in size and thickness. Afterwards, because her pneumologist did not observe any change in sarcoidosis lung involvement and to stop progression of scalp alopecia, the authors prescribed a therapy of systemic triamcinolone acetonide at the dosage of 40 mg intramuscularly, one injection per month for 3 months and then a half injection per month for 2 months.

Systemic steroids were used to obtain a quicker halting of the progression of the disease. During the 2 years of follow-up, the disease remained stable in the scalp, without trichoscopic sign of activity, and remained unchanged during this time.

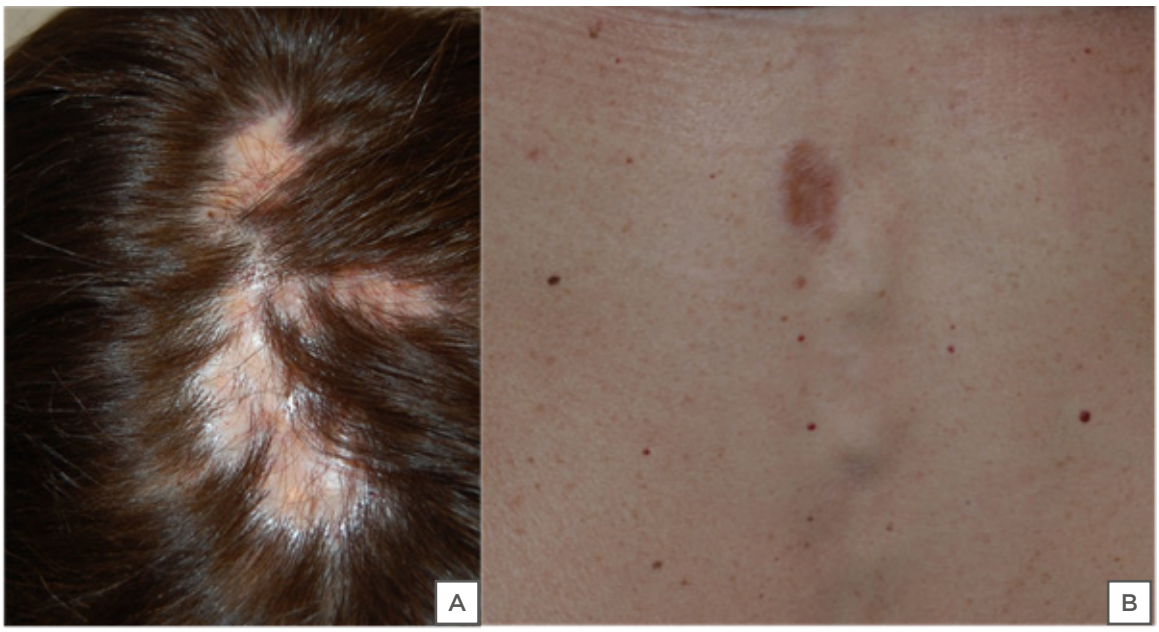

Figure 1: Clinical examination of the patient.

Clinical examination of our patient revealed (A) irregularly round and confluent patches of alopecia localised at the vertex that were red-orange in colour and (B) the presence of a small atrophic red-brown plaque of the back.

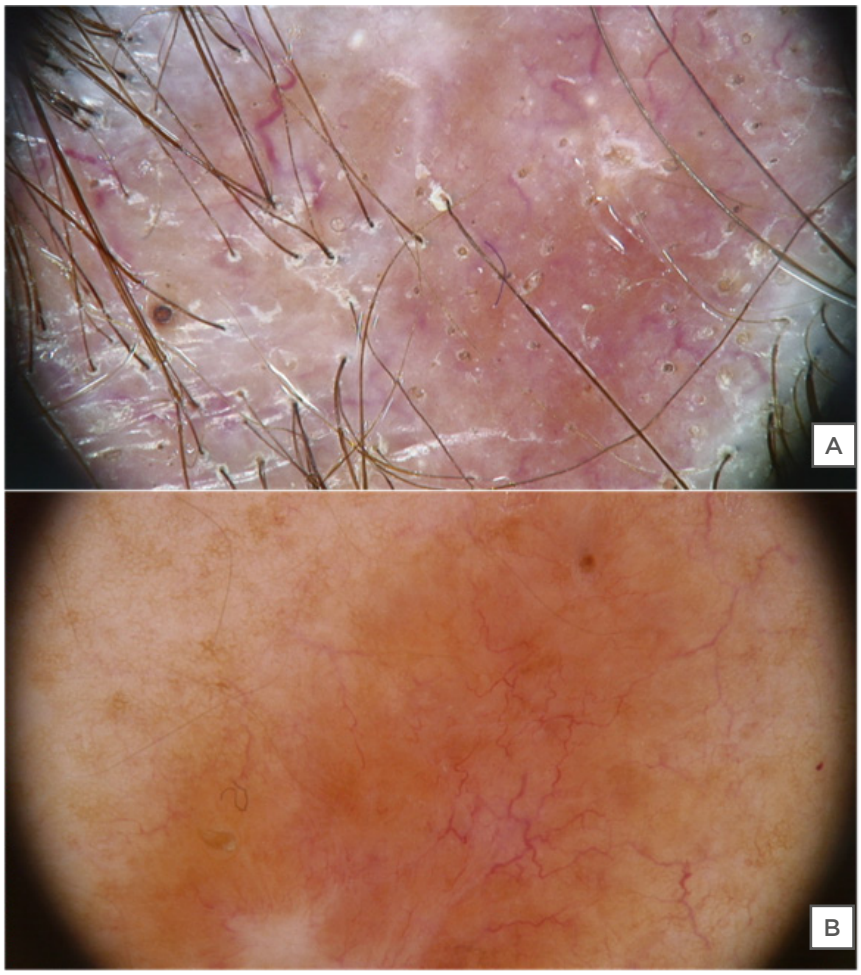

Figure 2: Trichoscopy of the scalp alopecia and dermoscopy of the skin plaque.

(A)Trichoscopy of the scalp alopecia showed loss of follicular ostia, a reddish-orange discoloration of the skin, and capillary telangiectasias (20x magnification); (B) dermoscopy of the skin plaque on the back showed a red-orange hue and dilated vessels (20x magnification).

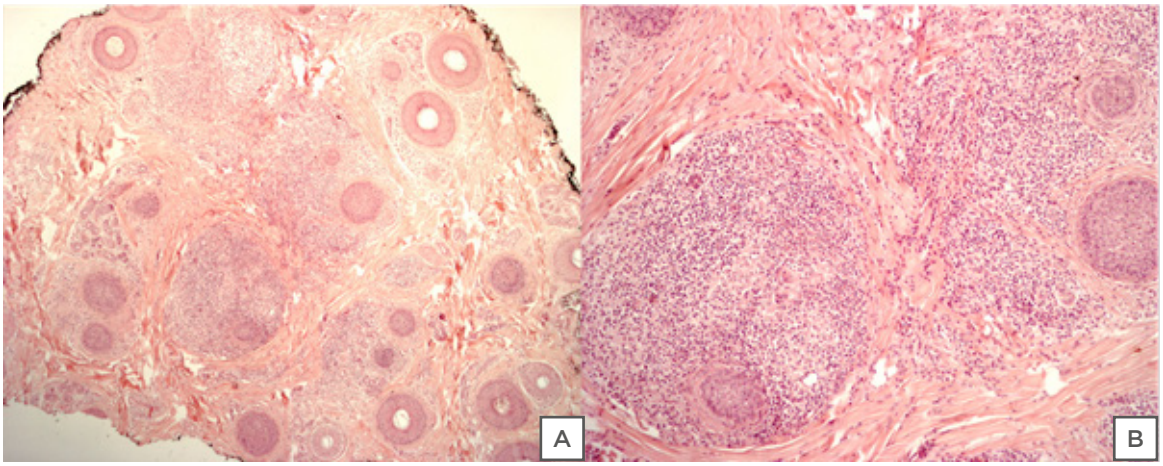

Figure 3: Pathology showed sarcoidal granulomas in the dermis, composed by epithelioid cells, with a few multinucleated giant cells, and a dense infiltrate of lymphocytes (A,B).

DISCUSSION

Sarcoidosis is an idiopathic chronic disease characterised by the presence of non-caseating granulomas that can involve different organs but is usually localised in the lungs, lymph nodes, and the skin.[4] Involvement of other sites, such as the liver, heart, nervous system, and kidneys, can also occur.[5] The skin is affected in about 20–30% of sarcoidosis patients, with papular sarcoidosis usually evolving on the face, especially around the eyelids and nasolabial folds, or maculopapular eruptions on the neck, trunk, extremities, and mucous membranes. Many cutaneous manifestations of sarcoidosis appear mostly at the onset, often as presenting symptoms. Histopathologically, classic sarcoid granulomas are non-necrotising with a central area composed of macrophages, epithelioid cells, multinucleated giant cells, and lymphocytes. The central areas are surrounded by CD8 and CD4 positive T lymphocytes surrounded by fibrosis. The proportions of lymphocytic infiltrate and fibrosis surrounding the granulomas vary depending on the patient and disease duration. Systemic therapy is chosen depending on severity of the systemic involvement, such as lung or other organs, since skin signs are usually treated with topical steroids.

The scalp is rarely affected by the sarcoidosis and only 46 cases of scalp sarcoidosis have been reported in the literature, with high prevalence in African-American women with associated systemic involvement.[6] In most of these cases, cicatricial alopecia was described, though a few cases of non-scarring alopecia as well as diffuse form of hair loss were also reported.[7,8]

Sarcoidal alopecia of the scalp may present as an atrophic and scaling patch that is red in colour, or as a scarring alopecia mimicking other causes of scarring alopecia, above all discoid lupus erythematosus (DLE) or lichen planopilaris. The difficulty in differentiating sarcoidal alopecia from DLE is well documented: in agreement with Henderson et al.,[9] the authors always recommend performing a skin biopsy to confirm a suspicion of DLE of the scalp.

It is important to keep in mind the possibility of necrobiosis lipoidica as another entity for the differential diagnosis; nonetheless, it rarely affects the scalp. This chronic granulomatous dermatitis can present itself with non-scaling plaque, characterised with erythematous violaceous borders and linear arborising vessels in a yellow background colour.[10] Diagnosis of sarcoidal alopecia is generally achieved through histopathological examination, which typically shows sarcoidal granulomas in the dermis. The local destruction and scarring of the follicles in scalp sarcoidosis may cause a permanent alopecia, which can be indistinguishable from pseudopelade of Brocq.[11]

Torres et al.[3] have recently proposed trichoscopy as a useful tool in the diagnosis of scalp sarcoidosis. They described the presence of 'orange spots', corresponding to the granulomas in the superficial dermis, and of prominent telangiectasia in the scalp, due to the vasodilatation in the papillary dermis. Dystrophic hairs may be present, in correlation with the granulomas activity that induces a scarring alopecia. Nonetheless, the sarcoidal hairless patches are generally described as red, well circumscribed, and pruritic. Cheraghi et al.[12] reported that they become hypopigmented and asymptomatic in time, further complicating the clinical diagnosis. The authors detected these features both in the scalp and in the back skin of the patient. Thus, the orange hue with telangiectasia should be considered a specific sign of skin sarcoidosis, as already stated by Pellicano et al. in 2010.[13] The particular anatomy of the scalp makes dermoscopic signs richer than in non-hairy skin, because hair shafts and follicular units have to also be considered. In scalp sarcoidosis, together with the dermoscopic signs seen on the skin, presence of, especially at the margin of the alopecic area, dilated ostia bigger than the well-known yellow dots typical of alopecia areata and dystrophic hairs that are not specific and are possibly seen in both scarring and nonscarring alopecia can be detected. These features are very important because they represent signs of an active state of the disease and so they can give information about the disease progression and prognosis. The loss of follicular ostia is, on the other hand, a sign of scarring, meaning a permanent loss of hair.

According to Katta et al.,[1] scalp sarcoidosis is usually resistant to treatment. Therapeutic options include antimalarial therapy; topical, systemic, and intralesional corticosteroids; and other immunosuppressive drugs, such as azathioprine,

but with a poor response. High potency topical steroids in ointment vehicle were ineffective in the authors' patient's scalp sarcoidosis after 2 months and systemic steroids were fundamental in stopping the progression of alopecia.

CONCLUSION

Trichoscopy is highly helpful for diagnosis of hair and scalp disorders and should always be used to properly evaluate any lesion on the scalp in patients with alopecia. This is especially true in cases of suspected scarring alopecia, where a prompt diagnosis is very important to start therapy and stop the progression of the disease. Trichoscopy gives important diagnostic clues in scalp sarcoidosis, i.e., a reddish-orange discoloration of the skin associated with wide telangiectasia, but the histological examination remains indispensable to confirm the diagnosis. Finally, clinicians should always remember that a scalp involvement in sarcoidosis should require a systemic treatment, even if the disease at the level of another internal organ is stable.

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A Versatile, Low-Cost, Three-Dimensional-Printed Ultrasound Procedural Training Phantom of the Human Knee

Authors: *Alvin Lee Day,[1] John Bradley Barger,[2] David Resuehr[2]

1. Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, Alabama, USA
2. Department of Cell, Developmental and Integrative Biology, School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA

*Correspondence to dopplerday@gmail.com

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Abstract

The use of musculoskeletal ultrasound is expanding in many medical disciplines, and simulation trainers have been successfully employed to help practitioners learn various ultrasound techniques. While there are fewer commercial trainers in musculoskeletal ultrasound than other ultrasound modalities, the ones that do exist can be prohibitively expensive. Several less expensive phantom trainers have been described in the literature, including those made of ballistic gelatine. The authors present a three-dimensional printed knee phantom that was overlaid with ballistic gelatine as a viable option for training.

INTRODUCTION

The use of ultrasonography has been increasing across many specialties, including rheumatology.[1,2] Interest in ultrasonography by clinicians, termed point-of-care ultrasound, has risen dramatically; this is attributable to evidence for improved patient safety, more rapid diagnostic assessments, and assistance with procedural guidance.[3] Among point-of-care ultrasound modalities, musculoskeletal ultrasound (MSUS) has been of particular interest to rheumatologists. Although MSUS is used by rheumatologists in the USA less frequently than in Europe, standardised training programmes have been developed to narrow the utilisation gap.[4,5]

In a recent survey of MSUS instruction in adult rheumatology fellowship programmes in the USA, 80% of programmes that offered MSUS training included ultrasound-guided injections and procedures as a programme topic.[5] The knee is the most common joint that may require synovial fluid aspiration in rheumatology practice, regardless of the use of ultrasound-guidance.[6] Across specialties, knee injections and aspirations have typically been performed using anatomic landmarks to determine needle placement.

Effusions of the knee joint have various causes. With the knee in extension, fluid accumulates in the suprapatellar pouch. The collection of fluid in the suprapatellar pouch allows for aspiration of joint fluid, and arthrocentesis is typically performed with a medial or lateral approach.[7]

When fluid collections are smaller, careful redirection of the needle may be required to access the suprapatellar pouch. Redirection of the needle can increase patient discomfort and chance of trauma to the femoral and patellar cartilage, as well as prolong the duration of the procedure.

Ultrasound guidance of intra-articular knee aspiration and injection increases accuracy compared to the landmark-based approaches.[8,9] Overall, an ultrasound-guided aspiration is similar to the landmark-based approach, although techniques vary between clinicians. Using a linear ultrasound probe, the suprapatellar pouch with effusion is identified in long-axis to the quadriceps tendon. The probe is then turned 90 degrees to identify the effusion in short-axis to the quadriceps tendon. A needle is inserted and visualised entering the effusion, and the contents are aspirated. Additionally, the injection of corticosteroids, or other agents, can be performed once the needle is visualised in the suprapatellar pouch.

Ultrasound training programmes have been developed across medical specialties and in many medical schools, and simulation training phantoms have been used to supplement education.[10-12] Rather than attempt a previously unperformed procedure on a patient, a trainee can practice procedures on a procedural phantom prior to attempts on patients. Fidelity of the model is paramount. The use of simulation phantoms improves competency,[13] but the cost of such phantoms may be unaffordable for many training programmes. Blue Phantom, a commercial simulation training company, has developed a knee ultrasound training model at the cost of $3,799.[14]

As a result of the high cost of commercially available phantoms, many clinical ultrasonographers have developed low-cost, homemade phantoms.[15,16] Various materials have been used for these phantoms, including gelatine, agar, and food substances. Ballistic gelatine has recently been described as a reusable material well suited for procedural training.[17] For anatomical guidance, pre-moulded models have been purchased and embedded in ballistic gelatine.[18]

A functional knee joint model can cost from $30 to >$100.[19,20] While clearly less expensive than the commercial knee ultrasound model, this cost could still be excessive to some. In addition, the presence of metal screws may create undesirable artefacts. Three-dimensional (3D) printing has been introduced as an inexpensive way to create prototypes of numerous objects and has been used in the printing of anatomical models.[21] To the best of the authors' knowledge, no literature exists on the use of 3D-printing to create an ultrasound procedural phantom of a human joint.

This article proposes a 3D-printed anatomical knee model embedded in ballistic gelatine as a low-cost ultrasound phantom for procedural guidance of suprapatellar needle placement.

MATERIALS AND METHODS

Knee models were created using open-source digital image manipulation software, a 3D printer, and common household materials.

1. The initial digital CT model of the knee bones was acquired from the open-source platform Thingiverse[22] and edited using Blender.

2. Cruciate ligament models were acquired from a Digital Imaging and Communications in Medicine (DICOM) MRI dataset.[23] The ligaments were selected from the MRI data using InVesalius (CTI 2017). A 3D model was created from the DICOM slices using InVesalius. The 3D rough model was exported to Blender for cleaning and preparation for use with the 3D printer. After cleaning in Blender, the finalised digital file was exported as a .STL file to Cura (Ultimaker 2016), which created instructions for the 3D printer.

3. The 3D printer (LULZBOT TAZ6, Aleph Objects 2016) used the instructions from Cura to render the model in plastic.

4. The bony model was affixed with the ligament models using heated metal pins to melt the two structures together into a functional knee joint with accurate placement of cruciate ligaments.

5. The printed knee was fitted with menisci and collateral ligaments, which were made from hot glue.

6. The pre-femoral, quadriceps, and Hoffa's fat pads were moulded using modelling putty, and

the suprapatellar effusion was created using negative space between the putty. Nitrile glove strips were secured over the putty to create the anterior portions of the quadriceps and patellar tendons.

7. Ballistic gelatine was mixed in a 10% consistency as previously described.[17]

- 200 g of Vyse professional grade ballistic gelatine (Gelatin Innovations Inc., Schiller Park, Illinois, USA) was dissolved in 1.8 L of water to create a 2.0 L mixture.
- A heated magnetic stirrer with stir bar was used to heat the ballistic gelatine mixture to a boil.
- Four drops of defoamer (Gelatin Innovations Inc.) was added to prevent foaming.
- Congealing was prevented by turning off the heating element of the stirrer while the stir bar continued to stir. Red food colouring was added.

8. The 3D-printed knee model was embedded in liquefied ballistic gelatine within a cylindrical food storage container, and an ice bath was used to surround the container to prevent melting of modelling putty. Once cooled and solidified, the phantom was removed from the container (Figure 1).

Ultrasound images of the phantom were acquired with a GE Venue 40 ultrasound machine and compared to a human suprapatellar effusion (Figures 2 and 3).

RESULTS

The sonographic appearance of the 3D-printed knee phantom was comparable to those observed from a human with suprapatellar effusion. The ballistic gelatine soft tissue component worked well for repeated needle insertions and was easy to remove, melt, and re-cast as desired. Needle placement into the suprapatellar pouch was performed with good technique correlation to human anatomy. The approximate cost of the materials to create the phantom was $50.

DISCUSSION

The major strengths of this knee phantom were its fidelity to a human knee effusion, low cost, and reusability. For a procedural phantom to be effective in improving the competency of trainees, it must be highly comparable to its source. By using a digital knee joint model created from a CT image, the size and contours of the bony components were precise, and there were no undesirable artefacts, such as screws, found on pre-made knee models.

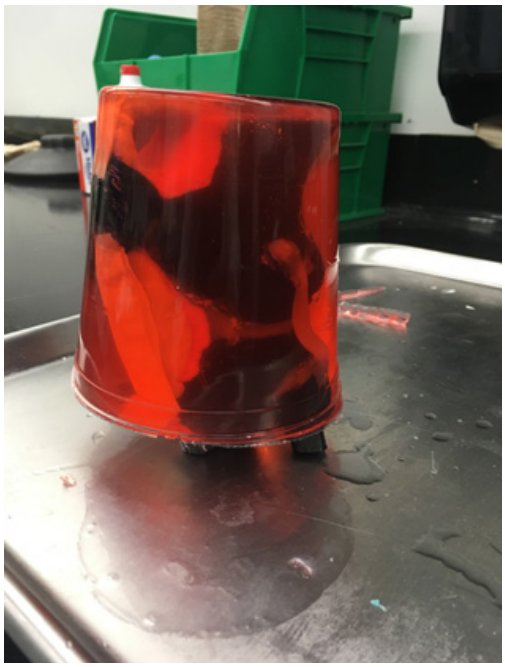

Figure 1: Completed phantom embedded in ballistic gelatine.

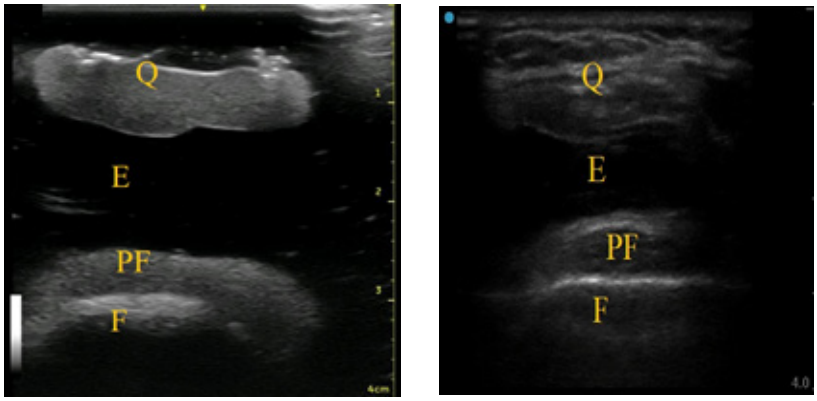

Figure 2: A) Suprapatellar short-axis view of knee phantom; B) human suprapatellar effusion ultrasound anatomy in short-axis.

E: effusion; F: femur; PF: pre-femoral fat pad; Q: quadriceps tendon, quadriceps fat pad.

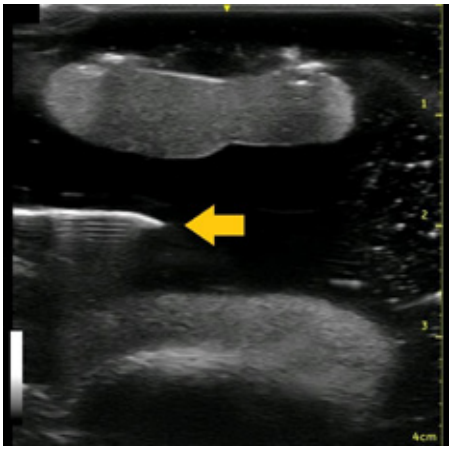

Figure 3: Knee phantom in suprapatellar short-axis view with effusion.

Arrow: needle placement.

Ultrasound imaging of suprapatellar pouch in short-axis showed soft-tissue landmarks comparable to those in a human knee with effusion. Finally, the anechoic effusion of the phantom was nearly identical to that seen in a human knee effusion. The technique required to visualise needle placement into the effusion was also comparable to that used on patients.

When compared to the $3,799 cost of the Blue Phantom commercial knee ultrasound training model, the $50 materials cost of the 3D-printed phantom was significantly less expensive. Many universities have 3D printers available for use, which would eliminate the need to purchase a 3D printer. All software used was open-source. The reusability of the 3D-printed knee phantom also allowed for further cost savings. Because the only area of the phantom that could be punctured was the ballistic gelatine outer component, the 3D-printed knee bones and soft-tissues remained intact. The outer ballistic gelatine could be removed, reheated, and reapplied without the need to purchase additional equipment.

LIMITATIONS OF THE THREE-DIMENSIONAL-PRINTED KNEE PHANTOM

The 3D-printed knee phantom was not without limitations, however. When compared with the commercial knee phantom, there was no outer covering to replicate skin and the musculoskeletal landmarks used for palpation. Ideally, a phantom would provide the ability for both landmark-based and ultrasound-guided aspiration and injection. The cylindrical design of the 3D-printed phantom

was created by the food storage container to allow for embedding of the knee within the ballistic gelatine, which limited its ability to be used for landmark-based procedures.

An additional limitation was the transparency of the outer gelatine layer. The ballistic gelatine used in the outer layer was dyed red, but the bones and soft tissues remained visible despite the dye. To ensure a trainee is unable to visualise the needle without the ultrasound, a darker dye is needed to force reliance solely on ultrasound visualisation of the effusion.

Finally, the effusion on the phantom was created using negative space that was filled with ballistic gelatine during the embedding process. While the anechoic gelatine was visually consistent with a human suprapatellar effusion, there was no fluid to aspirate. Creation of a reusable, fluid-filled pouch would have been technically challenging and increased both the complexity and the cost of the phantom. Commercial phantoms typically have fluid-filled compartments that can provide the haptic feedback of accessing the suprapatellar effusion.

CONCLUSION

Ultrasound phantoms have high value as learning tools for both novices and more experienced ultrasonographers because of their versatility. Since the advent of 3D-printing and the ability to create custom phantoms derived from scans of patient anatomy, the opportunity for the clinician to practice a procedure prior to performing it on the patient exists and provides the potential for a lower degree of errors with greater patient safety. This article demonstrated a versatile and fairly simple method for producing a low-cost, reusable knee phantom. This method can be tailored and modified to most, if not all, joints of the human body, and the resultant designs can be made available online and shared globally in an open-source manner. Additionally, 3D-printed ballistic gelatine phantoms for ultrasound-guided joint procedures could be used to more quickly increase the competency of trainees.

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Cholestasis in the Baby and Infant

Authors: *Mithat Gunaydin,[1] Asudan Tugce Bozkurter Cil[2]

1. Avicenna Hospital, Department of Pediatric Surgery, Istanbul, Turkey
2. Medicana International Samsun Hospital, Department of Pediatric Surgery, Samsun, Turkey

*Correspondence to mithatgunaydin@yahoo.com

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Abstract

Cholestasis in children is a serious condition due to various aetiologic factors. If children with jaundice present with acholic stool, dark urine colour, or direct hyperbilirubinaemia, the patient should be evaluated urgently. Early and timely diagnosis and initiation of appropriate treatment are extremely important determinants of morbidity and mortality. In the neonatal period, idiopathic neonatal cholestasis, alpha-1 antitrypsin deficiency, cholestasis from infections, and biliary atresia are the most common causes of cholestasis. Nowadays, with the development of genetic and molecular biological studies, the diagnosis of many diseases that have previously been evaluated as 'idiopathic' can be made. It is the aetiological factor that determines the prognosis. The treatment plan is created in accordance with aetiological causes and in response to symptoms such as pruritus and malabsorption: this can be surgical treatment across a diverse spectrum, from biliary diversion to liver transplantation. In this study, the aetiology, diagnosis, and treatment of cholestasis in babies and infants are reviewed in the light of current literature.

INTRODUCTION

Cholestasis is seen in every 2,500–5,000 live births. In full-term infants, jaundice that lasts >2 weeks should be evaluated; however, it should be noted that prolonged jaundice may be observed in 15–40% of healthy infants receiving breast milk.[1]

Cholestasis is a clinical condition in which bile production from hepatocytes or excretion through intra/extrahepatic bile ducts is impaired, resulting in bile accumulation within hepatocytes or bile ducts. Excretion of bile-excreted substances and absorption of substances that are absorbed via bile are all effected.[2-5]

The bile accumulating in the hepatocytes and bile ducts causes damage to these tissues. Lipids are normally degraded by pancreatic lipases with the help of bile acids, but this does not happen during cholestasis, and digestion and absorption of lipids are impaired. As a consequence of elevated serum bile acid levels, bile acid synthesis from cholesterol is depleted resulting in hypercholesterolaemia.[5,6]

Cholestasis can result in chronic liver dysfunction, liver transplantation, or even death. Therefore, early diagnosis, timely evaluation, and appropriate treatment are important.[2,7-9]

This review will especially focus on neonatal and infant cholestasis.

AETIOLOGY

In children and neonates, cholestasis can be caused by a wide spectrum of disorders. It may develop as a result of perinatal infections, congenital anomalies involving biliary tree, genetic or metabolic disorders, and multifactorial causes. [5,7,10,11] Cholestatic aetiology is summarised in Table 1.

CHOLESTATIC LIVER DISEASES

The incidence of cholestatic liver disease (CLD) in newborn infants is 1 in 2,500 births. The most common causes of cholestatic jaundice in the first months of life are biliary atresia (BA), viral infections, and alpha-1 antitrypsin deficiency (A1AT).[5] Cholestasis beyond the neonatal period includes a wide spectrum of congenital and acquired aetiologies.

Table 1: Aetiology of cholestasis.

Idiopathic	Idiopathic neonatal cholestasis
Metabolic disorders	**Disorders of carbohydrate metabolism** Galactosaemia Fructosaemia Glycogen storage disease type IV **Disorders of aminoacid metabolism** Tyrosinaemia Hypermethioninaemia **Disorders of lipid metabolism** Niemann-Pick disease Gaucher disease Wolman disease Cholesterol ester storage disease **Disorders of bile acid metabolism** 3 beta-hydroxy-C27-steroid dehydrogenase/isomerase deficiency Trihydroxycoprostanic acidaemia **Peroxisomal disorders** Zellweger syndrome Adrenoleukodystrophy **Miscellaneous metabolic disorders** Alpha-1 antitrypsin deficiency Cystic fibrosis Neonatal iron storage disease
Infectious diseases	**Bacterial causes** Sepsis Urinary tract infections TORCH infections *Syphilis* *Tuberculosis* *Listeriosis* **Viral causes** Hepatitis A, B, and C Adenovirus Cytomegalovirus Coxsackievirus Human herpesvirus 6 Epstein-Barr virus Parvovirus Human immunodeficiency virus

Table 1 continued.

Idiopathic	Idiopathic neonatal cholestasis
Chromosomal/genetic diseases	Trisomy 21 (Down's syndrome), 13 (Patau's syndrome), 18 (Edwards' syndrome) PFIC Type 1, 2, 3 BRIC 1,2 Citrin deficiency Aagenaes syndrome (cholestasis-lymphedema syndrome) Cystic fibrosis Alagille syndrome
Anomalies of biliary tract	**Intrahepatic** Alagille syndrome Limy bile syndrome **Extrahepatic** Biliary atresia Neonatal sclerosing cholangitis Choledochal cyst Pancreaticoduodenal junction anomalies Caroli disease
Autoimmune diseases	Autoimmune hepatitis Giant cell hepatitis with autoimmune haemolytic anaemia (<1 years old) Fulminant hepatitis Primary sclerosing cholangitis
Vascular diseases	Budd-Chiari syndrome Cardiac failure Perinatal asphyxia Multiple haemangioma
Bile acid synthesis disorders	3-ß-hydroxy-Δ-5C27-steroid dehydrogenase/isomerase deficiency Δ-4-3-oxosteroid 5-ß-reductase deficiency Dihydroxyalkanoic 24, 25 cleavage enzyme deficiency
Endocrine causes	Hypothyroidism Hypopituitarism
Toxic causes	Drugs Total parenteral nutrition Fetal alcohol syndrome
Other causes	Cholelithiasis Choledocholithiasis Hypoperfusion Shock Intestinal obstruction Neonatal haemochromatosis/gestational alloimmune liver disease Multifactorial cholestasis

BRIC: benign recurrent intrahepatic cholestasis; PFIC: progressive family intrahepatic cholestasis; TORCH: toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus.

In older children, the most common cause of biliary obstruction that leads to cholestasis is cholelithiasis. But, as mentioned above, this review will primarily focus on the differential diagnosis of cholestasis in neonates and infants. BA, choledochal cysts, tumours, and cholelithiasis result in extrahepatic cholestasis, while hepatobiliary infections; metabolic, genetic, and endocrine diseases; prematurity; drugs; parenteral nutrition; graft-versus-host disease; and post-liver transplantation pathologies can lead to intrahepatic cholestasis.

The diseases that are important causes of cholestasis are reviewed individually below.

In all chronic cholestatic disorders impaired copper secretion and accumulation can be seen.

Idiopathic Neonatal Cholestasis

Idiopathic neonatal cholestasis (INC) is the most common cause of cholestasis during the neonatal period and is responsible for approximately one third of cases. The condition appeared at a rate of 60–70% in a differential diagnosis of neonatal cholestasis in the 1980s and has now decreased to 15–20%.[12] The disease is characterised by loss of appetite, jaundice, and growth retardation. The degree of cholestasis is variable and may not be differentiated from extrahepatic causes in 10% of cases. INC can be diagnosed by the presence of giant cells in liver biopsies, of which diagnostic findings are shown after 4 weeks. Patients may also have intrauterine growth retardation and prematurity. Hepatomegaly with or without splenomegaly can also be present. Before the diagnosis of INC, the integrity of the biliary tract should be evaluated and any surgical pathology should be excluded.[5,12,13]

Prognosis is related to the duration of cholestasis. Progressive liver disease and cirrhosis are indispensable in cholestasis lasting >6–12 months. As a result, acidity develops and the patient progresses to liver failure requiring liver transplantation.[5,12]

Metabolic and genetic diagnostic tools evolve, diseases once diagnosed as INC may in fact be reidentified as one of the following: primary bile acid synthesis diseases, progressive family intrahepatic cholestasis (PFIC), familial haemophagocytic lymphohistiocytosis, neonatal sclerosing cholangitis, syndromic or non-syndromic intrahepatic biliary tract paucity, neonatal haemochromatosis, or metabolic/genetic diseases such as citrine deficiency and transient neonatal cholestasis.[7,9,14]

Extrahepatic Billary Atresia

BA is a rare disease in which progressive inflammation and fibrosis results in cirrhosis and end-stage liver failure unless treated within the first 2 years of life. It is possible to provide bile flow with early portoenterostomy and to reduce liver damage within the first 45 days of life. Despite surgical treatment, liver injury can continue and liver transplantation may be required in 80% of patients.[15,16]

BA is the most common cause of liver transplantation in children; however, patients have a good 10-year survival rate after transplantation. Recent studies have focussed on therapies to prevent liver fibrosis in these patients.[7,17,18]

Extrahepatic BA is a cause of cholestasis in 35–41% of patients. Fibrosis and progressive obliteration of the extrahepatic biliary tract occur and lead to damage of the parenchyma and intrahepatic biliary tract. This ultimately leads to cirrhosis and death before 3 years of age. The prognosis improves with surgical management, provided that it is performed within the first 45–60 days of life.[7,9,10,18,19]

There are three types of atresia defined according to the alteration in the hepatobiliary system, the subtypes of which are choledochal atresia, atresia of the common hepatic duct, cystic and bile duct atresia, and atresia of the hepatic duct onwards towards the intrahepatic biliary tract, which is present in 90% of patients and is not correctable.

Usually, healthy full-term newborns begin to have jaundice and light-coloured stools between the 2nd and 6th weeks of life. They later develop firm hepatomegaly and splenomegaly. Between the 2nd and 3rd months of life, the patient's health deteriorates with portal hypertension that progresses to cirrhosis and hepatic failure.[7,17] Elevation of gamma-glutamyl transferase (GGT) is the main biochemical marker. Other laboratory findings include elevation of total bilirubin and direct bilirubin, alkaline phophatase, transaminases, and the alanine aminotransferase/aspartate aminotransferase ratio.[10]

Choledochal Cysts

Choledochal cysts are the congenital cystic dilatations of the biliary tree. Although they are benign, they may show malignant transformation and have serious complications such as cholangitis, pancreatitis, and cholelithiasis. Current treatment is cyst excision and hepaticojejunostomy. Despite the excision of the cyst, the potential for malignant transformation in these patients is higher than the normal population. Accordingly, long-term follow-up is necessary.[10,12]

Bile Acid Synthesis Disorders/Inborn Errors of Bile Acid Metabolism

More than 14 enzymes are involved in the synthesis of bile acids from cholesterol precursor molecules. Deficiencies in 3-ß-hydroxy-Δ-5C27-steroid dehydrogenase/isomerase, Δ-4-3-oxosteroid 5-ß-reductase, or dihydroxyalkanoic 24 or 25 cleavage enzymes result in the absence of synthesis of primary biliary acids resulting in cholestasis, jaundice, and early onset pruritus. Some, however, may have a more indolent presentation later during childhood.[7,10,11,20] Bile acid synthetic disorders are rare forms of cholestasis, but in most cases are treatable. These conditions often present with normal or low gamma-glutamyltransferase levels. Total serum bile acids are usually low in contrast to other cholestatic disorders. Molecular techniques may identify the specific mutations in genes encoding the enzymes responsible for bile acid synthesis. Treatment with the end products of bile acid synthesis, cholic acid, and chenodeoxycholic acid, is often curative for several of the bile acid synthetic disorders.[7,10,21]

Metabolic Diseases

Tyrosinaemia appears as a result of cholestasis and clotting time prolongation that are not corrected after initial administration of vitamin K. It is a consequence of deficiencies of succinyl and acetone, with elevation of serum tyrosine and phenylalanine. Galactosaemia appears as malnutrition, with hypoglycaemia and reduced sugars in urine with lactose intake. It is diagnosed by measuring the levels of galactose-1-phosphate uridylyltransferase in red blood cells (without prior transfusion of red blood cells). Neonatal haemochromatosis is indicated by hepatomegaly, cholestasis, and saturation elevation of transferrin and ferritin, and is confirmed by hepatic biopsy. Wolman disease is indicated by diarrhoea, dyslipidaemia, adrenal calcifications, and cholestasis, and is diagnosed by measuring acid lipase in a skin biopsy.[7,10]

Alpha-1-Antitrypsin Deficiency

A1AT deficiency causes 10–34% of cholestatic jaundice in the newborn period. It occurs as the result of a mutation on chromosome 14 that leads to alteration in the production and accumulation of the A1AT protein. A1AT is a glycoprotein synthesised mainly by the liver and is the major circulating protease inhibitor that acts against neutrophil elastase. Jaundice due to autosomal recessive A1T1 deficiency usually occurs between 3 and 12 weeks. However, cholestasis is only seen in a percentage of the patients with A1T1 deficiency. The diagnosis is made by determining the serum A1AT level and is confirmed by genetic studies. There is no need to perform a liver biopsy when A1AT levels are <100 mg/dL.[19,22] There is no specific treatment apart from trying to prevent the complications of chronic liver disease. Accumulation of intracellular mutant A1AT results in hepatocyte death, inflammation, fibrosis, and cirrhosis.

Infections

Some bacterial (e.g., gram-positive and gram-negative bacteraemia, urinary tract infections caused by *Escherichia coli*), viral (e.g., herpes simplex virus, cytomegalovirus, rubella, Epstein Barr virus), or parasitic (e.g., toxoplasmosis) infections can cause cholestasis.

Bacterial toxins have direct cholestatic action. Further release of cytokines such as IL-1 and TNF-α decrease the transport of such toxins. These molecules are fibrogenic and they directly affect the liver.

In infants, classical hepatitis viruses are not a cause of cholestasis except when there is liver failure attributable to hepatitis B virus (usually after 45 days of life).[7,11,19]

Genetic Diseases

PFIC is an autosomal recessive disease family with mutations in several different genes, presenting with severe pruritus and moderate jaundice commonly before 6 months of age.[23-25]

PFIC (Type)-1 (Byler disease) occurs from a mutation of the assumed aminophospholipid transporter gene (*FIC1/ATP8B1*) on chromosome 18 resulting in impaired hepatocellular bile salt secretion. PFIC-1 manifests during the infantile period with episodes of cholestasis (average onset of 3 months of age), leads to liver cirrhosis, and rapidly progresses to end-stage liver disease.

PFIC-2 occurs as a result of a mutation of the major canalicular bile salt export pump (BSEP) gene on chromosome 2 (ATP-binding cassette, sub-family B member 11 [*ABCB11*]).[25] Expression

of this gene is limited to the liver; therefore, although the clinical course of PFIC-2 is similar to that for PFIC-1, extrahepatic manifestations are absent.

PFIC-3 occurs due to a mutation of adenosine triphosphate-binding cassette subfamily B member 4 gene (*ABCB4*) encoding the multidrug resistance Class III (MDR3) protein related with the bile phospholipid export pump.[26]

GGT are low or normal in PFIC-1 and 2, whereas high in PFIC-3.

Another subtype of PFIC is defined by mutations in the nuclear receptor subfamily 1 group H member 4 (*NR1H4*) gene, which encodes the farnesoid X receptor (FXR), a bile acid-activated nuclear hormone receptor that regulates bile acid metabolism and balances the production and circulation of bile acids. It is presented as the master regulator of bile acid homeostasis. FXR also provides protection against hepatocarcinogenesis.[23,27,28]

Cholestasis is also seen in the benign recurrent intrahepatic cholestasis (BRIC) disease group, intrahepatic cholestasis of pregnancy, and erythropoietic protoporphyria.

BRIC1 may be accompanied by pancreatitis while BRIC2 may be accompanied by gallstones. The clinical features of benign recurrent intrahepatic cholestasis include early onset of recurrent attacks of cholestatic jaundice, lasting up to months in duration and eventually resolving spontaneously. It is a benign disease and fibrosis of liver cells does not occur despite repeated episodes.[23]

In cystic fibrosis, the liver is frequently involved and may re-present as cirrhosis, steatosis, portal hypertension, or neonatal cholestasis. However, symptoms related to cystic fibrosis-associated liver disease appear late, mainly during puberty when damage to the hepatobiliary system is already advanced.[7,10]

Alagille Syndrome

Alagille syndrome is a multisystem disorder related with defects in components of the Notch signalling pathway, mostly attributable to mutations in the gene *JAG1*. It presents with chronic cholestasis due to paucity of intrahepatic bile ducts.

Alagille syndrome is associated with cardiac alterations, butterfly vertebrae, posterior embryotoxon/pigmentary retinopathy, dysplastic kidneys, and characteristic facial deformations including hypertelorism, bulging foreheads, and prominent chins (triangular face). GGT is high in Alagille syndrome, and there is no specific treatment. Patients are at increased risk of early development of hepatocellular carcinoma, and 50% of children will require transplantation.[29,30]

Endocrine Disorders

Both hypothyroidism and hyperthyroidism are associated with hepatic alterations, and thyroid diseases should be excluded in patients with transaminase elevation of unknown cause. Oestrogens are related to cholestatic liver damage.[31] Congenital panhypopituitarism has been recognised to cause neonatal cholestasis. Recently, isolated severe cortisol deficiency presenting with neonatal cholestasis and hypoglycaemia had been reported in which the resolution of cholestasis by hydrocortisone replacement therapy suggested a relationship between cortisol deficiency and the development of neonatal cholestasis.[32]

EVALUATION

History

In addition to a detailed history, diagnosis of cholestasis includes clinical findings, physical examination, laboratory studies, imaging studies, and liver biopsy. An early-as-possible diagnosis and appropriate treatment is very important; urgent complete blood count, serum direct-indirect bilirubin, blood urea nitrogen levels, electrolyte and glucose levels, prothrombin time, blood, and urine cultures should be checked.[5,10,12]

Clinical and Physical Findings

The most common findings of neonatal cholestasis include prolonged jaundice, acholic stool, and darkened urine. In patients with extrahepatic cholestasis, acholic stool usually appears early and pursues. If acholic stool lasts >10 days, extrahepatic aetiology should be suspected. Stool colour may show changes in intrahepatic cholestasis. Therefore, the stool colour of patients with cholestasis should be followed.[33,34]

Some newborns with cholestasis can present with serious problems such as intracranial haemorrhage as a result of vitamin K deficiency. Hypocalcaemia can be seen as a consequence of vitamin D deficiency and metabolic diseases (such as galactosemia, fructosemia, glycogen storage diseases), the latter of which may lead to convulsions. Panhypopituitarism can also present with cholestasis and hypoglycaemia, and lethargy and feeding problems may also be observed in metabolic disorders.[5,12,34]

Jaundice is the most important finding in physical examination. Hepatomegaly is usually present and may be associated with splenomegaly in advanced liver diseases.

Growth retardation as a result of intrauterine infections and facial dysmorphism as an outcome of syndromic causes may also be considered as physical examination findings.[10,13,34] Choledochal cysts can show up as a mass in the right upper quadrant of the abdomen.[12]

Laboratory Studies

Direct bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase are mostly elevated in CLD. Aminotransferases (aspartate aminotransferase, alanine aminotransferase) may also elevate when cholestasis persists.

If a jaundiced newborn has direct hyperbilirubinaemia, the causes of neonatal cholestasis, including BA, should be investigated. Direct hyperbilirubinaemia that is >1 mg/dL or >15% of the total bilirubin is pathological and its causes should be investigated. Indirect hyperbilirubinaemia may occur as a result of excess bilirubin production (e.g., haemolysis) or when the capacity of liver to conjugate the bilirubin is exceeded.[7,10]

Diagnostic Imaging Studies

Chest X-ray, abdominal ultrasonography, hepatobiliary scintigraphy, single photon emission CT, magnetic resonance cholangiography, percutaneous transhepatic cholangiography, and endoscopic retrograde cholangiography are among the diagnostic imaging methods used. The examination should start with noninvasive methods.[10,35-37]

Abdominal Ultrasonography

Abdominal ultrasonography, an easy and noninvasive method, is the first choice for diagnostic imaging. Ultrasonography can detect any obstructing pathologies of bile ducts, choledochal cysts, presence of gall bladder, stage of liver disease, and vascular and splenic abnormalities.

Many hepatic sonographic parameters have previously been suggested to help in the diagnosis of BA, such as the triangular cord sign (hepatic hyperechoic), abnormal gall bladder morphology, lack of gallbladder contraction after oral feeding, nonvisualisation of the common bile duct, hepatic artery diameter, hepatic artery diameter to portal vein diameter ratio, and subcapsular blood flow.[14,35,36,38]

Hepatobiliary Scintigraphy

Hepatobiliary scintigraphy is used to distinguish BA from other causes of cholestasis by showing the patency of the biliary tract. If 5 mg/kg/day phenobarbital is started at least 5 days prior, biliary excretion of the isotope is enhanced but has delayed diagnosis. If uptake of the labelled substances by the hepatocytes is delayed, neonatal hepatitis should be considered. Yang et al.[14] compared different diagnostic methods for differentiating BA from idiopathic neonatal hepatitis (INH). In this study, liver biopsy is reported as the most reliable method to differentiate INH from BA. Among imaging methods for cholestasis, hepatobiliary scintigraphy single-photon emission computer tomography (HBS SPECT) is reported to be a more reliable diagnostic method compared to magnetic resonance cholangiography, HBS (hepatobiliary scintigraphy), or ultrasonography.[14,37] Magnetic resonance cholangiography is a noninvasive technique for evaluating the intrahepatic–extrahepatic bile ducts and the pancreatic duct.[39,40] Endoscopic retrograde cholangiopancreatography requires an experienced team (e.g., endoscopist, paediatric anesthesiologist) and specific infant endoscopy equipment.[41]

Intraoperative Cholangiography

Diagnostic laparotomy/laparoscopy and intraoperative cholangiography is the gold standard for diagnosis of BA.[10] It also permits

therapeutic irrigation of inspissated bile from the ductal system. If the gallbladder is visibly highly atretic, the surgeon may proceed with the Kasai procedure without the need for cholangiography. However, percutaneous transhepatic cholecysto-cholangiography can be a preferable modality for excluding BA in neonates that are critically ill or have significant comorbidities and are at a high risk during open surgery and general anesthaesia.[17]

Liver Biopsy

Liver biopsy is crucial in neonatal cholestasis. Bile duct proliferation, bile plugs, portal area oedema, and fibrosis at histopathological examination are typical findings of BA while bile duct paucity is seen mainly in non-BA diseases. Liver consistency is increased in BA. Within the non-BA group, lobular irregularity and prominent giant cell transformation is most prevalent in INH cases and moderate to marked interlobular bile duct injury can distinguish cases of Alagille syndrome from other non-BA cases. Nonspecific findings such as microvesicles can suggest metabolic disease.[10,38,42]

TREATMENT

Treatment of cholestatic patients aims to treat the underlying problem itself (either surgically or medically) and to prevent and treat the secondary complications of cholestasis. Diets, medications, and vitamins are used for medical treatment and external or internal biliary diversions are applied for surgical treatment.

Medical Treatment

Medical treatment is primarily arranged according to the aetiology. Appropriate anti-biotherapy in bacterial infections, sepsis, urinary tract infections; appropriate diet therapy in metabolic processes such as galactosaemia and tyrosinaemia; L-thyroxine treatment in congenital hypothyroidism; steroid and immunosuppressive (e.g., azathioprine, mycophenolate mofetil) treatment in autoimmune hepatitis; chelation therapy in Wilson's disease; and effective intensive care therapy in fulminant hepatitis should be set before the patients deteriorate and have malnutrition.

The purpose of medical treatment in cholestasis can be summarised as follows: to enhance the bile flow and inhibit the accumulation of metabolites in the liver (choleresis); to treat toxic effects of bile re-entering the systemic circulation; to avoid the malabsorption of fat and fat-soluble vitamins; and to prevent acute and chronic malnutrition and ensure continuity of growth. Accordingly, the general principles of treatment regarding the secondary complications in children with CLD should include nutritional support (enough energy, protein, and medium-chain fatty acids), fat-soluble vitamins (vitamins A, D, E, and K), choleretic therapy (e.g., ursodeoxycholic acid, phenobarbital, corticosteroids), treatment of pruritus, and treatment of hypercholesterolaemia. Calcium intake and adequate exposure to sunlight are also essential.

There are many drugs used to avoid pruritus and other symptoms. Autotaxin (ATX) enzyme, a potent neuronal activator, plays a key role in the pruritogenic signalling cascade in cholestatic paediatric patients afflicted by itch in recent studies. Serum ATX activity correlated with itch intensity in children with cholestatic diseases. Lysophosphatidic acid is further formed from lysophosphatidylcholine by ATX. Serum LPA levels were found to be increased in cholestatic patients who had pruritis; however, there is no study regarding serum LPA levels in children with cholestasis.[10,13, 43-46]

Ursodeoxycholic acid dissolves cholestasis and is a successful treatment. Some reports suggest that, at a dose of 10–30 mg/kg per day, it could reverse the potential hepatotoxicity of the accumulating endogenous bile acids. It regulates bile acid distribution, reduces the amount of cholesterol in the bile, and provides mitochondrial integrity. It has choleretic, immunomodulatory, antioxidant, antiapoptotic, and cytoprotective effects.[47-49]

Cholestyramine is an oral bile acid binding resin used to resolve pruritus. It forms nonabsorbable micelles with the bile acids in the intestines and prevents bile acids from entering the enterohepatic cycle. It should be taken at least 1 hour before, or 4–6 hours after, meals, 1–4 g/day. It induces liver enzyme activity and increases bilirubin excretion. In patients with reduced serum bilirubin levels, pruritus also regresses.[44]

Rifampicin acts by upregulating detoxification enzymes and exporting pumps through FXR-dependent mechanisms. Ricfampicin indirectly induces hydroxylation of bile salts that are further glucuronidated and excreted in urine. It also induces conjugation and excretion of bilirubin through uridine diphosphate-glucuronosyl transferase. It is used at a dose of 5–10 mg/kg/day. [44,50]

Phenobarbital is used to induce the CYP/CYP450 system in the treatment of neonatal hyperbilirubinaemia and chronic cholestasis with low bilirubin levels at a dose of 3–10 mg/kg/day.[5]

4-phenylbutyrate is another drug used in PFIC types. At a dosage of 350 or 500 mg/kg/day taken orally, it significantly relieves the intractable itch. In patients with decreased cell-surface expression of BSEP among PFIC-2, 4-phenylbutyrate therapy has partially restored BSEP expression at the canalicular membrane, as well as significantly improved liver tests and pruritus at a dosage of 500 mg/kg/day.[15,51]

Antihistaminic agents, opiate antagonists, ondansetron, steroids, propofol, and carbamazepine are included within the other medical therapy options.

Surgical Treatment

Surgery is carried out in patients whose underlying pathology causing cholestasis requires surgical manipulation, such as BA and choledochal cysts.

The most popular surgical method in BA is hepatoportoenterostomy known as the Kasai operation. This operation should be performed taking care of the micron-wide bile canaliculus at the porta hepatis. Therefore, the first 2 months are vital. Cystectomy and/or choledochoenterostomy should be performed in choledochal cysts.[19,52,53]

Other surgical approaches include internal or external biliary diversion surgery to relieve symptoms such as excessive pruritus. In patients who have no response to medical therapy, it is aimed to reduce the accumulation of bile by external or internal drainages. [16,53]

Liver transplantation is inevitable in patients with cirrhosis and liver failure. Today, in many centres, successful donation of liver transplantation is still difficult because of religious or legal reasons.

CONCLUSION

In a large-scale systematic literature review, Catzola and Vajro[54] stated that a large number of rare hepatobiliary diseases in newborns and at childhood cause cholestasis. They emphasised that, even if there is no specific or curative treatment available, immediate medical treatment and regulation of nutrition are very important to avoid complications. They also stressed that studies on inherited versus acquired cholestasis would help to develop more effective specific therapies. They emphasised that the disease-specific medical and surgical treatment approaches are life-saving.[54]

In conclusion, cholestasis is a fatal condition which may result in cirrhosis, liver failure, and liver transplantation. The authors believe that the most important factor affecting morbidity and mortality is the aetiology of cholestasis. Early diagnosis, timely evaluation, and appropriate treatment are important for prognosis.

As a result of the advancement in genetic and molecular biology studies, patients formerly diagnosed with INH and neonatal cholestasis have turned out to have an underlying pathology, therefore the diagnoses of INC has decreased.

After ruling out any aetiology that would require surgery, patients should be directed to medical treatment. Medical treatment should be regulated according to the symptoms and aetiology.

In short, the evaluation and treatment of cholestasis in children, and especially in newborns, is a dynamic process, and the progression of molecular biology and genetic studies will accelerate this.

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Acquired Sperm DNA Modifications: Causes, Consequences, and Potential Solutions

Authors: Jan Tesarik

MARGen Clinic, Granada, Spain
Correspondence to jtesarik@clinicamargen.com

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Abstract

DNA of human spermatozoa can be subject to various kinds of modifications acquired throughout life. Put simply, two basic types of acquired sperm DNA modifications can be distinguished: genetic and epigenetic. Genetic modifications cause alterations of the DNA sequence and mainly result from the formation of breakpoints leading to sperm DNA fragmentation. Epigenetic modifications include a vast spectrum of events that influence the expression of different genes without altering their DNA sequence. Both the genetic and the epigenetic modifications of sperm DNA can negatively influence embryonic development, cause miscarriages, and be the origin of different health problems for the offspring. As to sperm DNA fragmentation, reliable diagnostic methods are currently available. On the other hand, the detection of potentially harmful epigenetic modifications in spermatozoa is a much more complicated issue. Different treatment options can be chosen to solve problems associated with sperm DNA fragmentation. Some are relatively simple and noninvasive, based on oral treatments with antioxidants and other agents, depending on the underlying cause. In other cases, the recourse to different micromanipulation-assisted *in vitro* fertilisation techniques is necessary to select spermatozoa with minimal DNA damage to be injected into oocytes. The treatment of cases with epigenetic DNA modifications is still under investigation. Preliminary data suggest that some of the techniques used in cases of extensive DNA fragmentation can also be of help in those of epigenetic modifications; however, further progress will depend on the availability of more reliable diagnostic methods with which it will be possible to evaluate the effects of different therapeutic interventions.

INTRODUCTION

Spermatozoa are vehicles whereby the male genome is transported to the oocyte to participate in syngamy with the female genome and form the resulting embryonic genetic makeup. Compared with the oocyte, sperm DNA needs to be 'packed' into the very small sperm nucleus preparative for its delivery to the oocyte. This process requires complex changes in the DNA-associated proteins (sperm chromatin)[1] during which sperm DNA is particularly exposed to environmental factors, which can affect its subsequent function in the fertilised oocyte and the resulting embryo.[2]

In addition to delivering the paternal genetic material to the oocyte, the fertilising spermatozoon

is also the source of two developmentally important non-genetic contributions: the factor(s) responsible for the reactivation of the oocyte's cell cycle, so far arrested at metaphase of the second meiotic division (oocyte activation);[3,4] and the centre of microtubule aster formation, required to develop mitotic spindles during the subsequent embryonic cell divisions.[5] Dysfunction of any of these factors causes secondary alterations in zygotic and embryonic DNA with possible consequences for further development.

Sperm DNA modifications that affect DNA sequence are called genetic modifications and can be transmitted to the offspring as genetic or 'hard' inheritance; however, there are also transmittable acquired sperm DNA modifications that do not affect the DNA sequence but can change the expression pattern of various genes.[6,7] These alterations are responsible for what is termed epigenetic or 'soft' inheritance. This latter type of inheritance in particular is garnering increasing interest nowadays, and the number of the known epigenetically inherited abnormalities and diseases is growing rapidly.

In this paper, both kinds of acquired sperm DNA changes are addressed, with particular attention given to their causes, pathological consequences, and methods for their prevention and treatment. Due to its clinical focus, a huge body of literature on preclinical research, though important for current knowledge in the field, is omitted.

HOW AND WHERE DO SPERM DNA MODIFICATIONS OCCUR?

Sperm production in the adult human testis begins with the maturation of spermatogonia, the still diploid sperm precursor cells, into primary spermatocytes which enter the first meiotic division, resulting in the formation of secondary spermatozytes. Each secondary spermatocyte subsequently divides, during the second meiotic division, into two haploid round spermatids. The meiotic events leading to the transformation of the diploid spermatocytes into the haploid spermatids are similar to those occurring in oocytes during their maturation. It is only after the completion of meiosis, at the round spermatid stage, when DNA of the future spermatozoon starts undergoing unique changes different from those occurring in oocytes. These changes, necessary to achieve the 'packaging' of sperm DNA required for its transportation into the oocyte during fertilisation, make sperm particularly exposed to different kinds of environmental factors which can produce both genetic and epigenetic DNA modifications.[2,7]

BIOLOGICAL BACKGROUND OF ACQUIRED SPERM DNA MODIFICATIONS

Sperm DNA, similar to oocyte DNA, needs to undergo the process of 'haploidisation' whereby the initially diploid precursor cells halve their DNA content in order to be able to restore the normal, diploid chromosomal constitution in the embryo resulting from their fusion at fertilisation. Some harmful sperm DNA modifications can be acquired during the process of haploidisation but most are due to events taking place thereafter, while the haploid round spermatids are being transformed into mature spermatozoa. This is partly because this final phase of sperm development occurs after the release of the sperm precursor cells from Sertoli cells, 'nursing' cells that can protect the developing germ cells from adverse external effects and eliminate those germ cells that have been irreversibly damaged.[8] Consequently, maturing spermatozoa are increasingly exposed to both genetic and epigenetic modifications during this period.

GENETIC MODIFICATIONS: ALTERATION OF SPERM DNA SEQUENCE

DNA fragmentation (breakage) is the most commonly acquired sperm genetic modification. Fragmentation is caused by distinct mechanisms and can involve only one of the two complementary strands forming the DNA double helix, thus producing single-strand breaks (SSB), or both of them, resulting in double-strand breaks (DSB). DSB of the DNA molecules occur as a physiological process in prophase of the first meiotic division, when primary spermatocytes deliberately produce DSB to allow DNA recombination between homologous chromosomes.[9] These physiological DSB subsequently activate a DNA repair machinery, acting through the protein kinase ataxia-telangiectasia mutated, which repairs the

free ends and therefore generates the chiasma.[10] Ataxia-telangiectasia mutated is also responsible for preventing the formation of new DSB once the recombination process is accomplished.[10] Failures of this DNA repair mechanism at this stage can result in the persistence of DSB which are poorly repaired later during meiosis or after fertilisation.[11] In fact, the ability to repair DNA lesions declines dramatically during final stages of spermatogenesis.[12] Meiotic interstrand DNA damage that escapes paternal repair can cause chromosomal aberrations in the zygote by maternal misrepair taking place after fertilisation.[13]

In addition to the physiological, recombination-related DNA breakage, DNA of the developing male germ cell can undergo SSB or DSB through different pathological mechanisms, especially those related to excessive production or insufficient scavenging of reactive oxygen species (ROS).[14] Excess ROS can originate both from germ cells themselves and from non-germ cells, and the germ cell chromatin is particularly sensitive to their action during the process of chromatin packaging in spermatids.[15]

The process of chromatin condensation in late spermatids is accompanied by replacement of DNA-associated histones with protamines (PRM1 and PRM2 in mammals), another type of nuclear protein specifically expressed in haploid male germ cells, whose presence and relative proportion protects sperm DNA from negative influences by environmental factors, and has recently been suggested as a new checkpoint to control sperm chromatin quality.[16] Abnormal protamine expression leads to defects in chromatin condensation, occasionally restricted locally.[16] The DNA within incompletely condensed areas of sperm nuclear chromatin, which can be visualised as intranuclear vacuoles by electron microscopy (Figure 1), appears to be the most vulnerable to oxidative damage, especially after late spermatids separate from Sertoli cells (spermiation) leading to the loss of these nursing cells' protective action.[8]

According to several recent reviews,[17-19] including one meta-analysis,[20] sperm DNA lesions have been shown to have negative consequences for embryogenesis after *in vitro* fertilisation, reflected by impaired morphological quality of Day 3 embryos,[17,18] increased incidence of embryonic cell apoptosis,[19] and decreased blastocyst formation, implantation, and pregnancy rates.[17,18,20]

There are a number of aetiological factors that can contribute to the pathogenesis of sperm DNA fragmentation. Different studies have suggested an association of sperm DNA breakage with age;[21] lifestyle habits such as dietary preferences,[22] smoking,[23-26] chronic alcoholism,[27] or recreational drug addiction;[28,29] environmental factors;[30] various diseases such as varicocele; [31,32] infections;[33-35] spinal cord injury;[36,37] diabetes[38] and obesity;[39] and drug therapies, including cancer chemotherapy[40,41] and antidepressants.[42,43] Most of these factors act via an excess of ROS, as first demonstrated in 1987.[44]

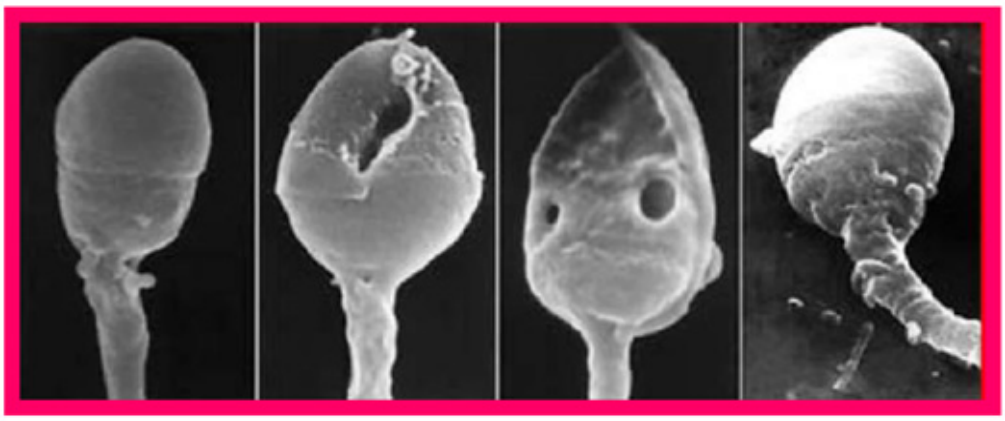

Figure 1: Scanning electron microscopy (SEM) image of human heads. The two middle images show the presence of intranuclear vacuoles.

Even though small amounts of ROS are normally produced by spermatozoa themselves and are essential for the fertilisation process, their excessive production by abnormal spermatozoa or by leukocytes in response to inflammation and infection, or their insufficient scavenging by antioxidant agents naturally present in human semen, result in ROS imbalance which leads almost invariably to increased sperm DNA damage.[45] On the other hand, some pathological conditions can also cause excessive sperm DNA fragmentation independently of ROS.[46]

EPIGENETIC MODIFICATIONS: ALTERATION OF SPERM DNA FUNCTION

Sperm DNA function can be altered, while keeping its sequence intact, by the action of complex mechanisms referred to as epigenetic modifications.[15] The most widely investigated epigenetic modifications include DNA methylation taking place at the 5' position of cytosine in CpG dinucleotides and histone acetylation.[6,47] However, a number of other epigenetic factors have been described and are critical in regulating gene expression, including chromatin structure and the expression of small non-coding RNA, such as microRNA, small interfering RNA, and Piwi-interacting RNA.[7,48]

Epigenetic information carried in sperm in the form of histone modifications, transcriptional factors, and chromatin three-dimensional architecture are other players in this complex game.[49] It is now clear that some of these epigenetic signatures persist after fertilisation, through different mechanisms, and influence future zygote, embryonic, fetal, and offspring development.[50-54]

These considerations are particularly important in the current era of assisted reproductive technologies (ART). Nowadays, ART can facilitate the transmission of these kind of factors by enabling fertilisation with abnormal spermatozoa. In the near future, however, newly emerging techniques will be likely to reduce the risk of epigenetic disease transmission by quality control of spermatozoa selection for fertilisation via detection of epigenetic biomarkers.[55] Out of the 6,871 sperm proteins identified so far, 560 have been found to be involved in modulating gene expression by transcription regulation, DNA methylation, histone post-translational modification, and non-coding RNA biogenesis.[56] It is in this group of proteins in which suitable candidates for such epigenetic biomarkes can be searched for, however direct analysis of DNA and RNA isolated from spermatozoa and seminal plasma is also a promising approach.[57]

The demonstration of the existence of epigenetic inheritance explains a number of clinical observations which would be hardly attributable to genetic inheritance only. For instance, in spite of an irrefutable evidence for an elevated germline mutation rate in patients directly exposed to ionising radiation, the results of numerous studies suggest that the incidence of the observed effects on genomic instability in the offspring is too high to be explained merely by radiation-induced mutations, which occur at a substantially lower rate.[58] Epigenetic inheritance through gametes can also explain both previously published and more recent data on transgenerational transmission of obesity,[59-61] diabetes,[62] and some types of cancer.[63,64] In addition to these 'metabolic' issues, recent research data show that early life stress in humans (e.g., maltreatment, violence exposure, loss of a loved one) and in rodents (e.g., disrupted attachment or nesting, electric shock, restraint, predator odour), a condition associated with increased risk of developmental psychopathology, can also be transmisible to subsequent generations via DNA methylation marks.[65]

CLINICAL CONSEQUENCES OF SPERM DNA MODIFICATIONS

Both genetic and epigenetic acquired sperm DNA modifications can have serious consequences for the survival and health status of embryos, fetuses, and the offspring.

Genetic Modifications

Alterations of the sperm DNA sequence can disturb different, developmentally important genes. Some of these alterations can be repaired by mechanisms acting in the germline or in the zygote after fertilisation.[66] The repair capacity of the testicular germinal epithelium appears to be related to the correct function of Sertoli cells. In

fact, Sertoli cells not only participate in the germ cell DNA repair but also remove, by phagocytosis, those germ cells that cannot be repaired.[67] If Sertoli cell function is disturbed, more germ cells with damaged DNA can escape this output control, complete spermatogenesis, and eventually be released in the ejaculate.[67] *In vitro* depletion of testosterone in culture media was shown to induce Sertoli cell apoptosis in human seminiferous tubules obtained from men with obstructive azoospermia and normal spermatogenesis.[68,69] This observation can explain the mechanism of action of some aetiological factors causing sperm DNA damage mentioned above, many of which are associated with decreased testicular testosterone production.[70]

Those DNA-damaged spermatozoa that escape the intratesticular quality-control mechanisms can be found in the ejaculate. In many cases sperm DNA damage is associated with other sperm functional disturbances which decrease their capacity to fertilise the oocyte by proper means. However, fertilisation with such spermatozoa is more likely when fertilisation is carried out by intracytopasmic sperm injection (ICSI). Sperm DNA damage can still be repaired after fertilisation through different mechanisms acting in the zygote.[66] It was noted that embryos resulting from fertilisation with spermatozoa from men with the same degree of DNA damage are more likely to develop to term when injected into the oocytes of very young women, such as oocyte donors whose maximum age is 25 years at the author's clinic, compared with older women.[8] Consequently, the capacity of the zygotic DNA repair appears to depend on the age-related oocyte quality.

If sperm DNA damage is not repaired, fertilisation usually takes place and the embryonic development goes on normally during the first 3 days after fertilisation.[71] The absence of apparent effects of unrepaired DNA damage on the early embro is likely to be related to the relatively late onset of paternal gene expression which occurs between the 4-cell and 8-cell stage in human embryos.[72-74] Moreover, oocyte-derived transcripts continue participating in the control of embryo development even during later stages of preimplantation development, including blastocyst formation.[75] On the other hand, sperm DNA fragmentation is associated with embryo implantation failure and miscarriage, referred to as the late paternal effect.[76] These problems occur at stages when the embryo becomes exclusively dependent on its own gene expression, and the timing of the onset of the late paternal effect clearly depends on the type and number of genes affected by sperm DNA fragmentation. Embryo aneuploidy, resulting from mismatch zygotic repair of sperm DNA strand breaks,[13] can also contribute to the late paternal effect. A recent study has suggested that DSB of sperm DNA are more likely to cause a delay in embryo development and to cause implantation failure as compared with SSB.[77]

Epigenetic Modifications

The first studies into transgenerational epigenetic inheritance were focussed on imprinted genes.[78-81] Imprinted genes are those that have to be expressed or silenced in a parent-of origin manner in order to allow normal dvelopment.[82] Several human diseases, such as Beckwith–Wiedemann syndrome, Silver–Russel syndrome, Prader Willy syndrome, and Angelman syndrome are caused by imprinted gene disruption leading to their abnormal expression.[82] Of these, an increased incidence of Beckwith–Wiedemann syndrome and Silver-Russel syndrome appears to be associated with assisted reproduction.[83,84]

A systematic review and meta-analysis of the literature has revealed a higher risk of congenital disorders caused by disruption of imprinted genes in children conceived through ART compared with those conceived naturally.[84] There is evidence from human studies that a number of assisted reproduction procedures, including superovulation, gamete micromanipulation, *in vitro* maturation of oocytes, and embryo culture can cause epigenetic disruption which is likely to involve both imprinted and non-imprinted genes.[79] The epigenetic risks associated with ART are thus not limited to abnormalities of imprinted genes but can concern a wide range of non-imprinted genes, leading to their abnormal expression and ensuing pathologies.[85]

In contrast with genetic modifications, epigenetic modifications of sperm DNA usually affect embryo development from its very beginning and are referred to as the early paternal effect.[71,76] In many cases, the early paternal effect can be detected as early as the one-cell zygote stage, based on the analysis of the assembly of

nucleolar precursor bodies (NPB) in the male and the female pronucleus.[86] Ultrastructural and autoradiographic studies on NPB assembly in human zygote pronuclei[87,88] detected the presence of new RNA synthesis in the chromatin regions adjacent to the NPB, and this early zygotic RNA synthesis was required for the NPB formation to go ahead.[89] Because, as mentioned in the previous section, the expression of the human embryonic genome starts much later during preimplantation development,[71-74] it is reasonable to suppose that the RNA synthesised in human zygotes might correspond to non-coding RNA species, such as microRNA, small interfering RNA, and Piwi-interacting RNA. These non-coding RNA are known to be involved in epigenetic events modifying gene activity.[7] This might explain the observed relationship between abnormalities of NPB in human zygotes and subsequent developmental fate of the respective embryos.[86,90] Moreover, the regulatory and amplifying activities of non-coding RNA acting during the early stages of embryonic development appear to be conditioned by factors present in the female genital tract.[91] Clearly, the possible repair mechanisms involving these factors during early zygote and preimplantation embryo development are precluded when the early embryos develop under *in vitro* culture conditions.

Previous findings have shown that the assembly of NPB coincides with the assembly of microtubule organising centres in human zygotes,[92,93] a process whose perturbations can cause abnormal chromosome segregations during embryo cleavage divisions, leading to aneuploidy. This can explain the observation that abnormal patterns of NPB assembly can also predict blastocyst transfer outcome[94] and chromosome constitution.[95] As previously mentioned, epigenetic mechanisms also appear to be responsible for transgenerational transmission of obesity,[60,61] diabetes,[62] and some types of cancer;[63,64] however, the mechanism of these phenomena remains to be elucidated.

PREVENTION OF HARMFUL SPERM DNA MODIFICATIONS

Both the genetic and the epigenetic sperm DNA modifications responsible for human disease have some common features. A recent study using cattle as the model showed that increased exposure of spermatozoa to oxidative stress causes changes in the sperm chromatin that are associated with both DNA fragmentation and aberrant DNA methylation affecting epigenetic reprogramming in early embryonic development.[96] Studies conducted with human spermatozoa showed that oxidative sperm DNA damage does not affect all of the sperm DNA in the same way. DNA located in compacted regions of the sperm nucleus, in which the process of sperm chromatin condensation has been completed sucessfully, is less exposed to oxidative damage compared with that located in incompletely compacted chromatin regions. This applies both to DNA fragmentation[97] and abnormal DNA methylation.[98] As oxidative stress affects sperm DNA both directly and indirectly, through impairing sperm chromatin condensation (Figure 1), eliminating the sources of excess ROS is the main preventive measure (Table 1).

Table 1: Summary of preventive measures and treatment modalities which can improve fertility in cases of acquired genetic and epigenetic sperm DNA modifications.

Preventive measures	Treatment modalities
Change in dietary preferences	Treatment of varicocele
Smoking cessation	Treatment of urogenital infections
Avoidance of alcohol and drug abuse	Control of diabetes
Avoidance of profesional toxicant exposure	Oral antioxidant treatments
Reduction of BMI	ART with surgically retrieved sperm
Avoidance of unnecessary drug therapies	ART using specific sperm selection methods

ART: assisted reproductive technologies; BMI: body mass index.

Excessive ROS can be of both endogenous (sperm-derived) and exogenous in origin.

The aetiological factors leading to excessive ROS production include unhealthy dietary preferences,[17] smoking,[18-21] chronic alcoholism,[22] recreational drug use,[23,24] varicocele,[26,27] infections,[28-30] spinal cord injury,[31,32] diabetes,[33] obesity,[34] and drug therapies including cancer chemotherapy[35,36] and antidepressants.[37,38] If any of these conditions are present in an infertile patient showing abnormally high levels of sperm DNA fragmentation, the first measure to be taken is to try and treat the underlying cause.[99] If this is not possible, or the measures taken do not give an expected result, the negative impact of ROS can be reduced by the use of scavenging agents, mainly antioxidants such as vitamin C, vitamin E, L-carnitine, coenzyme Q10, zinc, selenium, vitamin B9, vitamin B12, glutathione, docosahexaenoic acid, and folic acid.[100] Exogenous melatonin supplementation also prevents oxidative stress-evoked DNA damage in human spermatozoa.[101] If the patient shows abnormal levels of reproductive hormones, especially testosterone, follicle stimulating hormone, or luteinising hormone, a condition that can disturb the function of Sertoli cells may manifest, leading to an insufficiency of intratesticular germ cell DNA repair mechanisms. Hormonal replacement therapy can also be considered. In cases of infection, revealed by the detection of germs or excess leukocytes in the ejaculate, the use of specific antibiotics, sometimes accompanied by nonsteroidal anti-inflammatory drugs, can be of help. Appropriate personalised treatment of diabetes can improve the diabetes-associated sperm DNA modifications. If the use of none of these preventive measures improves the degree of sperm DNA alteration, or if none of the above potential aetiological factors can be detected, more radical treatment options (see below) can be taken into consideration. However, it is evident that the physician cannot efficiently control all of the factors related to excessive sperm DNA damage, and in some conditions, such as chemotherapy or antidepressant/antipsychotic medications, the physician's decision should be guided by the '*primum non nocere*' (first, do not harm) principle, in agreement with the Hippocratic Oath. The possible risks and benefits of the interruption of the treatment in course have to be weighed. If the interruption of sperm DNA-damaging medication is not advisable, the recourse to ART, employing specific *in vitro* treatment techniques to improve outcomes in cases of excess DNA damage, can be envisaged.

TREATMENT POSSIBILITIES

In some cases in which the appropriate treatment of the pathologies underlying the extensive sperm DNA damage (Table 1) does not lead to a significant improvement or in which no such aetiological factors can be identified, the treatment with high doses of oral antioxidants during several months can also be of help.[102] The resulting improvement can be sufficient to allow natural fertilisation but the recourse to ART is often required. The decision of whether or not assisted reproduction should be used in different clinical scenarios is sometimes difficult and depends on a number of associated factors concerning both the male and the female partner of the infertile couple. Recently, a guideline for the choice of the optimal assisted reproduction techniques according to differerent criteria has been suggested, including the basic sperm parameters, the duration of infertility, the number and type of previous unsuccessful assisted reproduction attempts, and the female age.[103] The techniques available include conventional ICSI, intracytoplasmic morphologically selected sperm injection (IMSI),[104,105] 'physiologic ICSI',[106] or a combination.[103] In case of failure of these noninvasive techniques, based on laboratory workup, ICSI with surgically retrieved testicular spermatozoa can solve the problem.[107]

As to the treatment of harmful epigenetic sperm DNA modifications, they are often associated with sperm chromatin abnormalities, and IMSI appears to be the method of choice to select spermatozoa with normal epigenetic profile to be used for fertilisation.[98] Hence, the use of IMSI appears to be particularly interesting for the prevention of both genetic and epigenetic acquired sperm DNA alterations. In fact, IMSI allows the detection of regions with defective sperm chromatin condensation, appearing as intranuclear vacuoles (Figure 2) with a precision similar to that achieved by electron microscopy (Figure 1). The processing of specimens for electron microscopy is incompatible with cell survival, whereas IMSI can be used to select living, vacuole-free spermatozoa to be injected into oocytes, with a resolving power highly superior to that of the conventional ICSI procedure (Figure 3).

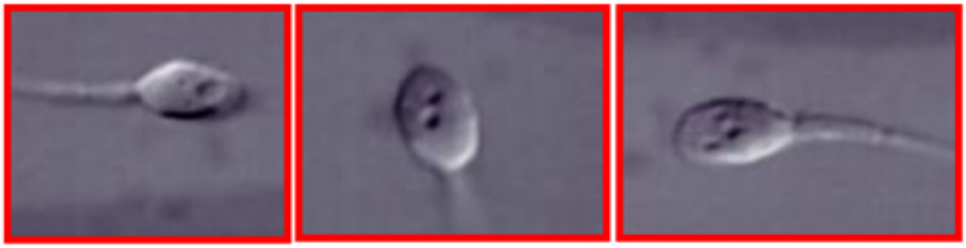

Figure 2: Sperm intranuclear vacuoles observed in living human spermatozoa at the magnification of x6,500, as used in intracytoplasmic morphologically-selected sperm injection (IMSI).

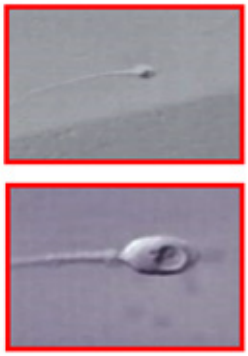

Figure 3: A human spermatozoon observed at the conventional magnification used in intracytoplasmic sperm injection (ICSI) (upper panel), and at high magnification used in intracytoplasmic morphologically-selected sperm injection (IMSI) (lower pane). The large intranuclear vacuole seen in the IMSI system is not detectable using the conventional ICSI magnification.

All of the above techniques have been reported to be successsful in some cases of excess sperm DNA damage.

However, the usefulness of each of them, in the context of different clinical scenarios, still remains to be confirmed by larger clinical studies.

FUTURE PERSPECTIVES

In spite of the wide range of diagnostic and therapeutic methods currently available to be used in cases of sperm DNA fragmentation, there are relatively few techniques for the management of epigenetic alterations of sperm DNA. As several waves of microRNA and tRNA fragments, molecules known to be involved in epigenetic modifications of DNA,[7] have been shipped to sperm during post-testicular maturation in the epididymis,[108] it remains to be determined whether the epigenetic status of human ejaculated spermatozoa differs from that of testicular spermatozoa. If RNA of epididymal origin responsible for epigenetic damage can be identified, they can be used for the development of diagnostic tests which might point out the cases of epigenetic sperm DNA damage in which the use of testicular spermatozoa would be of help.

Other, more sophisticated technologies, such as injecting specific microRNA molecules capable of repairing specific epigenetic defects into the early zygote,[108] or induction of DNA methylation of the genes of interest by a Dnmt3-type *de novo* DNA methyltransferase targeted to the corresponding sperm DNA sequence by a nuclease-inactivated CRISPR variant (dCas9),[109] may also be explored.

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Allergen-Specific Immunotherapy for Immunoglobulin E-Mediated Food Allergy

Authors: *Fernanda Chiera,[1] Irene Viola,[2] Antonietta Spinuzza,[2] Lucia Caminiti,[2] Giuseppe Crisafulli,[2] Ilenia Panasiti,[2] Giovanni Battista Pajno[2]

1. Department of Pediatrics, Ospedale San Giovanni di Dio, Crotone, Italy
2. Department of Pediatrics, Allergy Unit, University of Messina, Messina, Italy

*Correspondence to fernandachiera@hotmail.it

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Abstract

Food allergy is a potentially life-threatening condition and a significant public health concern worldwide. The current management includes food avoidance and use of emergency medications. The growing prevalence of food allergy drives research towards specific allergen immunotherapy (AIT), which represents a potential disease-modifying approach. AIT consists of the progressive administration of amounts of the offending allergen in order to induce food desensitisation, creating an increase in reaction threshold with regular exposure to the allergen. AIT can be performed through oral, sublingual, epicutaneous, and subcutaneous routes. The target is to achieve post desensitisation effectiveness: a long-lasting condition allowing patients to introduce food without reactions, even after a period of discontinuation of the offending food.

INTRODUCTION

Food allergy (FA) is a potentially life-threatening condition and has become increasingly common in children over the last two decades. FA affects up to 10.0% of the general population: approximately 5.0% of adults and 8.0% of children. The condition is considered a major public health issue in westernised countries, having a negative impact on quality of life, nutrition, and healthcare costs.[1] Currently, standard management includes food avoidance and the use of emergency medications. A food elimination diet is a troublesome and difficult task since the most common sources of allergens are widespread in daily diet: cow's milk (CM), hen's egg (HE), and peanut. These issues stimulate research on active and disease-modifying treatment for FA. Specific allergen immunotherapy (AIT) is a potentially active therapy indicated in those patients affected by persistent IgE-mediated FA in which food avoidance was ineffective, troublesome, or caused impairment of quality of life.[2] Other next-generation approaches, including probiotics, modified proteins, Chinese herbal supplements, biologics, and DNA vaccines, are under investigation.

ALLERGEN IMMUNOTHERAPY IN CLINICAL PRACTICE

The main goal of AIT is desensitisation, which is the ability to increase the amount of food the patient can tolerate without any reaction during

treatment. Desensitisation could achieve disease remission since the underlying allergic state persists but is temporarily modulated to a higher threshold and strictly dependent on regular consumption of the offending food.

AIT is potentially curative but does not guarantee permanent disease improvement or development of tolerance after stopping the desensitisation. The goal of FA-AIT is to achieve a post-discontinuation effectiveness, known as sustained unresponsiveness, so that a patient can eat a normal serving of the trigger food without being exposed daily to maintain desensitisation.[3] At present it remains unknown whether sustained unresponsiveness is equivalent to tolerance.[4]

Before starting any active treatment, the European Academy of Allergy and Clinical Immunology (EAACI) guidelines recommend confirmation of IgE-mediated FA and the threshold of reaction through allergy test (skin prick test, sIgE) and oral food challenge (OFC). AIT is recommended for persistent allergies to CM, HE, and peanut. In preschool age, especially when CM and HE are the culprit foods, it is preferable to wait for the natural resolution of FA and to start treatment at 4–5 years of age.

In AIT regimens, increasing amounts of allergen are delivered through oral immunotherapy (OIT), sublingual immunotherapy (SLIT), or epicutaneous immunotherapy (EPIT) routes at a scheduled time. The route of administration influences the amount of allergen delivered and, consequently, efficacy and safety. The best results in terms of efficacy have been reported with OIT, but they are counterbalanced by frequent adverse reactions, even if these are mild grade and not systemic.

Typically, an OIT protocol includes three phases: initial dose escalation, build-up, and maintenance. The first one consists of the consumption of predetermined and progressively increasing amount of an allergen source over several hours. During the build-up phase, the amount of allergen is periodically increased, usually weekly, until the target maintenance dose is achieved. Then, the maintenance dose is continued daily for months.[5] Dilutions of unprocessed products, raw extracts, and flours are used as allergen source.

SLIT efficacy is limited by the small volume of liquid antigen delivered sublingually, but conversely, adverse events (AE) do not occur as frequently as with OIT. In EPIT, smaller doses of antigen are delivered using a patch applied onto the skin. There are few pieces of evidence regarding the use of this route in FA-AIT; a recent randomised controlled trial showed positive results for peanut EPIT.[6] However, at the present time, SLIT and EPIT are not recommended for FA-AIT. The safety of OIT still represents one of the most important clinical aspects. The occurrence of AE remains quite frequent and common during the OIT maintenance phase. Home management presents additional safety issues. To improve the safety and efficacy, novel approaches investigated OIT combined with an immunomodulatory agent or an adjuvant, such as biologics, probiotics, and Chinese herbal supplements. Omalizumab is a recombinant, monoclonal antibody that selectively binds to freely circulating human IgE, but not mast cell or basophil-bound IgE. Its administration in addition to OIT or before starting the treatment decreases AE and can significantly reduce the time required to reach maintenance dosing. The rationale for the co-administration of probiotics is that they are potent stimulators of Th_1 immune response. Chinese herbal supplements have *in vitro* immunomodulatory effects and inhibit Th_2 cytokine response in murine models.

AIT is a safe approach, but it is not recommended in cases of comorbidities that could worsen during that treatment, such as uncontrolled or severe asthma, active malignant neoplasia, active systemic or autoimmune disorders, active eosinophilic oesophagitis, or other gastrointestinal eosinophilic disorders. Caution is needed when FA-AIT is proposed to patients with severe medical conditions such as cardiovascular diseases, systemic autoimmune disorders in remission (i.e., thyroiditis), uncontrolled active atopic dermatitis/eczema, chronic urticaria, mastocytosis, pharmacological treatments with beta-blockers, or ACE inhibitors. These are considered relative contraindications since the risk of AE has been demonstrated in other types of AIT.[3]

The setting of infection, exercise, or menses are described as frequent causes of acute AE, or temporary relapse of FA, during the maintenance or post desensitisation phases.[7] All patients should receive an emergency action plan and auto-injectable adrenaline. Therefore, according

to the available experimental data, a proper information and a structured written instruction plan (for example, avoiding physical activity within 2 hours of food intake, and reducing or interrupting the food intake during febrile illness) significantly reduce the risk of possible adverse reactions during the maintenance phase of food desensitisation, still maintaining the beneficial effect of treatment.

ORAL IMMUNOTHERAPY FOR COW'S MILK ALLERGY

After peanuts and tree nuts, CM is the third most common cause of anaphylactic reaction, and is the most common FA among children, affecting 0.6–2.5% of preschoolers and 0.3% of older children and teens.[7] CM contains >25 different proteins, but the allergenic source is represented by αS1-casein (Bos d 9), α-lactalbumin (Bos d 4), and β-lactoglobulin (Bos d 5), respectively, belonging to the casein and the whey fraction. Milk is one of the major components of children's diet, making it difficult to implement an elimination diet. The majority of children allergic to CM tend to naturally achieve tolerance by the age of 3 years, but lately, a higher incidence of persistence has been reported in adolescents, especially in cases of high sIgE concencetration.[8] Active immunotherapy is a good solution to this problem when the natural resolution does not occur.[9] AIT for the treatment of IgE-mediated FA has been discontinued for some decades. The current evidence shows that OIT is a good therapeutic approach to CM allergy and can successfully induce desensitisation. The Cochrane systematic review, including five randomised control trials, reported that 62.0% of children, following OIT, could tolerate a full serving of milk (approximately 200 mL), while 25.0% could tolerate a smaller amount (10–184 mL). In the control group, just 8.0% showed tolerance to a full serving, and none of them to a partial serving.[10]

Different protocols have been described, generally differing in the time needed to achieve the target dose (150–200 mL). Meglio et al.[11] demonstrated a desensitisation rate of 71.4% with a 6-month protocol. Starting with one drop of whole milk diluted 1:25 with water, increasing doses of CM were administered every 7 days for 10 weeks until the dose of 2 mL was scheduled every 16 days.[11] Pajno et al.[12] proposed a 4 month protocol with weekly increasing milk doses, starting with one drop of whole milk diluted 1:25. They reported that 77.0% of treated patients were successfully desensitised.[12] Berti et al.[13] proposed early OIT to be started in the first year. The target of the protocol was achieved in 97.0% of patients in a median time of 5.5 months.[13] The study included children who did not react to a low dose OFC performed at the baseline, so the authors could not exclude that some children would have developed tolerance spontaneously.

One of the major concerns about OIT protocols is safety. In the Cochrane review,[10] 91.5% of the treated patients experienced adverse reactions. In order to reduce the incidence of adverse reactions, new approaches are under investigation.

Up to 80.0% of CM allergic subjects can tolerate cooked milk. Heating destroys many conformational epitopes and reduces allergenicity of some foods. CM proteins may additionally be altered by their interactions with other substances, such as wheat, and their distribution in a food matrix. Casein is heat-resistant, while the whey fraction proteins are heat labile. Regular ingestion of baked milk has been demonstrated to induce immunologic changes and earlier development of tolerance to regular milk, suggesting its potential action as OIT.

Recent evidence showed that the combination of CM OIT with omalizumab shortens OIT protocol to achieve the maintenance dose and reduce the risk of associated adverse reactions, even in high-risk subjects. A pioneering study followed by other trials examined the combination of CM OIT with omalizumab. Omalizumab was started 9 weeks before oral rush desensitisation and discontinued at Week 16 during the maintenance phase. The authors concluded that OIT can be escalated more rapidly with omalizumab as an adjuvant therapy.[14] This approach, if confirmed by large double-blind placebo-controlled studies, could become an effective strategy for severe FA.

ORAL IMMUNOTHERAPY FOR HEN'S EGG ALLERGY

HE allergy is the second most common FA in infants and young children, following CM allergy.[15] The prevalence of HE allergy is up to 2.0% in young children.[16]

Over 50.0% of children with HE allergy develop natural tolerance at the age of 5 years; but for some, HE allergy can persist beyond adolescence[17] with a direct correlation to baseline levels of specific IgE. Subjects with sIgE above 50 kU/L (ImmunoCAP®) have a very low probability of resolving HE allergy before the age of 18.[16] Five major allergenic proteins from the egg of the domestic chicken (*Gallus domesticus*) are responsible for IgE-mediated reactions; these are designated Gal d 1-5.[18] Most of the allergenic HE proteins are found in egg white, including ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4), and ovomucin. Ovomucoid is the dominant allergen in egg (i.e., the allergen to which the most patients are sensitised), although ovalbumin is the most abundant protein in HE white. Two additional proteins, lipocalin-type prostaglandin D synthase and egg white cystatin, with IgE reactivity in individuals with HE allergy, have been identified.[19] Chicken serum albumin, or alpha-livetin (Gal d 5), is the major allergen in egg yolk. A minority of patients with HE allergy are also reactive to chicken meat and to other bird eggs (turkey, duck, goose, seagull, and quail) because of serologic and clinical cross-reactivity. Chicken serum albumin (Gal d 5) is responsible for this cross-reactivity.[20] Some medical products use egg proteins during production or as an ingredient. These drugs have the potential to cause allergic reactions in egg-allergic individuals. The yellow fever vaccine is prepared in egg embryos and allergic reactions to this vaccine have been reported.[21] Influenza vaccines contain a very small amount of HE proteins, but both the injectable inactivated influenza vaccines and the live attenuated influenza vaccine intranasally administered, can be safely administered to recipients with HE allergy without special precautions.[22]

The best treatment for HE allergy is OIT that induces a state of desensitisation increasing the threshold reaction. Unfortunately, 30.0–75.0% of patients lose the achieved desensitisation after a period of food withdrawal.[23,24]

One of the most popular OIT protocol consists of weekly administration, in a hospital setting, of increasing amounts of dehydrated egg white, diluted in sterile saline, starting with 0.1 mg. The dose is doubled every week until Week 16, to achieve a cumulative dose of 4 g in approximately 4 months. The children who tolerate the maximum dose of 4 g dehydrated egg white receive one cooked or one boiled egg, according to the child's preference, and continue the protocol at home. This kind of protocol appears to be safe and effective, and the whole procedure lasts approximately 4 months.[23]

As previously mentioned, processing foods may decrease or increase protein allergenicity in different ways, inducing, for example, a destruction of conformational epitopes, causing a lower accessibility of the epitopes to the immune system due to links between the proteins, fats, and sugars in the matrix or giving rise to the formation of new epitopes (Maillard reaction). Ovomucoid has a higher heat stability compared to ovalbumin, ovotransferrin, and globulin. A significant proportion of egg-allergic children are tolerant to egg in its baked forms: about 88.0% of children tolerate donuts, 74.0% baked omelettes, and 56.0% boiled egg.[25] The incorporation of baked egg, after an OFC confirming no clinical reactivity to extensively heated proteins, appeared to accelerate the development of unheated egg tolerance, compared to its strict avoidance.[26]

PEANUT ALLERGY

Peanut allergy is one of the most common allergies in paediatric age in westernised countries, with a rising prevalence from 1.2% in 2002 to 2.1% in 2008.[27,28] These data range in various countries based on peanut consumption. Peanut allergy, differing from CM and HE allergy, does not generally resolve spontaneously with age.[29] Peanut belongs to Leguminosae family (which includes peas and lentils), and its allergens are derived from different protein families that can cross-react with other members of the Leguminosae family, but also with other foods such as tree nuts. Botanically related or unrelated families have very similar homologous allergens, so IgE directed towards different allergens can cross-react and patients with peanut allergy may have a positive skin prick test or serum test to tree nut extract as a result of cross-sensitisation.[30,31] A study involving 278 patients with tree nut allergy found that only 9.0% outgrew their allergy. However, as with peanut allergies, sIgE correlate with prognosis, as 63.0% of patients with sIgE to tree nuts less than 2 kUA/L and 75.0% of patients

with negative sIgE outgrew their allergy.[32,33] For both peanuts and many tree nuts, the amount required to trigger an allergic reaction (50.0% of the maximum response, or elicitation dose 50) is very low compared with other major food allergens.[33] Peanut allergens include storage proteins (seed storage proteins), oleosins, defensins, lipid transfer proteins, pathogenesis-related proteins (PR-10), and profilins. Allergenic proteins, except profilins and PR-10, are heat stable and their allergenicity is not modified by food processing. At least three broad categories of seeds storage proteins have been identified as potentially important in FA: 2S albumins, vicilins (7S globulins), and legumins (11S globulins). In peanuts, Ara h 1 is a vicilin, Ara h 2 is a 2S albumin, and Ara h 3 is a legumin; Ara h 2 is responsible for most anaphylactic reactions.[31,34,35]

The analysis of IgE towards the molecular components is particularly useful in the diagnostic algorithm of peanut allergy, considering the high prevalence of sensitisation to Ara h 2 and its early onset.[36] Peanut allergy treatment consists of strict avoidance of peanut-containing products and a recommendation for parents to carry out a careful reading of food labels. Patients should be provided with a personalised emergency plan, which may include self-injectable adrenaline, antihistamines, corticosteroids, and bronchodilators. The most promising active treatment of peanut allergy is represented by allergen-specific immunotherapy. Two new drugs, AR101 and Viaskin patch, employed for peanut OIT and EPIT respectively, were submitted for U.S. Food and Drug Administration (FDA) licensing. They will likely be integrated into therapeutic management soon. Studies on safety and efficacy of AR101, a new drug containing defatted roasted peanut flour for OIT, documented that AR101 significantly improved patients' symptoms by reducing its severity during a double-blind, placebo-controlled challenge and increasing the amount of peanut protein tolerated after treatment.[37] Experimental trials on Viaskin, compared two doses (VP 100 mcg and VP 250 mcg) to placebo in a group of children and young adults. After 1 year of therapy, 45.8% of VP100 patients, 48.0% of VP250 subjects, and 12.0% of placebo group passed the double-blind, placebo-controlled challenge with 5,044 mg of peanut proteins or with a dose 10 times higher than the basal one. Treatment was more effective in patients <11 years of age.[38] Tang et al.[39] conducted a double-blind placebo-controlled trial on the association of *Lactobacillus rhamnosus* CGMCC and probiotic and peanut OIT (PPOIT) in peanut-allergic children. The primary outcome was the achievement of the post desensitisation effectiveness from 2–5 weeks after treatment suspension: 82.0% in the active group compared to 3.6% in the placebo group, acquired a possible post-desensitisation effectiveness. Moreover, patients in the active group presented an improvement in the quality of life when evaluated 3 and 12 months after the end of the treatment.[39,40]

POST DESENSITISATION STRATEGY

In the last decade, immunotherapy for FA has been shown to successfully induce desensitisation, but after stopping treatment, a daily ingestion of the culprit food is required to maintain tolerance. In children desensitised to CM, a twice weekly regimen has proved effective in maintaining tolerance.[41] Post discontinuation effectiveness is easier to achieve for CM and HE allergy, but recently a study found that most patients treated with combined PPOIT 4 years after completing the protocol were symptom free after peanut ingestion.[42]

CONCLUSION

Immunotherapy, especially through the oral route, is a promising treatment for food allergies in children. It represents a next-generation active approach to FA that is still under investigation, but has already shown many significant clinical results. If the elimination diet is merely a passive, conservative approach, in whom no allergen means no allergy, AIT represents an active treatment that, through the continuous exposition to the allergen, increases tolerance.

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Primary Sclerosing Cholangitis: A Clinical Update

Authors: *Sridhar Sundaram,[1] Vaneet Jearth[2]

1. Department of Gastroenterology, Seth GS Medical College and KEM Hospital, Mumbai, India
2. Department of Digestive Diseases and Clinical Nutrition, Tata Memorial Centre, Mumbai, India

*Correspondence to drsridharsundaram@gmail.com

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Abstract

Primary sclerosing cholangitis (PSC) is a rare cholestatic disorder of the liver, with strictures in the bile ducts leading to cirrhosis of the liver in a proportion of patients. PSC is commonly associated with inflammatory bowel disease and increased risk of cholangiocarcinoma, gall bladder cancer, colorectal cancer, and hepatocellular carcinoma. Medical therapies are primarily aimed at symptom management and disease-modifying therapies are limited. Endoscopic therapies are used in patients with dominant strictures and liver transplantation is a last resort. In this article, the authors aim to comprehensively review the epidemiology, diagnosis, and management of PSC with emphasis on risk of malignancies and management of PSC. The authors also survey the advances in pathogenesis understanding and novel medical therapies for PSC.

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a rare cholestatic disorder of the liver associated with inflammation and injury with fibrosis in the intrahepatic and extrahepatic bile ducts. A large proportion of patients have associated inflammatory bowel disease (IBD) with PSC.[1-3] PSC presents with features of cholestatic liver disease; however, patients can present with decompensated liver disease over the long term. PSC is associated with a predisposition to malignancies, including cholangiocarcinoma (CCA), gall bladder cancer, hepatocellular carcinoma (HCC), and colorectal cancer (CRC).[4] Diagnosis is largely based on imaging with magnetic resonance cholangiopancreatography (MRCP); however, liver biopsy may be needed in a small number of cases. Medical therapies are limited to management of cholestasis in patients with PSC, while endoscopic therapy remains the mainstay when dominant strictures are present.[5] Liver transplantation remains the last resort in decompensated liver disease, although recurrence post transplantation is frequent.[6] Despite the advances in imaging and diagnostics, therapy options for PSC are still limited, with most patients diagnosed with end-stage disease.

EPIDEMIOLOGY, GENETICS, AND PATHOGENESIS

PSC is a rare disease with an incidence rate in northern Europe and the USA of 0.4–2.0 per 100,000 people per year.[5] An increasing incidence of PSC has been reported, mainly attributed to the use of MRCP leading to an increase in early diagnosis.[7] The median age of presentation is 30–40 years, with a male to female ratio of 2:1.[8] In patients of northern European descent with PSC, 62–83% have associated IBD while 20–50% patients from southern Europe and Asia have associated IBD.[9] The ratio of ulcerative colitis (UC) to Crohn's disease (CD) is 6:1 in patients with PSC. A distinct phenotype termed as PSC–IBD has recently been described in association with PSC with features of both UC and CD.[10]

PSC is a complex multifactorial disease with known genetic associations, both HLA (HLA B8 and HLA DR3) and non-HLA associated. There is known familial association, with the risk being 0.7% amongst first-degree relatives and approximately 1.5% amongst siblings, both 100-times higher than that in the general population.[11] Non-HLA associations are found with killer immunoglobulin-like receptor (KIR), major histocompatibility complex (MICA and MICB), intracellular adhesion molecule-1 (ICAM-1), chemokine receptor 5 (CCR5), G protein coupled bile acid receptor 1 (GPBAR1) TGR5, multidrug resistance gene-1 (*MDR-1*), steroid and xenobiotic receptor (SXR), and its analogue pregnane X receptor (PXR) polymorphisms.[12] Despite the common association of PSC with IBD, the HLA association of the two disorders is not characteristic, as demonstrated in a Scandinavian cohort.[13] Although PSC is rare, the HLA subtypes associated with risk are rather common and hence the probability of false genetic association remains a problem in study design. This problem is not related to statistical power alone but to the possibility of allelic complexity at a susceptibility locus.[14]

The leaky gut hypothesis was initially used to describe the pathogenesis of PSC. Due to mucosal injury, increased bacterial translocation into the portal venous system leads to an inflammatory response in the bile ducts, leading to cholangitis and subsequent wound healing by periductal fibrosis. However, a Scandinavian study on this hypothesis showed no significant difference in small intestinal bacterial overgrowth or intestinal permeability between PSC patients and controls.[15] The gut lymphocyte homing hypothesis was proposed because PSC usually runs a course independent of IBD. Memory T lymphocytes primed in the inflamed gut may trigger and continue inflammation in the periportal region.[16] Both these hypotheses are proposed for patients with IBD and PSC. These hypotheses, however, failed to describe PSC patients without IBD and those who develop PSC prior to the development of IBD. The autoimmune hypothesis was proposed due to the presence of autoantibodies like perinuclear antineutrophil antibodies (pANCA) in association with 80% of PSC patients and known overlap syndrome between autoimmune hepatitis and PSC. This hypothesis is based on molecular mimicry of biliary antigens on cholangiocytes and immune response to the same.[17] In 2010, the biliary umbrella hypothesis was proposed. There is a loss of the biliary bicarbonate umbrella in patients with PSC, which may increase permeation of protonated bile acids, leading to the injury of cholangiocytes.[18] It is also referred to as the toxic bile hypothesis.

The pathogenesis for PSC remains complex, with need for further studies. In addition, various ongoing animal studies may help further elucidate the pathogenic mechanisms and potential therapeutic targets for both early and advanced PSC.[12]

CLINICAL FEATURES, DIAGNOSIS, AND DIFFERENTIALS

Fifteen to forty percent of patients are asymptomatic at diagnosis. Fatigue and pruritus are the most common symptoms of disease. Ascending cholangitis due to transient biliary obstruction may be a presenting manifestation in patients with fever, chills, and right upper quadrant pain. Patients may present with recurrent episodes of jaundice. In patients with cirrhosis and portal hypertension, decompensation is evident in the form of ascites, hepatic encephalopathy, or bleeding.[19] Laboratory anomalies include increased bilirubin and alkaline phosphatase. Transaminases <300 U/L are

seen in patients with PSC. Albumin is normal in early stages and decreases with progression of liver disease. Hypergammaglobulinaemia and elevated IgG levels are seen in patients with PSC. Antimitochondrial antibody (AMA) is rarely positive in patients with PSC.[20] Autoantibody testing is not a contributing factor in the diagnosis of PSC. Although pANCA is commonly seen in patients with PSC, it is not specific. Antinuclear and smooth muscle antibodies are seen in patients with PSC in low titres. In patients with autoimmune hepatitis–primary sclerosing cholangitis (AIH–PSC) overlap, the antibodies are seen in higher titres.[21] Although AMA is rarely positive in patients with PSC, overlap between primary biliary cholangitis (PBC) and PSC is rarely reported.[22]

Cholangiography is the gold standard for diagnosis of PSC. MRCP is the investigation of choice for diagnosis. MRCP avoids radiation and contrast media associated with endoscopic retrograde cholangiography (ERCP). However, MRCP provides suboptimal visualisation of the intrahepatic bile ducts. Multifocal short segmental strictures are seen on MRCP suggesting a 'beads on string appearance'.

Both intrahepatic and extrahepatic ducts are involved in most of the patients (Figure 1).

Isolated intrahepatic ductal involvement is seen in approximately 25% of patients. Isolated extrahepatic involvement is rare (<5%). Also gall bladder, cystic duct, and pancreatic ducts may show involvement in PSC.[23] MRI can also help in prognostication in patients by assessment of relative enhancement using hepatocyte-specific contrast (gadoxetate disodium) with a sensitivity of 73% and specificity of 92% and correlates with clinical outcomes.[24]

Histologic features in PSC include damage and loss of medium and large-sized bile ducts both within and outside the liver. This picture is not classically evident on liver biopsy and ductopenia may be seen in the small sized bile ducts due to obstruction. Onion skin fibrosis of the bile ducts is a classical feature, but is evident in only 15% patients following liver biopsy.[25] Staging systems for PSC have been described on liver biopsy, which include Stage 1) cholangitis with portal hepatitis, Stage 2) periportal fibrosis, Stage 3) septal fibrosis or bridging necrosis, and Stage 4) biliary cirrhosis.[26] Liver biopsy is performed to rule out other diseases, to look for AIH overlap, or in cases of suspected small duct PSC.

VARIANTS OF PRIMARY SCLEROSING CHOLANGITIS

Overlap Syndrome

Suspicion of overlap with AIH should be high in situations with raised transaminases (>5-times the upper limit of normal) with raised serum Ig levels (IgG >2-times the upper limit of normal) with features of PSC on imaging and evidence of cholestasis (raised alkaline phosphatase and gamma-glutamyl transferase) on biochemistry. Biopsy is required for confirming the diagnosis. Overlap of PSC with AIH is known to occur in 7–14% of patients with AIH. Treatment with immunosuppressants is useful, although their impact on fibrogenesis and cirrhosis is not as pronounced as with AIH without PSC.[27] PSC is also known to have overlap with PBC in few case reports.[22] A genetic risk locus has been described for coexistence of PSC with PBC (chromosome 1p36).[28]

Small Duct Primary Sclerosing Cholangitis

Involvement of small ducts only can be seen in 5–15% of patients. Liver biopsy is required for diagnosis of small duct PSC. Small duct PSC progresses to large duct PSC in 25% of patients. Small duct PSC in the absence of large duct involvement is not associated with predisposition to CCA. The diagnosis of small duct PSC is difficult to make, especially in settings without concomitant IBD. Small duct PSC is also more commonly associated with Crohn's disease.[29] Progressive familial intrahepatic cholestasis Type 3 is often similar in clinical presentation and histopathology to small duct PSC.[30]

DIFFERENTIAL DIAGNOSIS

Causes of secondary sclerosing cholangitis need exclusion prior to diagnosis of PSC (Table 1).[31]

Dominant Strictures and Risk of Malignancy

Various classifications have been used to

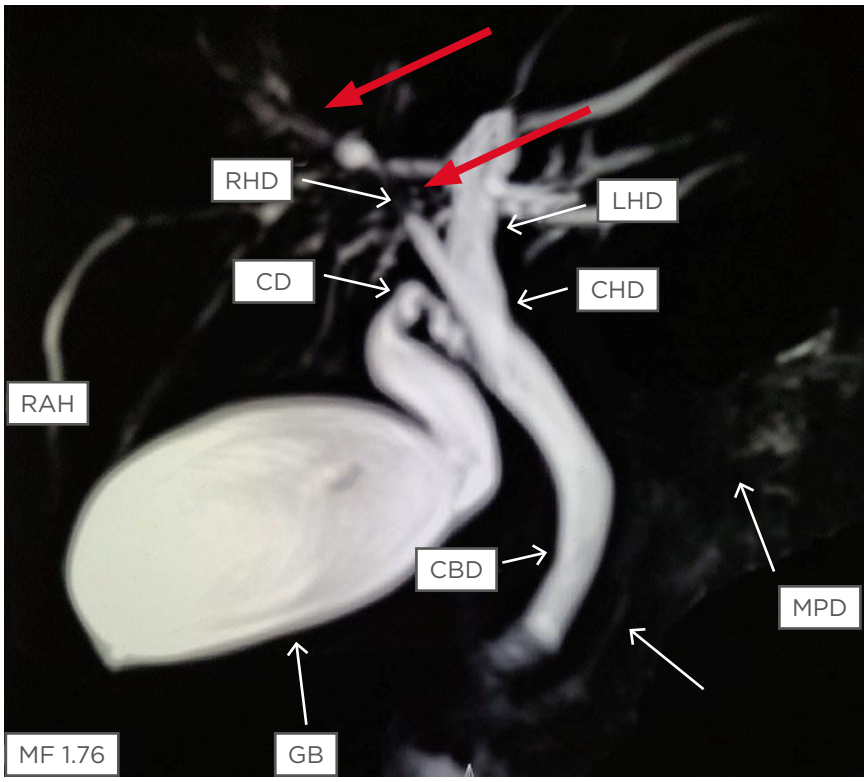

Figure 1: Magnetic resonance cholangiopancreatography showing stricture in the right hepatic duct (red arrows) with beading in the smaller ducts in the right lobe of the liver (white arrows).

CBD: common bile duct; CD: cystic duct; CHD: common hepatic duct; GB: gall bladder; LHD: left hepatic duct; MPD: main pancreatic duct; RAH: right anterior hepatic duct; RHD: right hepatic duct.

describe the cholangiographic findings in PSC. The one commonly used and correlating with prognosis is the Amsterdam Classification given by Ponsioen et al.[32] These criteria are not specific for PSC and must be interpreted in the context of patient demographic data and clinical features. Dominant strictures have been defined in patients with PSC as stenosis of <1.5 mm in the common bile duct and <1.0 mm in the hepatic duct within 2 cm from the main hepatic confluence. This definition primarily applies to stenosis seen on ERCP (Figure 2). MRCP is not used to define dominant strictures considering the insufficient spatial resolution and lack of hydrostatic pressure.[33] Multiple dominant strictures may be seen in up to 12% of patients. Due to the risk of malignancy, in settings with worsening clinical symptoms (such as jaundice and cholestasis), rising liver function tests, or the development of a new stricture as identified by MRC, it is best to consider stricture dilatation with sampling via brush cytology to rule out malignancy. Risk of malignancy in one cohort with dominant strictures was 6%.[33] In the same cohort, endoscopic intervention in dominant stricture via dilatation resulted in improved transplant-free survival at 5 years (81%) and 10 years (52%).

PSC is a risk factor for the development of CCA, gall bladder cancer, HCC, and colon cancer. The estimated lifetime risk of CCA is 8–36% in patients with PSC. About 50% of patients have CCA diagnosed during the first presentation or during the first year following diagnosis of PSC. MRI can be used for diagnosis of CCA in PSC. Carbohydrate antigen 19-9 levels are useful for diagnosing CCA; however, these may be elevated in patients with cholangitis with obstructive jaundice in PSC. A cutoff of 20 IU/mL may be used for diagnosing early CCA in PSC patients; however, the sensitivity is 78% and specificity 67%. Higher cutoffs lead to loss of sensitivity. Biliary brush cytology during ERCP is useful for diagnosis with 100% specificity; however, sensitivity is low at 43%.

Table 1: Causes of secondary sclerosing cholangitis that mimics primary sclerosing cholangitis.[30]

Infection	Bacterial/parasitic cholangitis
	Recurrent pyogenic cholangitis
Immunodeficiency syndromes	Congenital immunodeficiency
	Combined immunodeficiency
	Acquired immunodeficiency (HIV)
Mechanical/toxic/ischaemic	Cholelithiasis/choledocholithiasis
	Surgical bile duct injury
	Intra-arterial chemotherapy
	Vasculitis
	Portal cavernoma cholangiopathy
	Hepatic allograft arterial insufficiency
Pancreatic diseases	Chronic pancreatitis
	Autoimmune pancreatitis Type I/IgG4 cholangiopathy
Others	Caroli disease
	Sarcoidosis
	Hodgkin's lymphoma
	Neoplastic/metastatic disease
	Hypereosinophilic syndrome

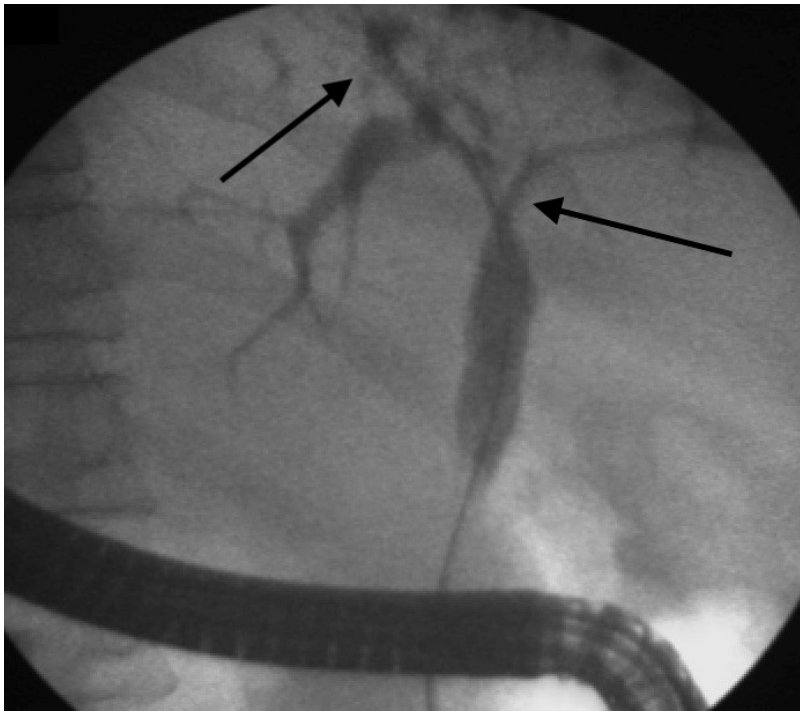

Figure 2: Endoscopic retrograde cholangiography showing presence of dominant stricture at hilum extending into the right and left hepatic duct (arrow) with beaded appearance of intrahepatic ducts (arrow).

The addition of fluorescent *in situ* hybridisation (FISH) to biliary sampling increases sensitivity to 51%. Presence of polysomy on FISH is highly suggestive of CCA.[34] Yearly carbohydrate antigen 19-9 levels tests and MRI are suggested for the surveillance of CCA in patients with PSC.

Lifetime risk of gallbladder cancer is 3–14% among patients with PSC. Patients with gall bladder polyps >8 mm in size are more likely to harbour malignancy and hence warrant cholecystectomy. Cirrhosis in patients with PSC predisposes them to the development of HCC. Lifetime risk of HCC is approximately 0.3–2.8%, hence HCC surveillance in cirrhotic patients with PSC is warranted.[35] Pancreatic cancer risk in PSC patients has been reported to be 14-times higher than in the general population as per a study from Sweden in 2002.[36] Another study from the USA reported >10-times increased risk of pancreatic cancer in patients of PSC with IBD.[37] There is still debate on the diagnostic overlap between CCA and pancreatic cancer. Pancreatic ductal changes are known in PSC; however, the exact mechanism of carcinogenesis is not known.

CRC risk is 4-times higher in patients with PSC and IBD than those with IBD alone. The subset of PSC patients without IBD does not have increased CRC risk.[38] Patients who develop CRC with a background of PSC–IBD have a younger age of symptom onset with a predilection for right-sided colonic malignancies. Risk factors in the presence of IBD include duration of disease, extent of disease, and presence of PSC. In view of the quiescent nature of IBD in patients with PSC, colonic mapping biopsies are warranted in patients with PSC for diagnosis of IBD. In the presence of PSC, patients with IBD should undergo yearly colonoscopies for CRC screening.[39]

RISK STRATIFICATION

As with cirrhotic patients, the Child–Pugh score and Model for End-Stage Liver Disease (MELD) are used for risk stratification. Alkaline phosphatase (ALP) is an independent predictor of outcomes in patients with PSC. ALP can be considered a continuous variable or single measurement for prognostication. The most commonly used risk score is the revised Mayo Score, which includes age, albumin, bilirubin, variceal bleeding, and AST levels. The score has good prediction of survival; however, it also has poor utility in early disease.[5] The use of the revised Mayo Score obviates the need for biopsy in risk stratification. Another score called the Primary Sclerosing Cholangitis Risk Estimation Tool (PREsTo), consisting of nine variables (bilirubin, albumin, ALP, SGOT, platelets, haemoglobin, sodium, patient's age, and years since diagnosis of PSC), is better than other available risk stratification tools (MELD and revised Mayo Score) for prediction of decompensation in patients with PSC.[40]

MANAGEMENT OF PRIMARY SCLEROSING CHOLANGITIS

Medical Management

No single drug or treatment has been proven to prolong transplant-free survival in PSC. The hydrophilic bile acid, ursodeoxycholic acid (UDCA), has been extensively studied. However, evidence showing the long-term benefit of UDCA is unclear and its use remains controversial. A study of 105 patients using low-dose of UDCA (13–15 mg/kg/day) showed biochemical improvement but lacked evidence of clinical improvement.[41] A landmark, large, multicentre study[42] using a high dose of UDCA of 28–30 mg/kg/day showed that the patients receiving the drug fared worse when compared to placebo, with substantially more adverse, clinically important outcomes, such as the need for transplantation and the development of varices. At this point, no indication exists for using the higher doses of 28–30 mg/kg/day of UDCA. Nonetheless, UDCA remains widely used, typically at moderate doses of 15–20 mg/kg/day. The chemoprotective effect of UDCA is not verified, with small retrospective studies showing benefit in chemoprevention of CRC in patients with PSC. On the other hand, few studies have shown increased risk of CRC in PSC patients who receive high doses of UDCA.[43]

The European Association for the Study of the Liver (EASL) guidelines recommend UDCA for use in high-risk settings for CRC prevention in patients with long duration and extensive disease with IBD. The American Association for the Study of Liver Diseases (AASLD) guidelines on

the other hand do not endorse use of UDCA for prevention of CRC.[34,44,45]

Immunosuppressive and Other Agents

Other treatments that have been tested without any proven clinical benefit or improvement of liver biochemistries include:

- Azathioprine[46]
- Budesonide[47]
- Docosahexaenoic acid[48]
- Metronidazole[49]
- Minocycline[50]
- Mycophenolate mofetil[51]
- Nicotine[52]
- Pentoxifylline[53]
- Pirfenodone[54]
- Prednisolone[55]
- Tacrolimus[56]
- Vancomycin[57]
- Methotrexate[58,59]

Novel Therapies

Farnesoid X receptor (FXR) agonists (e.g., obeticholic acid, 6α-ethyl chenodeoxycholic acid) and 24-norursodeoxycholic acid, a side-chain-modified UDCA derivate resistant to amidation which undergoes cholehepatic shunting, may be novel treatment options. A Phase II study in patients with PSC demonstrated the plausible effectiveness of the 24-norursodeoxycholic acid derivative with significant improvement in AP and bilirubin levels. The side effects were unremarkable with no change in IBD activity.[60] Large Phase III studies are ongoing into the use of 24-norursodeoxycholic acid in PSC. Obeticholic acid is currently being evaluated in a Phase II study in PSC.[61] Obeticholic acid reduces toxic bile production and induce secretion through FXR mediated pathways. Obeticholic acid also reduces FGF19 levels to decrease fibrogenesis.[62] Fibrates, with their global pleiotropic and anti-inflammatory properties, may be an alternative novel treatment strategy with reduction in production of toxic bile and stimulation of canalicular secretion. Two pilot studies have evaluated the role of fibrates in PSC. Twenty-one patients treated over 6–12 months showed benefit in biochemical markers; however, there is a need for larger placebo-controlled studies.[63] Various antibiotics have been tried in PSC in an attempt to change the microbiological milieu in the intestine; however, there have been concerns regarding emerging resistant organisms and hence their chronic use is not encouraged. Apical sodium bile acid transporter inhibitors like maralixibat have been studied in a Phase II trial in PSC, showing improvement in bile acid levels but no significant improvement in ALP and bilirubin levels.[64] Faecal microbiota transplantation remains a feasible option for patients in theory, but no studies are currently available to attest to its benefit in this setting.

ENDOSCOPIC MANAGEMENT

Patients with dominant strictures, even if CCA is excluded, have a substantially worse prognosis than those without. Endoscopic intervention for dominant biliary strictures in patients with symptomatic disease appears beneficial.[33] Guidelines recommend balloon dilatation as first-line endoscopic treatment.[65] Several studies have shown that short-term stenting is not superior to balloon dilatation alone.[66,67] Routine stenting after dilation of a dominant stricture is not required, whereas short-term stenting (1–2 weeks) may be required in patients with severe stricture. Long-term stent placement is associated with an increased risk of complications and a need for unscheduled stent exchanges. The role of metal stents, which are fully covered, removable, and self-expandable, in PSC is yet to be established. Patients undergoing ERCP should have antibiotic prophylaxis to prevent post-ERCP cholangitis. Patients with PSC are also at higher risk of pancreatitis and the routine use of diclofenac suppositories is warranted in these patients prior to or immediately after ERCP.[65]

Screening for varices should be performed if evidence suggests cirrhosis or suspicion of portal hypertension. Patients with PSC can develop varices even before cirrhosis develops. A total colonoscopy with mapping mucosal biopsies is advised to exclude IBD once PSC is diagnosed. For patients with confirmed colitis, annual colonoscopic surveillance for colorectal dysplasia or neoplasia is recommended. Percutaneous biliary drainage for treatment of dominant strictures can be performed in PSC patients with

altered anatomy that prevents successful ERCP, such as Roux-en-Y choledochojejunostomy or gastric bypass, or as a rescue therapy after failed endoscopic access. It should not be attempted as first-line therapy because of the risk of complications, including hepatic arterial injury, haemobilia, and cholangitis.[68-70]

SURGICAL MANAGEMENT

Biliary reconstruction by biliary-enteric drainage allows prolonged clinical improvement, with the resolution of jaundice and cholangitis, but is associated with increased risk of cholangitis and mortality. Postoperative scarring also increases the difficulty of liver transplantation. Surgical drainage procedures have largely been discontinued in favour of transplantation due to the superior outcomes of liver transplantation.[71]

LIVER TRANSPLANTATION

Liver transplantation remains the only curative treatment for PSC, albeit with a substantial risk of disease recurrence. PSC is a well-established indication for liver transplantation in patients with decompensated liver disease (MELD >14), intractable pruritus, or recurrent bacterial cholangitis.[72] PSC accounts for <5% of transplant indications in the USA and 15% of transplants in Scandinavia. Outcomes of liver transplant in PSC patients are better than with other indications considering the young age at which the patients are transplanted: 5-year survival rates are approximately 85%.[73] PSC patients have predilection for peritransplant hypercoagulability, although no specific prophylaxis is required for this. Another important consideration in these patients is the type of biliary anastomosis, with duct-to-duct anastomosis being better than bilioenteric anastomosis in PSC. This preference is largely based on case control studies, which demonstrate a higher rate of cholangitis in patients with bilioenteric anastomosis.[74] No specific guidance for immunosuppression post-transplant for PSC is available and triple drug regimens are used. At least 20% of transplanted patients will develop recurrent disease after transplant. Colectomy prior to liver transplant is associated with lower rates of recurrence. No single factor has consistently been associated with increased recurrence risk post-transplant.[75]

SYMPTOMATIC TREATMENT

The most troublesome symptom is pruritus. Dominant strictures should be sought and actively managed. Medical management of pruritus is directed by the severity of the underlying pruritus. Mild pruritus may be treated with skin emollients and possibly antihistamines. First-line therapy for severe pruritus is with the bile acid sequestrant cholestyramine. Second-line therapies include rifampicin, naltrexone, sertraline, and phenobarbitone. At present, no specific therapies for fatigue exist. Patients with PSC have an increased risk of osteoporosis and might also be deficient in vitamin D and other fat-soluble vitamins. Patients are advised to undergo weight-bearing exercise and take calcium and vitamin D supplementation.[44]

CONCLUSION

PSC is a chronic cholestatic liver disease, often associated with cirrhosis with increased risk of malignancy. There is a need for surveillance strategies for detection of various cancers. Medical therapies are sparse, with endoscopic therapies available for dominant strictures. Liver transplantation remains an option in decompensated liver disease, intractable pruritus, and rarely in settings with CCA. Although the clinicians continue to treat PSC, ongoing research in many areas, including effective medical therapies, diagnostic tools, genetics, pathogenesis, and biliary and bowel complications after liver transplantation may open doors to better management of this enigmatic disease.

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Polymyalgia Rheumatica and Seronegative Elderly-Onset Rheumatoid Arthritis: Two Different Diseases with Many Similarities

Authors: *Ciro Manzo,[1] Amir Emamifar[2,3]

1. Internal and Geriatric Medicine Department, Rheumatologic Outpatient Clinic Hospital "Mariano Lauro", Sant'Agnello, Italy
2. Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
3. Diagnostic Center, Svendborg Hospital, Odense University Hospital, Svendborg, Denmark

*Correspondence to cirmanzo@libero.it

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Abstract

Polymyalgia rheumatica (PMR) and seronegative elderly-onset rheumatoid arthritis (SEORA) are two of the most frequent inflammatory rheumatologic diseases in elderly patients. At first presentation, there are many similarities between PMR and SEORA, that may lead to a real diagnostic conundrum. The most relevant similarities and differences between PMR and SEORA are discussed in this review. In addition to the acute involvement of the shoulder joints, important features characterising both diseases are morning stiffness longer than 45 minutes, raised erythrocyte sedimentation rate, and a good response to low doses of prednisone. Some findings (such as erosive arthritis or symmetrical involvement of metacarpophalangeal and/or proximal interphalangeal joints) can help to make the diagnosis of SEORA, whereas shoulder and hip ultrasonography and 18-FDG PET/CT seem to be less specific. However, in several patients only long-term follow-ups confirm the initial diagnosis. A definite diagnosis of PMR or SEORA has significant therapeutic implications, since patients with PMR should be treated with long-term glucocorticoids, and sometimes throughout life, which predisposes the patients to serious side effects. On the contrary, in patients with SEORA, short-term treatment with glucocorticoids should be considered when initiating or changing disease modifying antirheumatic drugs, followed by rapid tapering.

INTRODUCTION

Polymyalgia rheumatica (PMR) is considered the most frequent inflammatory rheumatic disease in the elderly.[1,2] Its highest prevalence is between 70–80 years of age, with a slow increase until the age of 90 years.[3] Classic symptoms are bilateral pain and aching and stiffness in the shoulders and pelvic girdle, associated with morning stiffness lasting >45 minutes. In some patients, an inflammatory pain in the neck is also present.[4,5] Inflammatory markers (such as erythrocyte

sedimentation rate [ESR] and C-reactive protein concentrations) are usually raised. However, normal ESR and C-reactive protein concentrations should not be a reason to exclude PMR.[6-8]

Elderly-onset rheumatoid arthritis (EORA) is, by definition, a rheumatoid arthritis (RA) developing in persons >60 years of age.[9,10] Recent studies have confirmed that RA is among the most common inflammatory disease in older age groups, with a 2% prevalence.[11] When rheumatoid factor (RF) and anticitrullinated peptide/protein antibodies (ACPA) are absent, seronegative EORA (SEORA) is diagnosed. In some patients, a clinical onset that mimics PMR is possible.[12-14]

SEORA can be considered the most frequent PMR mimicking-disease. In some studies, >20% of patients changed the first diagnosis of PMR to SEORA during follow-ups.[15-17] In 1992, Healey[18] suggested that PMR and SEORA might be the same entity. Recently, this point of view has been put forward again.[19]

In this review, the authors discuss the diagnostic and classification criteria of these two inflammatory rheumatic diseases. Furthermore, the authors highlight the main differences and similarities between seronegative and seropositive RA, EORA, and young-onset RA (YORA), and EORA and PMR-like EORA. Finally, the authors discuss therapeutic differences and similarities: both steroid and non-steroid therapeutic options are discussed.

DIAGNOSTIC AND CLASSIFICATION CRITERIA

The diagnosis of PMR is challenging, due to lack of any specific diagnostic test and the presence of other conditions mimicking PMR, mainly elderly onset seronegative RA.[20-22] Due to uncertainty related to the diagnosis of PMR, a prompt response to glucocorticoid (GC) treatment has been commonly used to establish the diagnosis. However, only about half of patients respond completely to GC after 3 weeks of treatment with 15 mg oral prednisolone.[23] Additionally, response to GC can be observed in other mimics of PMR.[24]

To date, several diagnostic and classification criteria sets for PMR have been defined in the literature; they have some features in common, such as an age cut off, elevated markers of inflammation, and pain and/or stiffness in the shoulder and/or hip girdles.[1,25-28]

To improve the criteria specificity for PMR, the 2012 provisional European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria for PMR used, for the first time, findings of shoulder (subdeltoid bursitis, biceps tenosynovitis, and/or glenohumeral synovitis) and hip (synovitis and/or trochanteric bursitis) ultrasound (US) along with clinical presentations.[28] In this regard, the absence of positive RA serology (RF and ACPA) and peripheral synovitis is in favour of diagnosis of PMR and aims to distinguish PMR from RA. Although autoantibodies such as RF and ACPA are mostly seen in RA, diagnostically insignificant low levels of RF can be detected in elderly people.[29] It is worth noting that these classification criteria are designed to discriminate patients with PMR from other mimics of PMR and are not meant for diagnostic purposes.

In a single-centre study, Macchioni et al.[30] compared the performance of 2012 EULAR/ACR classification criteria for PMR with prior criteria sets in new-onset, confirmed PMR patients using ultrasonography findings. They found that the EULAR/ACR classification criteria had the highest ability to discriminate PMR from RA and other inflammatory articular diseases.[30] However, in a study by Ozen et al.[31] comparing the performance of different criteria sets in patients >50 years of age, presenting with new-onset bilateral shoulder pain with elevated acute phase reactants who fulfilled the EULAR/ACR classification criteria, the sensitivity of the EULAR/ACR classification criteria for PMR was high but its ability to discriminate PMR from other inflammatory/noninflammatory shoulder conditions, especially from seronegative RA, was suboptimal.[31]

The 1987 ACR[32] and, more recently, the 2010 ACR/EULAR[33] classification criteria for RA provide criteria for the classification of patients as having RA as opposed to other joint diseases. The specificity and sensitivity of the old criteria was not adequate for the classification of patients with early inflammatory arthritis as

having RA.[34] To detect early RA, on the other hand, the new criteria give much weight to RA serologic biomarkers, which may result in failure to identify individuals with seronegative RA.[35] Thus, the difference between proportions of patients fulfilling the new or old criteria sets highlights the importance of both criteria, especially in cases of seronegative RA.

SEROPOSITIVE VERSUS SERONEGATIVE RHEUMATOID ARTHRITIS

Based on the status of RA serology (i.e., RF and ACPA), RA can be categorised into seronegative RA and seropositive RA. This classification is valuable for diagnosis, making the treatment decision, and predicting the prognosis of RA, where the presence of RF and ACPA have been regarded as poor prognostic factors.[35-37] In comparison with seropositive RA, seronegative RA has been considered a less severe disease with less radiographic damage progression.[38-42] Less intensive treatment has been suggested in previous literature, but the necessity of changing to another conventional synthetic disease modifying antirheumatic drugs (DMARD) was underlined by the 2016 update of the EULAR treatment recommendations, also applying to patients with seronegative RA when they do not achieve the treatment target.[36]

Findings in recent literature in respect to the influence of seronegative status on clinical course and treatment are controversial. In a recent study by Nordberg et al.,[43] wherein 234 patients with RA (15.4% seronegative) who fulfilled the 2010 ACR/EULAR classification criteria were recruited between 2010 and 2013, the authors found that ultrasonography scores for joints and tendons, number of swollen joints, disease activity score, and Physician's Global Assessment were significantly higher in seronegative patients compared with seropositive patients, representing a higher level of inflammation in seronegative RA patients.[43] These findings may pinpoint the need for a high number of involved joints for seronegative patients to fulfil the 2010 ACR/EULAR classification criteria for RA.[44,45] Furthermore, after a 2 year follow-up, the authors found similar disease activity measures, radiographic progression, and remission rates, but a slower treatment response in seronegative RA, despite higher levels of inflammation in seronegative RA at baseline.[43] In contrast, seropositive RA has been suggested by other authors to represent a more aggressive subset of disease with significant radiological joint damage, which needs to be treated more intensively.[46-49]

Table 1: Main differences between elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis.

Features	EORA	YORA
Female:male ratio[9,11]	2:1	4:1
PMR-like onset[10,12]	25%	N.E.
Comorbidities[9,35,50]	50–75%	<10%
Polypharmacology[50,51,52]	>70%	<10%
Treatment[52-55]	Influenced by comorbidities. Usually not aggressive.	More frequent use of synthetic and biological DMARD.
Prognosis[10,45]	Lower remission rates, more radiographic progression, higher HAQ scores. Mortality and disabilities are more frequent.	

DMARD: disease modifying antirheumatic drugs; EORA: elderly-onset rheumatoid arthritis; HAQ: Health Assessment Questionnaire; N.E.: not evaluated; PMR: polymyalgia rheumatica; YORA: young-onset rheumatoid arthritis.

DIFFERENCES BETWEEN EARLY-ONSET AND YOUNG-ONSET RHEUMATOID ARTHRITIS

Similar to other diseases, RA has some significant differences in different age group populations. The main differences between EORA and YORA have been summarised in Table 1.

For example, there is a PMR-like EORA, a clinical feature that can occur in 25% of cases.[10] On the contrary, PMR-like YORA is very exceptional. Indeed, YORA is classically a symmetric polyarthritis involving the small articulations of hands, wrists, and/or feet, without involvement of shoulders.[12,13]

The effect of age on RF positivity is well known: in older persons, it can be expression of an age-related dysregulation of the immune system, with no diagnostic significance.[56] As already highlighted, recent studies showed that seronegative RA is not a mild form of the disease, requiring intensive treat-to-target therapy similar to treatment of seropositive RA, it can have more inflammatory activity compared to patients with seropositive RA, and it can result in a worse prognosis regardless of age.[43,45]

Comorbidities are commonplace in patients with EORA,[51] and their interference has to be properly assessed.[52] Moreover, in elderly patients, polypharmacology and an age-related impairment of organ functions can favour drug side effects more than in the younger patient or when using a less aggressive treatment.[53,54]

EARLY-ONSET RHEUMATOID ARTHRITIS AND POLYMYALGIA RHEUMATICA-LIKE EARLY-ONSET RHEUMATOID ARTHRITIS: CLINICAL AND INSTRUMENTAL DIFFERENCES AND SIMILARITIES

EORA is considered the most enigmatic PMR-mimicking disease. In addition to the acute involvement of the shoulder joints, characteristic features of both diseases are morning stiffness for >45 minutes, raised ESR, and a good response to low doses of prednisone. In clinical practice, even if physicians should look for diagnoses other than pure PMR when a steroid treatment with prednisolone 12.5–25.0 mg per day does not result in significant improvement,[20] the possibility that the PMR patient can have a favourable response using a GC different from prednisone or prednisolone should be taken into account.[12]

Constitutional manifestations such as fever, loss of appetite, weight loss, and malaise can be present both in EORA and in PMR.[15] On the other hand, patients with PMR may show distal synovitis, presenting as monoarthritis or an asymmetrical oligoarthritis involving mainly wrists and knees. In PMR, synovitis is usually transient and mild, non-erosive, and will be resolved completely after initiation of corticosteroid treatment or increasing the prednisone dose.[15,16,57,58]

In contrast to EORA, most relapses present as a monoarthritis. When arthritis in the wrist is associated with at least one metacarpophalangeal or proximal interphalangeal joint at disease onset, the probability of PMR diagnosis is minimal. This was calculated to be 4.8% in a 5-year prospective study.[59] Diffuse swelling of the distal extremities with pitting oedema and carpal tunnel syndrome can be present both in patients with PMR and in patients with EORA.[60,61] In particular, remitting seronegative symmetrical synovitis with pitting oedema, characterised by symmetrical distal synovitis, pitting oedema of the dorsum of the hands, elevated acute phase reactants, seronegativity of RF, and rapid response to low-dose steroids is a frequent finding in patients with PMR.[60,62]

The clinical utility of ACPA in the differential diagnosis of EORA and PMR has been highlighted, and their presence in a patient with clinical symptoms of PMR must be interpreted as highly suggestive of EORA.[63] On the other hand, as already underlined, the presence of RF should not be an exclusion factor for PMR, since RF can be detected in approximately 10% of the healthy population aged >60 years.

Shoulder and hip US examinations can give important contributions as proposed by EULAR/ACR classification criteria. However, their usefulness is counterbalanced by the absence of

pathognomonic findings.[64,65] As highlighted by the EULAR/ACR collaborative group, patients with PMR were more likely to have abnormal US findings in the shoulder (particularly subdeltoid bursitis and biceps tenosynovitis), and somewhat more likely to have abnormal findings in the hips (particularly trochanteric bursitis or synovitis) than comparison subjects as a group. However, PMR could not be distinguished from RA on the basis of US, but instead from non-RA shoulder conditions and subjects without shoulder conditions.[28]

Differentiation between PMR and EORA using 18F-fluorodeoxyglucose PET/CT has also been proposed.[66] However, differences in the numbers of area considered by the investigators, the lack of standardisation for the technique, and the limited access to PET in the clinical practice, are critical points to consider.[67]

The possibility that both PMR and SEORA can be present in one patient[19] is a further confirmation of great diagnostic difficulty.

Early-Onset Rheumatoid Arthritis and Polymyalgia Rheumatica: Pathophysiological Similarities and Differences

Some considerations about pathogenesis of these two diseases can be useful. The possibility that SEORA and PMR can have similar patterns of human leukocyte antigen association has been highlighted in population studies.[68] However, the association between human leukocyte antigen-DRB1 genotypes and susceptibility to PMR and SEORA is still controversial.[69] Toll-like receptors (TLR) play an important role in the activation and regulation of the innate and acquired immune response, through recognition of pathogen-related molecular patterns and endogenous peptides. TLR are expressed on many cells, including macrophages and dendritic cells, and are highly present in RA synovium as well as in peripheral mononuclear blood cells in patients with active PMR. Coding variants in the *TLR4* gene have been often associated with inflammatory and infectious diseases, but when two of these polymorphisms (Asp299Gly and Thr399Ile, capable of altering the function of the receptor) were studied in patients with PMR and in patients with SEORA, no significant difference in allele frequency or genotype was found.[69]

The role of triggers in the pathogenesis of PMR and SEORA is also unclear. A number of infectious and environmental agents have been suggested, including immune adjuvants present in vaccines,[70] but data in the literature are mostly anecdotal and should be confirmed using large cohorts.[69]

Of greater interest is the characterisation of the type of inflammatory cells occurring in PMR and in SEORA. PMR is characterised by the presence of macrophages and T lymphocytes, with few neutrophils, and no B cells or natural killer (NK) cells.[71] Instead, NK cells and B cells are present in the synovial fluids of RA patients and a strong correlation with severity and disease duration has recently been confirmed.[72] NK cells are considered to be important in bone destruction that is, by definition, absent in PMR.[56,57]

THERAPEUTIC DIFFERENCES AND SIMILARITIES

Steroid Treatment

Treatment with oral prednisolone, with a starting dose of 12.5–25.0 mg, is the main step in the management of PMR.[73] However, the above suggested initial doses should be adjusted individually according to the patient's BMI, presence of comorbid diseases, and risk of steroid-related side effects.[74] Although the EULAR/ACR recommendations for the management of PMR suggest using single rather than divided daily steroid doses, based on some experts' opinions, patients may benefit from divided doses of prednisolone in some occasions. The starting doses for treatment of PMR and subsequent tapering regimens have not been studied thoroughly; however, maintaining the initial doses for 3–4 weeks before following up with tapering doses to 10 mg prednisolone within 4–8 weeks has been recommended.[73,74] Subsequently, it has been recommended that prednisone be decreased by 2.5 mg every 2–4 weeks until the patient is at 10 mg daily, followed by the reduction of the daily dose of prednisone by 1 mg per month. In cases of relapse, defined by recurrence of PMR symptoms together with an increase in acute phase reactants, oral prednisolone dose should be increased to pre-relapse dose and tapered afterwards to the dose at which the relapse occurred within 4–8 weeks.[73]

In some patients, small doses of GC are necessary for years or the entirety of the patient's life.[4,20]

The role of systemic GC in the management of EORA is controversial.[75] In accordance with the 2016 update of EULAR recommendations for the management of RA, short-term treatment with GC should be considered when initiating or changing synthetic DMARD, followed by rapid and clinically feasible tapering regimens.[36] On the other hand, elderly patients are more susceptible to GC-related side effects, including but not limited to diabetes, hypertension, osteoporosis, and eye diseases. Therefore, the risk-benefit of steroid treatment for the individual patient should be assessed by the clinicians before initiation of the treatment.[4,10,12,51]

Non-Steroidal Treatment Options

In recent years, some non-steroidal treatment options have been proposed as GC-sparing drugs for PMR therapy, especially in patients with insufficient response or with relapsing disease. Methotrexate (MTX) has been studied in both randomised controlled trials and retrospective studies. In the 2015 EULAR/ACR recommendations, MTX in small doses (7.5–10.0 mg/week) is proposed as an initial therapy concomitantly to oral GC in case of risk factors for relapse, prolonged therapy, or serious adverse events, and during follow-up in cases of relapse, insufficient response to GC, or GC adverse events.[73]

Mizoribine (MRZ) is an oral immunosuppressive drug that has a mechanism of action similar to mycophenolate mofetil, with an inhibitory effect on inosine monophosphate dehydrogenase. MRZ was evaluated as not inferior to MTX as a GC-sparing drug both in patients with PMR and in those with SEORA. Additionally, it was evaluated as having a higher safety profile.[76] In patients with SEORA, MRZ can be an additional regimen to MTX therapy, especially when the latter is not very effective.[77] However, MRZ is not available in several countries, with its predominate use being in Japan where it has been approved since 1984.

Data on other synthetic DMARD, such as hydroxychloroquine, azathioprine, or leflunomide, are very scarce in patients with PMR. They are currently not considered as a therapeutic option for PMR. Similarly, the 2015 EULAR/ACR recommendations strongly advise against the use of TNFα inhibitors for the treatment of PMR.[73,74] On the contrary, the effectiveness of synthetic and biological DMARD is well-documented in patients with SEORA.[36,78] These therapeutic differences are a further, relevant point to the fact that PMR and SEORA are two distinct and separate diseases.

Finally, great attention has been paid to tocilizumab (TCZ), which is the first humanised anti-IL-6 receptor monoclonal antibody. To date, studies in PMR patients are still limited and TCZ cannot yet be recommended for routine treatment of isolated PMR.[79] On the contrary, starting from 2008, TCZ was used in RA patients, and five randomised, double-blind, controlled, multicentre, Phase III, pivotal, clinical trials demonstrated its efficacy and safety in a range of patient populations, including inadequate responders to MTX and inadequate responders to TNF inhibitors. These trials were central to the approval of TCZ by regulatory authorities in the European Union (EU) and the USA for the treatment of RA.[80]

CONCLUSIONS

PMR and SEORA are two of the most frequent inflammatory rheumatologic diseases in the elderly patient. At first presentation, there are many similarities between PMR and SEORA, which may lead to a real diagnostic conundrum. Moreover, several prospective studies re-evaluated the final diagnosis in patients with PMR and found >20% of patients being diagnosed at a later date as having RA. Consequently, it is understandable that PMR and SEORA have been considered as components of a single disease process. In fact, PMR and SEORA must be considered two different diseases with many similarities. Some features (such as an erosive arthritis or the symmetrical involvement of metacarpophalangeal and/or proximal interphalangeal joints) can help differentiate these two inflammatory diseases, whereas findings of shoulder and hip US as well as 18F-fluorodeoxyglucose PET/CT seem to be less specific.

As highlighted, a correct diagnosis also has relevant therapeutic implications. In PMR, GC are used for several months, and in some patients throughout life, and their side effects can induce significant comorbidities. On the contrary, in

patients with SEORA, short term treatment with GC should be considered only when initiating or changing DMARD, followed by rapid tapering. Finally, most synthetic and biological DMARD are scarcely or totally ineffective in PMR and effective in SEORA.

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Exercise and Rhinitis in Athletes

Authors: *Pavol Surda,[1] Chrysostomos Tornari,[1] Matus Putala,[2] Abigail Walker[1]

1. Department of Otorhinolaryngology, Guy's and St Thomas' University Hospital, London, UK
2. Department of Physical Education and Sports, Comenius University, Bratislava, Slovakia

*Correspondence to pavol.surda@gmail.com

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Abstract

Peak sporting performance requires optimal levels of health and fitness. Rhinitis, with its proven detrimental effects on sleep and mood, and its association with asthma, can clearly compromise athletic ability. Nasal health is therefore of key importance to the athlete. While not a limiting factor in a single exercise effort, the effects of nasal dysfunction can have repercussions in the post-exercise recovery period. Furthermore, it is linked with the development of asthma and may increase susceptibility to upper respiratory tract symptoms. This review aims to investigate the physiology of the nose during exercise, examine the relationship between exercise and nasal dysfunction, and consider the impact that dysfunction may have on an athlete. Lastly, the authors describe the diagnosis and treatment of rhinitis in athletes.

PREVALENCE OF RHINITIS IN ATHLETES

The reported prevalence of allergic rhinitis (AR) in the normal population differs from country to country. In a study using the Allergic Rhinitis and its Impact on Asthma (ARIA) definition for the European population, the prevalence was found to be around 25% and ranged from 17.0–28.5%.[2] Moreover, an increasing trend in the prevalence of AR was observed over the last decades of the past millennium, but in the last 25 years this trend seems to have tailed off.[3,4] The prevalence of non-allergic rhinitis (NAR) in the normal population is not as well studied as AR, but it has been reported to account for 17–52% of all cases of rhinitis in adults.[5,6]

Rhinitis in athletes has frequently been studied in combination with asthma and, although there are many literature estimates of prevalence in the athletic population, the estimates of frequency of rhinitis vary widely, ranging from 27–74%.[7-9] This variation might suggest heterogeneity of either the population or sampling methods. Recently, a systematic review examined the prevalence of rhinitis in athletes in three separate subgroups (land, water, and cold air) according to the environment where they spent the most training hours.[7] After doing so, the range narrowed and was easier to interpret: track and field athletes were not affected by rhinitis significantly more than the general population, regardless of whether they were endurance or sprint specialists;[10] contrary to this, 48.6% of the athletes who spent training hours in cold environments reported rhinitis,

with the distinctive, and often severe, symptom being rhinorrhoea (96%).[11]

There are several studies examining the prevalence of rhinitis in swimmers.[12-15] Surda et al.[12] studied a large cohort in which rhinitis was reported significantly more often in elite swimmers (45%) than in non-elite swimmers (31%), non-swimming athletes (32%), and controls (24%). AR prevalence was similar in all groups, but the prevalence of NAR was significantly higher in elite swimmers (33%) and non-elite swimmers (22%) compared to non-swimming athletes and controls.[12] An interesting new finding was that while AR prevalence was not affected, NAR prevalence increased in these patients. This study's findings do not support those of a Dutch study on 2,359 swimming children aged 6–13 years, which indicated that sensitisation to common allergens may be correlated with increased swimming frequency, especially during pool attendance in the first 2 years of life.[16] This is in agreement with a prospective longitudinal study in British children that did not show an increased risk of developing allergic symptoms or asthma in swimming children.[17]

PATHOPHYSIOLOGY

The acute effects of exercise on the nose have been well delineated: vasoconstriction of the capacitance vessels results in a measurable increase in nasal volume.[18] In aerobic exercise, nasal minute ventilation increases absolutely but proportionately contributes less than at rest, because the low resistance oral airway is used preferentially.[19] The impact of repeated exercise training on nasal physiology is less well established. Many of the environments and endeavours which athletes immerse themselves in have the potential to harm the nasal mucosa. For example, an exercise which takes place in cold environments (e.g., skiers, snowboarders, ice hockey) induces glandular hypersecretion and nasal discharge in normal subjects (under parasympathetic control) and this response shows increased severity in rhinitis patients.[20] Furthermore, during regular training, athletes are repeatedly exposed to allergens, cold air, and pollutants, and these can have a significant effect on their allergic diseases and respiratory physiology.[21] For instance, the nasal obstruction of rhinitis patients shifts the pattern of nasal breathing to oral breathing, increasing the exposure of lower airways to allergens, pollutants, or other adverse environmental factors. Some studies have shown that nasal breathing significantly reduced exercise-induced asthma,[22] due to the nose's role in humidifying inspired air.[23]

There is still a debate about the causative factor of rhinitis in elite swimmers. The most commonly accepted theory is that repeated exposure to chlorination byproducts, such as trichloramines (potent oxidants known to disrupt epithelial tight junctions), can facilitate the penetration of allergens or pollutants and the migration of inflammatory cells across the epithelial barrier.[13,24-27] It has been reported that 1 hour spent in a chlorinated swimming pool was sufficient to increase airway epithelium permeability in swimmers, whereas no change was observed after attending a copper-silver disinfected pool.[28] Compared with other sport athletes, swimmers demonstrate specific features in their breathing patterns characterised by a low breathing frequency but high tidal volume, which may favour a hypothesis of significant mechanical stress to the airways.[29] Fornander et al.[30] studied indoor swimming pool personnel and reported that 17% of the subjects suffered with airway symptoms. This difference in prevalence might have shed more light on the significance of other factors like high minute ventilation.

Major rhinosinusal problems experienced by boxers are certainly of traumatic origin. The so called 'boxer's nose' is typically the result of an osteo-cartilaginous nasal fracture with detachment of the distal tip of nasal bones associated with vertical fractures of the cartilaginous septum. Moreover, recurrent trauma, often associated with an exasperated use of haemostatic pencils, can lead to significant and important alterations at the rhinosinusal mucosal level in boxers. Specifically, post-traumatic oedema, associated with the reflex glandular hyper-secretion, can induce significant alterations of the mucociliary transport system, the consequence being increased risk of rhinosinusal infections in these subjects.[31]

Exercise-induced changes in nasal secretions during exercise have had surprisingly little attention from researchers in the field, far less than that given to salivary markers of immune function. Early studies have confirmed that the volume of nasal secretions produced increases during submaximal exercise.[32,33] There is a wealth of evidence supporting acute and chronic reductions in salivary IgA and antimicrobial protein levels during maximal exercise and heavy training periods.[34-36] By extrapolation, it might be expected that nasal secretions would follow a similar pattern. However, further investigation into the adaptations in nasal mucosal immunity in response to exercise is required to make robust conclusions.

NASAL CHANGES ASSOCIATED WITH EXERCISE IN ATHLETES

A recent systematic review has well described nasal changes associated with rhinitis in athletes.[37] Nasal mucosal changes triggered by sport activity can be reflected in predominant neutrophilic infiltration with reduced phagocytic activity, deterioration of olfaction, reduced ciliary beat frequency, and prolonged mucociliary transport time (MCTt).[13,31,38,39] These changes can be chronic or acutely related to a strenuous training exercise, and are also influenced by the type of activity and environment. MCTt was found to be prolonged in swimmers, which can be attributed to chlorine irritation.[31,38] Deterioration of MCTt and reduced ciliary beat frequency can also be observed in runners after a 20 km race. The examination of the nasal lavage obtained immediately after the competition showed increased neutrophil counts with reduced phagocytic activity.[39,40] Acute nasal mucosal changes induced by strenuous exercise in the runners recovered to the baseline within 3 days of the competition. In elite swimmers, a decrease in neutrophilic infiltration and improvement of clinical symptoms were described after 2 weeks of training cessation or 30 days-use of a nasal clip.[13] Several authors have studied changes in nasal patency judged by peak naval inspiratory flow before and after exercise. Interestingly, there has been no significant difference observed.[13-15,38]

UPPER RESPIRATORY TRACT INFECTIONS IN ATHLETES

Upper respiratory tract infections are an enormous burden to athletes. They are the most frequent reason for presentation to sports physicians and are the most common medical problem encountered at both Winter and Summer Olympics.[35,41,42] The J-curve of mucosal immunity, which proposes a depression in immunity with intensive exercise, has been suggested as a model to explain the increased frequency of upper respiratory tract infections in athletes following competitions.[43] This has been supported with clinical observations following extreme endurance events, with participants being up to five-times more likely to experience upper respiratory tract infections following the event than non-participating control subjects.[44,45] However, studies investigating specific pathogens have failed to identify an infectious agent in as many as 50% of athletes reporting upper respiratory tract infection symptoms.[46,47] This had led to a non-infectious hypothesis that supposes that many of the upper respiratory tract symptoms classically linked with infections (sneezing, blocked or runny nose, and coughing) are secondary to airway epithelial injury, cytokine release, and mucosal oedema that arise from intense exercise. AR may therefore actually predispose the athlete to the symptoms of upper respiratory tract infection, which has the associated cost to training and wellbeing.

QUALITY OF LIFE IN ATHLETES WITH RHINITIS

Active individuals with nasal symptoms suffer a considerable detriment to overall quality of life (QoL), as demonstrated by Walker et al.,[48] who showed that a mixed cohort of athletes had significantly increased SNOT-22 scores when compared to controls. Left untreated, nasal disease represents a significant burden to these individuals and could potentially limit performance in competitive athletes.[49,50]

Unfortunately, there are only a few studies describing the impact of rhinitis on QoL.[9,12,13,48,51] The most extensively studied group are swimmers.[9,12,13] This was firstly described by

Bougault et al.[9] who showed significantly increased Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) nasal domain scores in swimmers when compared to controls. However, this difference in overall score was not deemed significant. Interestingly, the scores improved after 2 weeks of training cessation, which attests to the irritational properties of chlorine and the effects of prolonged exposure. Of note, Gelardi et al.[13] also observed a significant improvement in nasal symptoms judged by visual analogue scale score after 30 days of nose-clip use. Recently, Surda et al.[12] showed a significant effect of swimming on the total RQLQ scores and all subdomains except the eye. The hours spent in the pool had a significant effect on total RQLQ and some subdomains.

Similar findings were also observed in non-swimming athletes.[48,51] One study that measured the impact of daily intranasal budesonide in athletes with rhinitis demonstrated significantly improved self-assessed performance scores after just 8 weeks of treatment.[51] It is not known whether these improvements translate into an objective competitive gain, but they nonetheless highlight the importance of diagnosing and treating nasal disease in this population.

RHINITIS AND ASTHMA IN ATHLETES

The last decade has seen an increased understanding of the functional complementarity of the upper and lower airways as a single 'unified airway'. As such, rhinitis and asthma frequently co-exist, with >80% of asthmatics also having rhinitis and 10–40% of rhinitics also having asthma.[52] Extensive and repeated athlete studies have returned an increased prevalence of asthma, with participants in endurance sports reporting the highest rates of asthma.[53,54] The last decade has seen widespread acceptance of two distinct asthma phenotypes: exercise-induced asthma and exercise-induced bronchoconstriction. The distinction lies in background respiratory function. Exercise-induced asthma implies a background of airway hyper-responsiveness that may be exacerbated by exercise. In contrast, exercise-induced bronchoconstriction implies airway hyper-responsiveness that is solely triggered by exercise. One postulated mechanism for exercise-induced bronchoconstriction is airway drying in response to hyperpnoea, with dehydration of airway epithelium leading to injury that can be demonstrated by the increased shedding of epithelial cells and the release of inflammatory mediators.[52] Common sense might suggest that athletes will follow a similar pathophysiological pattern to the general population, but the confirmation of rhinitis co-existing with exercise-induced asthma and/or exercise-induced bronchoconstriction remains largely uninvestigated.

DIAGNOSIS AND TREATMENT

An ARIA document in collaboration with GA^{2}LEN suggested a comprehensive management plan for athletes with rhinitis:[1]

1. Early recognition and diagnosis.

2. Allergy testing.

3. Recognition of associated or subclinical asthma through adequate pulmonary function tests.

4. Avoidance of exposure to relevant allergens (if any) and pollutants during exercise.

Treatment to improve nasal symptoms and prevent exercise-induced bronchoconstriction without affecting athletic performance while complying with anti-doping regulations.

Early recognition and diagnosis should include a thorough history check with specific focus given to the identification of the symptom-inducing trigger (e.g., allergen, infection, cold air, swimming pool, or exercise itself), anterior rhinoscopy or endoscopy, and consideration of further imaging diagnostic methods. Every athlete should be screened for allergies as a causal factor of rhinitis. This validated Allergy Questionnaire for Athletes (AQUA) is often used as a screening tool to identify athletes with allergic disease, and a score >5 has a positive predictive value of 0.94.[55]

State-of-the-art guidelines, such as the ARIA guidelines, provide clinicians with evidence-based treatment algorithms for chronic rhinitis. These algorithms consist of a stepwise approach based on symptom duration and severity. Primary treatments are divided into several categories: decongestants, antihistamines, chromones, antileukotrienes, anticholinergics,

corticosteroids, and immunotherapy. Nowadays, the most effective treatment modalities are topical corticosteroids (intranasal corticosteroids) or a combination of topical corticosteroids and antihistamine nasal sprays depending on the presence of AR.[50]

Surgical intervention should only be considered if aggressive medical therapy has failed to control a patient's symptoms. Currently, no single modality has evolved as the gold standard for the treatment of rhinitis.

Table 1: The Prohibited List of the World Anti-Doping Agency, effective from 1 January 2019.

Treatment	WADA Rules	Notes
Antihistamines	Permitted	None
Antileukotrienes	Permitted	None
Oral steroids	Prohibited in competition; require 'Use Exemption' approval	None
Topical steroids	Permitted	None
Decongestants	Permitted	None
Oral beta-2 agonists	Prohibited	None
Inhaled salbutamol, terbutaline, formoterol, salmeterol	Permitted	• Inhaled salbutamol: maximum 1,600 µg over 24 hours in divided doses not to exceed 800 µg over 12 hours starting from any dose. •Inhaled formoterol: maximum delivered dose of 54 µg over 24 hours. •Inhaled salmeterol: maximum 200 µg over 24 hours.
Ephedrine, methylephedrine	Permitted	• Ephedrine and methylephedrine: prohibited when the concentration of either in urine is >10 µg per mL. • Pseudoephedrine: prohibited when its concentration in urine is >150 µg per mL.
Immunotherapy	Permitted	

WADA: World Anti-Doping Agency.

The mainstay of surgical intervention targets the inferior turbinate to control prominent nasal congestion. Complications such as atrophic rhinitis or empty nose syndrome have driven practitioners away from radical turbinectomy. Minimally invasive techniques are more favourable because they have fewer complications and they preserve ciliary anatomy.[50]

A study by Walker et al.[48] showed a high disparity between prevalence of rhinitis and use medication: 70% of active participants described one or more nasal symptoms on most days of the year, potentially illustrating a huge body of diseases within the active population. Despite this, medication was seldom-used, with over half of the active participants with regular nasal symptoms using no medication at all. The most commonly used nasal medication by athletes was a decongestant. Resorting to over-the-counter decongestants to relieve symptoms may be a latent indicator of self-medication of rhinitis by athletes. This may, in part, be because of a fear of using prescription

medications that may fall foul of anti-doping regulations. However, interestingly, the current World Anti-Doping Agency's list of prohibited medications (Table 1)[56] makes no specific reference to corticosteroids that are delivered intranasally.[48]

DIRECTIONS OF FUTURE RESEARCH

There are several lines of inquiry to be taken to further research into the field, including but not limited to:

- Comparing allergic conditions between outdoor and indoor sports.
- Large scale studies focussing on prevalence in different types of athletes using a validated questionnaire and objective measurements.
- Studies describing the impact of rhinitis on QoL and performance in non-swimming athletes.
- Further studies examing the possibility the prophylactic treatment of rhinitis could reduce the post-exercise upper respiratory tract infection symptoms in athletes.

CONCLUSION

Nasal health is clearly of key importance to the athlete. While not a limiting factor in a single exercise effort, the effects of nasal dysfunction can have repercussions in the post-exercise recovery period. Nasal dysfunction is associated with worse sleep quality, mood scores, and QoL. Furthermore, it is linked to the development of asthma and may increase susceptibility to upper respiratory tract symptoms. Based on the evidence presented, the authors recommend that both athletes and sports physicians remain mindful of the importance of maintaining nasal health.

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What's New

Adolescents' Kidney and Liver Function Impacted by Fluoride

EXPOSURE to fluoride commonly found in public water systems may alter liver and kidney function in adolescents in the USA. According to the results from a clinical trial, complex changes to liver and kidney function in young adults, due to higher water fluoride concentrations, include alterations to bodily fluoride absorption and metabolic processes. Children excrete fluoride at a lower rate than adults. The team of researchers aimed to investigate the effects of chronic low-level fluoride exposure on the young adults who participated in the survey.

In the USA, 74% of water systems have fluoride added to them for the improvement of oral health. The kidneys accumulate a vast quantity of fluoride and the association between fluoride exposure in adults and damage to the liver and kidney has been previously investigated. "While the dental benefits of fluoride are widely established, recent concerns have been raised regarding the appropriateness of its widespread addition to drinking water or salt in North America," said Dr Ashley J. Malin, Icahn School of Medicine at Mount Sinai, New York City, New York, USA.

"This study's findings suggest that there may be potential kidney and liver health concerns to consider when evaluating fluoride use and appropriate levels in public health interventions."

Data was taken from the National Health and Nutrition Examination Survey: a group of studies that assess health and nutritional wellbeing in the USA. The plasma fluoride concentration in blood samples of 1,983 adolescents and the fluoride content of tap water in 1,742 households with adolescents were analysed. Parameters testing the liver and kidney function were compared with fluoride exposure and concluded that fluoride exposure in the participating adolescents may result in complex changes with kidney or liver dysfunction.

Dr Malin continued: "This study's findings suggest that there may be potential kidney and liver health concerns to consider when evaluating fluoride use and appropriate levels in public health interventions. Prospective studies are needed to examine the impact of chronic low-level fluoride exposure on kidney and liver function in the U.S. population."

The study suggested that the hepato and nephron-toxicity of fluoride indicates that adolescents with poorer kidney or liver function may also absorb more fluoride. Further research is vital and significant findings may dictate the need to account for children's kidney and liver function in drafting public health guidelines and recommendations.

What's New

A Novel Toolkit for the Detection of Anaemia in Pregnancy

IRON deficiency (ID), if left undiagnosed or untreated, can lead to health implications including ID anaemia (IDA) and long-term developmental issues in children. Although pregnancy-associated ID, a common problem that can affect both maternal and fetal health, can effectively be treated with iron supplementation, ID and IDA are often unrecognised and untreated because of a lack of knowledge of its implications and competing clinical priorities. In a recent study, a research team at St. Michael's Hospital, Toronto, Canada, developed a novel quality-improvement toolkit to enhance screening and management of ID and IDA in pregnancy.

Co-lead author of the study Dr Michelle Sholzberg, a haematologist at St. Michael's Hospital and a researcher at the hospital's Li Ka Shing Knowledge Institute, noted that "screening for iron deficiency in pregnancy is recommended by health agencies but with low awareness of its implications and competing priorities in busy obstetric clinics, it doesn't happen as often as it should." This was the primary reason for investigating a way to provide guidance and support for women to be encouraged to speak to their care providers and ensure that they are receiving enough iron, which resulted in the lab's quality-improvement toolkit. Included in the paper-based toolkit, which is known as IRON MOM (IRON Deficiency in Pregnancy with Maternal IrOn OptiMization), are clinical pathways for diagnosis and management, educational resources for clinicians and patients, templated laboratory requisitions, and standardised oral iron prescriptions. The impact of IRON MOM was assessed using the retrospectively extracted laboratory data of women seen in both the obstetrics clinic and the inpatient delivery ward settings of the hospital to compare various measures pre and post-application of the toolkit.

From the electronic patient record, ferritin tests and antenatal haemoglobin results were extracted pre and post-intervention. One year after IRON MOM implementation, the team discovered a 10-fold increase in the rate of ferritin testing in the obstetric clinics at the hospital. This was also linked to a significantly lower risk of anaemia and patients requiring blood transfusions before and after pregnancy to improve their red blood cell count. Although this study lacked a control group, the researchers concluded that the implementation of a standardised toolkit decreased the incidence of anaemia and provided support for expanding IRON MOM into other clinics and institutions. The team is now working on an IRON MOM smartphone application to increase the availability of the toolkit. Regarding this, Dr Sholzberg stated: "It's widely accepted that many women will develop anaemia in pregnancy as the result of ID, but this doesn't have to be the case. Treating ID requires a culture change and IRON MOM addresses that need."

"Gold-silica nanoshells infusion allows for a focussed therapy that treats the cancer, while sparing the rest of the prostate, thus preserving a patient's quality of life."

Gold Nanoparticle Treatment Demonstrates Effective Therapy for Prostate Cancer

PROSTATE cancer is the second most common non-skin cancer in the USA, and the second leading cause of death by cancer. Biocompatible gold nanoparticles were shown to ablate low-to-intermediate grade tumours within the prostate, producing no known side effects. The gold nanoparticles were designed to convert near-infrared light to heat, offering a treatment option with the potential to maintain the form of the prostate, avoiding side effects associated with whole-gland treatment, such as prostatectomies. Complications of whole-gland treatment include urinary incontinence and erectile dysfunction; several studies have demonstrated the options for focal therapies with fewer side effects.

"Gold-silica nanoshells infusion allows for a focussed therapy that treats the cancer, while sparing the rest of the prostate, thus preserving a patient's quality of life by reducing unwanted side effects, which could include erectile dysfunction and/or the leakage of urine," said Dr Ardeshir Rastinehad, Icahn School of Medicine at Mount Sinai, New York City, New York, USA. The researchers tested the effectiveness of AuroLase® Therapy (Nanospectra Biosciences, Texas, USA). This therapy uses gold-silica nanoshells (GSN) designed to absorb energy from near-infrared light and convert it to heat. The causal effect is selective hyperthermic cell death and adjacent healthy tissue is spared.

A total of 16 men, aged 58–79 years, with low-to-intermediate grade prostate cancer (Gleason score of 4+3) received infusion with GSN. The participants were diagnosed and treated with magnetic resonance-ultrasound fusion imaging, a targeted biopsy technique, which uses magnetic resonance imaging (MRI) technology for tissue extraction from the invasive tumour. Approximately 48–72 hours after GSN infusion and high-precision laser ablation, the patients received an MRI of their prostate. They also underwent MRI-targeted fusion biopsies at 3 and 12 months, and a standard biopsy at 12 months. At 1 year of follow-up, GSN-mediated focal laser ablation was shown to be successful in 87.5% of lesions.

This research is promising for the future of prostate cancer treatment. "Dr Rastinehad's gold nanoparticle research shows that patients are not only benefiting from this treatment, but also experiencing minimal side effects," concluded Dr Ash Tewari, Department of Urology, Icahn School of Medicine at Mount Sinai.