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Welcome

A very warm welcome to the final 2017 edition of the European Medical Journal's flagship eJournal. Inside, we have drawn together some of the most important developments across a variety of therapeutic areas. Peer-reviewed articles and feature articles form the content of the journal and, together, provide highly valuable and insightful developments across a range of therapeutic areas, ready and waiting for your attention.

Among the many peer-reviewed articles is an intriguing study by Munhoz da Silveira Campos et al., which looks into groups of obese young adults undergoing long-term weight loss therapy. The two groups, defined as insulin resistant and non-insulin resistant, were analysed for inflammatory biomarkers, fibroblast growth factor-21, and their metabolic profile. The results are fascinating and are sure to spark further research and developments in the field. Another thought-provoking article, penned by Flavin et al., describes the perioperative influences on patient renal physiology in those undergoing robotic urological procedures. Robotic surgery is the current pinnacle of modern medicine, and the authors highlight important considerations for the care of patients undergoing the procedure. Keep reading for even more exciting developments in modern medicine.

> "We hope you enjoy the content included within *EMJ 2.4* and that the topics covered will inspire you to delve further into these research areas."

Also included within this issue is a feature article by de Reijke which provides an interesting and insightful discussion regarding the implementation of clinical guidelines. Clinical guidelines are a hugely important aspect of medical care, and, as the author describes, deviations from them can result in harmful effects on patient care and the efficiency of a medical establishment. De Reijke concludes the feature with a section on his own opinions regarding what is useful in clinical practice, including the use of an application for recommendations in clinical consultations, since the guidelines discussed may not be practical in this scenario.

We hope you enjoy the content included within *EMJ 2.4* and that the topics covered will inspire you to delve further into these research areas. Each article has been chosen for its high-quality content and relevance to the therapeutic field; as such, we are confident that they will prove informative and insightful for all readers. We are already eagerly awaiting the next edition of our interdisciplinary flagship eJournal for more breakthrough research from a plethora of therapeutic areas in early 2018.

Kind regards,

Spencer Gore

Director, European Medical Journal

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Foreword

Prof Ahmad Awada

Jules Bordet Institute, Belgium, Germany.

Dear Colleagues,

It is with great delight that I introduce to you the final issue of the *European Medical Journal* for 2017, *EMJ 2.4*.

This issue of our quarterly flagship journal covers a range of topics with, in my opinion, the underlying theme of self-reflection within the medical community: how we, as medical professionals, can refine our methodologies and techniques to improve treatment and care for our patients. Nowhere is this sentiment better expressed than in de Rekjie's special feature on the development and implementation of clinical guidelines, in which he explains the current process and touches upon the ways in which they can be improved.

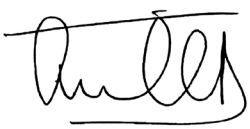
For the Editor's Pick of this issue, I am thrilled to present you with an article on screening for pancreatic cancer, penned by Puri et al. This thorough overview describes current screening standards and highlights potential directions for the future. In a disease with such poor prognosis as pancreatic cancer, this discussion is critical. Oncologists will also be happy to note the inclusion of an intriguing paper by Ruwali, discussing the effect of genetic variations on the outcome for head and neck cancer patients.

Turning to the field of nephrology, this edition contains a pair of excellent articles that approach kidney disease from two very different directions; the first, by Flavin et al., discusses medical technology with regard to the perioperative influences on renal physiology during robot-assisted laparoscopy procedures, while the second study, authored by Maoujoud et al., takes a broader viewpoint, considering the demography of chronic kidney disease in low and middle-income countries, specifically Morocco.

Additionally, in an article sure to be of interest to both dermatologists and urologists, Dreyfus provides this issue with a fascinating case study on chronic urticaria after vasectomy, leading him to suggest that autoreactive proteins with enzymatic activity may have increased propensity to generate autoreactive immunoglobulin E.

With further topics including hepatopulmonary syndrome, haemochromatosis, weight loss in obese diabetic adolescents, and more, this eJournal is certain to contain articles to interest and inspire all medical practitioners. I hope you enjoy reading the final 2017 edition of the *European Medical Journal*.

Best wishes,



Ahmad Awada

Head of the Medical Oncology Clinic, Jules Bordet Institute, Brussels, Belgium.

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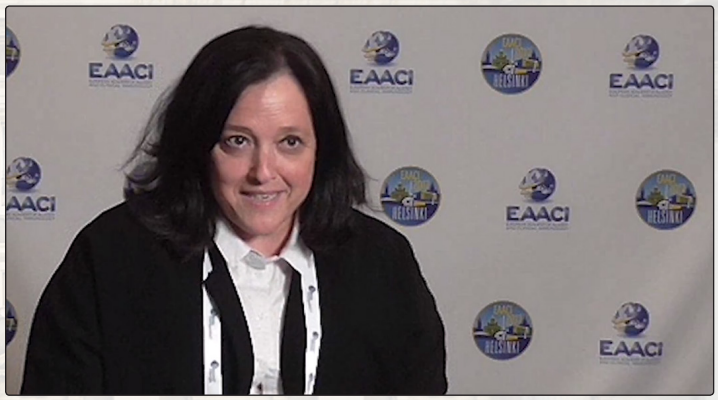

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HOW TO IMPLEMENT GUIDELINES INTO CLINICAL PRACTICE

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National and international guidelines are available for almost all common diseases, and these should be applied when counselling patients. Deferring from the guidelines and the reasons for doing so must be discussed with the patient, and should be documented within the patient's file. It is equally important to know how these guidelines have been constructed; whether the guidelines follow evidence-based medicine or are a consensus-based guideline. The general consensus now is that a guideline has to comply with the rules of evidence-based medicine. This means the following:

- All professionals dealing with the specific disease should be involved in the composition of the guideline, and preferably also a patient representative. These professionals should have a mandate from their professional specialty.
- For each of the topics, a 'problem, intervention, comparator, outcome' (PICO) is composed and, once there is a consensus on a PICO, an epidemiologist performs a systematic literature search (e.g. using Cochrane Library, Medline, and Embase) from a predefined time period, and all relevant articles and references are scored and graded for their level of evidence.

The level of evidence for a manuscript concerning intervention, prevention, or therapy is as follows:

A1) Systematic review
A2) Randomised clinical trial
B) Randomised clinical trial of intermediate quality, or non-randomised trial
C) Non-comparative studies
D) Expert opinion

Based on these levels of evidence, a grade of recommendation should be provided:

1. Based on investigation at A1 level or two independent studies at A2 level
2. Based on A2 level or two independent studies at B level
3. Based on studies at Level B or C
4. Based on expert opinions

At the end, the committee describes considerations based on patient preferences, availability of investigations or medications, logistic issues, and costs. This puts evidence into perspective and adapts to daily practice. Once all the items have been discussed between the committee members and there is agreement, the draft is sent to all participating scientific organisations for their approval and/or suggestions for adaptation. Following this process, the guidelines are implemented.

Furthermore, it is important to evaluate the implementation of these guidelines in daily clinical practice and this should be done by publication and distribution of the guidelines through the participating scientific organisations. At the end, indicators have to be developed to see how the guidelines are really followed.

Guidelines are not a legal document, but they help to provide the patient with qualitative good healthcare. However, it is not a one-size-fits-all document, and personalisation is important. This is where the multidisciplinary team has its role. The guidelines should form the basis of the discussion among the multidisciplinary team of professionals, and ideally all the data from the patient should be recorded beforehand in a database. Based on the guidelines and input from the meeting, the electronic system provides the proposed investigation(s) or treatment options for patients. We are currently working on such a system, which we expect to help the multidisciplinary team, and the investigations and treatments will be more uniform, providing there is adherence to the guidelines. Of course, in the individual patient, the ultimate decision on the treatment or investigation can differ, because the patient is the most important partner in the decision regarding what can and should be done. Another problem is that Level 1

evidence and high grade of recommendation is usually not present in the guidelines due to the lack of high quality studies, thus, again, the discussion with and involvement of the patient is of utmost importance.

WHAT IS HELPFUL IN CLINICAL PRACTICE?

I think each professional working in a specific field should be aware of the existing local and international guidelines. In most healthcare systems, the local guidelines are the most important, because healthcare providers base their reimbursement for investigations and treatments on the guidelines (at least in the Netherlands). The guidelines are, however, not practical tools to use during consultations. For prostate cancer, a tool has been developed (Mirrors of Medicine) that can help the physician by using an App. By introducing patient and disease characteristics, recommendations are provided (using a traffic light appearance) of what should be done according to the guidelines. The prerequisite is that this tool should be updated once new evidence becomes available. Here we pinpoint a problem with the guidelines, because if each update has to go through a system of evidence-based medicine (PICO, literature search, approval of all scientific organisations) it takes a long time before the guideline can be updated and healthcare providers will allow reimbursement. How this issue can be tackled is still not well defined, but usually real-life practice is ahead of the guidelines. Because patients are aware of the new developments, they may ask for the investigation or treatment.

THE TREATMENT APPROACH OF IDIOPATHIC INFLAMMATORY MYOPATHIES

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INTRODUCTION

This commentary reflects our personal approach to the treatment of idiopathic inflammatory myopathies (IIM), also commonly referred to as myositis. Despite substantial ongoing research, there remains a large gap in our understanding of the pathogenesis, variability of organ involvement, predictors of response to treatment, and, consequently, in optimising outcomes for an individual patient. Given the rarity of IIM, it is understandable that there are few randomised controlled or head-to-head trials to draw upon when making treatment decisions. Furthermore, myositis spectrum disorders (including polymyositis, dermatomyositis, overlap syndrome, antisynthetase syndrome, immune-mediated necrotising myositis, inclusion body myositis, and juvenile dermatomyositis) are heterogeneous, which makes a unified approach to treatment challenging.

This review is influenced by an awareness of the research as it currently stands and our approach to integrate these study results into clinical practice. This is not intended to be a comprehensive guideline but, instead, a clinically focussed discussion supported, where possible, by evidence. This commentary is limited to the cases of adult-onset IIM that are responsive to immunosuppression, and the reader is, therefore, directed elsewhere for a discussion on the management of juvenile dermatomyositis and inclusion-body myositis.[1,2]

IMPORTANT CAVEATS

Non-Pharmacological Treatment

It would be remiss not to highlight the role of non-pharmacological treatments at all stages of the management of IIM. For example, exercise regimens tailored to the patient's functional level, meticulous nursing care, including the management of aspiration risk if present, as well as access to psychological and social support are of utmost importance.

Accurate Diagnosis

An accurate diagnosis of IIM is essential to ensure that other disorders, including those less readily modified by immunotherapy, are not missed. Late presentations of inherited or acquired myopathies can closely mimic IIM, as can other systemic causes of the disorder, such as endocrinopathies or drug toxicity.[3] In particular, it is important to recognise that a presentation of sporadic inclusion body myositis, an IIM clinical subtype that affects older patients and can mimic polymyositis in the early stages, unfortunately does not respond to immunotherapy.[2]

Paraneoplastic Disease

It is also important to stress the association of IIM, particularly dermatomyositis, with a range of malignancies; study results have shown an approximate prevalence of cancer in 10% of adult-

onset IIM cases.[4] In such cases, treatment of the muscle disease or other associated organ involvement is usually unsatisfactory until the underlying malignancy is identified and treated as effectively as possible.

Infection Risk

All patients are screened for latent or occult infections and appropriate vaccinations are administered as early as possible to minimise potential harm from treatment.

Extramuscular Manifestations

IIM are systemic conditions with a range of potential extramuscular manifestations, including dermatologic, pulmonary, cardiovascular, and articular conditions. In some patients, these manifestations are the predominant feature of their condition and treatment should be tailored appropriately.

THE TREATMENT TARGET

Pharmacological treatments at a biologic level are intended to arrest immune-mediated end-organ damage to allow restitution and repair of the affected tissues. At a clinical level, the aim is to halt progression of the significant disability that almost invariably accompanies these conditions and maximise the recovery of function as quickly as possible. It is from these principles that we suggest a model of treatment structured as remission induction and remission maintenance, similar to the approach used to treat antineutrophil cytoplasmic antibody-associated vasculitis.[5] Without effective treatment, patients can quickly develop permanent damage, such as fatty infiltration of muscles or pulmonary fibrosis.

A large unmet need in rheumatic autoimmune disease is predicting which patients will respond to particular therapies; the concept of disease 'pathotypes' is gaining traction to better target interventions.[6] However, in current clinical practice, it is not uncommon to need to trial a number of different (second or third-line) agents, either sequentially or in combination, before the optimal response is achieved. It is essential to have in mind a measure of response; relying solely on serial measurements of muscle enzymes is not appropriate. We carefully measure a patient's strength at each clinic appointment and have found that changes in their physical function are the single most useful guide to treatment decisions. The International Myositis Assessment and Clinical Studies (IMACS) Group criteria are used to standardise the assessment of response to treatment.[7]

INDUCTION TREATMENTS

Although there are no associated placebo-controlled trials, corticosteroids remain the backbone of our induction treatment because they can provide rapid responses and have a familiar, if not ideal, side effect profile. In particular, we carefully assess the risk of steroid-induced osteoporosis and appropriateness of antiresorptive therapies in every patient. We use doses of 0.5–1.0 mg/kg prednisolone at induction and consider pulsed parenteral doses initially in patients who are severely ill. The high oral doses are usually continued for 4–6 weeks to establish disease control before starting a tapering regimen. A typical taper involves patients treated with a dose of ≤20 mg prednisolone 2–3 months after the initiation of treatment and <10 mg at 6 months.

Overall, >80% of patients will respond to corticosteroids alone, but the majority do not return to full strength when treated with monotherapy. We always aim to include a second induction agent from the outset to minimise the cumulative corticosteroid dose and establish a more appropriate medium-to-long-term treatment regimen.[8] The treatments we would consider include methotrexate, azathioprine, mycophenolate, tacrolimus, or cyclosporin. Cyclophosphamide and intravenous immunoglobulin can be used for induction treatment in cases of severe disease features, including certain extramuscular end-organ involvement, profound muscle weakness, and dysphagia, and/or corticoresistant cases. An additional agent that plays a role in induction treatment is rituximab.

Methotrexate

Retrospective studies have reported response rates to methotrexate of approximately 80%, even in patients who initially failed on corticosteroid treatment.[9] The advantages of methotrexate therapy include a convenient dosing schedule, extensive knowledge of the side effect profile, and the option to continue treatment long-term when necessary as maintenance therapy. Methotrexate is likely to take weeks to reach its full effect and, therefore, we usually administer it to patients with less severe disease activity who are likely to respond well to corticosteroids. We aim for doses between 20 and 30 mg weekly, either orally or subcutaneously.

Azathioprine

One small prospective study of azathioprine showed improved clinical outcomes and reduced corticosteroid requirements.[10] In our experience, it can take months to reach a clinical response and there is a significant incidence of side effects. Thiopurine methyltransferase levels are measured before starting azathioprine with a typical maintenance dose of 2–3 mg/kg. Again, we use azathioprine in patients with relatively mild disease and it has the advantage of being an appropriate maintenance treatment if successful and well tolerated. Azathioprine is also one of only a few potentially effective therapies that can be continued safely throughout pregnancy.

Cyclophosphamide

Cyclophosphamide is reserved for patients with severe or life-threatening disease manifestations; the drug can provide rapid and significant response rates but has a substantial side effect profile, including serious infection, concerns about iatrogenic malignancy, and an effect on fertility. The Euro-Lupus or CYCLOPS medication regimens are commonly selected and continued for approximately 6–10 treatments;[11,12] patients should also receive *Pneumocystis jirovecii* antifungal drugs prophylactically. Cyclophosphamide is used more frequently in patients with interstitial lung disease as there is good evidence for its efficacy in lung involvement associated with connective-tissue disease.

Intravenous Immunoglobulin

Intravenous immunoglobulin therapy can be a very effective immunomodulatory induction treatment and usually has a rapid onset of action. It is a scarce resource and, therefore, we typically only choose to use it first-line if other options are clearly inappropriate (for example, in cases with concurrent sepsis or pregnant women). Two notable trials demonstrated a response in the large majority of patients and most patients with oesophageal involvement experienced improvement, often a relatively resistant disease manifestation.[13] A total dose of 2 mg/kg is given over 2–5 days, which can be repeated at 4-weekly intervals if necessary.

OUTCOMES OF INDUCTION TREATMENT

There are three possible outcomes from induction treatment: a) the patient achieves a successful and sustained remission, b) they achieve a remission only to have a disease relapse, or c) they are refractory to induction treatment. Patients who achieve sustained remission progress to a treatment strategy that maintains disease control; if suitable, continuation of the induction treatment into maintenance treatment is preferable because it avoids the risk of disease relapse by changing treatment. Patients treated with other induction regimens are typically switched to either methotrexate or azathioprine once their induction treatment is concluded as we have the greatest experience with these drugs long-term. Approximately one-third of patients will have a monophasic illness and be able to withdraw from all treatment; however, the majority will require longer periods of immune-targeted treatment and the minimum dose of medications necessary to maintain remission is used, with emphasis on minimising and ideally stopping corticosteroid treatment.

Treatment decisions in patients who relapse are difficult to summarise because the potential approaches vary considerably. In general, if relapse occurs early after the initiation of induction therapy on relatively large doses of corticosteroids (i.e. >20 mg/day prednisolone), we treat the patient as a refractory case and use alternative induction agents. If relapse occurs at doses of corticosteroids below this level then options include adjusting the existing medications, changing to an alternative maintenance agent, or using combination treatment whilst typically employing an increased dose of corticosteroids to regain disease control. At this stage, we may consider other medications that we reserve as second-line therapies because there is less evidence on their efficacy or less familiarity with their use long-term; these include the following treatments.

Mycophenolate

A number of retrospective studies detail success with mycophenolate, including in disease refractory cases.[14] We aim to achieve a dose of 1–3 g in divided doses daily (different preparations of mycophenolate have different dosing recommendations). Mycophenolate is an attractive choice in patients with interstitial lung disease since it avoids the familiar quandary of the patient on methotrexate whose lung disease progresses.

Calcineurin Inhibitors

Calcineurin inhibitors, including cyclosporine and tacrolimus, have displayed evidence of efficacy and, in particular, there are promising data for

tacrolimus in a group of patients with interstitial lung disease related to antisynthetase syndrome.[15] We use oral doses of cyclosporine ranging 2.5–4.0 mg/kg. Tacrolimus is prescribed at a starting dose of 4 mg in two divided doses (0.04–0.08 mg/kg/day) and a maintenance dose of 4–10 mg daily (0.10–0.25 mg/kg/day), with monthly trough drug levels. Patient blood pressure should also be monitored, and these agents can be used carefully in patients with comorbid renal failure where other treatments are inappropriate.

If the patient does not respond adequately to the initial choice of induction treatment or relapses early after treatment, we consider them treatment refractory. The management choices for these patients are, again, potentially numerous and largely unsupported by rigorous evidence. In any patient with treatment refractory disease, it is essential to revisit the evidence that supports their working diagnosis, asking the question: is it possible they, in fact, have a late-presenting inherited myopathy or an alternative diagnosis, such as paraneoplastic myositis? If satisfied that the patient still has a primarily autoimmune process, then an alternative induction regimen may be appropriate; the treatment choice is between the aforementioned agents but would also increasingly include rituximab.

Rituximab

A randomised trial of rituximab in patients who were refractory to corticosteroids and at least one other immunotherapy agent has been conducted. Although the primary endpoint (time to clinical improvement between two trial arms) was not met, taken collectively, 83% of patients met the definition of improvement during the trial period.[16] Post-hoc analysis suggested that the presence of antisynthetase antibodies or anti-Mi-2 (a myositis-specific antibody associated with a dermatomyositis phenotype) was associated with a more favourable response.[17] In a retrospective case series and a Phase II trial of antisynthetase syndrome patients with interstitial lung disease, there was an improvement in muscle and/or pulmonary involvement in the majority of patients.[18,19] As per the rheumatoid arthritis regimen, two doses of 1 g rituximab (750 mg/m^2 to a maximum of 1 g) are administered 2 weeks apart.

Biologic or Experimental Treatments

Although there is much interest in targeted therapies, in particular biologics in the treatment of IIM, there is currently little supportive evidence and we consider these a third-line therapy; as a result, these treatments are only to be used in exceptional circumstances. The published results from the use of anti-tumour necrosis factor-alpha agents are not convincing, although there may be some activity in patients with resistant calcinosis. In addition, the data for interleukin-6 pathway blockade is sparse, although trials are ongoing. T cell coreceptor blockade is an attractive potential treatment target; in a Phase IIb study of abatacept (an anti-CTLA4 antibody) in treatment refractory polymyositis and dermatomyositis, 8 out of 19 patients achieved the definition of response.[20] There is Phase Ib data that support the use of Type-1 interferon blockade with sifalimumab, although research into this product in myositis was subsequently terminated during a Phase II study.[21] In a study of patients treated with anakinra (an interleukin-1 receptor antagonist) for 12 months, 7 out of 15 participants had a clinical response.[22] There are also case reports only for janus kinase inhibition, although a trial of tofacitinib is ongoing.[23,24] Lastly, there are registered ongoing trials into basiliximab (an anti-interleukin-2 receptor antibody), interferon-kappa, belimumab (an anti-B cell activating factor antibody), h5G1.1-mAb (a monoclonal antibody targeting the complement terminal attack complex), MEDI7734 (targeting plasmacytoid dendritic cells), IMO-8400 (a toll-like-receptor 7–9 antagonist), and BAF312 (a sphingosine 1-phosphate receptor modulator inhibiting lymphocyte egress from lymph nodes). Our attributed third-line status of biologics may, therefore, change in the near future.

CONCLUSION

Therapeutics in autoimmune connective tissue disease is a changing landscape. Options for more effective treatments and the understanding of which patients are likely to respond to particular therapies will evolve greatly over time. We hope that this review provides a useful framework for the application of currently available treatments for these challenging conditions.

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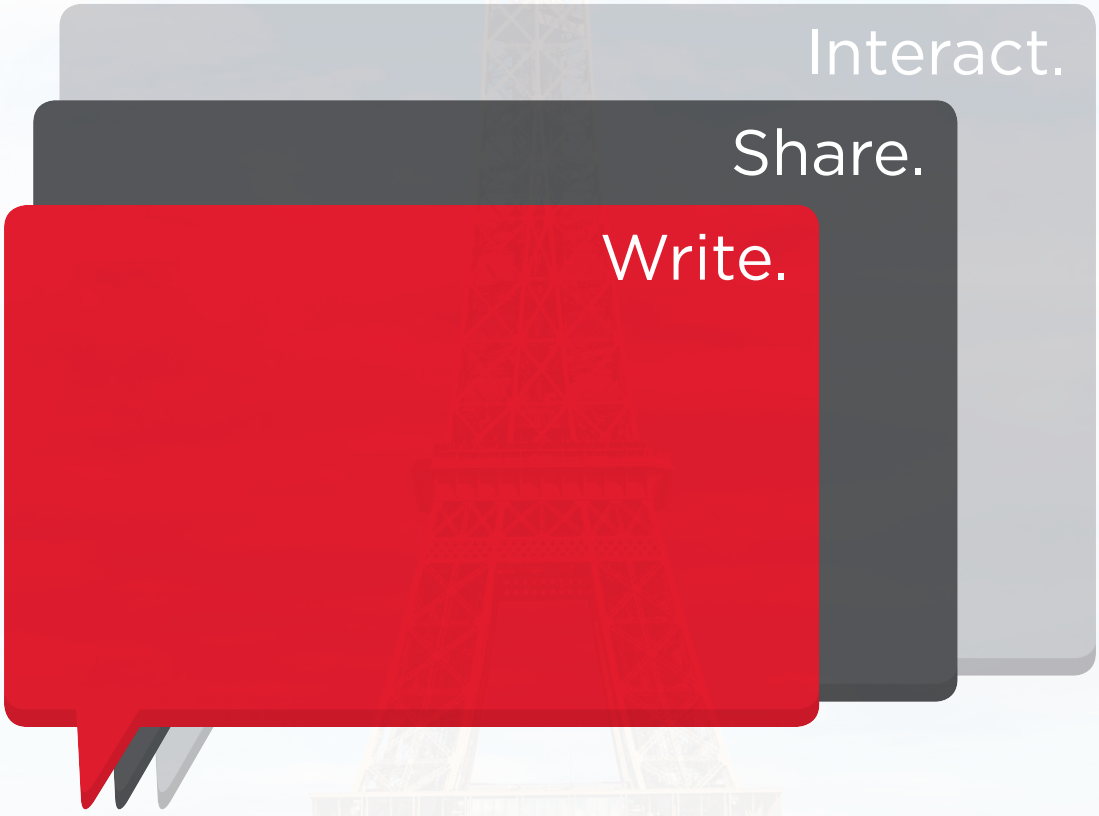

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SUSTAINING CANCER CARE THROUGH COLLABORATIVE STEWARDSHIP

This symposium took place on 9th September 2017, as part of the European Society for Medical Oncology (ESMO) meeting in Madrid, Spain

Chairperson
Matti Aapro[1]
Speakers
Muir Gray,[2] Geoffrey Henning,[3] Matti Aapro,[1] Jatinder Harchowal,[4] Bengt Jönsson[5]

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Disclosure: Prof Aapro is, or has been, a consultant for Amgen, BMS, Celgene, Clinigen, Eisai, Genomic Health, GlaxoSmithKline, Helsinn, Hospira, Johnson & Johnson, Novartis, Merck, Merck Serono, Pfizer, Pierre Fabre, Roche, Sandoz, Tesaro, Teva, and Vifor; he has also received honoraria for lectures at symposia for Amgen, Bayer Schering, Biocon, Cephalon, Chugai, DLR, Eisai, Genomic Health, GlaxoSmithKline, Helsinn, Hospira, Ipsen, Johnson & Johnson, OrthoBiotech, Kyowa Hakko Kirin, Merck, Merck Serono, Novartis, Ono Pharmaceuticals, Pfizer, Pierre Fabre, Roche, Sandoz, Sanofi, Tesaro, Taiho, Teva, and Vifor. Jatinder Harchowal is a member of advisory boards for Amgen and Sandoz. Prof Sir Gray, Geoffrey Henning, and Prof Jönsson have declared no conflicts of interest.
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MEETING SUMMARY

Cancer care has undergone rapid changes in recent years, providing dramatically improved outcomes for many patients. However, these changes have resulted in substantial increases in the costs of care in some situations. This symposium brought together a multidisciplinary faculty of experts in oncology, patient advocacy, hospital pharmacy, and health economics to discuss current issues of affordability and improving patient access to oncology medicines. The aim of the symposium was to understand what value truly means with regard to cancer care, consider what could happen when the cost of cancer care becomes unsustainable, and propose solutions to ensure optimal cancer care now and in the future.

In healthcare, it is no longer sufficient to demonstrate the effectiveness and cost-effectiveness of treatment. Now, issues of value, evidence-based decision-making, and quality must also be considered. The emerging paradigm of population and personalised healthcare was discussed by Prof Sir Gray, who highlighted the basic concepts of value-based healthcare and the need for improvement through collaborative systems and networks. The right of all patients to have equitable access to the best treatments and care was discussed by Geoffrey Henning. Among the potential solutions available, patient knowledge and empowerment will be of utmost importance, and co-ordinated campaigns by, and on behalf of, patients have the potential to change legislation for the benefit of patients.

Prof Aapro considered how cost savings from the increased use of biosimilar medicines might be re-invested to improve access to other medications, and Jatinder Harchowal provided examples of

how pharmacists can improve system-wide efficiencies, thus establishing and embedding value at a fundamental level. Finally, Prof Jönsson provided an overview of the burden, cost, and cost-effectiveness of cancer management, highlighting the growing importance of appropriate economic evaluations in the new paradigm of value-based healthcare.

The session demonstrated that through the actions of patients and healthcare professionals as equal partners, a shift towards value-based healthcare and a culture of stewardship can be achieved. Importantly, these changes are necessary to safeguard the future sustainability of cancer care.

Introduction

Currently, affordable cancer care is at a crossroads. Unprecedented advances in cancer detection and treatment, together with growing and ageing populations, mean that healthcare budgets can no longer keep pace with escalating costs. To ensure that the costs of cancer care do not outweigh the benefits, existing resources must be optimally deployed to deliver value-based care. This requires collaboration across disciplines, balancing patient needs against those of the hospital and wider healthcare system, and the elimination of tests and interventions that are of little or no value.

The Urgent Need for Value-Based Cancer Care

Professor Sir Muir Gray

Society currently faces three major healthcare problems: unwarranted variation, the underuse of high-value interventions, and the overuse of lower or zero-value interventions.

Wide variation is present in many aspects of healthcare. If that variation is harmful for patients, their families and carers, and the health services that support them, it is unwarranted and unacceptable. Due to the complexities of healthcare systems, unwarranted variation in healthcare cannot be explained by the type or severity of illness or by patient preferences alone.[1] Unwarranted variation must be addressed if high-value health services are to be provided within a set budget. Investigations of the causes of unwarranted variation have revealed two main problems. The first is the underuse of high-value interventions, especially among poorer populations, which can result in preventable disability, death, and inequity. The second is the overuse of lower or zero-value interventions, which can result in a waste of resources and, ultimately, patient harm (Figure 1).

It is estimated that the demand for cancer care will increase by about 20% over the next decade. To reduce this need and improve efficiency, measures should be put in place to prevent disease, disability, dementia, and frailty; improve outcomes by providing only effective evidence-based interventions; and increase productivity by reducing cost. However, the most important focus should be on increasing value. Three different types of value should be considered:

- Allocative value: have we allocated resources to different groups equitably and in a way that maximises value for the whole population? This is determined by how well assets are distributed to different subgroups that can be defined by clinical condition, such as cancer, or by a characteristic, such as having multiple morbidity and frailty. Decisions need to be more explicit not only regarding the amount of resource allocated to patients with cancer but also within the cancer budget. One way to allocate resources is by type of cancer, reviewing whether the allocation is optimal between, for example, services for patients with breast cancer versus patients with gastrointestinal cancer. The other allocative decision is by treatment modality: is there optimal allocation in the budgets for chemotherapy, radiotherapy, and surgery? In an era of growth, these may be implicit, but in an era in which need and demand outstrip resources, the value from different patterns of investment needs to be calculated and debated.
- Technical or utilisation value: determined by how well resources are used for all the people affected within a population. Improving the quality and safety of healthcare increases the value derived from resources allocated to a particular service.
- Personalised value: basing decisions on the best current evidence, a careful assessment of an individual's clinical condition, and an individual's values. These are the values they place on good and bad outcomes, because even

the highest quality healthcare can do harm; therefore, patients need to be provided with full information about the risks and benefits of the intervention being offered. For example, there would be little value for patients to gain a few weeks of life at the expense of extreme nausea.

The introduction of 'triple-value healthcare' has the potential to cultivate a culture of stewardship, whereby clinicians can realise and hold themselves accountable for value-based healthcare principles. Such principles include shifting resources from budgets where there is evidence of overuse or lower value to budgets for populations in which there is evidence of underuse and inequity, ensuring that specialist services are reserved for those who would benefit most, and using the cost-savings generated from reduced spending on interventions that are of limited value to fund innovation and increase the use of high-value interventions (Figure 2).

While there is a need to continue evidence-based decision-making, prevention, and quality improvement, these decisions should also involve individuals to ensure that they are right for the particular values and condition experienced by each patient. Maximising value for populations and the individuals within them will require time and multi-stakeholder engagement to ensure that all perspectives are accounted for.

Putting the Patient First: Showing the Unacceptable Levels of Inequality of Access Across Europe

Geoffrey Henning

Universal access to health services is a commitment made by all European Union (EU) member states, yet this principle has not prevented substantial inequalities in access to healthcare across Europe. In fact, health and access to healthcare in Europe are strongly determined by socioeconomic status.

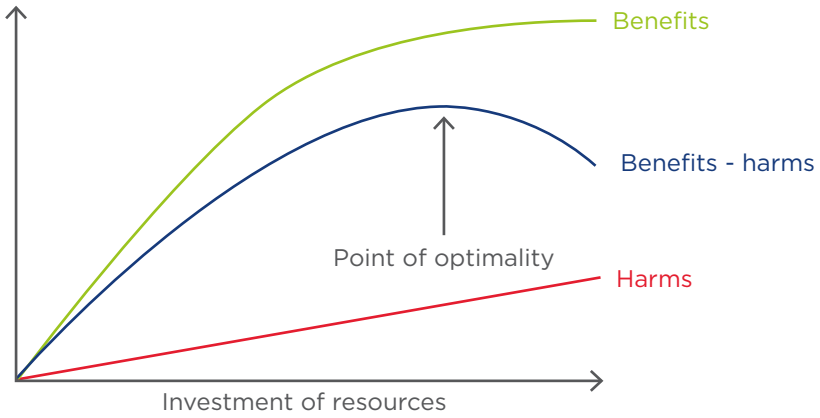

Figure 1: The pattern of benefits and harms following increasing investment of resources.
The benefits of high-quality healthcare increase with the investment of resources until optimal benefits are achieved, after which benefits decline.[2]

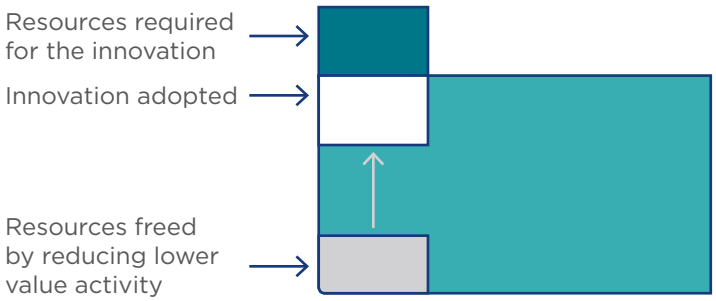

Figure 2: Population-based systems that implement high-value innovations funded by reduced spending on lower-value interventions in the same programme budget.

According to the Organisation for Economic Cooperation and Development (OECD) statistics, expenditure on drugs and other perishable goods for 2014 ranged from a high of $618 per capita in Germany to as low as $114 per capita in Poland.[3] Indeed, patients with metastatic breast cancer in Eastern Europe have far less access to medicines than similar patients in Western Europe. Between 2012 and 2014, while around 11,800 patients in Poland were diagnosed with *HER2+* breast cancer, only 5,100 women had access to the appropriate medicine.[4]

Biosimilars represent a real opportunity to increase patient access to safe and effective medicines, but patient concerns will need to be addressed for the nature and benefit of these medicines to be realised. Patients have a right to know which medicines they are prescribed and need to be informed when their medicine has been switched or substituted. Appropriate discussions are needed and full information about biosimilars should be made available for all patients so that they understand the issues surrounding them, along with the opportunities they offer. The challenge will be pricing and whether biosimilars will be available for all patients or remain out of reach for some.

Copayments are an option in many European countries; however, there is a danger that these put increased financial pressure on families, potentially leading to worse outcomes. A distinction regarding copayment could potentially be made according to value, with therapies considered of high value automatically funded and those of lower value considered for copayment.

In terms of changing the current situation, patient empowerment is crucial. Together, clinicians and patients represent a formidable force for change in health systems, and their united voice should be heard where inequalities exist. Through knowledge and collaboration, co-ordinated campaigns have the power to change legislation, helping to ensure access to the best treatment and care for all EU citizens.

As Oncologists, What Can we do to Improve Value?

Professor Matti Aapro

There is no evidence that spending more on cancer consistently improves outcomes. In 2010, a study found that Sweden and Finland had almost identical 5-year colorectal cancer survival rates (approximately 59%). However, according to the calculations presented in the paper, the total expenditure per colorectal cancer case was approximately €10,000 in Sweden versus €172,000 in Finland, equating to a 17-fold difference in expenditure per case with no evidence of benefit.[5]

According to the European Society for Medical Oncology (ESMO), the value of any new therapeutic strategy or treatment is determined by the magnitude of its clinical benefit balanced against its cost.[6] However, whereas costs vary from country to country, the magnitude of clinical benefit, as derived from well-designed clinical trials, is a relative constant. The aim of the ESMO Magnitude of Clinical Benefits scale, therefore, is to assess the clinical benefit of different cancer medications. This will allow stakeholders to distinguish between treatments that bring substantial improvements in the duration of survival and/or quality of life of cancer patients and treatments with benefits that are more modest, limited, or even marginal. In short, it aims to identify which interventions are of high value: knowledge that should help to minimise the use of low-value treatments. In doing so, therapies that provide little, or no, patient benefit can be avoided and the overuse of treatments and/or misuse of tests reduced.[7,8]

One approach to increasing value-based healthcare is through the use of more affordable medications. Biosimilar medicines have equivalent efficacy and safety to already approved reference medicines, but may be more affordable. In the UK, the availability of biosimilar filgrastim resulted in a 30% increase in overall filgrastim use from 2008 to 2010, contributing to a shift in treatment practice from secondary prophylaxis towards increased primary prophylaxis.[9] Switching to biosimilar filgrastim in a community setting resulted in an increase from 36% to 52% in the use of filgrastim as primary prophylaxis.[10] Furthermore, between 2006 and 2013, the treatment volume of filgrastim per capita increased by an average of 44% across the EU.[11] These data suggest that the use of more affordable medicines may give more patients access to important treatments.

Cost-savings generated from the use of biosimilar medicines, and other more affordable treatments such as small molecule generics, can also be reinvested to improve patient access to other,

more expensive, treatments. For example, it has been estimated that a 100% switch to a biosimilar epoetin for oncology indications in seven European countries (France, Italy, Spain, Germany, Romania, UK, and the Netherlands) in 2010 would have resulted in a total of $188 million being saved annually.[12] This may enable increased access to potentially life-saving drugs. A saving of $188 million would, for example, have supported rituximab treatment for approximately 9,000 extra patients.[12]

The Pharmacist as an Agent of System-Wide Change

Jatinder Harchowal

The current pipeline for oncology medicines will result in a plethora of new agents coming to market, with an associated increase in overall costs. Conversations around value-based pricing based on outcomes have, therefore, become commonplace, with questions arising on how value should be defined. In such situations, collaborative leadership is required, and all aspects of the patient pathway considered.

At the Royal Marsden Hospital, London, UK, there are approximately 50 pharmacists, almost 20 of whom have an advanced practice role, which enables them to review and prescribe chemotherapy agents and review supportive medications. Such pharmacists add value to patient care by optimising the use of medicines as part of a multidisciplinary team. They play a key role in reducing medication errors, inappropriate polypharmacy, preventable medication-related harm, and poor adherence. In many cases, non-adherence is the result of adverse events; therefore, monitoring safety is an especially important component of the pharmacist's role.

Pharmacists are also increasingly involved in the implementation of system-wide efficiencies, with the aim of introducing value-based care. An NHS England initiative on the standardisation of chemotherapy, for example, introduced national dosing bands for the 50 most commonly used cancer drugs. Compared with the previous system, where dose calculations varied slightly according to each cancer centre's protocol, the new standardised approach provides significant efficiencies. These include doses being stocked as batches, the ability to offer off-the-shelf treatment to new patients or patients in urgent need, less wastage, and fewer delays to the patient receiving treatment.

In 2015, the Cancer Vanguard programme was launched to test and fast-track innovative models of cancer care, as recommended by the NHS National Cancer Strategy. Pharmacy teams from the main sites of the Cancer Vanguard programme worked collectively to produce a centralised repository of information to help support the introduction of biosimilar rituximab across NHS England. This included a co-ordinated educational approach, developing an interactive PDF to educate staff, an information sheet for patients, and policy guidance for the entire country. Most patients have now been switched from reference rituximab to biosimilar rituximab. In 2016, £186 million was spent in England on rituximab across all its indications. It has been estimated that NHS England will save at least £65 million in oncology alone by switching to the biosimilar medicine over the next year. Such savings can be used to fund the purchase of additional medicines, thus increasing patient access, and to employ additional staff that can help manage the overall patient journey.

Leveraging Heath Economic Data

Professor Bengt Jönsson

To assess the sustainability of cancer care, data are needed on three key economic variables: the burden of cancer, the cost of cancer, and the cost-effectiveness of cancer management. Between 1995 and 2014, the incidence of cancer increased by 30%, largely due to population growth and ageing[13] and as such, the burden of cancer has also increased. One measure of the overall burden of a disease is the disability-adjusted life year (DALY), which equals 1 year of healthy life lost. In 2012, 19% of DALY in Europe were due to cancer, second only to cardiovascular disease (21%).[14] In many countries, cancer has recently overtaken cardiovascular disease as the leading cause of DALY, suggesting that in the near future, cancer is likely to be the main contributor to disease burden across Europe. The proportion of spending on cancer, however, does not reflect its disease burden. In the EU, health expenditure on cancer increased from €35.7 billion in 1995 to €83.2 billion in 2014, with spending on cancer drugs increasing from €7.6 billion in 2005 to €19.1 billion in 2014.[13] Despite these increased costs, the share of total health expenditure devoted to cancer (around 6%) has changed little over the last 30 years in both Europe and the USA. This may be explained by other expenditures, such as inpatient care, having decreased.

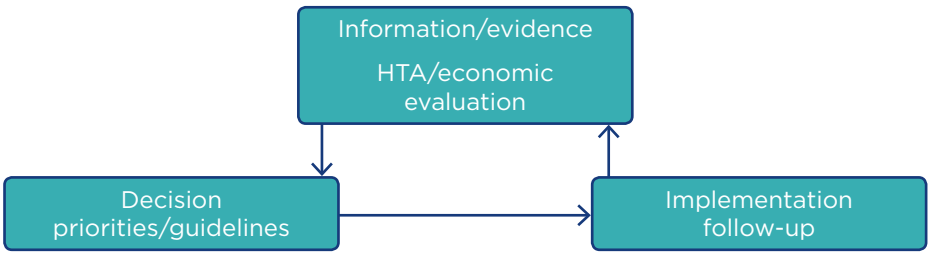

Figure 3: The circle of follow-up to ensure value for money.
An illustration that the right decision is necessary but not wholly sufficient.
HTA: health technology assessment.

Wide variations in the cost of cancer care exist across Europe, even between countries of similar economic strength. For chronic myeloid leukaemia, data show that Spain spends €20,000 per case, Germany €16,000, France €14,500, Italy €13,000, and the UK €10,000.[14] These data suggest some countries are overspending and others underspending. Furthermore, for tyrosine kinase inhibitors, which have dramatically changed outcomes in chronic myeloid leukaemia, data show that the defined daily dose given to patients varies according to a country's gross domestic product (GDP). The defined daily dose for tyrosine kinase inhibitors was 375 mg for countries in the lower GDP tier, 500 mg for those in the mid GDP tier, and 590 mg for those in the upper GDP tier.[14] Therefore, when prices decrease, it will be countries with the lowest incomes that will benefit most.

With limited healthcare resources, greater emphasis is needed on achieving value for money. To understand the value of treatments, data on health economic outcomes must be captured in clinical trials and must also be generated post-launch. To help support decisions on the value of medicines, information and evidence from health technology assessments must be fed into decisions around priorities and guidelines, and the impact of these measures continuously reviewed and updated (Figure 3).

The need for long-term follow-up of reimbursement decisions is supported by the UK Cancer Drug Fund (CDF), which, between 2010 and 2016, cost £1.27 billion. Of the 47 CDF approved indications, a statistically significant benefit in overall survival was only seen in 18 (38%), with a median survival of 3.1 months (1.4–15.7 months).[15] Furthermore, when assessed according to clinical benefit scales, only 23 (48%) and 9 (18%) indications met American Society of Clinical Oncology (ASCO) and ESMO criteria for clinical benefit, respectively. The National Institute for Health and Care Excellence (NICE) had previously rejected 26 (55%) of the CDF approved indications, because they were not considered cost-effective; however, no additional data on patient outcomes were collected, which highlights the need for real-world evidence on outcomes and resource use.

Conclusion

The symposium provided a great opportunity to demonstrate that the future of sustainability in cancer care requires ongoing effort from multiple stakeholders throughout the healthcare system, from patient to health economist. With collaborative effort, increasing implementation of value-based healthcare can, and must, be achieved.

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TACKLING THE INFLAMMATORY BURDEN OF PSORIASIS: A MULTIDISCIPLINARY APPROACH

This symposium took place on 15th September 2017, as part of the 26th European Academy of Dermatology and Venereology (EADV) Congress, in Geneva, Switzerland

Chairperson

Giampiero Girolomoni[1]

Speakers

Giampiero Girolomoni,[1] Naveed Sattar,[2] Frank Behrens,[3] Krisztina Gecse[4]

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Disclosure: Prof Girolomoni has received fees for participation on advisory boards from AbbVie, Abiogen, Almirall, Amgen, Avantgarde, Bayer, Biogen, Boehringer Ingelheim, Celgene, Eli-Lilly, Galderma, Genzyme, Janssen, Hospira, Leo Pharma, Menlo Therapeutics, Merck, MSD, Mundipharma, Novartis, Pfizer, Regeneron, Rottapharm, Samsung, Sanofi, Shiseido, and Sun Pharma; and has received fees for consultations from Bioderma, Ducray, Insiderma, Pierre Fabre, Roche, and Sandoz. Prof Sattar is involved in the European League Against Rheumatism (EULAR) guidelines on cardiovascular disease, the European Society of Cardiology (ESC) cardiovascular disease prevention guidelines, and has consulted for Amgen, Sanofi, Roche, and UCB. Dr Behrens has received research support from Abbvie, Pfizer, Roche, Chugai, Prophylix, Bioline, Novartis, Janssen; consultant/speaker fees from Abbvie, Pfizer, Roche, Hugai, UCB, BMS, Celgene, MSD, Novartis, Biotest, Janssen, Genzyme, Lilly, Sandoz, and Hexal; and is an advisory board member for Abbvie, Pfizer, Roche, Chugai, UCB, Celgene, Novartis, Biotest, Janssen, Genzyme, Hexal, Lilly, and Boehringer-Ingelheim. Dr Gecse reports having received consultancy fees and/or speaker's honoraria from Amgen, AbbVie, Boehringer Ingelheim, Ferring, Hospira, MSD, Pfizer, Sandoz, Takeda, and Tigenix.

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MEETING SUMMARY

Prof Girolomoni provided an overview of psoriasis, considering how patients are frequently affected by other comorbidities. Cost, he explained, can be a constraint for optimal anti-tumour necrosis factor (TNF) treatment, with biosimilars representing an important opportunity for providing more patients with effective therapy. Data from X-ray crystallography studies, neutralisation studies, and clinical trials were presented, demonstrating that biosimilars have comparable efficacy to reference treatments.

Prof Sattar explained how to define the overall cardiovascular disease (CVD) risk score in psoriasis; the standard risk score should be multiplied by 1.5 for patients with young onset or more severe disease. Throughout the presentation he stressed that all CVD risk factors need to be taken into consideration. Just because someone has severe psoriasis does not mean they are necessarily at high risk of CVD, and just because someone has mild psoriasis does not mean they are at low risk. In the second part of his talk, Prof Sattar reviewed evidence suggesting that psoriasis and obesity are interlinked, and discussed benefits of weight loss.

Dr Behrens considered the hypotheses for psoriatic arthritis (PsA) genetic predisposition in patients with psoriasis. He reviewed data suggesting that psoriasis and PsA are different diseases, with psoriasis acting as a trigger for PsA. Dr Behrens went on to discuss predictors of PsA in patients with psoriasis and the importance of individualising treatment to phenotype.

Dr Gecse reviewed the aetiology, disease course, prognostic factors, and characteristics of inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC). She explained how the prevalence of CD and UC is four-times higher in patients with psoriasis versus the general population, with the highest rates occurring in patients with both psoriasis and PsA. She went on to present studies showing how interleukin (IL)-17 inhibitors, which show promising effects in psoriasis, worsened in IBD.

Introduction

Professor Giampiero Girolomoni

Psoriasis is a common disease affecting 3% of the population,[1] said Prof Girolomoni. Around 10–20% of patients with psoriasis need systemic treatment[2] and 5–30% have PsA.[3,4] Psoriasis is a life-long disease with a chronic relapsing course, requiring long-term therapy for most patients.[5] Biological treatments are associated with high costs which limits optimal treatment for many patients.[6] Psoriasis, said Prof Girolomoni, affects all aspects of patient life including emotional,[7] financial,[8] work,[9] and leisure.[10]

Patients with psoriasis frequently have associated disorders, which may be due to common pathogenesis resulting from shared genetic loci and cytokines (PsA and CD)[11-13] or result from chronic inflammation (CVD, metabolic syndrome, and Parkinson's disease).[11,12,14-16] Finally, psoriasis has a psychosocial impact, and may result in depression, anxiety, smoking, and alcoholism.[11,12]

Current Treatment Landscape in Moderate-to-Severe Psoriasis

Professor Giampiero Girolomoni

Many factors influence psoriasis severity, said Prof Girolomoni, including body surface area involvement, erythema, infiltration and scaling, lesions in sensitive areas, impact on quality of life, non-response to treatments, disease activity, frequency, and severity of relapses.[17] Such factors can be perceived differently by patients and doctors. One study, involving 2,513 patients and 391 dermatologists, showed patients believed itching to be the most important factor contributing to psoriasis severity (43%), while dermatologists said location and size of skin lesions (53%).[18,19]

With regard to psoriasis severity, it is important to take comorbidities into consideration. Severe psoriasis is an independent risk factor for metabolic comorbidities such as obesity, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), hypertension, dyslipidaemia, hyperuricaemia, and diabetes. The nature of associations is not well understood, but may be due to a common gene(s) and/or the effect of persistent severe skin inflammation. One study showed psoriasis shares predisposing genes with obesity, rheumatoid arthritis (RA), Type 2 diabetes mellitus, and Alzheimer's disease.[20]

The Italian guidelines on systemic treatments for moderate-to-severe psoriasis recommend a holistic treatment approach taking into consideration disease (severity and frequency of relapses), the patient (contraindications, likelihood of adherence), and treatment (short and long-term effectiveness, practicability, flexibility, safety, tolerability).[17]

Factors in choice of biological treatment include overall efficacy, need for rapid response, and presence of concomitant diseases benefitting from the same treatment;[17] however, the most common reason for not initiating biological therapy, said Prof Girolomoni, was cost. This view is supported by a recent survey where 48.8% of dermatologists and 45.9% of rheumatologists responded that cost was the main reason for not initiating biological therapy.[6]

Biosimilars, said Prof Girolomoni, have a place to ensure early, optimal, and equal access to anti-TNF treatment in psoriasis.[21] Biosimilars can provide earlier access to effective treatment options and have the potential to improve treatment continuity and reduce regional variability in access across Europe. A recent paper provided an overview of the current status of anti-TNF biosimilars, showing the number of biosimilars that have been registered and the number of agents currently investigated in preclinical and the number clinical trials (Figure 1). Although two etanercept and six adalimumab biosimilars have been tested in psoriasis, no infliximab biosimilars have been tested in these patients.[22]

A Phase III study comparing the adalimumab biosimilar ABP 501 with reference adalimumab showed that at Week 16, the mean Psoriasis Area Severity Index (PASI) percent improvement was comparable between the two groups.[23]

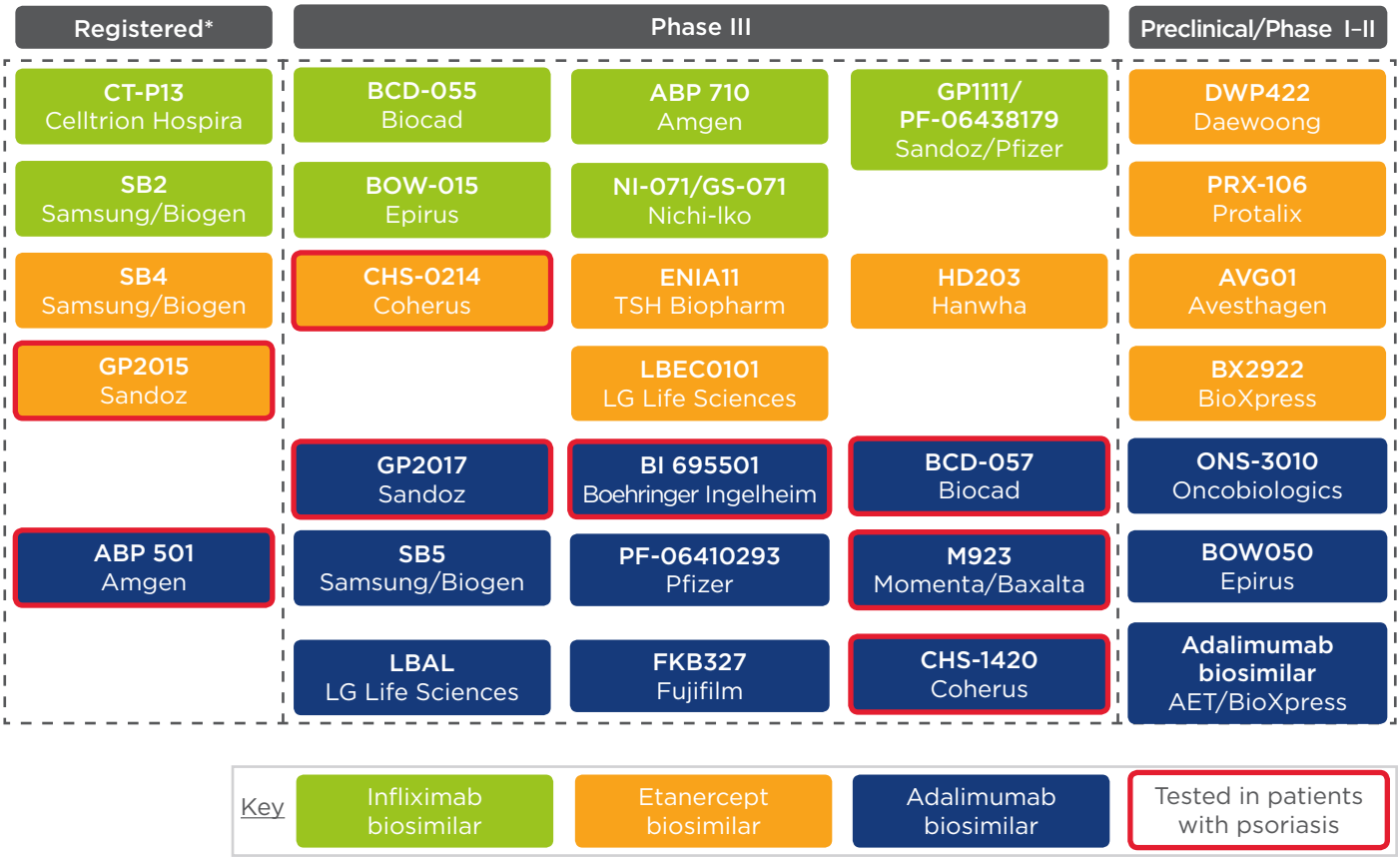

Figure 1: Principal anti-tumour necrosis factor biosimilars available or in development.
*Authorised by the European Medicines Agency (EMA), current status as of July 2017.
Adapted from Braun and Kay.[22]

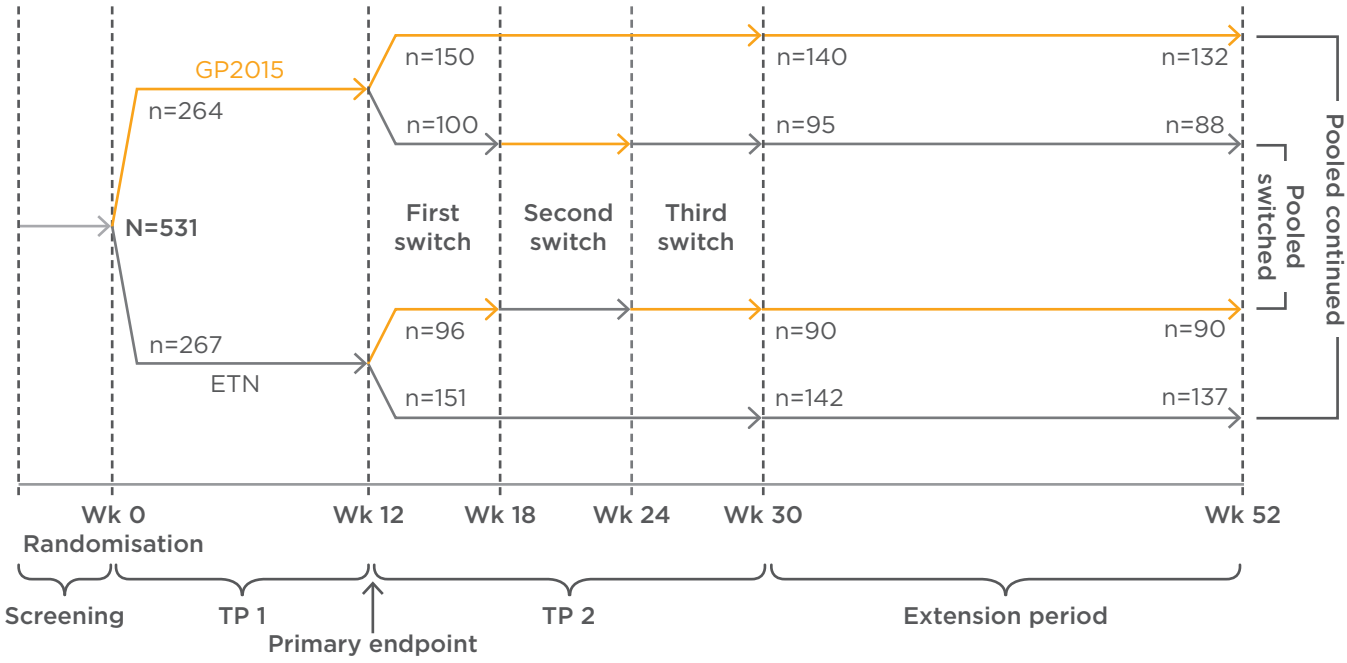

Figure 2: Bioequivalence of GP2015 was confirmed in patients with psoriasis in a multi-switch study (EGALITY).
ETN: reference etanercept; TP: treatment period; Wk: week.
Adapted from Griffiths.[25]

Several studies have shown that biosimilar and reference medicines have similar levels of efficacy and comparable safety, said Prof Girolomoni. X-ray crystallography analysis of Sandoz's etanercept biosimilar GP2015 found that the higher order structure of GP2015 and reference etanercept were indistinguishable.[24] In addition, similar protein content (essential for clinical efficacy of a biologic) and TNF binding and neutralisation were comparable between GP2015 and reference etanercept sourced from the European Union (EU) and the USA.[24] The EGALITY study[25] showed comparable efficacy, safety and immunogenicity between GP2015 and reference etanercept over 52 weeks in patients with psoriasis; switching between GP2015 and reference etanercept multiple times did not impact on efficacy and safety. Furthermore, the primary endpoint for equivalence, comparable PASI 75 response rates at Week 12, was met. (See Figure 2 for multiple-switch study design).

The EGALITY study[2] showed comparable efficacy, safety, and immunogenicity (note: bioequivalence was confirmed at Week 12, primary endpoint) of GP2015 and reference etanercept over 52 weeks in patients with psoriasis; switching between GP2015 and reference etanercept multiple times did not impact on efficacy and safety. Furthermore, the primary efficacy endpoint for equivalence, comparable PASI 75 response rates at Week 12, was met (see Figure 2 for multiple-switch study design).

Cardiometabolic Risks in Psoriasis and Psoriatic Arthritis

Professor Naveed Sattar

Studies indicate patients with psoriasis are at increased risk of CVD, said Prof Sattar;[26] but risk is only meaningfully increased in a subgroup, usual risk factors matter. In a prospective population cohort study of 556,995 controls, 127,139 patients with mild psoriasis, and 3,837 with severe psoriasis, relative risk (RR) for myocardial infarction (MI) was higher in younger patients.[27]

For 30-year-old patients, the adjusted RR for having a MI was 1.29 for mild disease, versus 3.10 for severe disease; while for a 60-year-old the adjusted RR for MI was 1.08 for mild psoriasis, versus 1.36 for severe psoriasis. Severe early-onset psoriasis is the group at the highest risk. The study cannot be considered definitive in terms of assessing independent risk, said Prof Sattar, since it did not fully adjust for residual confounding factors. Nevertheless, if risk is increased meaningfully, it is in younger patients with severe psoriasis.

In their CVD risk calculator, the Joint British Societies for the Prevention of Cardiovascular Disease use a 1.4 multiplication factor for patients with RA, after adjusting for other predictors.[28] Similarly, EULAR guidelines recommend CVD risk prediction models are adapted for patients with RA and PsA using a 1.5 multiplication factor.[29] The same approach can be undertaken for severe, early-onset psoriasis.

Two Case Studies

- Patient A: A 50-year-old man with no other conditions, BMI 23, cholesterol 6.5 mmol/L, systolic blood pressure 146 mmHg, who smoked 30 cigarettes per day, and had a family history of CVD.
- Patient B: A 50-year-old man with severe psoriasis for 38 years, BMI 34, cholesterol 4.8 mmol/L, systolic blood pressure 135 mmHg, who was a non-smoker, with no CVD family history.

When risk scores were calculated, Patient A (without psoriasis) had a 22% risk of having a cardiovascular event in the next 10 years compared with Patient B (with psoriasis), who had a 7% risk. The risk for Patient B was initially calculated as 4.6%, explained Prof Sattar, but became 7% when multiplied by 1.5 to take psoriasis into consideration (which in this case was severe and of early onset). These case studies highlight that just because a patient has early-onset psoriasis and a high BMI, they are not automatically at a higher risk of CVD. It is important, he said, to take all risk factors into consideration, since many other specific factors can place patients at high risk, irrespective of the nature of their psoriasis.

The best evidence for lowering CVD risk, said Prof Sattar, comes from statin trials. The Joint Societies risk calculator shows statins lower CVD risk by 20–25% per mmol/L reduction in low density lipoprotein-cholesterol.[30] The risk calculator can be used to demonstrate how much longer patients can expect to live if they take preventive actions. Statins should be considered if patients are above the risk threshold, and blood pressure should be treated according to national guidelines. Helping patients stop smoking, Prof Sattar added, provides huge benefits with a wide variety of techniques available (patches, tablets, and cognitive behaviour therapy).

Turning to diabetes and metabolic risks, Prof Sattar said that evidence suggests that psoriasis and obesity are interlinked; of people with a BMI >27, 28% have PsA versus 15% with RA. There is evidence that obesity is 47% more common in patients with severe versus mild psoriasis, and that response of patients to systemic therapy is inversely proportional to BMI.

Prof Sattar presented the case of a 38-year-old male non-smoker who had 5 years of mild psoriasis with no joint disease. Blood pressure was 138/78, weight 88 kg, height 1.7 m, and BMI 31.2. Test results showed haemoglobin A1c was 43 mmol/mol, alanine transaminase 53, aspartate transaminase 32, cholesterol 4.9 mmol/L, high-density lipoprotein-c 1.1, and triglycerides 3.5 mmol/L.

The patient has a higher risk of developing diabetes than CVD since haemoglobin A1c was at a prediabetic level, and he has evidence of NAFLD. His CVD risk, however, was lower due to his youth and being a non-smoker with normal blood pressure. If psoriasis and PsA patients are overweight or obese, said Prof Sattar, they should undergo cholesterol (including triglycerides and high-density lipoprotein cholesterol) and liver function testing.

Weight reduction, said Prof Sattar, delivers a variety of benefits, including improved quality of life, direct or indirect improvement of psoriasis, and reduced risk of diabetes, NAFLD, and CVD.[26] New National Institute for Health and Care Excellence (NICE) guidance suggests a 3% weight loss is realistic.[31] It was far easier to lose weight through dietary changes than physical exercise. The focus, he added, should continue to be on smoking cessation and sensible alcohol intake. Remember, Prof Sattar concluded, to be kind to patients and not to give them a hard time, because weight loss can be difficult to sustain; doctors should also remember most patients do not wish to be overweight or obese, so they deserve our empathy and help.

In the question and answer session Prof Sattar was asked if treatment of psoriasis reduces CV risk. Prof Sattar said the CANTOS trial provided proof of concept that damping systemic inflammation reduces CV risk. Additional support comes from observational data on anti-TNF. Other risk factors, such as smoking, will need to be taken into consideration.

Understanding the Rheumatic Comorbidities of Psoriasis

Doctor Frank Behrens

Data suggest, said Dr Behrens, that 70% of patients with pre-diagnosed psoriasis go on to develop PsA, 15% have PsA prior to skin disease, and 15% have concomitant onset of PsA and skin disease. So how do clinicians decide whether an individual patient with psoriasis is at risk of developing PsA?[30]

A cross-sectional observational study demonstrated that patients who have had psoriasis for 27 years have the same probability of developing PsA in one year as those who have had psoriasis for 1 year (Figure 3).[32] For clinicians, said Dr Behrens, the bottom line is you have to ask about joint complaints at every consultation, because patients have the same risks of PsA regardless of whether it is their first or twenty-first visit.

Such data suggest that psoriasis and PsA are the same disease, with PsA occurring later in the disease course. A paired genetic analysis of synovial and skin tissue revealed that gene expression profiles for PsA synovial joints were more tightly linked to skin psoriasis than RA.[33] Such links, said Dr Behrens, indicate it may be better to select drugs for PsA that work in psoriasis, opposed to RA.

Currently there are two alternative hypotheses for PsA genetic predisposition. The first is that all risk alleles for skin diseases also cover the total heritability of PsA peripheral joint manifestations and PsA axial manifestations. The second is that risk alleles for skin disease and different phenotypes of PsA only partially overlap.[34]

With its limited genetic pool, Iceland provides valuable insights into risks of family members developing different diseases. In one study, national identification numbers of 220 Icelanders known to have PsA were linked with genealogy databases and risk ratios were estimated for first to fifth-degree relatives matched to unaffected controls from the Icelandic population.[35] Results showed RR for developing PsA from first to fourth-generation were 39.0, 12.0, 3.6, and 2.3, respectively (all $p<0.0001$); whereas the fifth-degree relatives had an RR of 1.2 ($p=0.236$). These results therefore indicate a strong genetic component.

If psoriasis and PsA were more or less the same disease, said Dr Behrens, similar numbers of patients would be found in second, third, and fourth

generations, which was not the case. First-degree relatives of patients with psoriasis have between 4 to 10-fold increased risk of developing the skin condition, far lower than the 39-fold increase in first-degree relatives of PsA patients. PsA, it appears, has a higher genetic component than psoriasis.[35]

In a genome study, significant associations with the *PTPN22* loci were found for PsA susceptibility, but not for psoriasis.[36] Such data, said Dr Behrens, suggest PsA and psoriasis are different diseases but are linked. A model where mice spontaneously develop arthritis after 6–12 months (due to *STAT 3C* overexpression) showed that when psoriasis is induced experimentally, mice immediately develop PsA.[37] Psoriasis appears to act as a trigger for developing PsA in those with a genetic predisposition.

With such links between psoriasis and PsA, dermatologists are in a good position to identify PsA early. In a logistic regression analysis of patients with psoriasis, the strongest predictors for concomitant PsA were nail involvement (odds ratio: 2.93) and inpatient hospital treatment (odds ratio: 1.63).[38] The current recommendation for screening patients with psoriasis for PsA is once a year using screening questionnaires.

To meet Classification Criteria for Psoriatic Arthritis (CASPAR), patients must have inflammatory articular disease (joint, spine, or entheses) with ≥3 points from five additional categories.[39] Dr Behrens cautioned that it can take 6 months to 1 year for swollen joints, osteoproliferation, and erosions to develop, and that they were not seen in every patient. Patients may be free from joint swelling, but found to have periostitis and bone marrow oedema on magnetic resonance imaging (MRI). Other patients who are negative on clinical examination may show slight inflammation with fluorescent optimal imaging or ultrasound.

According to the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) treatment recommendations for patients with peripheral arthritis, axial disease, enthesitis, and dactylitis, conventional synthetic disease-modifying antirheumatic drugs, such as methotrexate, should be used in peripheral arthritis and dactylitis, but not in axial disease and enthesitis.[40]

There is a need, said Dr Behrens, to individualise PsA treatment to phenotype. For patients with skin, dactylitis, and enthesitis manifestations, and arthritis, there is evidence of efficacy with leflunomide, apremilast, methotrexate, cyclosporine, and sulfasalazine. However, for patients with skin, enthesitis, and dactylitis manifestations, but not arthritis, evidence suggests going directly to biologics (Behrens F. Personal communication).

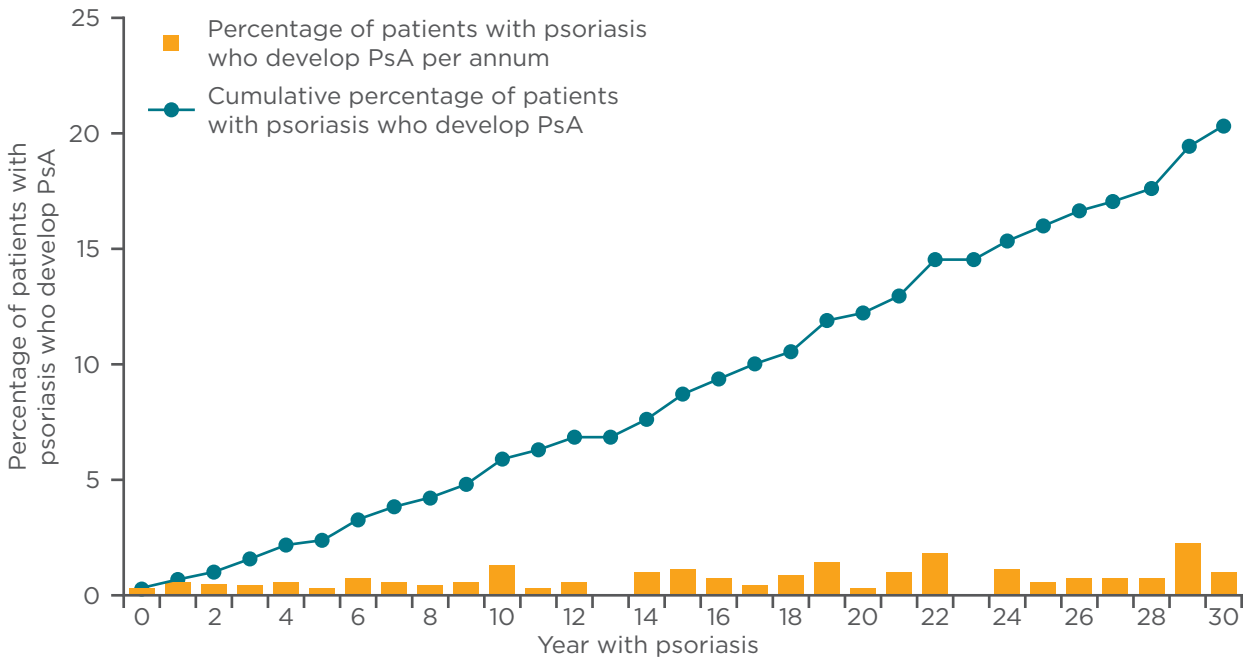

Figure 3: Prevalence of PsA in patients with psoriasis: Incidence and cumulative prevalence of PsA among patients with psoriasis treated by dermatologists.

PsA: psoriatic arthritis.

Adapted from Christophers et al.[32]

In rheumatology, only around 30% of patients achieve an American College of Rheumatology score of 50% symptom improvement (ACR 50), making it important to optimise outcomes. A study by Behrens on optimisation of anti-TNF monotherapy showed 43% of patients achieved good quality of life scores, 56.6% achieved good skin scores, and 72.5% good arthritis scores; however, only 24.3% achieved simultaneously good responses in all three domains. The addition of conventional synthetic disease-modifying antirheumatic drugs increased the percentage of patients achieving good skin scores to 75.5%; although, this was at the expense of arthritis scores and made no difference to the achievement of good scores in all three domains (Behrens F. Data on file).

The challenge for the future, Dr Behrens concluded, is to optimise outcomes by finding the right treatments for individual patients.

The Link Between the Skin and the Gut: Inflammatory Bowel Disease in Patients with Psoriasis

Doctor Krisztina Gecse

IBD, such as UC and CD, said Dr Gecse, are defined as a chronic inflammation in the gastrointestinal (GI) tract induced by "inappropriate and continuing inflammatory response to commensal microbes in a genetically predisposed individual."[41]

IBD aetiology includes genetic susceptibility, environmental triggers, luminal microbial antigens and adjuvants, and aberrant immune responses.[41] The natural course of CD shows most patients start with inflammatory disease and later experience stricturing or penetrating complications (abscesses and fistulas).[42] However, a significant proportion of CD patients already present with penetrating or stricture disease. With UC, approximately 20% of patients will have undergone colectomy after 20 years of disease duration.[43]

The disease course of IBD follows four principal scenarios, of which the most common are remission or mild severity of intestinal symptoms after initial high activity (affecting 43% of CD patients and 59% of UC patients) and chronic intermittent symptoms (affecting 32% of CD patients and 31% of UC patients).[43]

A number of prognostic factors have been associated with complicated CD including young age at disease onset, smoking, severe upper GI disease, extensive small bowel disease, perianal disease, need for steroids at diagnosis, weight loss, and deep ulcerations upon index endoscopy.

CD can affect any segment of the GI tract, most commonly the terminal ileum, as indicated by its Latin name, *ileitis terminalis*.[44] UC, in contrast, is a diffuse, continuous disease which typically starts in the rectum and spreads upwards, with no ileal involvement (except backwash ileitis) and is not associated with perianal lesions.[45] Signs and symptoms for CD include abdominal pain, weight loss, chronic diarrhoea, malaise, anorexia, and fever;[44] while signs and symptoms for UC most commonly include bloody diarrhoea, rectal bleeding, tenesmus, abdominal pain, urgency, faecal incontinence, nocturnal defecation, fatigue, anorexia, and weight loss.[45] Clues to IBD diagnosis include chronic symptoms, systemic symptoms, young age, and family history of IBD. However, there is no single gold standard method for diagnosing IBD, therefore a combination of clinical evaluation, biomarkers, endoscopy, histology, and imaging should be taken into account.[44,45]

The link between the skin and the gut can be approached from three directions; psoriasis and IBD as true comorbidities, psoriasis as paradoxical reaction to anti-TNF medication, and IL-17 inhibitors in the treatment of psoriasis. IBD patients, said Dr Gecse, are routinely screened for extra-intestinal manifestations including psoriasis. Prevalence rates of CD and UC are approximately four-times higher in the psoriasis population than in the general population, with the highest risks occurring in patients with both psoriasis and PsA. Psoriasis is more strongly associated with CD than UC, with Psoriasis-CD patients having milder psoriasis but earlier onset and more severe Crohn's phenotypes.[46]

Since the IL-17 pathway in the pathogenesis of IBD and psoriasis are shared, it seemed plausible to investigate IL-17 inhibitors for the treatment of IBD as well. However, both anti-IL-17A monoclonal antibodies secukinumab and brodalumab induced worsening of CD in moderate-to-severe CD, and showed the area under the curve analysis significantly favoured placebo ($p=0.043$).[47] A second Phase II study, this time using brodalumab in moderate-to-severe CD, showed the proportion of patients with worsening disease was lowest for placebo and the Crohn's Disease Activity Index (CDAI) higher in brodalumab.[48]

IBD patients undergoing anti-TNF treatment can show paradoxical skin reactions. The prevalence of new-onset psoriasis with anti-TNF is known to be 0.6–5.3%,[49,50] with the most frequent presentation being palmoplantar.[49-51] Skin lesions can appear at any time after initiation of anti-TNF treatment, most frequently after the third infliximab infusion.[51-53] In most cases, topical treatment is sufficient; however, stopping anti-TNF and initiating other systemic treatments may be necessary.[49]

IBD patients represent a broad disease spectrum in terms of disease location, severity, complications, and comorbidities. In an ideal world, biopsy samples, serum samples, or faecal stool tests would allow patient profiling and personalised medicine, determining the most optimal choice of treatment to reach mucosal healing, to avoid complications and clinical symptoms, and ultimately leading to improved quality of life.

In the question and answer session, when asked about symptoms and laboratory results that should alert rheumatologists to refer patients for IBD assessments, Dr Gecse said young patients with a family history of IBD, chronic abdominal, and possibly systemic symptoms should be referred to a gastroenterologist. Additionally, Dr Gecse added, faecal calprotectin is a good biomarker to distinguish between functional GI disorders and IBD.

Click here to view the full symposium.

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HEREDITARY ANGIOEDEMA: THE DAWN OF A NEW ERA OF HEREDITARY ANGIOEDEMA MANAGEMENT

This symposium took place on 19th June 2017, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Annual Meeting in Helsinki, Finland

Chairpersons

Marco Cicardi,[1] Timothy Craig[2]

Speakers

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MEETING SUMMARY

This symposium provided an overview of past, current, and future therapies and routes of administration for patients with hereditary angioedema (HAE). Prof Cicardi opened the symposium by welcoming attendees and introducing the main topics of the session. Prof Magerl then focussed on treatments that are currently used for acute and prophylactic management of patients with HAE and highlighted that there is an unmet medical need in terms of better prophylactic treatment options. Prof Craig summarised the clinical evidence gathered over the last decades and shared the key findings and insights that led to our current understanding of the disease and laid the foundations for current and future treatment approaches. Prof Zuraw presented the findings from the pivotal Phase III COMPACT trial that explored the efficacy and safety of a self-administered subcutaneous (SC) nanofiltered C1-esterase inhibitor concentrate (C1-INH[SC]) for the prevention of HAE attacks.

Unmet Medical Needs in Hereditary Angioedema Management

Professor Markus Magerl

In the current treatment landscape for on-demand treatment of HAE there are a number of options, such as intravenous plasma-derived C1-INH (C1-INH[IV]), recombinant human C1-INH, icatibant (a bradykinin B2-receptor antagonist), and ecallantide (a kallikrein inhibitor). However, prophylactic treatment options are very limited, with only androgens, tranexamic acid, and plasma-derived C1-INH(IV) concentrate available. Plasma-derived C1-INH is the only therapy licenced for long-term prophylaxis in adults, adolescents, and children, and is recommended by international guidelines as first-line therapy for long-term prophylaxis of HAE.[1] Androgens, such as danazol and stanozolol, are older drugs that are not approved for long-term prophylaxis of HAE in several European countries, which are still used in

doses of ≤200 mg/day and 2 mg/day, respectively, to suppress HAE symptoms.

Tranexamic acid is no longer recommended as a long-term prophylactic treatment option because of a very low preventive effect.[1] Clinical studies on the use of androgens for prophylactic treatment of HAE demonstrated that they can be quite effective in reducing the number of HAE attacks in some patients; however, their safety profile limits their use. The majority of patients experience adverse events (AE) during long-term use, and a substantial number of patients discontinue treatment because of side effects.[2,3] Side effects of androgens include virilisation, weight gain, menstrual disorders, psychological abnormalities, headache, myalgia, and acne.[4]

On the use of an C1-INH(IV) concentrate for long-term prevention, interim results from a European registry of 45 patients showed that the majority of patients were on a routine regimen of a 3–4-day interval between C1-INH(IV) administration, while about one-third were on an intensified 1–2-day interval regimen or a prolonged 5–7-day interval regimen. Patients who adhered to the routine regimen (1,000 units IV every 3–4 days) had a lower breakthrough attack rate (1.5 attacks per month) compared with those on an intensified or prolonged regimen (around 3–5 attacks per month). Most breakthrough attacks occurred on the intended day of dosing, which indicates that C1-INH(IV) concentrate fell short in the prevention of attacks during the whole dosing interval.[5]

In conclusion, real-world evidence shows that HAE is well-controlled in most patients using the approved C1-INH(IV) dosing regimen; however, some patients require more intensive therapy due to insufficient response.

Real-world evidence has also demonstrated the challenges that exist with the IV administration of C1-INH(IV). An observational, retrospective cohort of USA healthcare claims data (covering an observation period from 2006–2014) of 521 patients with HAE and accessible health records revealed that 18 patients had been using SC ports for IV administration of HAE medication. Of the 18 patients, 10 had at least one major complication that lead to replacement or repair of their port.[6,7]

In summary, current treatment options for prophylaxis are very limited. The use of androgens is restricted by numerous contraindications and side effects. Real-world and clinical evidence on the only approved treatment option, C1-INH(IV) prophylaxis, indicates that many patients still experiencing breakthrough HAE attacks would probably benefit from a more flexible dosing recommendation (higher dose of C1-INH or shorter injection intervals). In patients with IV access failures, the use of SC ports is associated with a high risk of port-related complications. Therefore, there is an unmet medical need for more effective and more easily administered therapy.

Experience with C1-INH Replacement Therapy for Hereditary Angioedema Management

Professor Timothy Craig

The C1-INH protein inhibits the complement protein, C1, and blocks several pathways of the kallikrein cascade. Mutations in the *SERPING1* gene coding for the C1-INH protein cause HAE Type I and II. Mutations that cause HAE Type I lead to reduced levels of C1-INH in the blood, while mutations that cause Type II result in the production of a C1-INH that functions abnormally. Without the proper levels of functional C1-INH, excess bradykinin is generated. Excessive accumulation of fluids in body tissues causes the episodes of swelling seen in individuals with HAE.[8,9]

Until the 1970s, the only treatment available for acute attacks of HAE was fresh frozen plasma, which had complications such as sensitisation, worsening of acute exacerbation of HAE, and increased risk of viral transmission. In 1973, a call for purified C1-INH was published[10] and was answered by Pickering and Hamblin,[11] and Brackertz and Kueppers.[12] The response from the latter reported successful treatment of two patients with HAE with a partially purified preparation of C1-INH at a dose of 1,200 'inactivator units' (produced by Behringwerke, Marburg, Germany).[12] A critical level of functional C1-INH was first postulated by Späth et al.[13] in an observational study of patients with HAE treated with oral prophylaxis. It was observed that attacks most frequently occurred when C1-INH antigen levels were <0.035 g/L, which corresponded to approximately 40% functional C1-INH, and attacks were absent when near-to-normal (≥0.075 g/L) C1-INH antigen levels were present. Bork and Witzke[14] were among the first to describe the prophylactic use of C1-INH(IV) concentrate in two patients with HAE, who received 500 units of C1-INH(IV)

every 4–5 days. Both patients experienced a marked reduction of attacks during treatment when C4 protein levels normalised and C1-INH levels increased above the critical threshold of C1-INH antigen (approximately 7 mg/dL).[14]

The first double-blind, placebo-controlled studies of C1-INH(IV) prophylaxis was carried out by Waytes et al.,[15] whereby six patients with a history of >5 attacks within 1 year received 25 plasma units/kg of C1-INH(IV) or placebo every third day for two 17-day treatment periods. The primary endpoint was the daily symptom score (highest severity scores [0–4] recorded for each symptom [abdominal, extremities, laryngeal, or genitourinary] for a 6-hour period and averaged over four consecutive 6-hour periods). The mean total daily symptom scores were significantly lower in patients treated with C1-INH compared with placebo ($p<0.001$).[15] C1-INH(IV) concentrate resulted in an increase in plasma levels of C1-INH to above the critical threshold (40% of normal). Following infusion, C1-INH levels fell to approximately the lower limit of normal within 24 hours but remained above baseline at 72 hours.[15]

A placebo-controlled crossover study compared the number of HAE attacks per month in 22 patients treated with C1-INH(IV) 1,000 international units (IU) twice-weekly or placebo. Patients treated with placebo had a mean attack rate of 4.6 per month, compared with 2.3 attacks per month in those treated with C1-INH(IV), which represents a 50% reduction in attack rate.[16] In an open-label study, the efficacy of C1-INH(IV) was associated with a better preventative effect with shorter intervals between injections. A dose interval of 2–3 days between IV infusions was deemed optimal for HAE attack prevention.[17]

The pharmacokinetics (PK) of subcutaneous C1-INH was first studied using 1,000 IU of C1-INH (approved for IV use [Berinert, CSL Behring, Marburg, Germany]) in 24 patients with HAE. The mean relative bioavailability of functional C1-INH(SC) was about 40%.[18] Based on these data, a Phase II study compared mean trough C1-INH activity in patients administered SC fixed doses of a volume-reduced C1-INH concentrate of 1,500, 3,000, and 6,000 IU. Patients who received the 1,500 IU dose achieved a mean trough C1-INH activity level of 32%, compared with 44% and 81% for those who received 3,000 and 6,000 IU doses, respectively.[19] An analysis of the PK data further revealed that SC dosing provided a more even distribution of C1-INH activity levels across the body weight spectrum than IV dosing. A PK simulation showed that C1-INH(SC) administered as a weight-based dose of 60 IU/kg provided a markedly lower peak-to-trough ratio compared with the IV dose of 1,000 IU (1.3 versus 1.9) and critical C1-INH levels were better maintained after follow-up injections.[20]

In summary, C1-INH(IV) replacement therapy is effective and can provide a good level of protection if trough C1-INH levels can be consistently maintained above a critical threshold of 40% functional C1-INH.[13] SC C1-INH administration as a volume-reduced formulation is feasible and provides a more consistent increase of trough C1-INH levels above the critical levels compared with IV C1-INH[18,19] and thus is expected to be associated with an improved preventive effect.

COMPACT: Evidence for Subcutaneous C1-INH for Routine Prevention

Professor Bruce Zuraw

The international, prospective, multicentre, randomised, double-blind, placebo-controlled, dose-ranging, crossover Phase III COMPACT trial evaluated the efficacy and safety of self-administered formulation of a volume-reduced C1-INH concentrate, CSL830, in patients with Type I or II HAE. Patients were required to have had ≥4 attacks in a consecutive 2-month period within 3 months before screening and were included if they were aged ≥12 years, had HAE Type I or II confirmed by a central laboratory, and were stable on oral HAE prophylaxis. Those with a history of arterial/venous thrombosis that required anticoagulant therapy, were at risk of thrombosis, or were not adequately managed with on-demand treatment were excluded. On completion of the screening and run-in period, patients were randomised 1:1:1:1 to 40 IU/kg, 60 IU/kg, or low or high-volume placebo twice-weekly for 16 weeks (2 weeks to reach steady state, plus 14 weeks efficacy assessment). Following the first treatment period, patients were switched so that those assigned to placebo received active treatment for a further 16 weeks, or vice versa (Figure 1).[21]

The primary efficacy endpoint was the time-normalised number of HAE attacks (investigator-reported); secondary efficacy endpoints included the percentage of patients who had a response (defined as ≥50% reduction versus placebo in

number of attacks) and the time-normalised number of times rescue medication was used. Other endpoints included severity of attacks, safety and tolerability, and PK/pharmacodynamics analysis.[21]

In total, 90 patients were randomised to receive treatment in the first period, of which 91% (n=82) proceeded to the second treatment period (Figure 1). Patient demographics were relatively well-balanced between groups; the average age was 40 years, 67% were female, and 93% were Caucasian.[21]

Among patients who received CSL830, the rate of HAE attacks was lower compared with the rate among those who received placebo. Patients in the CSL830 lower dose group experienced 1.2 HAE attacks per month, compared with 3.6 in the placebo group (mean difference of -2.42 attacks per month; p<0.001). Patients in the CSL830 higher dose group experienced 0.5 HAE attacks per month, compared with 4.0 in the placebo group (mean difference of -3.51 attacks per month; p<0.001). The median reduction in the normalised number of attacks versus placebo was 89% and 95% with 40 IU/kg and 60 IU/kg of CSL830, respectively (Figure 2).[21]

The mean normalised number of times rescue medication was used was reduced with both doses of CSL830 compared with placebo: -4.4 and -3.6 with 40 IU/kg and 60 IU/kg, respectively. The median reduction in the normalised use of rescue medication versus placebo was 89% for 40 IU and 100% for 60 IU. Of those treated with lower dose CSL830, 76% had a ≥50% reduction in the number of attacks, 67% had a ≥70% reduction, and 43% had a ≥90% reduction versus placebo. Of those treated with higher dose CSL830, 90% had a ≥50% reduction in the number of attacks, 83% had a ≥70% reduction, and 58% had a ≥90% reduction versus placebo. Overall, fewer patients in the pooled CSL830 group experienced severe HAE attacks compared with those in the placebo group (14% versus 71%, respectively). Most patients in the pooled CSL830 group experienced mild-to-moderate attacks (42%) compared with those in the placebo group (20%). In total, 39% of patients treated with CSL830 did not have any HAE attack versus 4% of placebo-treated patients (Figure 3).[21]

Levels of functional C1-INH and C4 protein were measured at screening and throughout the study. At baseline, the levels of functional C1-INH activity and C4 protein were similar for all three groups and increased following randomisation in a dose-dependent manner until steady state was reached at Week 3. Patients treated with the 60 IU/kg dose had a mean increase in functional C1-INH activity that neared the lower limit of normal (approximately 70%) and those treated with the 40 IU/kg dose showed an intermediate increase to just below 50% functional activity. Levels of C4 protein were normalised for both dose groups; levels were maintained above the lower limit of normal with the 60 IU/kg dose and just below the lower limit of normal with the 40 IU/kg dose.[21]

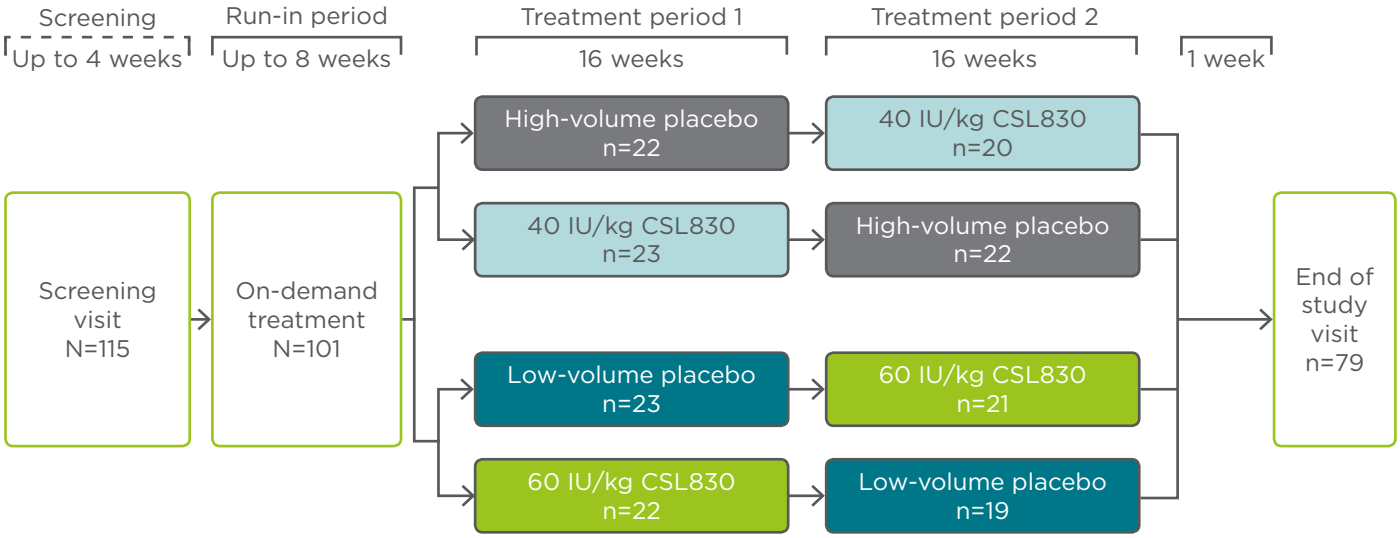

Figure 1: COMPACT study design.
IU: international units.
Adapted from Longhurst et al.[21]

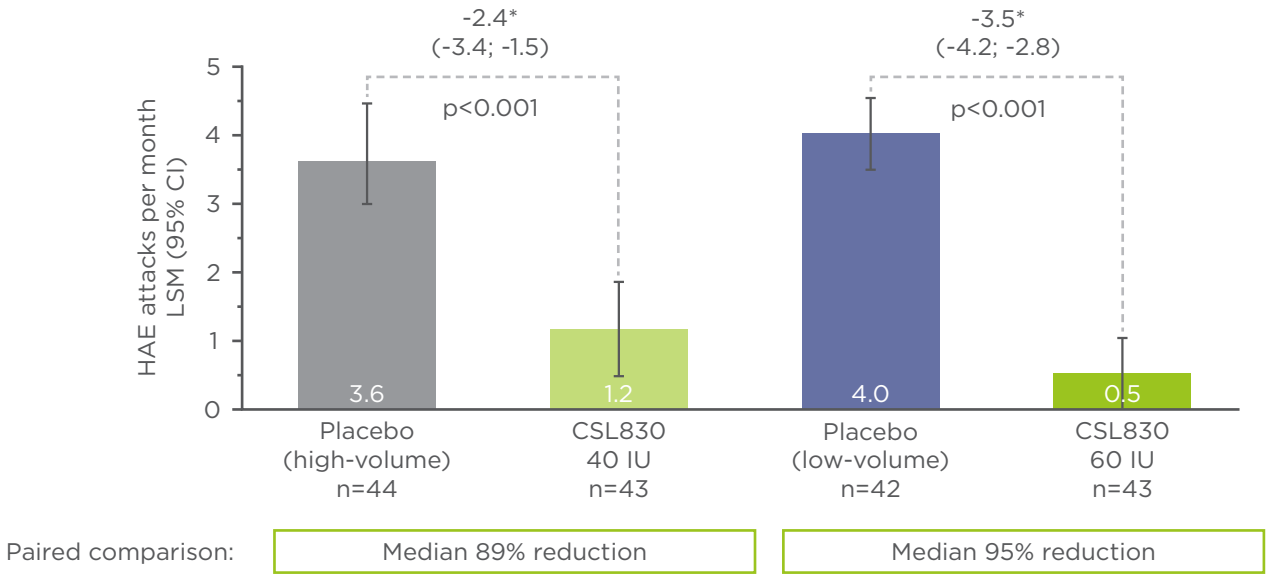

Figure 2: Time-normalised number of hereditary angioedema attacks per month.
*LSM (95% CI) estimate; error bars represent 95% confidence interval
CI: confidence interval; HAE: hereditary angioedema; IU: international units; LSM: least-squares mean.
Adapted from Longhurst et al.[21]

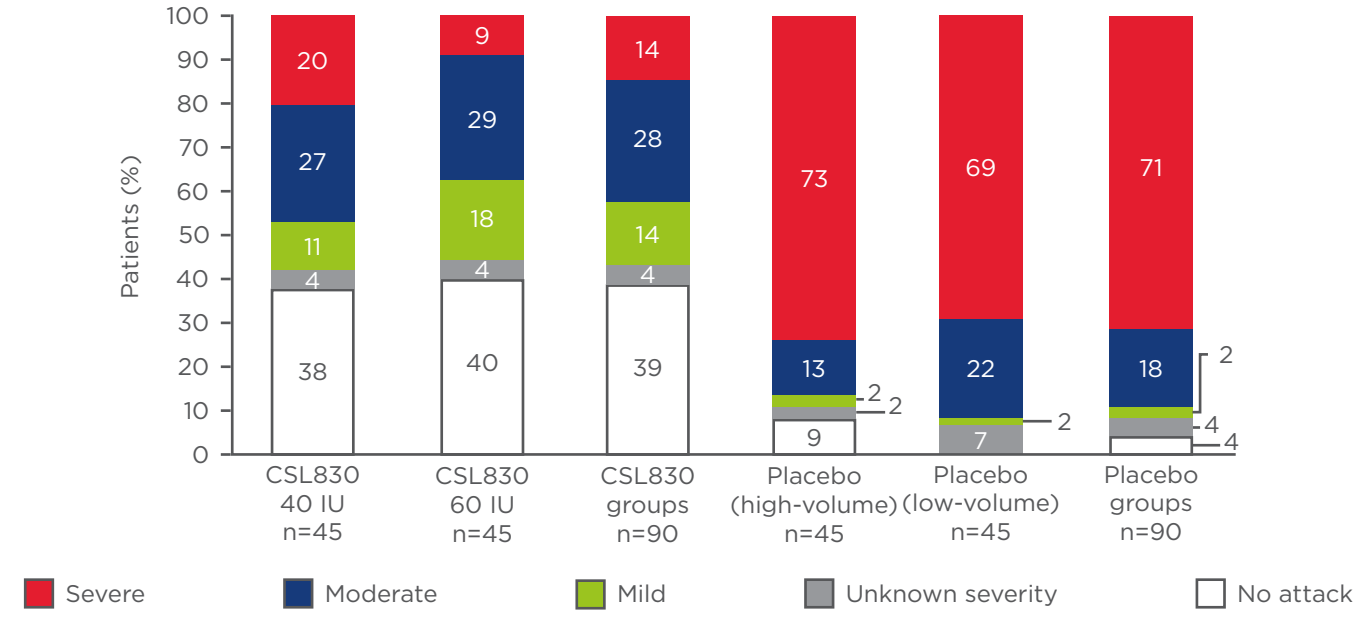

Figure 3: Attacks of hereditary angioedema according to the maximum severity.
IU: international units.
Adapted from Longhurst et al.[21]

Population-based exposure-response analysis revealed that there was an inverse relationship between the predicted functional C1-INH activity and the time of an attack, which suggests that if C1-INH activity is maintained close to the lower level of normal, the risk of a patient having an HAE attack approaches zero.[21]

Patient-reported quality of life (QoL) outcomes (European QoL-5 dimensions questionnaire; treatment satisfaction questionnaire for medication, hospital anxiety, and depression scale; work productivity and activity impairment questionnaire) were measured at screening and various times during the two treatment phases. Improvements were seen for all QoL outcomes versus placebo

for both lower and higher dose CSL830, and were statistically significant for the visual analogue scale, treatment satisfaction (effectiveness and overall satisfaction), anxiety, and presenteeism.[22]

Most reported AE were injection-site reactions, which occurred in 31% of patients treated with CSL830 (28% and 35% of patients in the 40 and 60 IU/kg groups, respectively) and 24% of placebo-treated patients. Of the injection-site reactions, 95% in the CLS830 and placebo groups were of mild severity and ≥83% resolved within 1 day of onset. Other reported AE were not deemed to be related to the study drug and included nasopharyngitis, upper respiratory tract infection, hypersensitivity (pruritus, rash, and urticarial), dizziness, fatigue, and back pain.[21]

Overall, both doses of CSL830 significantly lowered the rate of HAE attacks compared with placebo and were associated with improvements in QoL measures. The higher (60 IU/kg) dose was more effective at reducing the attack rate and medication use compared with the lower (40 IU/kg) dose, and both were more effective than placebo. AE were mostly injection-site reactions, generally mild and transient in nature, and occurred in similar proportions of patients in the active treatment groups and placebo. The two treatment periods were not designed to assess the long-term effects of SC CSL830; however, an open-label extension trial[23] is currently ongoing to assess safety and explore whether dose adjustments can further improve treatment response.

Question and Answer Session

Q: Should we personalise doses in clinical practice based on functional C1-INH levels?

A: Prof Craig replied that there is currently insufficient evidence to suggest that this is needed or useful, although it is an interesting research topic that deserves further exploration.

Q: Could we start at a low dose and increase it based on patient response?

A: Prof Magerl replied that there is variation from patient to patient in frequency of symptoms, frequency of relapse, the interval between doses, and the appearance of symptoms, so future efforts should identify patient characteristics that could guide physicians and help to individualise therapy.

Prof Zuraw added that the future target is not to start at a given dose and adjust it, but rather to be able to predict which drug for which patient is best; this is something that may become a reality as the repertoire of drugs increases.

Q: When starting a patient on SC C1-INH, would you first administer an IV bolus or would you start on SC?

A: Prof Magerl replied that after 2–3 injections, C1-INH plasma levels reach what is considered an 'effective' level and therefore no initial bolus should be needed. In his view, C1-INH(SC) can be administered from the outset as prophylaxis.

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DEVELOPMENTS IN THE TREATMENT AND MANAGEMENT OF PSORIASIS

These sessions took place on 13th–17th September 2017, as part of the 26th European Academy of Dermatology and Venereology (EADV) Congress, in Geneva, Switzerland

Chairperson

Lluis Puig[1]

Speakers

Andrew Blauvelt,[2] Kim Papp[3]

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MEETING SUMMARY

The oral session consisted of a talk on the 2-year efficacy and safety of guselkumab in the treatment of moderate-to-severe psoriasis from the Phase III VOYAGE 1 trial. This was followed by an oral session consisting of presentations regarding the clinical efficacy of tildrakizumab in patients with chronic plaque psoriasis over 2 years of treatment, presented from the two Phase III trials, reSURFACE 1 and 2. In the poster session, data were presented on the association between psoriasis and severity index measures,

the distribution of improvement measures in psoriasis, and the efficacy of guselkumab in treatment-experienced patients, as well as its impact on the comorbidities of anxiety and depression.

Two-Year Efficacy and Safety of Guselkumab for the Treatment of Moderate-to-Severe Psoriasis: Phase III VOYAGE 1 Trial

Professor Andrew Blauvelt

Guselkumab is a selective interleukin (IL)-23 blocker that blocks the P19 subunit of IL-23. It was approved in the USA in July 2017 and by the European Medicines Agency (EMA) in November 2017 for the treatment of adult patients with moderate-to-severe plaque psoriasis. VOYAGE 1[1] was a Phase III, randomised, double-blind, placebo and active comparator-controlled trial. Patients were randomised to one of three treatment groups: guselkumab 100 mg subcutaneously at Week 0, 4, and every 8 weeks (Q8W) thereafter (n=329); placebo at Week 0, 4, 12, and then guselkumab 100 mg subcutaneously at Week 16, 20, and Q8W thereafter (n=174); or adalimumab 80 mg subcutaneously at Week 0, 40 mg at Week 1, and every 2 weeks thereafter up to Week 47, followed by guselkumab at Week 52 and Q8W thereafter (n=334). The data presented pertains to patients included in the VOYAGE 1 trial up to Week 100. Data analysis and the methodology used is of particular importance when determining long-term efficacy of agents. In the VOYAGE 1 trial,[1] efficacy was prespecified to be assessed using non-responder imputation (NRI) through Week 48 and then application of treatment failure rules during Week 52–100. Treatment failure rules are not considered as conservative as NRI; however, they remain considerably more conservative than 'as observed' data.

All three treatment arms demonstrated very high Psoriasis Area Severity Index (PASI) 75 levels (approximately 95% of patients in all groups). Patients who were switched from placebo or adalimumab had the same outcomes as those patients who were treated with guselkumab for the entire 2-year period. Evaluating duration on treatment and durability analysis showed that 88% of patients who started on guselkumab remained on treatment at 2 years. Patients who switched to guselkumab from the placebo and adalimumab arms demonstrated a retention rate of 98%. Over 80% of all patients in all treatment arms achieved PASI 90 at 2 years. The number of patients who achieved PASI 100 was between 50% and 55%. The proportion of patients who achieved Investigator's Global Assessment (IGA) 0 or 1 over the entire 2-year period was between approximately 82% and 85%, and correlated well with the PASI 90 data. There was little difference in outcomes between the treatment arms. IGA 0 correlated well with PASI 100.

Using less conservative but standard 'as observed' methodology, the proportions of patients achieving PASI 75, 90, and 100 were consistently higher than with NRI analysis. This was also seen in the IGA responses. Arguably, the NRI method of analysis is the most conservative analysis that can be used, but it is not consistent with real-world use and may underestimate the true efficacy of drugs, Prof Blauvelt said. He said that the closest data to true life data will in fact be somewhere between the 'as observed' and NRI numbers. The Dermatology Life Quality Index data demonstrated that the quality of life of patients on guselkumab continued to increase from Week 48–100. This was of particular interest in consideration of the efficacy data that showed that efficacy remained relatively consistent during this period of time. Adverse event (AE) rates in the second year were consistent with the first year of treatment. Serious AE rates were low and remained stable over time. There were no new safety concerns identified in the second year of treatment. Furthermore, the event rates per 100 patient-years remained consistent between the first and second years of treatment. In conclusion, efficacy of guselkumab was maintained over a 2-year period at high levels and it was well-tolerated with an acceptable safety profile.

Clinical Efficacy of Tildrakizumab, an Anti-IL-23P19 Monoclonal Antibody, in Patients with Chronic Plaque Psoriasis Over 2 Years of Treatment: Results From Long-Term Extensions to Two Phase III Clinical Studies (reSURFACE 1 and reSURFACE 2)

Professor Kim Papp

Prof Papp presented data from the reSURFACE 1 and 2 trials,[2] demonstrating the maintenance

and response in different strata (PASI 75, 90, and 100) in patients treated with tildrakizumab over 2 years. The base studies were three-part, double-blinded, randomised controlled trials. Patients were ≥18 years of age with moderate-to-severe chronic plaque psoriasis. Inclusion criteria comprised body surface involvement of ≥10%, a Physician's Global Assessment (PGA) score of ≥3, and PASI ≥12. Patients were randomised to either tildrakizumab 100 or 200 mg or placebo in the dosing-finding reSURFACE 1 study (64 weeks) and reSURFACE 2 study (52 weeks), which compared tildrakizumab and etanercept.[2]

Patients who were identified as responders to tildrakizumab were enrolled in the extension studies. Patients needed to have achieved PASI ≥50 and have received an active dose within 12 weeks of the end of the study in the reSURFACE 1 extension trial. Open-label treatment continued at 100 or 200 mg every 12 weeks. In the reSURFACE 2 trial, responders to etanercept were not switched to tildrakizumab and were not enrolled in the extension study.

The primary efficacy population in the extension studies consisted of the full analysis set (patients with ≥1 dose of extension treatment based on assigned treatment). The primary safety population consisted of all subjects as treated (patients with ≥1 dose of extension treatment based on treatment received). The efficacy objective for the extension trials was to evaluate the maintenance of efficacy response levels, prespecified based on observed data. The safety objectives were to evaluate pre-specified adverse events of interest, and to determine yearly and cumulative incidence rates. The data presented were the interim 2-year data of the planned 5-year total extension trials.

The baseline characteristics and demographics were similar across both studies and between the treatment arms. Prof Papp suggested that the similarity in baseline characteristics would predict similar levels of maintenance and response between studies. Patients in reSURFACE 1, up to 2 years, demonstrated a maintained PASI 75 response at Week 48 in the extension period at both doses, compared with the end of the base study. Due to the low drop-out rate, the 'as observed' method was sufficiently robust to suggest the data is a good representation of the expected maintenance response, he said. Both trials demonstrated a gradual but minimal level of decline in PASI 75. The absolute efficacy values after 2 years of treatment were slightly different between reSURFACE 1 and 2. The maintenance response remained acceptable during this period. In reSURFACE 2, approximately one-third of patients maintained PASI 100 and approximately two-thirds of patients maintained PASI 90. The overall efficacy data from the reSURFACE 1 and 2 extension trials of PASI 75, 90, and 100 demonstrated a convincing maintained response over 2 years, he said.

The safety data from both extension trials showed an acceptable safety 'profile' at both doses. The 2-year cumulative number of patients with AE of interest remained low. Severe infections, which are of particular interest in treatments that modify, modulate, or suppress the immune system, also remained low at both doses. Prof Papp suggested the rate of severe infections appeared to be independent of dose, based on observations of the 2-year interim data presented.

Overall, the reSURFACE 1 and 2 extension trials 2-year interim efficacy data demonstrated convincing maintenance of efficacy in the treatment of chronic plaque psoriasis. Furthermore, the cumulative 2-year safety observations indicated that both the 100 and 200 mg doses were well-tolerated with low rates of AE of interest.

Question and Answer Session

Q: Are there any particular phenotypes of psoriasis that did not respond to treatment, because there still appear to be a few patients who did not respond?

A: Prof Papp replied: "The whole discussion surrounding phenotype is complicated. The complexity of the disease shows that phenotype drift can occur even within patients. The focus should be on the instruments used. The PASI score, for example, is incredibly useful yet deficient in its ability to capture the lower ranges of responses. This can translate into the exclusion of certain phenotypes, e.g., widespread psoriasis that is not very inflamed, thin, or scaly. An instrument that could identify the different phenotypes would be very useful; however, currently a tool that is sensitive enough does not exist."

Poster Presentation P1726: Association Between Psoriasis Area Severity Index and Physician's Global Assessment Responses in Moderate-to-Severe Chronic Plaque Psoriasis Studies of Tildrakizumab

Professor Doctor Kristian Reich

Tildrakizumab is an anti-IL23p19 monoclonal antibody under development for the treatment of moderate-to-severe chronic plaque psoriasis. It has been shown to significantly improve PASI 75, 90, and 100, and PGA response rates when compared with placebo.[2,3] PASI and PGA are the most commonly used measures of psoriasis severity but an association study between the two measures has not been conducted for treatment with tildrakizumab. The objective of the study presented was to determine if there was an association between the two measures using tildrakizumab data from two Phase III trials, reSURFACE 1 and reSURFACE 2.

The patients in the reSURFACE 1 and 2 trials were adults with moderate-to-severe plaque psoriasis and with ≥10% body surface area, a PGA score of ≥3, and PASI ≥12. The primary endpoints of both studies were the proportion of patients achieving PASI ≥75 and the proportion of patients achieving a PGA score of 'clear' (0) or 'minimal' (1) with a >2 grade reduction from baseline at Week 12. Secondary endpoints included the proportion of patients achieving PASI 90 and 100 at Week 12, and PASI 75 and a PGA score of 0 or 1 with a >2 grade reduction from baseline at Week 12 and 28 versus etanercept (reSURFACE 2).

Response was analysed using the Cochran-Mantel-Haenszel test stratified by weight and prior use of biologics for psoriasis. NRI was prespecified at Week 12. At Week 28, NRI was prespecified for PASI 75 and PGA response in reSURFACE 2. Analysis of observed data was prespecified for PASI 75, 90, 100, and PGA response in reSURFACE 1, and PASI 90 and 100 in reSURFACE 2. Data were pooled from patients in all treatment arms with both PASI and PGA data at baseline at Week 12 and 28.

A total of 772 and 1,090 patients were included in reSURFACE 1 and 2, respectively. There was a statistically significant association between PASI 75, 90, and 100 responses, and PGA 0 or 1 responses at Week 12 and 28 ($p<0.001$ for all). Furthermore, the association between PASI 100 and PGA response was higher compared with PASI 75 or 90 and PGA response (Table 1). In summation, there was a significant association between PASI 75, 90, or 100 responses, and achievement of PGA 0 or 1 in tildrakizumab-treated patients at Week 12 and 28.

Table 1: Association between the proportion of patients with Psoriasis Area Severity Index and Physician's Global Assessment response at Week 12.

PASI response	reSURFACE 1				reSURFACE 2			
	PGA response % No	PGA response % Yes	Odds ratio (95% CI)	p value[a]	PGA response % No	PGA response % Yes	Odds ratio (95% CI)	p value[a]
Week 12	n=374	n=372	-	-	n=512	n=510	-	-
PASI 75 response								
No	83.4	9.7	-	-	81.4	11.0	-	-
Yes	16.6	90.3	47.0 (30.3–72.8)	<0.001	18.6	89.0	35.6 (20.6–56.1)	<0.001
PASI 90 response								
No	97.9	43.0	-	-	96.5	44.7	-	-
Yes	2.1	57.0	60.6 (29.2–125.8)	<0.001	3.5	55.3	33.9 (20.6–56.1)	<0.001
PASI 100 response								
No	100	76.3	-	-	100	82.9	-	-
Yes	0.0	23.7	233.0 (14.4–3,770.3)	<0.001	0.0	17.1	211.8 (13.1–3,422.8)	<0.001

[a]p values not adjusted for multiplicity.
CI: confidence interval; PASI: Psoriasis Area Severity Index; PGA: Physician's Global Assessment.

Poster Presentation P1812: Distribution of Improvements in Psoriasis Area Severity Index from the Phase II Trial of Risankizumab in Moderate-to-Severe Plaque Psoriasis

Professor Bruce Strober

Risankizumab is a humanised immunoglobulin G1 monoclonal antibody, which is an IL-23 inhibitor. A Phase II trial demonstrated its superiority over ustekinumab in patients with moderate-to-severe plaque psoriasis.[4] PASI 90 is often used as a primary endpoint in clinical trials as a measure of treatment efficacy. It is suggested that additional visualisation of the cumulative distribution of responses can help assess the consistency of PASI at the population level. The objective of this study was to determine the distribution of PASI responses in patients treated with risankizumab compared with ustekinumab in a Phase II trial.

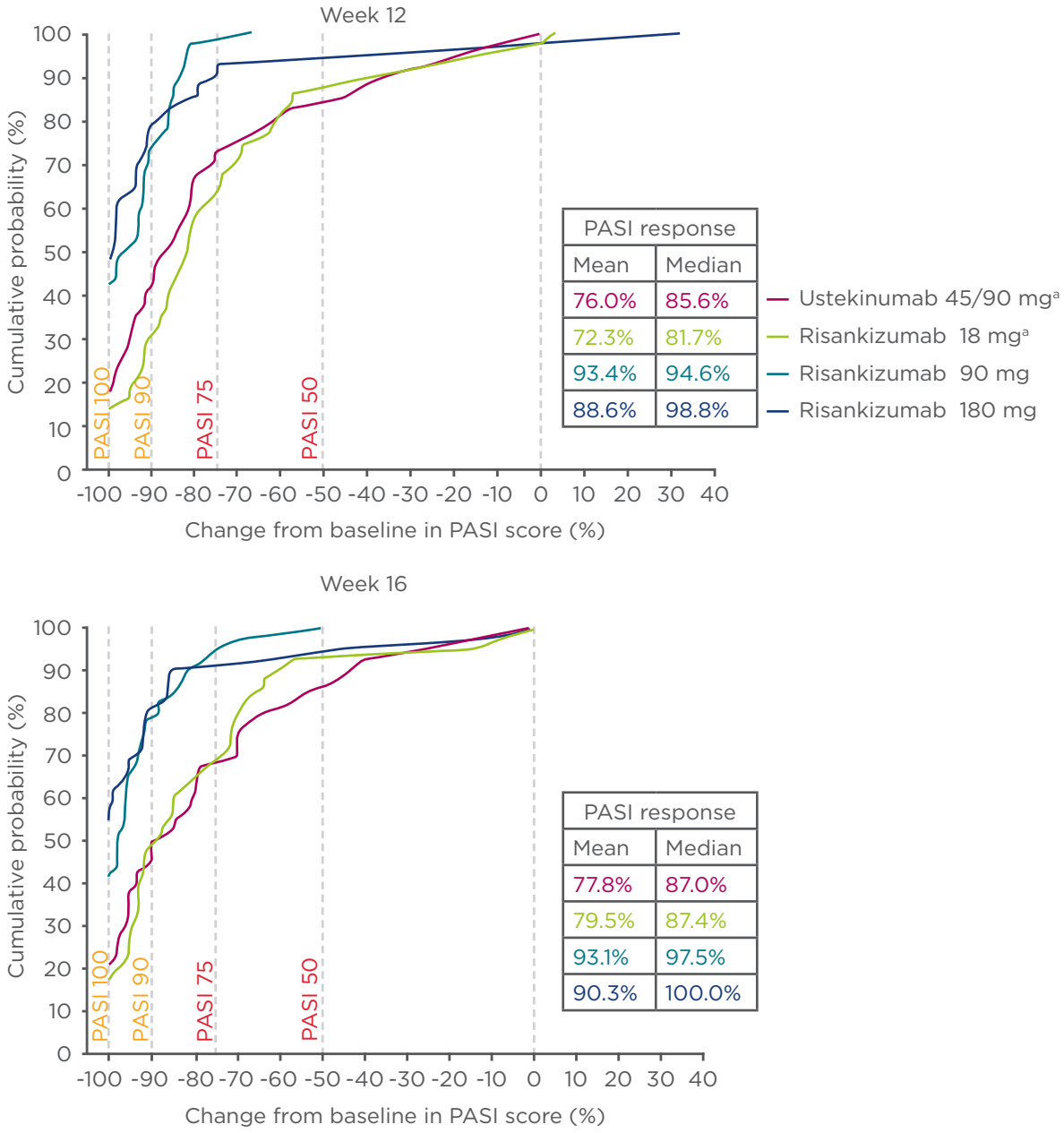

Figure 1: Cumulative probability of percentage change from baseline in Psoriasis Area Severity Index scores at Week 12 and 16.

[a]One patient each with missing response in risankizumab 18 mg and ustekinumab 45/90 mg groups, and were imputed as having no (0%) improvement.

PASI: Psoriasis Area Severity Index.

The Phase II trial consisted of patients with moderate-to-severe plaque psoriasis (n=166) randomised to receive subcutaneous injections of risankizumab (either 18 mg single dose, 90 mg, or 180 mg, at Week 0, 4, and 16) or ustekinumab (45 or 90 mg based on body weight at Week 0, 4, and 16). The proportion of patients who achieved PASI 75, 90, or 100 were assessed at Week 12 and 16 in an intent-to-treat population. Those patients with missing assessments were defined as non-responders. Cumulative probability plots were used to determine the distribution of changes in PASI from baseline across the treatment groups.

At Week 12, 30.2%, 73.2%, and 78.6% of patients achieved PASI 90 for 18, 90, and 100 mg risankizumab, respectively, compared with 40.0% in ustekinumab-treated patients. Patients who were treated with 90 or 180 mg risankizumab achieved higher response rates across all levels of PASI compared with patients treated with either 18 mg risankizumab or ustekinumab. The cumulative probability plot demonstrated that patients treated with 90 or 180 mg risankizumab had a higher probability of increased improvements in PASI scores from baseline compared with patients treated with 18 mg risankizumab or ustekinumab, as seen in Figure 1.

Poster Presentation P1813: Guselkumab Demonstrates Greater Reductions in Anxiety and Depression Symptoms Than Adalimumab in Psoriasis Patients

Professor Kenneth Gordon

Psoriasis is associated with anxiety and depression; improvements in psoriasis lesions have been shown to decrease these comorbidities.[5,6] Guselkumab has demonstrated efficacy and safety in the treatment of patients with moderate-to-severe plaque psoriasis.[1,7] The study presented here aimed to evaluate whether guselkumab improved the symptoms of anxiety and depression using the Hospital Anxiety and Depression Scale (HADS), compared with placebo and adalimumab-treated patients from the VOYAGE 2 trial. The study further aimed to evaluate any correlations between anxiety or depression scores and efficacy measures.

VOYAGE 2 was a Phase III, double randomised, double-blind, study. Patients were randomised to one of three treatment arms: guselkumab 100 mg subcutaneously at Week 0, 4, 12, and 20 (n=496); placebo at Week 0, 4, 12, followed by guselkumab 100 mg subcutaneously at Week 16 and 20 (n=248); or adalimumab 80 mg subcutaneously at Week 0, 40 mg at Week 1, and then every 2 weeks to Week 23 (n=248). Efficacy was assessed through Week 24. The anxiety and depression HADS subscales (HADS-A and HADS-D, respectively), consisted of seven questions scored 0–3. Total scores ranged from 0–21 with higher scores reflecting increased severity. Scores ≥8 are the instrument definition of anxiety or depression. PASI was used to assess psoriasis severity. Patients with HADS scores ≥8 were evaluated for correlations between change in anxiety or depression scores with percentage improvement in PASI scores.

A total of 992 patients were randomised at baseline. The mean HADS-A and HADS-D scores were 6.8 and 5.3, respectively; a total of 38.6% and 27.7% of patients had HADS-A and HADS-D scores ≥8, respectively. Baseline characteristics were similar between treatment arms. The findings showed that patients treated with guselkumab demonstrated significantly greater improvements in both HADS scores at Week 8 and 16, and in HADS-A scores in Week 24 ($p<0.001$ for all). Significantly greater proportions of patients in the guselkumab arm with baseline HADS scores of ≥8 reported HADS scores <8 compared with placebo at Week 16 ($p<0.001$ for both HADS scores) and compared with adalimumab at Week 24 ($p=0.028$ for HADS-A and $p=0.079$ for HADS-D). Overall improvements in PASI scores showed significant correlations with change from baseline in anxiety ($r=0.27$) and depression ($r=0.25$) at Week 24 ($p<0.001$ for all).

The findings from this study showed that guselkumab demonstrated greater improvements in the comorbidities, anxiety and depression, in patients with moderate-to-severe plaque psoriasis when compared with placebo and adalimumab. These improvements were correlated with improvements in psoriasis.

Poster Presentation P1830: Efficacy of Guselkumab in Previously Treated Patients with Moderate-to-Severe Plaque Psoriasis: An Analysis from VOYAGE 1 and VOYAGE 2

Professor Kenneth Gordon

Guselkumab therapy has demonstrated superior clinical responses ($p<0.001$) compared with placebo

and adalimumab in the Phase III clinical trials VOYAGE 1 and 2.[1,7] The study described here presented data from these trials evaluating the efficacy of guselkumab compared with placebo and adalimumab in psoriasis treatment-naïve and experienced patients. A total of 1,829 patients were randomised in VOYAGE 1 (n=837) and 2 (n=992) to the following treatment groups: guselkumab 100 mg at Week 0, 4, 12, and 20 (n=825); placebo at Week 0, 4, 12, followed by guselkumab 100 mg at Week 16 and 20 (n=422); or adalimumab 80 mg at Week 0, 40 mg at Week 1, and 40 mg every 2 weeks thereafter through Week 23 (n=582). Data from both trials were pooled for analysis.

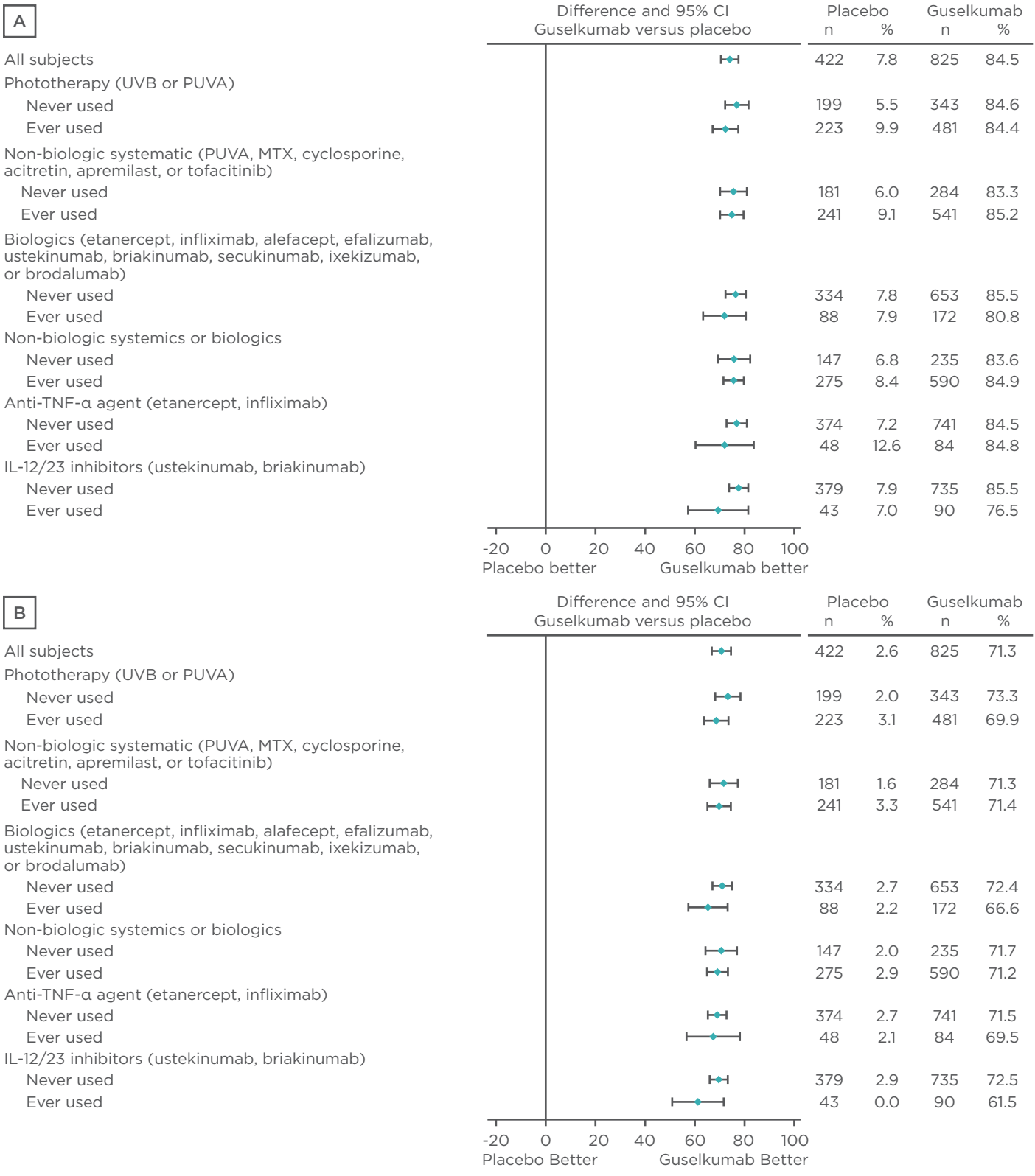

Figure 2: Proportion difference and 95% confidence intervals for comparing proportion of patients achieving Investigator's Global Assessment and Psoriasis Area Severity Index scores.

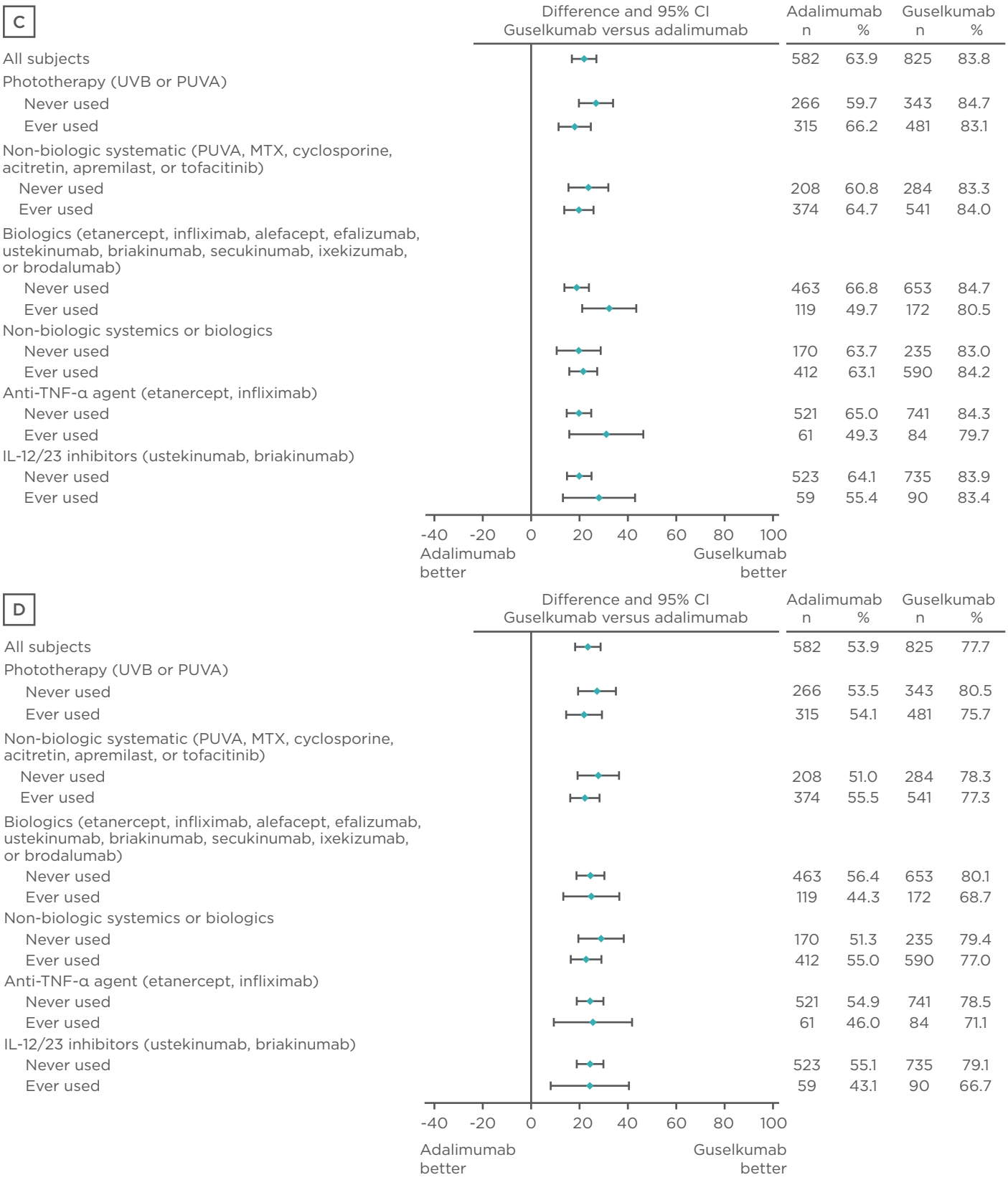

Figure 2 continued.

A) IGA score 0/1 at Week 16 by treatment experience (placebo versus guselkumab); B) PASI 90 at Week 16 by treatment experience (placebo versus guselkumab); C) IGA score 0/1 at Week 24 by treatment experience (adalimumab versus guselkumab); D) PASI 90 at Week 24 by treatment experience (adalimumab versus guselkumab).

CI: confidence interval; IL: interleukin; IGA: Investigator's Global Assessment; MTX: methotrexate; PASI: Psoriasis Area Severity Index; PUVA: psoralen and ultraviolet A; TNF-α: tumour necrosis factor-alpha; UVB: ultraviolet B.

Prior psoriasis therapies included non-biologic systemics, biologics, non-biologic systemics or biologics, anti-tumour necrosis factor, biologics etanercept and infliximab, and IL-12/23 inhibitors. Efficacy assessments performed at Week 16 and 24 included IGA scores of 0 or 1 and PASI 90.

Baseline characteristics were similar between treatment arms. The majority of patients had psoriasis for >17 years and PASI and IGA scores were consistent with a population with moderate-to-severe psoriasis. Over 50% of patients had used phototherapy and non-biologic systemics, approximately 20% had used biologic therapies, and approximately 10% had used anti-tumour necrosis factor biologics and IL-12 or IL-23 inhibitors. Regardless of previous psoriasis treatment experience, significantly greater proportions of patients in the guselkumab treatment arm achieved IGA scores 0 or 1 and PASI 90 compared with placebo ($p<0.001$) and adalimumab ($p<0.001$) at Week 16, as seen in Figure 2. Both IGA and PASI 90 outcomes were generally similar between all guselkumab-treated patients, regardless of previous treatment experience, at Week 16 and 24.

Overall, treatment with guselkumab was well tolerated and the safety results were comparable between treatment-naïve and experienced patients. Guselkumab demonstrated superiority to placebo at Week 16 and adalimumab at Week 24 regardless of previous therapy experience.

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LINKING PATHOPHYSIOLOGY TO CLINICAL OPTIONS IN PSORIASIS: NEW INSIGHTS INTO INTERLEUKIN-23

This symposium took place on 29th September 2017, as part of the 47th Annual European Society for Dermatological Research (ESDR) meeting in Salzburg, Austria

Chairpersons

Antonio Costanzo,[1] Carle Paul[2]

Speakers

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MEETING SUMMARY

Prof Costanzo welcomed attendees to the symposium and outlined the agenda before explaining the autoimmune pathophysiology that underlies psoriasis. Dr Caprioli discussed the role of the T helper (Th)17 cell lineage and its accompanying cytokines in autoimmune diseases. Prof Paul discussed the latest clinical trial data for therapies targeting the interleukin (IL)-17/IL-23 axis in patients with psoriasis, and how this is changing the treatment landscape. The symposium was followed by a question and answer session.

The Autoimmune Story of Psoriasis: A Focus on Autoantigens and Cytokines

Professor Antonio Costanzo

Psoriasis is a multifactorial autoimmune disease with various genetic and environmental stimuli contributing to its pathogenesis.[1] Therefore, identifying genetic markers, functional networks, and regulatory mechanisms that drive disease progression may aid the development of novel therapeutics.

Genome-wide association studies have identified significant associations between 63 genetic loci and psoriasis, accounting for approximately 28% of the estimated disease heritability.[2] Most of the genes identified in these studies are related to the immune system, particularly the regulation of lymphocyte differentiation, responses to viruses and bacteria, signalling, and pattern recognition.[2] For example, variants of the *HLA-Cw6* gene, which codes for a component of the major histocompatibility complex, have been implicated in early onset (Type I) psoriasis because

antigen-presenting cells that express *HLA-Cw6* variants are known to recognise and bind both the streptococcal M protein and keratin found in the skin.[3] Consequently, *HLA-Cw6* allele expression can result in an autoimmune response to fragments of keratin protein, activating both the innate and adaptive immune responses.[3] Furthermore, several other immune-related genes associated with psoriasis (e.g. the gene coding for the IL-23 receptor) have been linked to chronic autoimmune and inflammatory disorders, including Crohn's disease and ankylosing spondylitis.[3]

Keratinocytes are also believed to play a central role in psoriasis because changes in the expression of genes related to maintaining the epidermal barrier in these cells (e.g. *cJun* and *JunB*) have also been implicated in the pathology of psoriatic lesions.[4-6] Activated keratinocytes and other innate immune cells release cytokines that activate myeloid dendritic cells, generating innate and adaptive immune responses.[7] In addition, keratinocytes produce a range of antimicrobial peptides (AMP), such as LL-37 (a 37-amino acid cathelicidin AMP) and β-defensins, as the first line of defence against microbial infiltration through the skin.[6,7] Notably, AMP are highly expressed in psoriatic skin, activate proinflammatory pathways, and shape the immune microenvironment.[6,8]

LL-37 forms complexes with DNA released from injured cells, encouraging the formation of condensed DNA–LL-37 aggregates; however, in up to 75% of patients with moderate-to-severe psoriasis, circulating CD4+ and CD8+ T cells recognise LL-37 as an autoantigen.[8,9] Keratinocytes and/or epidermal plasmacytoid dendritic cells (pDC) possessing an *HLA-Cw6* allele may present LL-37 to autoreactive CD8+ T cells (e.g. Th1/Th17 cells and natural killer cells), which in turn release inflammatory cytokines, such as interferon (IFN)-γ and IL-17, activating the adaptive immune system.[9] In addition, mature pDC secrete IL-12 and IL-23, which induce naïve T cells to differentiate into Th1 and Th17 cells.[7,9]

IL-26, produced by Th17 cells, has direct bactericidal properties and can form insoluble complexes with self and bacterial-DNA released from dying cells;[10] IL-26–DNA complexes are thought to activate more Type I IFN-producing pDC in a similar manner to LL-37.[10] Consequently, self-DNA-bound IL-26 may contribute to a perpetual inflammatory feedback loop, resulting in autoimmunity.

CD8+ T cells isolated from psoriasis plaques also recognise a melanocyte-expressed molecule known as ADAMTSL5 when it is presented on the HLA-Cw6 protein, suggesting the existence of a second autoantigen.[11] Indeed, both ADAMTSL5 and LL-37 are highly expressed in cases of active psoriasis and often co-expressed within dendritic cells and keratinocytes.[12] Therefore, given the central role of LL-37 in the autoimmune response observed in patients with psoriasis, LL-37 offers a potential treatment target.

In conclusion, existing studies have demonstrated that psoriasis is an autoimmune disorder that results in inflammatory cytokine-induced skin and systemic damage.[1,3] While promising susceptibility markers for early onset psoriasis (e.g. *HLA-Cw6*) and autoantigens that stimulate immune cell activation (e.g. LL-37 and ADAMTSL5) have been identified,[3,7-9,11] further research is required to investigate further into the complex biochemical networks that drive autoimmunity in patients with psoriasis. Understanding the biochemical networks that underlie psoriasis may help deliver additional therapeutic targets and novel treatment options.

Lessons from Interleukin-23 in Immune-Mediated Inflammatory Diseases

Doctor Flavio Caprioli

According to the classical Th1/Th2 immune response paradigm, naïve T cells differentiate into Th1 or Th2 lineages, each with a unique cytokine secretion profile driving cell-mediated immune responses or antibody-mediated responses, respectively.[13,14] Therefore, human autoimmune diseases have generally been separated into two classes based on experimental models: Th1-predominant diseases, such as psoriasis and Crohn's disease, or Th2-predominant diseases, such as allergic asthma and ulcerative colitis.[13] Similarly, experimental models of autoimmune diseases have been classically divided into Th1 or Th2-mediated diseases. For example, administering myelin antigens and Freund's complete adjuvant to otherwise healthy mice results in experimental autoimmune encephalomyelitis (EAE), a severe and potentially fatal neurological disease resembling human multiple sclerosis.[15] In this model, the central nervous system is heavily infiltrated with the IFN-γ-producing CD4+ T cells, leading to the initial

hypothesis that EAE was a Th1-mediated disease. However, subsequent work with IFN-γ knockout mice resulted in a worsening of EAE, as opposed to the anticipated disease-resistance, suggesting a need for a shift in our understanding of the Th1/Th2 paradigm.[16]

As IL-12 is an important factor in the formation of Th1 cells, it was initially considered to be an essential factor in the EAE model.[17] However, following the discovery and cloning of IL-23, which is comprised of a p19 subunit and a p40 subunit that is shared with IL-12, it was instead determined that IL-23 was the critical factor driving this model.[17] Studies using IL-10-deficient mice confirmed that the presence of p19, but not p40, was essential in maintaining a chronic inflammatory state.[18] It was later elucidated that IL-23 was a critical factor to confer pathogenic features to Th17 cells, which differentiate from naïve CD4+ T cells under the combined action of transforming growth factor-β and IL-6.[19,20] Under homeostatic conditions, IL-23 is mainly expressed in the bone marrow and gastrointestinal tract (Figure 1).[21] Conversely, an overexpression of p19 subunit is detected in the gastrointestinal tract in patients with active inflammatory bowel disease (IBD) and in the skin of patients with active psoriasis.[22,23] The reasons behind IL-23 overexpression in these clinical conditions are still unclear, in spite of recent hypotheses about changes in the intestinal microbiota. Moreover, mutations in the IL-23 receptor increase susceptibility to several human autoimmune diseases, suggesting that a link between autoimmune inflammation and increased biological responsiveness to this cytokine exists.[24]

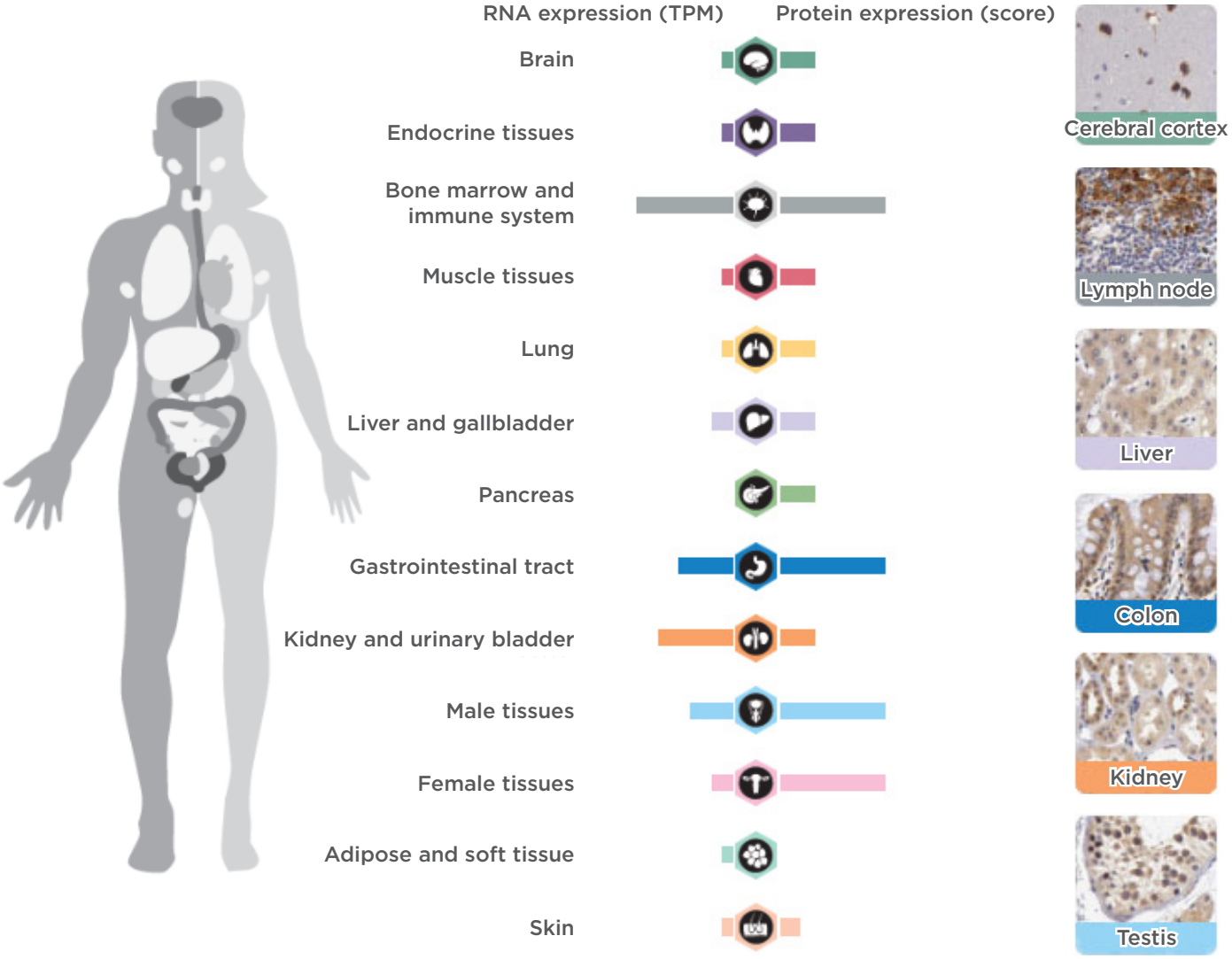

Figure 1: RNA and DNA expression of interleukin-23 in healthy humans.
TPM: transcripts per million.
Adapted from The Human Protein Atlas[21]

Cytokines secreted by Th17 cells, particularly IL-17, tumour necrosis factor (TNF), and IL-22, can have pleiotropic pathogenic effects in psoriasis and other autoimmune diseases. Specifically, IL-17 induces psoriasis-related gene transcription and activates inflammatory cells, encouraging neutrophils to infiltrate the skin, resulting in inflammation and reactive proliferation of both keratinocytes and blood vessels and ultimately leading to psoriasis plaque formation.[25] IL-17 is also responsible for mediating inflammation, enthesitis, and bone erosion in the joints of patients with psoriatic arthritis.[26-29] However, targeting IL-17 expression may be difficult because the cytokine is required for tight junction integrity in the intestinal epithelium and this may explain why anti-IL-17 treatments have not yet proven to be effective for IBD.[30]

In summary, the discovery of Th17 cells, IL-17, and IL-23 has changed our understanding of the underlying pathology of autoimmune diseases, such as psoriasis and IBD. Experimental data and ongoing clinical studies have highlighted the potential of the IL-17/IL-23 axis as a therapeutic target for treating patients with psoriasis and psoriatic arthritis.

Psoriasis: Linking Pathophysiology and Clinical Evidence

Professor Carle Paul

Until 1980, psoriasis was thought to be a keratinocyte hyperproliferative disease, but between 1980 and 1990, a link between psoriasis, cytokine expression, and keratinocyte proliferation was identified.[31,32] In particular, psoriasis was classified as a Th1-mediated disease because cyclosporine treatment was effective in some cases. It was not until 1994, when elevated TNF-α levels in psoriatic lesions were reported, that the first proof-of-concept study targeting cytokines could be performed.[31,32] This was followed by the identification of IL-12 and IL-23 in the decade from 2000–2010, and the Th17 cell lineage, which also facilitated a proof-of-concept study supporting targeting of the Th17 pathway in patients with psoriasis. Notably, over the last 12 years our understanding of the underlying pathophysiology of psoriasis has evolved, from psoriasis being the result of injury and then the activity of IFN-γ and Th1-mediated inflammation,[33] to the current model that considers the roles of dendritic cells in producing cytokines and multiple effector cells, particularly Th17 cells.[34]

Likewise, ongoing research is likely to further elucidate the pathological pathways that contribute to psoriasis, resulting in a rapid rate of treatment innovation. For example, traditional immunosuppressants were the only available therapy in the 20th century, whereas between 2000 and 2010, targeted anti-TNF-α therapies were developed. In addition, since 2010, five new biological therapies have been approved for the treatment of psoriasis in the USA, and an even wider range in Europe, while many other novel therapies continue to progress through Phase II and III clinical studies.

There are currently no clear biomarkers to guide treatment selection for patients with psoriasis, but clinicians tend to have a multidimensional approach to treatment, considering the efficacy and safety profile of each agent, patient comorbidities, and patient preference. For example, each of the anti-IL-17 agents (secukinumab, ixekizumab, and brodalumab) is generally efficacious, with the relative efficacy dictated by pharmacokinetics, i.e. differences in exposure to each drug. However, while up to 40% of patients will experience complete clearance of psoriasis, relapse will often occur soon after ceasing treatment.[35-38]

One anti-IL-23 therapy (guselkumab) has been granted marketing approval for the treatment of psoriasis in the USA, while tildrakizumab and risankizumab are under development. Administering guselkumab 100 mg at Weeks 0 and 4, followed by dosing every 8 weeks, resulted in almost 75% of patients achieving a 90% reduction in Psoriasis Activity Severity Index (PASI 90) compared with <3% of patients treated with placebo, and 85% of patients having an Investigator's Global Assessment (IGA) of 0 or 1 versus 7% for placebo (Figure 2).[39] In addition, the response was maintained for ≥2 years.[39] A greater proportion of patients also responded to guselkumab compared with adalimumab and no loss of response over time was seen with guselkumab (Table 1). Response rates are also increased by switching patients who have not achieved a full response after 16 weeks of ustekinumab treatment to guselkumab.[40] Complete clearance (PASI 100) at 2 years was also observed in approximately half of all patients treated with guselkumab and responses were maintained for >6 months after ceasing therapy in a relatively high proportion of patients.[41] Furthermore, the high level of response was achieved with guselkumab therapy without increasing the risk of infection, particularly opportunistic infections, such as *Candida* and *Staphylococcus aureus*.[39]

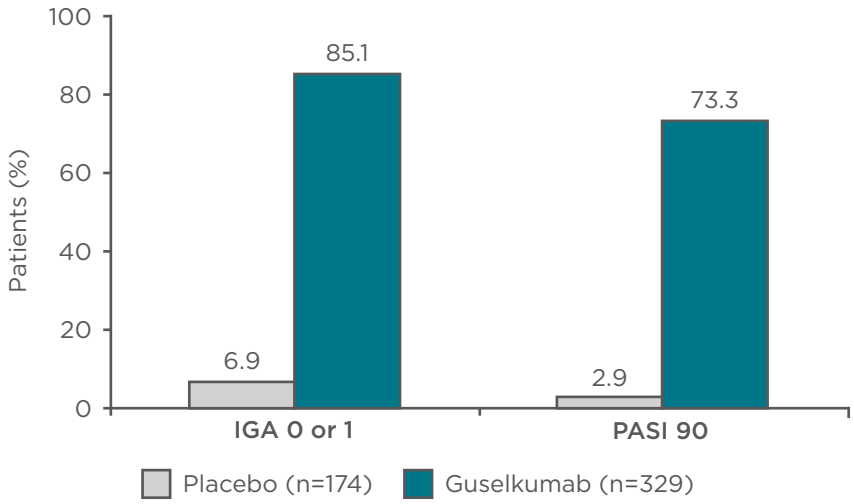

Figure 2: VOYAGE I Phase III results.[39]
Guselkumab is not approved for the treatment of psoriasis or psoriatic arthritis in Europe. Co-primary endpoints: IGA 0 or 1 and PASI 90 at Week 16. Eligible patients had moderate to severe plaque psoriasis (PASI ≥12; PGA ≥3; body surface area ≥10%). p<0.001.
IGA: Investigator's Global Assessment; PASI: Psoriasis Area Severity Index; PGA: Physician's Global Assessment.

Table 1: Proportion of patients who achieved Psoriasis Area Severity Index 90 response (Week 0–100).*

	Guselkumab	Placebo† switch to guselkumab	Adalimumab†† switch to guselkumab
Week 52			
N	329	165	280
Proportion of patients with PASI 90 response (%)	74.8	77.0	50.4
Week 100			
N	329	165	280
Proportion of patients with PASI 90 response (%)	72.3	79.6	78.8

*Data was inputed for non-responders; †Patients were treated with placebo and switched to guselkumab at Week 16; ††Patients treated with adalimumab for the first 48 weeks then switched to guselkumab.
PASI: Psoriasis Area Severity Index.

Risankizumab 180 mg has demonstrated similar results to guselkumab in Phase II studies, with 81% of patients achieving PASI 90, while Phase III studies are ongoing.[42] In contrast, approximately 60% of patients treated with tildrakizumab, an anti-IL-12/IL-23 antibody, achieved PASI 90 or IGA 0 or 1 in a Phase III study, but the response rates increased slightly after long-term follow-up.[43] In addition, following clinical research outcomes in other autoimmune diseases, such as IBD, a hypothesis paper has been developed suggesting that early and aggressive intervention for patients with psoriasis could modify the course of the disease and improve patient outcomes.[44] Anti-IL-23 therapies are suitable candidates for testing this hypothesis. In conclusion, anti-IL-23 therapy may offer similar efficacy to anti-IL-17 therapy, with the potential benefit of a sustained response after ceasing treatment. This not only provides the opportunity to increase dosing intervals for some patients, but to also investigate the disease-modifying potential of anti-IL-23 therapy in future studies.

Question and Answer Session

Q: If psoriasis is classified as an autoimmune disease, why is the condition observed in limited areas of the skin and not in all areas?

A: Dr Caprioli noted that this is an unresolved question. One explanation could be trauma as a trigger for psoriasis; differences in microbiota across the skin could be another explanation.

Q: Is there still a role for topical treatments, such as corticosteroids and vitamin B preparations, in treating patients with psoriasis?

A: Dr Caprioli suggested that topical treatments are appropriate for patients with mild psoriasis because there is no systemic involvement in these cases.

Q: Could combination therapy with two biological therapies be effective in treating patients with psoriasis?

A: Dr Caprioli outlined how biological combination therapies are being investigated in patients with highly refractory IBD, with a particular focus on patients with comorbidities. The efficacy of these treatment options in autoimmune disease has not yet been established, but the potential immunosuppression-related adverse events could be a barrier to routine use of biological combination therapy.

Q: Is there a neurological component to psoriasis?

A: Prof Costanzo highlighted the potential link between stress and psoriasis, while Dr Caprioli indicated that, in his opinion, there may be a neurological element to psoriasis which needs to be explored further.

Q: What is the biggest challenge for the treatment of patients with psoriasis in the future?

A: Prof Paul indicated that the goal is to discover treatments that are long-acting and/or disease-modifying so that the severity of the disease experienced by some patients can be permanently controlled.

Q: Are there any biomarkers that can be used to guide the treatment of psoriasis with targeted therapies?

A: Prof Costanzo replied that biomarkers are yet to be identified, but this is an area of active research.

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EDITOR'S PICK

This must-read paper from Kundrada et al. details the potential role of molecular and radiogenomic markers in the early detection of pancreatic cancer. It is thought that by 2030 pancreatic cancer will be the second leading cause of cancer-related deaths, but the fact that it can take more than a decade from the initiation of the disease to metastatic disease provides a critical window for early detection. Currently there is no consensus on the most appropriate screening protocol for early pancreatic cancer; however, there has been much progress made in available biochemical and molecular markers, which could potentially reduce pancreatic cancer-specific mortality.

Samantha Warne

SCREENING FOR PANCREATIC CANCER: CURRENT STATUS AND FUTURE DIRECTIONS

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ABSTRACT

Pancreatic ductal adenocarcinoma is a lethal disease for a multitude of reasons, including difficulty of early detection, early metastatic spread, and absence of more effective therapies. Even with the advent of newer systemic therapies, the 1-year survival for metastatic disease ranges from 17–23% and 5-year survival is <5%. This necessitates an urgent need for the development of more effective modalities for early detection, particularly due to the long latent period between the genomic cellular changes and the development of metastatic disease. Currently available biochemical and molecular markers have significant potential; however, they require further clinical validation. Endoscopic ultrasound is one of the most sensitive modalities used to both screen and sample lesions, but is limited to use in high-risk patients due to its invasive nature and associated risks. Although clinically meaningful progress has been made in screening the high-risk cohorts in terms of detection of pancreatic ductal adenocarcinoma, intraductal papillary mucinous neoplasms, and mucinous cystic neoplasms, leading to early diagnosis and treatment, nonselective population-based screening is not yet available for widespread use. Currently there is no consensus on the most appropriate screening protocol for early pancreatic cancer detection. In this review, we focus on understanding the potential role of molecular and radiogenomic markers in the early detection of pancreatic cancer.

Keywords: Biomarkers, imaging, pancreatic cancer (PC), pancreatic ductal adenocarcinoma (PDAC), screening, serum.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy that originates in exocrine pancreatic cells and accounts for about 95% of pancreatic cancers (PC). Despite a low incidence of around 3%, the mortality rate in the general population[1] is also close to this figure, thus making PDAC

a deadly disease. It was estimated that there would be 53,670 new cases of PDAC diagnosed in the USA during 2017, with approximately 43,090 deaths resulting from the disease, making it the fourth leading cause of cancer-related deaths. It is also estimated that by 2030, PC will be the second leading cause of cancer-related deaths in the USA.[2]

In the last decade, significant improvements have been made in the screening and therapy of multiple solid tumours, thus increasing the incremental chance for a cure in some of these cancer types. Despite these improvements, PDAC remains difficult to treat. More than 80% of PDAC cases are metastatic at the time of diagnosis, with a mean overall survival (mOS) between 3 and 6 months. Even with early diagnosis, the median survival is poor, ranging from 31.50% at 3 years to 11.86% at 5 years.[3]

Numerous clinical trials since the 1990s have minimally improved the mOS of patients with PDAC. Gemcitabine, a nucleoside analogue and the current standard treatment of PDAC, was tested against 5-fluorouracil in the 1990s and had an improved clinical response (24% versus 5%), mOS (5.6 versus 4.4 months), and 1-year survival (18% versus 2%);[4] these outcomes occurred despite the lack of objective response.[4] Other agents combined with gemcitabine have also been tested in advanced PC; however, despite higher objective response rates in some studies,[5] 33 randomised Phase III trials failed to demonstrate a survival benefit, with the exception of addition of erlotinib.[6] FOLFIRINOX as a single agent also improved mOS by 4.3 months over gemcitabine alone (11.1 versus 6.8 months; hazard ratio: 0.57; p<0.001).[7] In the MPACT Phase III trial, nab-paclitaxel plus gemcitabine, administered for the initial 3 weeks of a 4-week period, also prolonged mOS over gemcitabine alone (8.5 versus 6.7 months; p<0.001).[8] These results have prompted additional trials using backbones of FOLFIRINOX or nab-paclitaxel with mixed results.[9] Nevertheless, even with the advent of newer combination systemic therapies, the 5-year survival of patients with advanced disease is 8.2%, approximately a 3% improvement from 1975.[3] This is, in part, caused by the lack of visible and distinctive symptoms and reliable biomarkers for early diagnosis, as well as the aggressive nature of metastatic disease with poor response to treatments.

The poor response to current therapy emphasises the need for novel and effective strategies for early screening and diagnosis, such that more patients can undergo curative surgery. This is supported by the better mOS of patients with pancreatic adenocarcinomas discovered incidentally compared to those diagnosed based on clinical symptoms (30 versus 21 months; p=0.01),[10] as well as the significantly decreased complication rate of pancreatoduodenectomies, from 25–30% in the 1960s[11] to as low as 2.5% in high-volume centres with expertise.[12] Therefore, early screening can lead to better improvements in survival, as noted with colon and breast cancer patients, and an ideal screening strategy will have a higher sensitivity and a higher positive predictive value. Although several studies have been performed for both the asymptomatic and high-risk subject groups, the results have not been encouraging. In this review, we provide a broad description of the risk factors, currently available options for screening, and novel strategies that are presently being undertaken to improve outcomes for PDAC patients.

RISK FACTORS

Constitutional or Environmental

There are multiple constitutional and environmental factors that may influence the development of PDAC; the most common of these are listed in Table 1.

Genetic Syndromes

Many syndromes that occur in multiple family members carry an increased risk of developing PDAC and are a cause of ≤17% of all PC cases.[13] The risk for developing PDAC in these families is synergistically increased with environmental risk factors, such as smoking and alcohol use.[14,15] These syndromes increase the patient's life-time risk for PDAC by ≤40% with an odds ratio of ≤61.[14] The syndromes with most published literature are hereditary pancreatitis, cystic fibrosis, Peutz-Jeghers syndrome, familial atypical multiple mole melanoma, hereditary breast and ovarian cancer, hereditary non-polyposis colorectal cancer or Lynch syndrome, familial adenomatous polyposis, familial pancreatic cancer, Li fraumeni syndrome, and ataxia telangiectasia, with some of them increasing the risks of other cancers as well. Families with ≥2 first-degree relatives who have PDAC, which is not associated with a known cancer syndrome, are categorised as familial pancreatic cancer. They comprise of 80% of patients with an inherited predisposition[16] and *BRCA2* mutations were found in 17.2% of families with ≥3 relatives with PDAC.[17]

Table 1: Risk factors associated with pancreatic ductal adenocarcinoma.

Risk factors	Risk
Increasing age	Greatest risk factor[1,4]
Ethnicity	• African Americans, the Ashkenazi Jews, Pacific Islanders, and people of New Zealand Maori descent have an increased risk[58]
Active smoking	• Two-fold increased risk for developing PDAC[59] • Risk increases 3–5-fold with a >40 pack-a-year smoking history[60] • In high-risk cohorts, smoking can decrease the age of onset of PDAC by approximately 10 years[61]
Chronic Type 2 diabetes mellitus	• Relative risk of PDAC is 2.1-times compared to non-diabetics[15] • Some reports suggest that new onset of Type 2 diabetes mellitus in an older individual may herald to an impending diagnosis of PDAC[15] • The causality is controversial, with evidence supporting both the theories of diabetes predisposing a patient to PDAC and that of the cancer itself causing glucose intolerance[15]
Chronic pancreatitis	• Standardised incidence ratio of 8.2 (95% CI: 5.5–11.8) for PDAC[62]
Obesity and anthropometric variables	• A BMI >40 kg/m^2 increased the risk of PDAC 1.5-times in males and 2.8-times in females[63]
Diet	• Diets with a high content of meat and/or fat and lower fruit and vegetable content
Alcohol	• Increases the risk of PDAC by its association with pancreatitis and glucose intolerance • As per study, odds ratio of 2.7 was associated with PDAC and heavy alcohol consumption after being adjusted for other risk factors like BMI, smoking, and diabetes[64]
Occupational exposure	• Chlorinated hydrocarbons used in dry-cleaning and polycyclic aromatic hydrocarbons in dyes and pigments have been reported to increase PDAC risk
Male sex	• Incidence being 14.2/100,000/year in men compared to 11.1/100,000/year in women in all races[65]

CI: confidence interval; PDAC: pancreatic ductal adenocarcinoma.

Precancerous Lesions

One of the most common theories on cancer progression is the development of multiple mutations that progressively lead to invasive behaviour.[18] The adenoma-carcinoma sequence is an instance of this theory and has worked well for both colon and breast cancers, for which screening has resulted in a dramatic improvement in survival. In the pancreas, there are several lesions that demonstrate slow growth and are amenable for surveillance, including pancreatic intra-epithelial neoplasia (PanIN), mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasm (IPMN).

These slowly progressing lesions allow clinicians time for screening and surveillance before the lesion worsens. PanIN is the prototype of the adenoma-carcinoma sequence in the pancreas, with cells progressing from atypia, dysplasia, and then to carcinoma *in situ*. MCN is an epithelial neoplasm containing ovarian stroma that produces mucin but does not communicate with the pancreatic duct;[19] this lesion is more likely to be invasive in older patients and has nonspecific presentations.[19] Unlike MCN, IPMN are of ductal origin and produce copious amounts of mucin, which results in dilated pancreatic ducts.[20] The prevalence of cancer ranges from 57–92% in main-duct IPMN and 6–46% in branch-duct IPMN (BD-IPMN), as reported in a recent consensus statement.[21,22]

CURRENT SCREENING STUDIES

Biochemical Screening

CA 19-9 is a Lewis antigen that is not expressed in 5–10% of the population, implying that at least 5–10% of the population cannot be screened using this marker.[23] CA 19-9 also becomes elevated in patients with benign pancreatobiliary disorders, chronic pancreatitis, and other causes of cholestasis.[23] Although CA 19-9 is one of the best single markers in terms of sensitivity and specificity, the elevated values in benign conditions make CA 19-9 less useful as a screening marker. This has been exemplified in several of the screening studies conducted in Japan and Korea;[24,25] in these studies, which screened >80,000 subjects, the results were dismal for CA 19-9 as the single marker of PC. Although the sensitivity and specificity were excellent at 100% and 98%, respectively, the positive predictive value was poor at 0.03–0.09%.[24,25] These values make CA 19-9 excellent for ruling out, but not for ruling in, PDAC. If one were to act

on a positive CA 19-9 at the 37 U/mL threshold, significant unnecessary procedures would be performed, since only eight cancers were detected in the screened patients.[24,25] The conclusions drawn from these large-scale screening studies are that CA 19-9 screening of asymptomatic patients is ineffective, but it is an effective marker for symptomatic patients.

In high-risk patients, the use of CA 19-9 to screen is more effective; however, not all lesions identified are adenocarcinomas.[26] Using a combination of CA 19-9 and endoscopic ultrasound (EUS)-guided fine needle aspiration (FNS), Zubarik et al.[26] evaluated 546 high-risk individuals, of whom 27 had elevated CA 19-9. Follow-up EUS identified five individuals with pancreatic lesions, of which only one lesion was PDAC. In this small study, the positive predictive value was 3.7%, significantly higher than screening asymptomatic patients.

By adding additional markers to CA 19-9, it may be possible to improve the effectiveness of biochemical screening of asymptomatic patients.[27] In a longitudinal study of the prostate, lung, colon, and ovarian screening cohort, multiple markers were evaluated for detecting PDAC at >1 and <1 year prior to actual diagnosis. This produced a panel consisting of CA 19-9, carcinoembryonic antigen, and Cyfra 21-1. This combination produced a higher sensitivity of 30% compared to 17% for CA 19-9 alone, at a specificity of 95%. These studies conclude that a stand alone screening cohort for identifying a PDAC biomarker may be difficult to accrue but may be more easily recruited as part of a larger screening study for multiple cancers.

In a recent publication, Fric et al.[28] proposed a new algorithm for screening the sporadic PDAC in the general population based on the endocrine function of the pancreas, suggesting that glucose intolerance is a sign of early PDAC. According to their algorithm, the following patients aged >50 years should be screened for PDAC: patients with new-onset diabetes with decreasing body weight, low body mass, or unstable diabetes requiring insulin therapy, or long-term diabetic patients with new-onset resistance to prior treatment and weight loss.[28] These are logical applications based on the physiological function of the pancreas, but require further clinical validation.

Imaging Screening

Imaging studies have not been used to screen the general asymptomatic population due to the significant cost incurred with these studies.[1] To improve the screening yield, screening research has focussed on high-risk individuals. The definition of high-risk population has been stated by the International Cancer of the Pancreas Screening Consortium (CAPS).[29] These patients include those with at least one first-degree relative affected by PDAC at age <50 years and patients with genetic mutations and cancer syndromes.[26,30]

When screening these patients, the most common screening technique has been either EUS or magnetic resonance cholangiopancreatography. EUS is most sensitive at detecting pancreatic lesions and can detect lesions <1 cm in size, which were not detectable by computed tomography (CT) or magnetic resonance imaging (MRI).[29] The reported rates of detection were 43% for EUS, 33% for MRI, and 11% for CT.[31,32] EUS also has the advantage of being able to sample the lesion at the time of scanning; therefore, based on these studies, EUS is currently the recommended technique for screening high-risk individuals.

Table 2: Sensitivity, specificity, positive predictive value, and negative predictive value of various imaging modalities used in the diagnosis of pancreatic ductal adenocarcinoma.

Imaging modality	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Endoscopic ultrasonography	98[66]	96[67]	77[68]	100[69]
Computed tomography (CT)	90[70]	85[71]	70[72]	55[73]
Transabdominal ultrasound	70[70]	83[73]	94[74]	76[73]
Magnetic resonance imaging (MRI)	86[71]	82[71]	85[75]	98[75]
Magnetic resonance cholangiopancreatography	100[76]	88[76]	97[76]	100[76]

In high-risk patients, the incidence of abnormalities on imaging is around 30–45% when MRI is involved.[26,31,32] Magnetic resonance cholangiopancreatography, in addition to having high sensitivity, has positive and negative predictive values that are helpful in tumour staging and determining respectability. On the other hand, MRI provides excellent contrast resolution and detects small tumours on gadolinium-enhanced fat-suppressed images. In these studies, the identified lesions are predominantly cystic-related to either cystic or mucinous neoplasms (ranging between 50% and 90% of the abnormal findings) and PDAC was identified in between 0% and 4% of the screened patients. Mucinous lesions are present in 2–30% of the screened patients and dysplasia and early malignancy were frequently present in the mucinous lesions. Thus, even in high-risk patients, PDAC is present in a low percentage of patients, although the relative risk is significantly higher than the general population. Identifying the mucinous lesions early allows for early surgery to prevent eventual development of PDAC, and this may be the most significant benefit of screening the high-risk population. Table 2 outlines the sensitivities and specificities of different imaging modalities in the detection of PDAC.

Molecular and Genomic Screening

Reliable molecular markers can help in determining the nature of pancreatic lesions identified by imaging, especially during the screening of high-risk individuals. Due to the size and non-invasive nature, microscopic precancerous lesions (PanIN and IPMN) are likely to be insensitive to detection using serum markers.

Circulating Tumour Cells and DNA

Pancreatic juice contains high concentrations of DNA and other molecules released from PC; thus, molecular alterations can be readily detected as opposed to other specimens such as blood or stool.[33] The fluid can be collected during routine upper gastrointestinal endoscopy after secretin infusion, making it a potential specimen to analyse markers in patients with diffuse abnormalities of the pancreas by imaging; on the other hand, FNA is best used to sample focal lesions detected by imaging. A study showed that circulating tumour DNA (ctDNA) was detectable in 48% of patients with PDAC, making it a potential marker to be used in conjunction with other panels.[34] Other than improvement in sensitivity, it is important to standardise pre-analytical processes for ctDNA analysis, such as blood sample acquisition, plasma separation, ctDNA extraction, and quantification; ctDNA analysis will need further clinical validation in larger prospective studies in patients with early stage disease.

Mesothelin

Mesothelin is a glycoprotein that acts as a tumour antigen and can be detected in FNA from suspected pancreatic lesions.[35] Mesothelin-specific T cells can be induced in patients with PDAC, making it a potential target for immune-based interventions.[36] However, it still remains to be determined if the gene products could serve as diagnostic serum markers of PC for screening.

Macrophage Inhibitory Cytokine-1 and Other Protein Markers

Markers are elevated in the serum of patients with PDAC.[37] Macrophage inhibitory cytokine-1 (MIC-1) is a more sensitive marker of PDAC than CA 19-9[37] and is a distant member of the transforming growth factor-beta superfamily, originally identified in the setting of macrophage activation.[38] MIC-1 is overexpressed in several cancer types, including pancreas, colon, prostate, breast, and gastric cancers,[39-41] and has demonstrated *in vivo* and *in vitro* effects on tumour growth and/or apoptosis.[42] In a recent study[37] of 50 patients with resectable PC, 50 with chronic pancreatitis, and 50 healthy, age and sex-matched controls, MIC-1 performed significantly better than CA 19-9 at differentiating patients with PC from healthy controls (area under the curve: 0.99 versus 0.78; p=0.003). A total of 90% of the patients with resectable PC had MIC-1 levels >2 standard deviations above age-matched controls, whereas only 62% had elevated CA 19-9, and, unlike CA 19-9, MIC-1 elevations were independent of tumour, node and metastasis (TNM) stage; 6 of 7 patients with T1 or T2 cancers had elevated MIC-1, whereas only 2 of 7 had elevated CA 19-9. In contrast, MIC-1 was no better than CA 19-9 in distinguishing patients with chronic pancreatitis from those with PC,[37] an important feature since this distinction is difficult to make using clinical and radiographic criteria. These results suggest that serum MIC-1 could be helpful in the early detection of PDAC in high-risk cohorts as a part of their pancreatic screening protocols.

In other studies, serum markers like matrix metalloproteinase 7 and adenosine deaminase successfully distinguished pancreatic adenocarcinoma from chronic pancreatitis, but

showed no improvement in accuracy compared to CA 19-9 alone.[37,43] A new monoclonal antibody to mucin 1, a membrane-associated glycoprotein that is overexpressed in multiple cancers including PDAC and from which the CA 19-9 antigen is derived, satisfied both criteria to differentiate PDAC from normal state and chronic pancreatitis. However, its sensitivity and specificity were 77% and 95%, respectively, remaining below desirable accuracy levels.[44]

Mutant *KRAS*

KRAS mutations are present in approximately 90% of PDAC cases, limited to one codon, and can be readily detected using molecular assays.[33] However, *KRAS* mutations are not specific for PC and can also be present in smokers and patients with chronic pancreatitis and PanIN.[45,46] It is possible that quantifying mutant *KRAS* levels in the blood and pancreatic juice could improve the diagnostic utility of mutant *KRAS* and potentially be used as a screening tool.[47]

TP53

TP53 mutations occur relatively late in the neoplastic process towards invasive PC and are found in approximately 70% of invasive PDAC cases.[48] A few nucleotide hot spots of the *TP53* gene mutation are known, but mutations occur throughout the gene.[49] Thus, the detection of *TP53* mutations in pancreatic juice has the potential to be a useful diagnostic and screening strategy, particularly if improvements in mutation detection technology can enable accurate detection of such mutations at low concentrations.

Methylated Genes

Numerous genes undergo aberrant methylation during the neoplastic process and are rarely detected in non-neoplastic pancreatic tissues. These genes include *p16*, *ppENK*, *Cyclin D2*, *SOCS1*, *SPARC*, and *TSLC1* and can be detected via methylation-specific polymerase chain reactions, making them potentially attractive for early detection protocols.[33]

Other markers like SPAN-1, CA-50, DUPAN-2, elastase-1, tissue polypeptide antigen, and tissue polypeptide-specific antigen have been studied but have not performed well.[50] In addition, microscopic precancerous lesions like PanIN and IPMN are insensitive to detection using these serum markers due to their small size and non-invasive nature. Larger panels, with appropriate molecular marker combinations, can improve accuracy in pancreatic adenocarcinoma diagnosis.[51] Table 3 outlines the sensitivities, specificities, and other statistical significances of these novel molecular and genetic markers.

DIRECTIONS AND CHALLENGES

Primary PC contains a mix of distinct subclones, each containing hundreds of millions of cells that are present within the primary tumour years before the metastases become clinically evident.[52]

Table 3: Sensitivity, specificity, positive predictive value, and negative predictive value of the emerging biochemical, molecular, and genetic markers.

Marker	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
CA 19-9	80.0[77]	82.0–90.0[77]	69.0[78]	90.0[78]
Monoclonal antibody to mucin 1	77.0[79]	95.0[79]	-	-
Circulating tumour cells and DNA	92.3[80] 85.0[81]	80.0[82]	-	-
Mesothelin	75.0[83]	80.0[83]	-	-
Macrophage inhibitory cytokine-1	65.8[84]	96.4[84]	96.7[84]	63.6[84]
Mutant *KRAS*	100.0[80] 88.0[81] 87.2[34]	99.0[85]	86.0[85]	74.0[85]
TP53	67.4[86]	-	-	-
Metalloproteinase 7	72.0[87]	80.0[87]	0.031[87]	99.9[87]
Insulin-like growth factor II mRNA binding protein 3	85.0[83]	90.0[83]	-	-

There can be an interval of >1 decade for genetic progression of PC from initiation to the metastatic stage, suggesting that a window of opportunity for early detection exists.[53] Currently, several screening modalities have been implemented in high-risk cohorts to increase the pretest probability of testing.

One such computer-based risk assessment tool, PancPRO, assigns a quantitative risk score to any member of families with familial PC.[54] An Italian PDAC registry study evaluating this model demonstrated that having a PancPRO risk score >10 is one of the major criteria for enrolment in a screening programme.[55] High-risk patients are screened using a multimodality screening combination of a CT and an EUS; an abnormal EUS will be followed by an EUS-FNA and an endoscopic retrograde cholangiopancreatography.[56]

The objective of developing a robust screening programme is to provide early resection for the aforementioned precancerous lesions. Based on the size and internal features of the lesions, follow-up imaging with MRI should be performed every 1–2 years for lesions <3 cm in size and those without intracystic solid nodules. These benign-appearing lesions need to be monitored for ≥5 years.[22] While resection is recommended for main duct IPMN and MCN, high-risk stigmata and worrisome features like enhanced solid component, MPD size of >10 mm, cyst size of >3 cm, thickened enhanced cyst walls, non-enhanced mural nodules, MPD size of 5–9 mm, abrupt change in the MPD calibre with distal pancreatic atrophy, and lymphadenopathy have been defined to stratify the risk of malignancy in BD-IPMN to consider resection versus increased frequency of surveillance.[22] Some authors advocate continuation of surveillance every 6 months in view of the relatively high incidence of PDAC in patients with BD-IPMN; on the other hand, PanIN can only be identified reliably after surgical resection. Thus, suspicion of pancreatic lesions on imaging screening in these high-risk cohorts should be followed by a biomarker panel to suggest high-risk PanIN leading to resection, versus low-risk PanIN, which can be monitored by close surveillance.

Several issues currently limit standardisation of surveillance. One obstacle to surveillance is the lack of knowledge regarding the natural history of premalignant lesions and the outcome of these lesions in high-risk patients. In several reports of PDAC cases, the volume doubling time of PDAC once visible by a CT scan ranged from 20–1,351 days;[57] this could mean a potential surveillance frequency that ranges from 3–12 months. Another issue concernings lead time bias, when earlier detection of tumours via screening may seem to result in longer survival than those identified by clinical symptoms, although the natural history of the tumour may not have been altered. We also face challenges of tumours with aggressive biology where recurrence or metastases are seen even after resection.

CONCLUSION

PC is a deadly disease even though it has a low incidence in the general population. The fact that it can take more than a decade to progress from initiation to the metastatic disease provides us with a critical window of opportunity for early detection. Although we have made some clinically meaningful progress in screening the high-risk cohorts, non-selective population-based screening is certainly not ready for widespread use. Currently, there is no consensus on the most appropriate screening protocol for early PC; however, newer techniques using molecular and genomic modalities are promising. These tests, if validated, can provide an accurate and reliable modality to enable early diagnosis in patients that are asymptomatic. In addition, this will reduce PC-specific mortality and could prevent PC from becoming the second leading cause of cancer-related death in 2030.

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HEPATOPULMONARY SYNDROME: OXIDATIVE STRESS AND PHYSICAL EXERCISE

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ABSTRACT

Hepatopulmonary syndrome (HPS) may be defined by hepatic disease, gas exchange abnormalities that may lead to hypoxaemia, and the presence of pulmonary vascular dilations. The balance between the many substances involved in vasodilation and vasoconstriction is regulated by the liver; thus, liver damage may generate systemic changes throughout the body. The pulmonary tissue may be damaged by reactive oxygen species or nitric oxide. Dyspnoea is the most frequent pulmonary symptom, caused by tissue damage, and may become worse when an individual exercises. In experimental research the surgical model of bile duct ligation is the optimal model to simulate the typical lung alterations present in HPS, which results in an increase in oxidative stress in hepatic and pulmonary tissues. In liver injury, the muscular system may also be damaged, for example sarcopenia may seriously aggravate cirrhosis and is associated with cirrhotic patient mortality. Muscular changes can be explained by the actions of myostatin and insulin-like growth factor and the increase in body levels of ammonia. As a result of impaired cardiopulmonary and muscular conditions, HPS patients may exhibit a low exercise tolerance, low muscle strength, and low functionality. Liver disease can contribute to HPS oxidative stress and is one of the main factors responsible for the reduction of gas exchange. Physical exercise can be performed as a way of modifying this pathophysiological state. Studies that have investigated physical exercise as a therapy for cirrhosis suggest that this approach may be beneficial for cirrhotic patients, primarily with regard to muscular and cardiorespiratory injuries.

Keywords: Bile duct ligation (BLD), cirrhosis, hepatopulmonary syndrome (HPS), oxidative stress, physical exercise, sarcopenia.

INTRODUCTION

Hepatopulmonary syndrome (HPS) may be defined by hepatic disease, gas exchange abnormalities that may lead to hypoxaemia, and the presence of pulmonary vascular dilations.[1] The physiopathology of the hypoxaemia in HPS is multifactorial and involves intrapulmonary shunting, a low ventilation-perfusion ratio, low diffusion, and an unbalanced alveolar-arterial oxygen gradient ($AaDO_2$). The prevalence of HPS in cirrhotic patients is approximately 10–20% and contributes to an increased morbidity and mortality in these individuals.[1,2]

Hypoxaemic events are common in patients with HPS, and these alterations may be caused by intrapulmonary vascular dilations associated with hepatic disease. Lung capillary dilation is

a typical vascular alteration of HPS; it may be localised and is less frequently present in the pleural and arteriovenous lung communications.[3] The hypoxaemia in these situations may be explained by the unbalance of the ventilation-perfusion ratio, the presence of intrapulmonary shunts, and the decrease in the diffusion caused by intrapulmonary vasodilation.[4,5]

Vascular disorganisation results in the appearance of new alveolar units. These units have preserved ventilation; however, the presence of an arteriovenous shunt and intrapulmonary vasodilation compromises the maintenance of proper levels of arterial oxygen. The presence of biomolecular mediators produced by the liver, such as nitric oxide (NO), which is a powerful biological vasodilator derived from the endothelium, may be the main factor causing pulmonary vasodilation. Decreased oxygen flow to the blood capillaries does not occur because of the thickening of the alveoli-capillary membrane, it occurs because of poor vascular dilation, which is associated with increased cardiac output, a common alteration of cirrhotic patients with hyperdynamic circulation.[6-8] Liver disease can contribute to HPS, where oxidative stress is one of the main factors responsible for the reduction of gas exchange, and physical exercise can be performed as a way of modifying this pathophysiological state.

DIAGNOSIS, CLASSIFICATION, AND SYMPTOMS

The main features present in HPS patients are dyspnoea and platypnoea, (with an increase in dyspnoea when changing from lying to standing), which may be associated with fatigue, as well as the clinical findings of peripheral cyanosis and digital clubbing. Dyspnoea is considered the more frequent pulmonary symptom and may worsen when the patient exercises.[6] The diagnosis is made when the $AaDO_2$ is >20 mmHg or when there is hypoxaemia, which is defined by a partial pressure of oxygen (PaO_2) <70 mmHg. Intravascular lung dilations may be present, which facilitates confirmation of the diagnosis made by an echocardiogram.[8]

According to Krowka and Cortese,[9] HPS may be classified into two subtypes based on the PaO_2 and angiograph findings. HPS Type I is defined by the presence of precapillary diffuse dilations that respond well to oxygen therapy. HPS Type II is characterised by small vasodilatation and arteriovenous malformation. In contrast, Rodríguez-Roisin et al.[1] suggest a staging classification of HPS into four groups based on the gas exchange abnormalities, which are considered mild when the PaO_2 is >80 mmHg, moderate when the PaO_2 is 60–80 mmHg, severe when the PaO_2 is 50–60 mmHg, and very severe when it is <50 mmHg.

HEPATIC DISEASE AND OXIDATIVE STRESS

The liver is critical in regulating the balance between vasoconstrictor and vasodilator substances, and hepatic tissue injury can impair the regulatory functions that can lead to many abnormalities. Liver damage makes it more difficult for the organ to filter blood from the portal vein, which leads to the appearance of portosystemic shunts and a decrease in the hepatic phagocytic capacity. As a result, the lung filtrates systemic blood to compensate for the decrease in hepatic phagocytosis,[10] and the increase in the lung phagocytic activity results in macrophage accumulation in the pulmonary endothelium and increases cytokine and NO levels in the extracellular environment. NO acts as a molecular signal that mediates the vasodilation of pulmonary capillaries when it is produced in low concentrations by vascular endothelial cells. NO has a toxic oxidant action when it is produced in high concentrations by lung macrophages. Phagocytes also produce the superoxide anion radical, which oxides the plasmatic membrane and produces a substantial amount of reactive oxygen species (ROS).[11]

ROS and reactive nitrogen species are products of physiological cellular metabolism and have both noxious and beneficial effects on the organism. Many physiological functions are controlled by these molecules, including vascular tonus regulation, defence against invading micro-organisms and harmful cells, and monitoring of oxygen pressure in ventilation control and molecular levels.[12] An unbalance in this system can lead to tissue damage and can result in chronic-degenerative diseases.[13]

Tieppo et al.[14] assessed the lipoperoxidation of pulmonary tissue following the experimental model of common bile duct ligation (BDL); using the techniques of thiobarbituric acid reactive substances and chemiluminescence, the authors identified a significant increase in the lipid peroxidation, which may be explained by the action of phagocytic cells when fighting against the

process of bacterial translocation. The migration of macrophages to the lungs leads to the production of the superoxide anion radical and increases the antioxidant enzyme activity. Tieppo et al.[14] identified a significant increase in the enzyme superoxide dismutase in the pulmonary tissue of the cirrhotic group, which suggests increased enzyme activity as a result of the aggression induced by ROS. The researchers concluded that the experimental model of common BDL simulates the main symptoms of HPS, which suggests it is a good model to investigate oxidative stress, hepatic changes, and the respiratory system.

HEPATOPULMONARY SYNDROME AND EXPERIMENTAL MODELS

The experimental models more commonly used to investigate the abnormalities caused by HPS are the BDL and partial portal vein ligation in rats. In both models, NO develops an important rule regarding the pathogenesis of the experimental HPS, which causes vascular dilation and leads to hypoxaemia, similar to the findings of cirrhotic patients.

Experimental studies that used the BDL surgical model have indicated an increase in oxidative stress of hepatic and pulmonary tissues, as well as other lung alterations typical of HPS.[14] Long-term bile duct obstruction causes secondary biliary cirrhosis, which leads to acute inflammatory reactions, causing systemic changes.[15] Chang and Ohara[16] demonstrated that animals that had undergone a BDL surgery presented progressive worsening of the gas exchange, which is similar to the changes that occur in humans with HPS. The researchers determined that the cirrhotic animals had an $AaDO_2$ >100 mmHg, associated with moderate hypoxaemia (values <85 mmHg). These findings may be linked with intrapulmonary vasodilation caused by high levels of endothelial NO synthase present in pulmonary tissues.[17]

Tieppo et al.[14] identified a decrease in the PaO_2 and peripheral oxygen saturation in haemoglobin, and a significant increase in the partial pressure of carbon dioxide in the arterial blood in cirrhotic animals compared with the control group. The same researchers also identified a significant difference in the $AaDO_2$ between the BDL and sham groups, which is consistent with the findings of Luo et al.[18] that showed animals developed progressive hyperventilation and hypoxaemia in association with the increase in the $AaDO_2$.

Vercelino et al.[19] suggested that the experimental model of BDL best simulates the alterations present in HPS. The researchers investigated the gas exchange abnormalities of different experimental models of hepatic cirrhosis (Table 1), and the BDL model exhibited more changes in the gas exchange values. An increase in partial pressure of carbon dioxide in the arterial blood was identified in the BDL group compared with the control group, as well as alterations in the PaO_2, arterial oxygen saturation, and $AaDO_2$. In addition to the gas exchange abnormalities, the BDL group also exhibited a larger intrapulmonary vasodilation evidenced by histological analysis. Therefore, BDL is a method proven to be efficient at simulating vascular alterations and gas exchange abnormalities identified in HPS.

Berthelot et al.[20] identified an increase in the diameter of the lung arterioles of animals that had undergone BDL surgery, in which the ratio between the pulmonary weight and body weight increased in the cirrhotic group compared with the control group. These alterations are likely caused by pulmonary shunts and intrapulmonary vascular dilations, as a result of intrapulmonary capillary congestion. The intrapulmonary vasodilation in these cases may be associated with bacterial translocation, which occurs as a result of the absence or decrease of bile in the small intestine. This bile deficit decreases the emulsifier and antiendotoxic effect of the bile salts, which leads to an increase in the endotoxin levels in the large intestine, which are carried by the portal circulation to the pulmonary system.[21]

MUSCULOSKELETAL ALTERATIONS IN HEPATOPULMONARY SYNDROME

Striated muscle is a complex tissue that is versatile, heterogeneous, and composed of specialised multinucleate muscle fibres. These cells have a myonuclei placed in the periphery of the fibre immediately below the plasmatic membrane. Contractile units, also referred to as sarcomeres, are morphologically repeated and are comprised of muscle fibres. Each sarcomere is composed of many proteins, including the contractile proteins of myosin, a thick filament formed by the polymerisation of 200–300 molecules of Class II myosin, and actin, a thin filament associated with the regulator proteins of troponin and tropomyosin.[1]

Table 1: Serum enzyme levels, blood gases, and alveolar-arterial oxygen gradient in control and experimental groups of the four experimental models of cirrhosis.*

Variable	IN CCl4		IP CCl4		PPVL		BDL	
	Co	Ex	Co	Ex	Co	Ex	Co	Ex
AST (IU/L)	191.3±45.6	717.0±207.6	132.5±19.6	1,715.0±689.2†	133.7±22.3	108.5±19.4	105.2±21.5	500.5±45.1‡
ALT (IU/L)	124.8±46.4	759.6±232.2†	86.7±7.3	1,399.5±459.5†	79.0±9.1	84.5±16.9	78.7±18.8	162.7±17.7†
ALP (IU/L)	86.8±8.7	196.4±25.2‡	88.2±11.7	220.7±60.9	116.0±20.5	127.8±29.7	160.0±20.4	373.2±45.4‡
PaO_2 (mmHg)	85.3±0.8	63.8±3.8‡	107.0±4.0	97.8±7.4	69.7±0.9	64.3±5.2	85.2±4.0	49.9±11.3†
$PaCO_2$ (mmHg)	48.7±2.9	54.7±2.9	49.0±5.0	46.5±4.0	54.3±1.7	56.3±5.0	9.8±3.3	64.0±5.1†
SaO_2 (%)	95.3±0.3	78.8±9.3†	96.0±1.3	96.2±0.7	92.0±0.0	86.0±2.9	95.0±0.7	73.3±12.1†
$AaDO_2$ (mmHg)	23.1±12.8	56.3±2.8	12.9±4.3	22.7±5.8	12.1±2.9	15.0±3.9	30.4±3.5	62.6±10.5‡

*The results expressed as the means ± standard errors of the means; †p<0.05 versus the corresponding control group; ‡p<0.001 versus the corresponding control group.
$AaDO_2$: alveolar-arterial oxygen gradient; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BDL: bile duct ligation; Co: control; Ex: experimental; IN CCl4: inhaled carbon tetrachloride; IP CCl4: intraperitoneal carbon tetrachloride; PaO_2: arterial oxygen tension; $PaCO_2$: arterial carbon dioxide tension; PPVL: partial portal vein ligation; SaO_2: arterial oxygen saturation.

Similar to other chronic diseases, the muscular system is one of the most damaged in hepatic injuries and determines the patient's limitations regarding physical exercise. Striated muscle makes up 45% of the body weight, and is critical in the preservation of bioenergetics, homeostasis, once it is the main place of processing, and storage of energy, as well as being the final destination of the primary support systems involved in exercising, such as the cardiovascular and respiratory systems.[1] Sarcopenia, or muscle loss, is the most common muscle abnormality of liver diseases and only a limited number of studies have investigated the mechanisms involved in this process.[22] In cirrhosis, sarcopenia may aggravate the clinical condition of patients because it decreases functionality, contributes to a worse disease prognosis, and, as an independent factor, increases the mortality of the cirrhotic population.[23] Malnutrition in cirrhotic patients is also associated with a worse disease prognosis; when combined with low food intake and high metabolic activity, malnutrition may lead to cachexia, damaging the muscular system.[24]

Sarcopenia is the result of a reduction in protein synthesis and an increase in muscular proteolysis, as well as the proliferation and uncontrolled differentiation of satellite cells of the muscular system.[25] Muscular tissue alterations depend on molecular factors, such as myostatin and insulin-like growth factor 1, which are the main regulators of the muscle skeletal growth process.[26] Myostatin and insulin-like growth factor 1 also participate in protein synthesis regulation and an increase in proteolysis, with high levels of myostatin identified in cirrhotic patients with end-stage liver disease.[26,27]

Many other molecular mediators also contribute to sarcopenia in cirrhotic patients, with an excess of serum ammonia levels one of the main factors involved in this process.[28] Once the liver is the main organ responsible for controlling ammonia levels in the body, the presence of hepatic dysfunction results in increased serum ammonia levels.[28] Hyperammonaemia results in a process referred to as nitration, which damages protein cell functioning when the serum ammonia levels are increased.[28] In an attempt to degrade the damaged proteins of the skeletal muscle, a process of cellular autophagy may be initiated; however, the autophagy process may not work properly in specific diseases[28] and is associated with tissue damage in hepatic cirrhosis.

In contrast to sarcopenia, cachexia is defined as a concomitant loss of muscle and fat tissues.[22] Cachexia has also been associated with malnutrition, which is intensified by the high resting energy demand and is not supplied because of an inadequate absorption of nutrients in cirrhotic patients.[24]

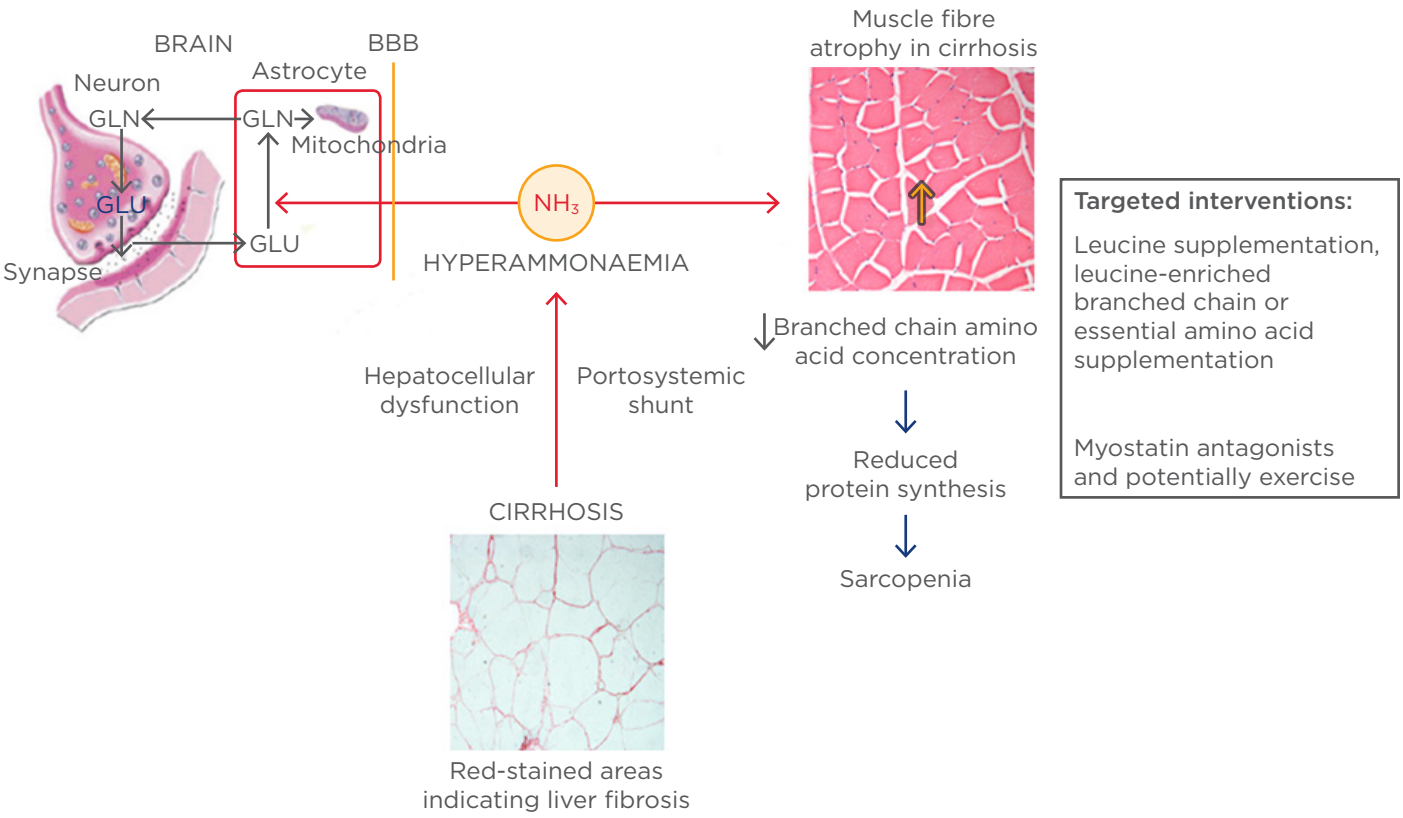

Figure 1: Alterations caused by ammonia in cirrhosis and its targeted interventions.
BBB: blood brain barrier; GLU: glutamate; GLN: glutamine.
Adapted from Dasarathy[22] and Córdoba and Mínguez.[29]

Furthermore, the physiological state of hypermetabolism in association with bacterial translocation episodes activates the production of proinflammatory cytokines in cirrhotic patients, leading to protein catabolism and decreases nutrient absorption.[24]

Figure 1, modified from Dasarathy[22] and Córdoba and Mínguez,[29] shows the increase in the serum ammonia levels as a result of hepatocellular damage. Ammonia may damage astrocytes when it crosses the blood-brain barrier, thereby damaging mitochondria and the transport and metabolism of glutamate and glutamine. The presence of ammonia in the systemic circulation may also damage the muscular tissue, thereby interfering in protein synthesis and causing sarcopenia. Physical exercise and the use of myostatin antagonists appear to be potential therapeutic approaches to revert sarcopenia, as well as leucine supplementation, once there is a low level of available amino acids in the body of patients with hepatic dysfunction.

PHYSICAL EXERCISE AND LIVER DISEASE

Gas exchange abnormalities are associated with cirrhosis and are evident in HPS patients, which, thus, influence their exercise tolerance. HPS patients exhibit a low maximal oxygen consumption (VO_{2max}), worse results in the 6 minute walk test, and lower respiratory muscular strength compared with cirrhotic patients who do not have an HPS diagnosis.[30] Furthermore, cirrhotic patients who have an increased $AaDO_2$ have earlier ventilatory thresholds and worse gas exchange during maximal efforts.[31]

The muscular and respiratory weakness of cirrhotic patients negatively affects their exercise tolerance and functionality. Andersen et al.[32] stated that patients with alcoholic cirrhosis have decreased lower and upper limb strength compared with healthy subjects. Tarter et al.[33] have also demonstrated that cirrhotic patients have lower strength capacities of the lower and upper limbs when both concentric and eccentric contractions were assessed. Moreover, the decrease in peripheral

muscle strength may affect the respiratory muscle strength. Kaltsakas et al.[34] and Abdel-bary et al.[35] identified a negative correlation between the respiratory muscle strength and dyspnoea in cirrhotic patients. Galant et al.[36] identified a correlation between the VO_{2max} and respiratory strength and a decrease in the quality of life, which suggests an interaction between physiological factors and decreased functionality in cirrhotic patients.

Different studies that investigated physical exercise as a therapeutic approach for cirrhosis suggest that these patients may benefit from exercise.[37-39] Child–Pugh Class A and B patients underwent an 8-week aerobic exercise programme on a cycle ergometer, which indicated an increase in the quadriceps mass via ultrasound analysis and VO_{2max} improvement.[37] Another study, which included cirrhotic patients from a waiting list for liver transplantation, demonstrated improvements in VO_{2max}, knee extensor maximal strength, and 6-minute walk distance following a 12-week programme of aerobic and strength exercises.[38] Furthermore, cirrhotic patients with a model for end-stage liver disease <25 improved their 6-minute walk distance, step test values, and muscle mass following a 12-week moderate exercise programme on a treadmill and bicycle, which was associated with leucine supplementation.[39]

CONCLUSION

Physiological alterations present in HPS patients are similar to the alterations present in experimental models of BDL. The clinical features of intrapulmonary vasodilation and gas exchange abnormalities are better understood when investigated in combination with the antioxidant system, inflammatory processes associated with bacterial translocation, and the presence of portosystemic and intrapulmonary shunts. HPS, and other diseases that affect the hepatic system, may contribute to the development of sarcopenia and cachexia because of hyperammonaemia, malnutrition, hypermetabolism, and proteolysis. Physical exercise is a potential therapeutic approach to revert existing impairments in the musculoskeletal and pulmonary systems and is an option for the treatment of HPS patients and other individuals with hepatic injury.

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EPIDEMIOLOGY, HEALTH ECONOMIC CONTEXT, AND MANAGEMENT OF CHRONIC KIDNEY DISEASES IN LOW AND MIDDLE-INCOME COUNTRIES: THE CASE OF MOROCCO

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ABSTRACT

Background: There is a significant emerging burden of chronic kidney disease (CKD) and end-stage renal disease (ESRD) in low and middle-income countries. Nonetheless, despite these trends, knowledge of CKD epidemiology and management remains incomplete. This review presents a critical analysis and comparison of the current data related to CKD epidemiology in Morocco and an overview of the health economic context of the management of ESRD.

Main text: In Morocco, the demographic transition occurring as a result of urbanisation, population ageing, and the global epidemic of diabetes exposes a growing number of people with CKD who are consuming a significant proportion of healthcare budgets. While the real prevalence of ESRD may be underestimated due to limited access to medical coverage for a fraction of the population, the growing costs in the face of limited resources may shortly compromise the healthcare system.

Conclusion: Based on the available data, the prevalence of CKD may grow during the coming decades, according to the increasing prevalence of its major risk factors (diabetes, hypertension, and older age). Thus, early diagnosis, treatment of the underlying cause, and implementation of preventive measures are fundamental for CKD patients.

Keywords: Chronic kidney disease (CKD), end-stage renal disease (ESRD), renal replacement therapies, incidence, prevalence, epidemiology, low and middle-income countries (LMIC), health economics.

BACKGROUND

There is a significant emerging burden of chronic kidney disease (CKD) and end-stage renal disease (ESRD) in low and middle-income countries (LMIC) such as Morocco.[1-3] Nonetheless, despite these trends, knowledge of CKD epidemiology and management remains incomplete.[4,5] This review presents a critical analysis and comparison of the current data related to CKD epidemiology in Morocco and an overview of the health economic context of the management of ESRD.

DISCUSSION

Prevalence of Chronic Kidney Disease

Based upon representative samples of the Moroccan population, researchers from the MAREMAR Study[6] have estimated the prevalence of CKD in the general population through the measurement of markers of kidney damage, such as elevated serum creatinine concentration, decreased estimated glomerular filtration rate using the modification of diet in renal disease equation, and the presence of albuminuria or haematuria. The screening was conducted in a sample of 10,524 adults, aged from 26–70 years, in two Moroccan cities: El Jadida and Khemisset. The study reported an overall CKD prevalence (not adjusted for age) of 6.6% and an adjusted prevalence according to the Moroccan population distribution of 2.9%, with the following age-specific rates: 26–40 years: 0.5%, 41–55 years: 1.3%, and 56–70 years: 6.6%. If these percentages are extrapolated to the overall Moroccan population of 33 million,[7] considering its age composition, the total number of people who have CKD could be estimated at 957,000.

Risk Factors of Chronic Kidney Disease in the Moroccan Population

Epidemiology reveals an association between a number of clinical characteristics and the development of CKD. For many potential risk factors, the supporting evidence is inconclusive, of poor methodological quality, or does not clearly establish a causal relationship.[1,8-10] In the MAREMAR study,[6] the main aetiologies of CKD were diabetes (32.79%), hypertension (28.2%), and nephrolithiasis (9.8%). The authors also reported that 32.8% of the participants were diabetic and 28.2% were hypertensive. Also, it is important to acknowledge that because of the cross-sectional design of the MAREMAR study, it cannot definitively indicate the real aetiology of CKD, rather, it highlights an association with the disease.

In the general population, the crude prevalence of diabetes within different communities in Morocco is 6.1%, and the prevalence of hypertension is estimated at 33.6% (30.2% for men and 37.0% for women), as reported in a national survey of cardiovascular risk factors in Morocco,[11] with an expected continuous and significant increase in the diabetes population in upcoming years. For this reason, in Morocco, with the collaboration of the World Health Organization (WHO), a national diabetes prevention and management plan has been launched in order to reduce diabetes-related morbidity and mortality. This is in accordance with the epidemiological data in Arab countries provided by the WHO. The prevalence of diabetes, hypertension, and other cardiovascular risk factors has increased significantly in the last two decades, from 3% prior to 1980, to a current prevalence >10% of the adult population in most Arab countries (United Arab Emirates [19.5%], Saudi Arabia [16.7%], Bahrain [15.2%], and Kuwait [14.4%]).[9,12]

Aetiologies of End-Stage Renal Disease in the Moroccan Population

Based on partial data reported in the Moroccan registry of renal disease (MAGREDIAL),[6,13] diabetes (25–43%), and hypertension and vascular diseases (11–21%) are the two major underlying renal diseases of ESRD in 2013, while glomerulonephritis represents only 4–10%, chronic interstitial nephritis 4–10%, and polycystic and other hereditary diseases account for <3%. However, unknown or missing causes of ESRD are more often reported (25–31%) and this implies the need for in-depth investigation to identify the exact pattern of primary diseases of ESRD.[14] During past years, Morocco has undergone rapid changes in major social and economic determinants of health and living standards, and experienced a rapid demographic transition resulting in an increase in the number of adults and elderly people.[7] This has led to a shift in the major causes of morbidity and mortality in the Moroccan population, from infectious diseases to chronic non-communicable diseases. Furthermore, the pattern of aetiologies of ESRD in Morocco is not different from that in developed countries,[1,4,5,9,15,16] where the prevalence and incidence of diabetes and cardiovascular disease are increasing; this is consistent with improvement in the living standards and urbanisation in Morocco. This pattern, which is similar to that of other North African and Arabic areas,[4,9,13,16,17] contrasts with that observed in the sub-Saharan countries,[18] where glomerulonephritis, hypertensive nephrosclerosis, and infectious nephropathies are the leading causes.[1,8,10,19]

Differences in ESRD aetiologies between Morocco and sub-Saharan African countries can also be explained by certain genetic factors; genetic polymorphisms of *APOL1* and *MYH9* genes are highly associated with kidney disease predisposition, initiation, and progression. These

are more common in people of sub-Saharan African ancestry in comparison to members of the North African population of Arab-Berber ancestry.[3]

Incidence and Prevalence of End-Stage Renal Disease and Renal Replacement Therapies

In Morocco, and in Northern Africa in general, national epidemiological data about CKD and ESRD are still lacking or incomplete.[3-5,8,9,16,20] Such information is essential because the data collected in the American and European populations may not be applicable to those in this geographic area. Data available on the exact prevalence of CKD in LMIC are very limited. In reviewing recent literature, it was found that there is no country with up-to-date information on the epidemiology of CKD. Most of the available information comes from small sample size and single-centre studies and is based on estimations rather than solid epidemiological investigations, which can be biased by obvious factors.[3,9] The reported age-standardised global prevalence of CKD in LMIC ranged from 5.3% in Pakistan and 7% in South Africa to 10% in Nigeria and 12% in Iran.[1-3] This wide range of prevalences may reflect not only the uncertain national statistics[20] but also the discrepancy regarding the methodology to define CKD between countries.[20]

Regarding the annual incidence of ESRD in Morocco, it has been estimated at 42 and 48 cases per million population (pmp) in the survey reported by the Moroccan Society of Nephrology (MSN), which was conducted in the cities of Tanger-Tetouan-Al Hoceima and Oujda, Morocco.[13] The annual incidence in most North African countries was reported and estimated to be 74–200 pmp[3,5,20] ranging from 98–198 pmp per year in registries from developed countries.[15,21,22] There is no reason to believe that the incidence of ESRD will be lower in Morocco than data reported from countries with a close resemblance in ethnic-genetic background, CKD risk factors, and socioeconomic standards.[3] These disparities can be explained by the fact that the reported ESRD incidence refers to patients on renal replacement therapy (RRT), which is often used as a proxy measure for ESRD;[21,23-25] however, this figure is an underestimate, as it does not include people who are being managed conservatively, or who do not have access to RRT facilities.[10,24,26,27] In addition, many patients with untreated and possibly undiagnosed ESRD are not counted. This could result in an underestimated incidence of ESRD. Taking a conservative estimate of ESRD incidence of 50–100 pmp/year and a population of 33 million,[7] an estimated 1,650–3,300 new patients are likely to reach ESRD every year in Morocco. This is consistent with the continuous increase of treated ESRD on dialysis, as reported in the MAGREDIAL registry; the dialysis prevalence rate has more than doubled over the last 10 years, from 100 pmp in 2000, to 161 pmp in 2005, and 323 pmp in 2010.[13] Therefore, according to the data provided by the MSN, there were ˜16,950 patients receiving RRT in Morocco at the end of 2014, giving a national population prevalence of 530 pmp. According to MAGREDIAL data,[6,13] in-centre haemodialysis is the most prevalent modality of RRT in Morocco (99%); the use of peritoneal dialysis is still very low (<0.5%) and can largely be explained by some non-medical, mainly economic, factors, such as reimbursement strategies, which are not well adapted for dialysis clinics and healthcare providers, but also by the limited numbers of peritoneal dialysis training centres.

From 2001–2013, the population of patients who have undergone renal transplantation was estimated at 250 patients and represented only 0.5% of the total RRT population (approximately 7 pmp), as opposed to 50% in Denmark,[15,28] 40% in France,[15,28] 24% in Brazil,[27] 43% in Iran,[17] and 44% in Sudan.[5,9] The low rate of kidney transplants in Morocco may be a consequence of the sociocultural characteristics of the population and variety of structural factors, such as limited education and awareness, and the lack of financial support for kidney transplantation until recently, with the willingness of the government to expand the renal transplant programme.[13] In Morocco, renal transplantation is available in only five academic centres, with the potential capacity to undergo 50 transplantations per centre per year,[6,13] which is far from an actual rate. To reverse the current trends, the implementation of suitable reimbursement strategies for renal transplantation is needed, as well as an increase in the investment in the training of young nephrology fellows and education programmes for patients and other non-nephrological healthcare providers. To achieve these goals, academic training centres, which often consider transplantation to be a high-level priority, must have more financial support to invest in research and training related to this modality of RRT.

Despite a probable heavy burden of ESRD in LMIC, relatively few patients receive RRT; the corresponding figures in Japan[17,21] and the USA[29] are 1,400 pmp and 1,100 pmp, respectively.

The average is 1,010 pmp in European countries,[15,28] 766 pmp in Tunisia,[13] 407 pmp in Algeria,[13] and 480 in Qatar,[9] which suggests that ESRD may be either underdiagnosed and/or undertreated in Morocco as in many LMIC.[1,3,4,30] International variation in the number of people receiving RRT might reflect underlying differences in CKD incidence,[1,8,16,29,31] due to differing risk factors, genetics, or environmental factors. However, it also may be explained by the limited economic capacity of some developing countries to provide highly expensive interventions such as RRT.[1,21,22,32] Indeed, several studies have suggested a relationship between some macroeconomic factors such as the gross domestic product (GDP) per capita (as a measure of national wealth) and either the incidence or prevalence of RRT.[21,33]

Financing Model of the Healthcare System and Economic Impact of ESRD

Apart from the morbidity, mortality, and poor quality of life engendered by CKD and ESRD, these diseases impose high direct and indirect costs to society.[29,34,35] The financing of the healthcare system in Morocco represents a total health expenditure, as the sum of public and private global expenses, of 6% of the GDP in the period 2011–2015[36] and a total expenditure on health per capita at $189 in 2013.[36] A considerable amount of this funding is spent for ESRD. Indeed, it was estimated that 12% of the health expenditure was used for reimbursement of dialysis sessions, while ESRD patients represent only 4% of the general population.[37] The Moroccan healthcare system includes a mix of public and private financing and delivery, and the government of Morocco introduced two reforms in 2005 to expand health insurance coverage.[38] The first is a payroll-based mandatory health insurance system, l'Assurance Maladie Obligatoire (AMO),[37] for public sector employees, including eight public-mutual insurers under the co-ordination of La Caisse Nationale des Organismes de Prevoyance Sociale (CNOPS), in addition to the compulsory social security system, La Caisse Nationale de Securité Sociale (CNSS), for formal employees in the private sector. The second system is Le Regime d'Assistance Medicale (RAMED), a publicly financed fund to cover services for the poor.[37,38] In addition, there are private-sector mutuals or insurances, which provide insurance to employees and their families, and cover ˜3% of the entire population. Another 3% is covered by private non-mutual insurance. In this context, the Moroccan dialysis care system is currently organised around two sectors, the public and private sector.

The public sector, with the healthcare resources of the Ministry of Health (MOH) as the most important provider, and the private sector, which is made up of two sub-sectors. One sub-sector is non-profit, grouping the health resources of the CNSS; the second sub-sector is for-profit, which made up of the healthcare structures of the free market sector, organised individually or grouped together, by nephrologists. Thus, there are ˜218 dialysis centres in Morocco, which provide treatment for 16,950 patients. The MOH offers the largest network of these facilities, with 105 centres and 5,300 patients, located in provincial hospitals in major cities. Over recent years, the private sector has registered an impressive growth in both treatment capacity and patients treated, with 9,400 patients treated across 113 centres. Dialysis in the private centres is accessible for dialysis patients who are included in:

- The AMO medical coverage (CNOPS, CNSS)
- The RAMED regime: publicly funded dialysis sessions through a public-private partnership
- Private health insurance and some mutual internal regimes
- Patients without medical coverage paying out of pocket

In addition, there are 45 not-for-profit centres, which provide dialysis for 2,300 patients who are unable to enter the public programme or afford private dialysis. Between 2004 and 2015, as a result of rapid economic growth in Morocco and the change in government policy,[5,6,13,37] the rate of medical coverage has been considerably improved, from 16% in 2005 to 53% in 2013. Furthermore, dialysis treatment access rates patients increased more than three-fold, and equipment, including the latest-generation medical devices, dialysis machines, and biocompatible synthetic dialysers is available in all public and private units.[13,25] However, there are no official data regarding the adequacy of dialysis schedules and therapy targets. Due to economic constraints, the most prevalent practice in public and charity centres is to provide two haemodialysis sessions per week. According to the survey conducted by the MSN in four cities,[13] only 53% of patients are on a three sessions per week schedule, and most patients without full health insurance are on a schedule of one to two sessions per week. Although haemodialysis is covered to some extent in some rural zones with no dialysis facilities, patients

may be responsible for indirect costs (travel cost, loss of wages), which may also limit their dialysis attendance. In the same study,[13] it was reported that the rate of erythropoietin use in prevalent haemodialysis patients is only 33.4%, with mean haemoglobin at 8.3 g/dL. These differences can be explained by the benefit package disparities between health coverage systems, as governmental support for dialysis via RAMED in public facilities or in private centres[37] is based on a basic medical coverage, which includes only dialysis with a cap of 10 sessions per month. This did not cover the care of comorbidities, including anaemia, until recently; hence, the management of anaemia in this category of patients was mainly based on blood transfusion. In contrast, for patients with CNOPS or CNSS coverage, or under some internal regimes mutual, erythropoietin is regularly provided without charges.

CONCLUSION

In Morocco, the demographic transition occurring as a result of urbanisation and population age increase, and the global diabetes epidemic, exposes a growing number of people to an increased risk of CKD, who are consuming a significant proportion of healthcare budgets.[4-6,13] While the real prevalence of ESRD may be underestimated because of limited access to medical coverage for a fraction of the population,[13,37] the growing costs in the face of limited resources may shortly compromise the healthcare system. As in most countries, measures for the prevention and treatment of chronic nephropathies and kidney disease risk factors are urgently needed.

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THE IMPACT OF VITRIFICATION IN ARTIFICIAL REPRODUCTIVE TECHNOLOGY PROGRAMMES

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ABSTRACT

Cryopreservation is an integral part of the current methods of assisted reproductive technology (ART). In the past two decades, slow freezing has been replaced worldwide by vitrification due to its association with improved survival rates and clinical outcomes comparable to fresh embryo transfers. Successful embryo vitrification programmes have led to a significant reduction in the incidences of two major complications of ART: ovarian hyperstimulation syndrome and multiple gestations. Multiple embryo transfer cycles from the single ovum aspiration cycle have had a cumulative effect on the numbers of live births. Oocyte vitrification has also helped women to delay their pregnancies for medical or social reasons. This has made oocyte banking a viable option for better synchronisation of oocyte donation programmes. The emerging field of ovarian tissue vitrification has made fertility preservation possible for women undergoing gonadotoxic therapy. In this review, we have discussed the basic principles and methodology of slow freezing and vitrification along with its need and impact on ART.

Keywords: Cryopreservation, embryo vitrification, fertility preservation, oocyte vitrification, ovarian hyperstimulation syndrome (OHSS), vitrification.

INTRODUCTION

The term vitrification is derived from the Latin word '*vitreum*' meaning 'glass'. It describes the process of cryopreservation using high initial concentrations of cryoprotectant and ultrarapid cooling to solidify the cell into a glass-like state without the formation of ice. Ever since the birth of the first baby from a frozen embryo in 1983, and the first pregnancy from a frozen oocyte in 1986, interest in cryopreservation techniques has grown tremendously. Over the past 30 years, two main techniques of cryopreservation have been used in clinical practice; namely, slow freezing (SF) and vitrification. Due to better success rates, SF has been replaced by vitrification in most centres across the world for the cryopreservation of embryos and oocytes, and, in certain circumstances, for ovarian tissue.[1-3]

Since the introduction of vitrification, the number of frozen embryo cycles has increased tremendously across the world, increasing the cumulative success rates of assisted reproductive technologies (ART) without the fear of complications such as ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. According to the 2010 report of The International Committee for Monitoring Assisted Reproductive Technologies (ICMART), the number of frozen embryo transfer (FET) cycles increased substantially during 2008–2010, from 204,427 cycles in 2008 to 260,861 cycles in 2010, amounting to a 27.6% increase.[4] The percentage of frozen thaw cycles compared to autologous initiated cycles (fresh and frozen) increased from 22.4% in 2008 to 26.5% in 2010. Japan, followed by the USA and Australia, conducted the largest number of FET cycles. In some countries, such as Switzerland, Finland, the Netherlands, Sweden, and Iceland, the proportion of cryopreserved embryo transfers is >50%.[4] The differences in the number of FET cycles among European countries are mainly due to policies requiring fewer numbers of embryos to be transferred which, in turn, have led to supernumerary embryos available for cryopreservation.

THE PRINCIPLE OF CRYOBIOLOGY

The basic principle of cryobiology is to shift the pendulum from cell death to immortality at low temperatures. This can be achieved by eliminating the two main causes of cell death (ice formation[5] and lethal concentrations of solutes[6]) while maintaining the functional capacity of intracellular organelles. There are a number of cryoprotectants that, upon exposure, facilitate the expulsion of intracellular water and thus reduce the intracellular ice formation. This is achieved either by permeating the cell membrane and displacing water via an osmotic gradient (using propanediol, dimethyl sulfoxide, or ethylene glycol), and/or by providing a continuous gradient without permeating the membrane (using sucrose). Permeating cryoprotectants also balance other intracellular solutes, which are lethal at high concentrations. These hyperosmotic, lethal conditions also occur in the domains between ice crystals in the extracellular environment as the temperature is reduced and, as a consequence, further dehydration of the cell occurs. The extent of dehydration depends on the rate at which the temperature is decreased[7] and the permeability of the cell(s) to water.[8] These are fundamental principles in cryopreservation that are applied in different ways in SF and vitrification.

SLOW FREEZING

In SF, dehydration of both oocytes and embryos is carried out without excessive shrinkage by exposure to permeating and nonpermeating cryoprotectants. During SF, the dehydration process is thought to continue until -30°C, after which any remaining water is super cooled.[9] In SF, equilibration of oocytes or embryos is carried out by exposure to one or more dehydrating solutions for 10–15 minutes before loading into plastic straws, which are then heat sealed and placed in the chamber of a programmable freezing machine that slowly (in increments of ˜0.3°C) reduces the temperature to ˜-30°C. During this process, ice nucleation (seeding) is induced manually between -5°C and -8°C. Upon reaching the temperature of -30°C, a rapid temperature reduction (at ˜-50°C/min) to -150°C is carried out before storing the straws in liquid nitrogen (LN_2). While thawing, a rapid temperature rise followed by rehydration with decreasing concentrations of permeating and non-permeating cryoprotectants is carried out.

VITRIFICATION

Vitrification is also based on the principle of dehydration. In contrast to SF, vitrification allows solidification of the cell(s) and the extracellular milieu into a glass-like state without the formation of ice. To achieve successful vitrification, a reduction in water content and a highly viscous cytoplasm are necessary.[10] This is achieved by exposure to high concentrations of permeating and non-permeating cryoprotectants, which leads to extreme cellular shrinkage. In order to minimise the impact of the hyperosmotic conditions, the exposure time is reduced to 1 minute.[11] This approach was first introduced in human embryology for cleavage stage embryos[12] and then for oocytes[13] and pronuclear stage embryos.[14,15]

The major concerns associated with exposure to a high concentration of a single permeating cryoprotectant are lethal effects[16] or impaired development,[17] and are dealt with by combining multiple cryoprotectants[18] to reduce the individual cryoprotectant toxicity while achieving a highly viscous solution.[16] In practice, vitrification is routinely achieved by exposure of oocytes or embryos initially to low concentrations of permeating cryoprotectants (commonly combinations of ethylene glycol, dimethyl sulfoxide, or propanediol), followed by a short (≤1 minute) exposure to high concentrations before loading onto a variety of carriers (Cryotop,[19] Cryoloop,[20] Hemi straw,[21] and Flexipet[22]) in a very small (0.1–2.0 mL) volume to ease the rapid cooling process. Rapid reduction in temperature (>10,000°C/minute) is achieved by immediate exposure to LN_2, in either an open or closed system. Equivalent rapid warming is also required, which is followed by stepwise rehydration.

In the open method, embryos and oocytes are directly exposed to LN_2 to increase the cooling/warming rates and thereby improving the efficiency of the procedure.[23] The concern with the open method is that embryos and oocytes are not safely protected from further contact and potential cross-contamination during storage. As an alternative, closed devices have been devised to avoid direct contact of the samples with LN_2 during vitrification and storage.[24,25] It should be noted that not all closed systems available commercially are completely free of any possible sources of contamination.[23] Furthermore, a slower cooling/warming rate in closed systems can have an effect on the survival and success rates. There is no report, for open or closed systems, showing

disease transmission during vitrification.[26] However, to ensure biosafety during cryopreservation, it is always advisable to use sterile approaches,[27] provided there is no compromise on adequate cooling and, particularly, warming rates.

Vitrification requires a long learning process and intense focus of the embryologist performing the task. Even though primarily there are just two steps, equilibration and vitrification, within each step timing control has proved to be critical. As the manual process has stringent skill requirements, and variation in success rate and cell survival rate is observed significantly across operators, the new era of research is being directed toward using a robotic approach for automated vitrification and thawing of embryos. In 2015, Liu et al.[28] reported the first robotic system, RoboVitri, for vitrification of mammalian embryos.

EMBRYO VITRIFICATION

Since the report of the first live birth using vitrified-warmed cleavage stage embryos in 1998 by Mukaida et al.,[12] followed by the first vitrified blastocyst pregnancy in 2000 described by Yokota et al.,[29] there has been continuous research and advances in the field of embryo vitrification.

Indications

Surplus embryos

When *in vitro* fertilisation (IVF) was first introduced, fresh embryo transfer (ET) was the norm. However, to avoid risk of multiple pregnancies and to prevent wastage of supernumerary embryos, cryopreservation became an essential part of ART.

Segmentation of cycle

One of the lethal complications of ovulation induction and ART is OHSS. A new strategy, proposed by Devroey et al.,[30] comprising of a planned 'freeze all' of all embryos in a fresh cycle and transfer in a subsequent frozen embryo cycle, has led to the concept of OHSS-free clinics.[31] The other indications for segmentation, when pregnancy rates are compromised in fresh transfer, are progesterone elevation on day of trigger, a poor or thin endometrium during stimulation, a significant endometrial polyp, or fluid in the endometrial cavity.

Preimplantation genetic screening/ preimplantation genetic diagnosis

Preimplantation genetic screening/preimplantation genetic diagnosis involves testing an embryo for chromosomal or specific monogenic disorders before ET. This requires a biopsy of an embryo on Day 3 or Day 5. It is indicated for use in patients with known genetic disorders, recurrent miscarriages, recurrent implantation failure, advanced maternal age, and severe male factor infertility. To avoid the probabilities of mosaicism in Day 3 embryos, trophectoderm biopsy of Day 5/6 embryos has become regular practice. Since the embryos may take more than 5 days to become suitable for biopsy and the biopsy results may take some time, freezing of all of the biopsied blastocysts with FET in a subsequent cycle is a preferred option.[32]

Cryo-pooling of embryos

In poor responders, especially those with advanced maternal age, embryos of 2–3 cycles are pooled to collect sufficient blastocysts that can be tested for preimplantation genetic screening. Transferring the genetically normal embryos increases the pregnancy rate.

'Freeze all' for all

The objective of the 'freeze all' policy is to replace the embryos in a more favourable intrauterine environment, without possible adverse effects of supraphysiologic hormone levels over the endometrial receptivity.[33] This concept is strengthened by the results of a recent meta-analysis by Roque et al.,[34] which concluded that the use of FET, compared with fresh ET, significantly improved clinical and ongoing pregnancy rates. The authors showed a substantial increase of 32% in the ongoing pregnancy rate when FET was performed compared to fresh ET. However, this conclusion should be cautiously interpreted as only three randomised control trials (RCT) were included in the meta-analysis, with a total of 633 cycles. Secondly, the cryopreservation technique and stage of vitrification differed in studies, and, thirdly, all studies included good prognosis patients. To date, there is a lack of high-quality RCT detailing 'freeze all' for all, but it seems to hold potential for the future in ART.[35] However, it does rely heavily on a good vitrification technique.

SUCCESS RATES OF EMBRYO VITRIFICATION

Vitrification is reported to be associated with a significantly increased post-thaw survival rate compared with SF in two literature reviews and meta-analyses.[27,36] A recent meta-analysis from

seven RCT revealed a significantly higher embryo cryosurvival following vitrification compared to SF (relative risk: -1.59; 95% confidence interval: 1.30–1.93).[26] With vitrification, the reported post-thaw survival rates for blastocysts were 94.5% compared to 21.4% with SF. Similarly, for cleavage stage embryos the post-thaw survival rates were 91.5% and 49.8% for vitrification and SF, respectively.[37]

In terms of pregnancy outcomes of vitrified-warmed versus slow frozen thawed embryos, a study by Stehlik et al.[38] showed comparable results for blastocyst transfers (53% and 51%, respectively), while Rama Raju et al.[39] showed more promising results for eight-cell embryos (35% and 17.4%, respectively). However, in 2014 Li et al.[40] reported a significantly higher clinical pregnancy rate (CPR) per thaw cycle with vitrified warmed blastocysts compared to slow frozen blastocysts (31.3% and 21.5%, respectively). In the study, the likelihood of a live delivery per thaw cycle was found to be 47% higher for vitrified blastocyst transfer cycles compared with SF blastocyst transfer cycles.[40] A recent meta-analysis, including three RCT reporting CPR, also found a higher CPR per cycle with embryo vitrification compared to SF (relative risk: 1.89; 95% confidence interval: 1.00–3.59).[26]

SAFETY OF EMBRYO CRYOPRESERVATION

Few studies have compared the impact of vitrified thawed ET on obstetric and neonatal outcomes.[41,42] Shi et al.[41] did not find any significant difference in obstetric and perinatal outcomes when vitrified ET were compared to fresh ET, but it was mentioned that mean birth weight was higher in the vitrified group compared to the fresh ET group.[41] Recently, Belva et al.[43] analysed 1,072 pregnancies occurring after embryo vitrification on Day 3 and Day 5 and concluded that neonatal health parameters, including the prevalence of congenital malformations (3.4% in the vitrified ET group and 3.9% in the fresh ET group), were similar to, or slightly better than, those after fresh ET.[43] Though the perinatal outcomes are promising after transferring vitrified warmed cycles, a few recent studies have raised concerns about an increased risk of 'large for gestational age' babies following FET.[44,45]

OOCYTE VITRIFICATION

The first report of a birth after oocyte cryopreservation was published 25 years ago.[46] However, for the last 15 years the overall efficiency has remained low, hampering its widespread application. The large size and spherical shape of oocytes interfere with even distribution of cryoprotective additives. Moreover, some subcellular structures are especially sensitive to cryoinjuries, and the fact that oocytes consist of one cell decreases the chance of recovery from a serious injury. Eventually, improvements in freezing techniques by vitrification did overcome most of these hurdles, now giving pregnancy rates comparable to those following the use of fresh oocytes.

INDICATIONS FOR OOCYTE VITRIFICATION

Oocyte Freezing for Fertility Preservation

Medical reasons

Chemotherapy and radiotherapy are generally gonadotoxic and can lead to premature ovarian failure in the future. Young women suffering from cancer can preserve their fertility by oocyte freezing if referred in time. Even for patients who have less time to start their chemotherapy, there are random start stimulation protocols that have similar results when compared to conventional protocols. Other than cancer, certain women who are carriers of *BRCA-1* and *BRCA-2* requiring prophylactic oophorectomy, who suffer from autoimmune disorders (systemic lupus erythematosus, Behçet's disease, etc.) affecting ovarian reserve, and those prone to premature ovarian failure (mosaic Turner's syndrome, fragile X permutation) may be candidates for oocyte freezing. Women undergoing gender reassignment procedures should also be counselled and given options for fertility preservation, as removal of the ovaries destroys the ability to have genetically related children and the masculinising medications used may lead to diminished fertility.

Social reasons

Oocyte freezing is a viable option for women who want to delay marriage and/or pregnancy for career-related reasons, before their biological clock starts running out. In many developed countries nowadays, companies provide sponsorship for egg freezing so that women can concentrate on their work. As the rate of miscarriage also increases after 30 years of age, freezing eggs gives a woman a chance of having her own healthy genetic child whenever she is prepared for it.

Oocyte Freezing for Ethical Reasons

Until recently, embryo vitrification was banned in Italy. Even though it is now legally allowed, it is not preferred and may be considered as an offence by some followers of certain religions or faiths, such as Catholics.[47] In such circumstances, oocyte vitrification is a viable option rather than discarding surplus embryos.

Non-Retrieval of Sperm

In cases of non-retrieval of sperm on the day of the egg retrieval process, oocytes can be vitrified and then thawed at a later date when sperm is obtained in subsequent testicular biopsies.

Donor Oocyte Programme/Egg Banking

Egg banking negates the need of synchronisation between donor and recipient and hence reduces the anxiety of both the patient and the treating physician. It also helps in giving the recipient wider options for selecting a donor without compromising pregnancy results. The clinical outcomes following fresh versus vitrified oocytes in egg donation programmes have been shown to be comparable in multiple studies, including one RCT.[48-50]

Oocyte Pooling in Poor Responders

In patients with a poor response to ovarian stimulation, oocytes from multiple stimulation cycles can be collected and vitrified. Once an adequate number of oocytes has been reached, intracytoplasmic sperm injection can be performed to create an adequate number of embryos, thereby increasing the inseminated cohort equivalent to that of normal responders, giving better pregnancy rates.[51,52]

SUCCESS RATES

According to current evidence, oocyte survival after vitrification and warming ranged between 84% and 96.7% in different studies.[25,48,49,53,54] The data from donor oocyte vitrification studies show fertilisation rates between 74% and 78%, implantation rates (IR) varying between 26.8% and 40.8%, and CPR per transfer between 33.3% and 65.0%.[48-50] The CPR per thawed oocyte was between 4.5% and 6.0%. Studies comparing clinical outcomes in infertile populations using fresh and vitrified oocytes have shown fertilisation rates in the vitrified group varying between 64.5% and 85.0%, IR between 17% and 41%, CPR per ET between 35% and 57%, and CPR per thawed oocyte between 6.5% and 12.0%.[25,53,54]

The Human Oocyte Preservation Experience (HOPE) Registry was set up in 2008 to measure the clinical outcome of using cryopreserved oocytes. It compared the outcomes of two techniques used for oocyte cryopreservation, SF versus vitrification, and a subgroup analysis was also carried out comparing results between donor and autologous oocytes. In the subgroup analysis using vitrified donor versus vitrified autologous oocytes, IR, CPR, and live birth rate were all significantly lower in the autologous oocyte group (45.8% versus 26.9%, 62.6% versus 30.0%, and 52.1% versus 17.4%, respectively).[55]

Most studies suggest that post-thaw survival rates of vitrified oocytes are superior to those that have undergone SF protocols.[55-57] Only one RCT directly compared pregnancy rates with SF versus vitrified supernumerary oocytes and demonstrated that vitrification resulted in improved oocyte survival (81% versus 67%), fertilisation rate (77% versus 67%), and CPR per thawed oocyte (5.2% versus 1.7%) compared to SF.[58] It has also been suggested that meiotic spindle recovery occurred faster in oocytes that had been vitrified rather than cryopreserved with a SF technique.[59]

A recent review and meta-analysis assessed the efficacy of oocyte vitrification in comparison to fresh oocytes and SF. Five studies were included in the meta-analysis and they concluded that rates of ongoing pregnancy, top-quality embryo, embryo cleavage, and fertilisation rates did not differ between the vitrification and the fresh oocyte groups.[60] The oocyte survival rate and fertilisation rates were higher in vitrified versus SF oocytes. Vitrification also resulted in a higher rate of top-quality embryos (22.4% versus 8.0%) and embryo cleavage rate (Day 2: 64.6% versus 47.7%; Day 3: 53.0% versus 33.3%) when compared with SF.[60]

SAFETY OF OOCYTE VITRIFICATION

Despite concerns regarding spindle abnormalities in cryopreserved oocytes, the incidence of chromosomal abnormalities in human embryos obtained from cryopreserved oocytes is no different than that of control embryos. A study of 200 infants born from 165 vitrified oocyte pregnancies revealed no difference in birth weight or congenital anomalies among those born from vitrified oocytes compared to children conceived after fresh IVF.[61] Though vitrified thawed oocytes have comparable pregnancy results, these results should be taken

with caution as most of the studies come from donor programmes and from patients with a good prognosis, which may not be applicable to all patients.

OVARIAN TISSUE CRYOPRESERVATION

Ovarian tissue can be cryopreserved as cortical tissue biopsies, strips, or the whole ovary. It is an option for patients who require immediate gonadotoxic treatment for aggressive malignancies, when there is no sufficient time to allow the woman to undergo ovarian stimulation, oocyte retrieval, and cryopreservation of oocyte and/or embryos. It is the only option available for fertility preservation in young prepubertal girls or in women with hormone-sensitive malignancies. It may be transplanted back to the original site in the pelvis near the primary blood supply of the ovary (orthotopic) or to an extra pelvic (heterotopic) site, commonly involving subcutaneous tissues such as the forearm and abdomen.

There are two methods of cryopreservation of ovarian tissue: SF and vitrification. Until recently, the method of choice has been SF. However, vitrification is gaining popularity, owing to good results obtained with vitrification of oocyte and embryos. In a systematic comparison of vitrification and SF of ovarian tissue followed by tissue culture to assess subsequent oocyte viability, vitrification was found to have a similar outcome to SF with preservation of the morphologic integrity of the ovarian tissue.[62] Although the survival of oocytes was similar between the two methods, granulosa cell survival and the integrity of the stroma were better with vitrification. To date, there are limited clinical studies on ovarian tissue vitrification, and further comparative studies are needed between SF and vitrification to draw any conclusions.

VITRIFICATION AND CUMULATIVE PREGNANCY OUTCOMES

The average probability of a frozen embryo resulting in a living child lies in the range of 19.7–24%, and, today, babies born from cryopreserved embryos represent approximately half of the total number of babies born from assisted reproduction.[4] It is unquestionable that successful cryopreservation of zygotes/embryos has greatly enhanced the clinical benefits and cumulative conception rates for couples following a single cycle of ovarian stimulation and IVF. Results in the literature, expressed as the augmentation of the delivery rate per oocyte harvest, are between 31% and 34%.[63] The data show that women who had transfers of fresh and frozen embryos experienced considerably improved live birth rates by using their cryopreserved embryos.[64]

SHORTCOMINGS OF VITRIFICATION

Even though there are multiple reports suggesting excellent clinical outcomes post-vitrification thaw transfers, there are many questions regarding probable cryoinjuries, such as spindle deformities, DNA damage, and aberrant genomic imprinting, while cells are being exposed to mechanical, chemical, and thermal stresses during the process.[65] Multiple reports have demonstrated that when possible subcellular damages were analysed, mature oocytes were less tolerant to cooling than embryos. This is due to spindle sensitivity to cryoprotectants and low temperature.[66] Unquestionably, the genomic imprinting of cryopreserved oocytes is affected by a complex relationship among the impaired fertility, ART, and cryopreservation.[67]

CONCLUSION

Vitrification has revolutionised ART practice. Optimised embryo and oocyte cryosurvival rates, and improved clinical outcomes achieved with this technique, have allowed us to give a personalised approach to patients. The concept of OHSS-free clinics and elective single ET, eliminating the chances of multiple pregnancies, has become realistic. Moreover, successful numbers of ET cycles from a single aspiration cycle have reduced the need of multiple stimulation cycles. In addition, oocyte vitrification has made fertility preservation and oocyte banking much more viable options.

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RENAL PHYSIOLOGY AND ROBOTIC UROLOGICAL SURGERY

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ABSTRACT

The use of robotic-assisted laparoscopic techniques has transformed the face of urological surgery in the last decade, with demonstrable benefits over both unassisted laparoscopic and traditional open approaches. For example, robotic-assisted partial nephrectomy is associated with lower morbidity, improved convalescence, reduced postoperative pain, shorter length of hospital stay, and a superior cosmetic result when compared to an open procedure. This review discusses the various perioperative influences on the renal physiology of patients undergoing robotic-assisted urological procedures.

Keywords: Cystectomy, laparoscopic, nephrectomy, prostatectomy, renal physiology, robotic surgery.

PREOPERATIVE INFLUENCES

Preoperative Fasting

In contrast to long-held beliefs, no demonstrable reduction in circulating volume has been shown following periods of prolonged fasting in patients without cardiorespiratory problems.[1] Furthermore, there has been mounting evidence over the last two decades that perioperative fluid restriction during major abdominal surgery improves outcome.[2] According to current recommendations from the European Society of Anaesthesiology (ESA),[3] patients are encouraged to drink clear fluids until 2 hours before induction of anaesthesia.

Drug Therapy

Alpha-adrenoceptor blocking drugs and phosphodiesterase type-5 inhibitors are commonly used to treat the symptoms of prostatism. They have no direct effect on renal function, but can cause hypotension[4] and may indirectly contribute to perioperative renal dysfunction.

PATHOPHYSIOLOGY OF UNDERLYING CONDITIONS

Obstructive Nephropathy

Any obstruction of the urinary tract, e.g. urological malignancy, causes an increase in ureteric and tubular pressure that results in a reduced glomerular filtration rate (GFR). There is a decline in medullary blood flow as a consequence of the increased tubular pressure, which is thought to cause prostaglandin release.[5] Prostaglandins reduce intrarenal vascular resistance and increase renal blood flow (RBF). The direct compression caused by the increased tubular pressure gradually increases the renal vascular resistance (RVR) and RBF declines. This is later exacerbated by an intrinsic preglomerular increase in RVR.[6]

Progressive chronic obstruction may result in tubular atrophy and nephron loss, causing chronic renal insufficiency, which often presents with polyuria due to impaired concentrating ability. Sudden complete obstruction is a potentially reversible cause of acute kidney injury (AKI) if it is recognised and treated early.

Renal Cell Carcinoma

There are common pathological changes in the non-neoplastic kidney in patients with renal cell carcinoma (RCC),[7] and this correlates with the higher rate of chronic kidney disease (CKD) in patients with RCC compared with the general population. It is unknown whether these changes are carcinogenic per se, or if CKD increases circulating carcinogens or produces an immunological defect that causes RCC.

Patients with RCC may have paraneoplastic syndromes that can affect renal function. These include hypertension, the cause of which is multifactorial, but often due to hyper-reninaemia; and hypercalcaemia, which may be the result of bony metastases, or due to paraneoplastic parathyroid hormone production, and can result in nephrogenic diabetes insipidus (NDI) as well as cardiac complications including arrhythmias.[8]

INTRAOPERATIVE INFLUENCES

Partial Nephrectomy

Partial nephrectomy offers superior renal functional outcomes and comparable oncological outcomes when compared with radical nephrectomy[9] in the management of localised small renal masses (i.e. <7 cm).[10] The swifter convalescence and comparative oncological safety of laparoscopic partial nephrectomy (LPN) have been proven, but it remains a technically challenging operation despite advances in techniques, skill, and instruments and as such has not been widely adopted.[11] Robotic-assisted partial nephrectomy (RAPN) has gained rapid popularity because of improved visualisation and relative technical ease. Several studies have suggested a trend towards superiority of RAPN above LPN[12] due to the associated lower morbidity, improved convalescence, reduced postoperative pain, shorter reduced length of hospital stay, and a superior cosmetic result.[13]

Warm ischaemic time during partial nephrectomy impacts negatively on postoperative renal function, and the tumour should be resected within 20 minutes regardless of surgical approach.[14] RAPN is associated with more favourable results than LPN in conversion rate to open or radical surgery, operating time, warm ischaemic time, intraoperative blood loss, change of estimated GFR, and length of stay.[15,16] Rates of detection of positive surgical margins and postoperative renal dysfunction are comparable[16] and long-term renal functional recovery is better following RAPN.

Cystectomy and Urinary Diversion

Traditional diversionary surgery involves the creation of an ileal conduit: an anastomosis is made between the cut ureters and a free section of ileum, which is then brought out to form a urostomy. In the last 30 years, orthotopic neobladder formation has become an increasingly popular approach, in which a spherical 'bladder' is fashioned from a free section of bowel (either ileum, colon, or sigmoid) and connected to the ureters and urethra in place of the native bladder.[17] Patients have to undergo neobladder training but have comparable or better sexual function than those with ileal conduits.

The intestinal mucosa is more permeable to electrolytes than the urothelium. Urinary potassium, chloride, and hydrogen ions may be exchanged with sodium and bicarbonate ions in the bloodstream, risking a hyperchloraemic, hyperkalaemic metabolic acidosis in association with salt loss.[18] Creatinine, urea, and ammonia are also reabsorbed by the intestine. The subsequent additional metabolic and excretory burden means that patients with pre-existing renal and liver dysfunction are precluded from neobladder formation.[19] A significant proportion of patients with neobladders develop hydronephrosis and associated decline in renal function, which should be monitored. However, baseline creatinine may increase even in the absence of hydronephrosis, although the significance of this is unclear.[20]

A recent meta-analysis of robotic-assisted radical cystectomy (RARC) over open radical cystectomy showed it to be a credible alternative with fewer perioperative complications, increased lymph node yields, less estimated blood loss, lower need for transfusions, and shorter length of stay.[21] Operative time is often greater in RARC.

Prostatectomy

Robotic-assisted radical prostatectomy (RARP) has surpassed the open technique as the most common extirpative treatment for prostate cancer. There has been extensive regionalisation of RARP, and an associated improvement in global outcomes in high-volume compared to low-volume centres.[22]

Bleeding or Hypovolaemia

The physiological response to haemorrhage or hypovolaemia involves four principal compensatory mechanisms, which are explained subsequently.

Cardiovascular

Stimulation of baroreceptors in the carotid sinus and aortic arch inhibits the tonic sympathetic activity of the rostral ventrolateral medulla (RVLM) via glossopharyngeal and vagal afferents, producing a reduction in heart rate (HR), myocardial contractility, and vasodilatation. Hypovolaemia-induced reduction in stroke volume is detected as reduced vascular distension by the baroreceptors, whose rate of firing decreases. The subsequent increase in sympathetic outflow from the RVLM increases HR and contractility, and causes venoconstriction of capacitance vessels (increased preload), and arteriolar constriction (increased systemic vascular resistance [SVR]).

Renal

The reduction in renal perfusion pressure (RPP) in hypovolaemia is detected by stretch receptors in the juxtaglomerular apparatus, which causes renin release. Renin has two actions, firstly to stimulate conversion of angiotensinogen to angiotensin, and secondly to stimulate antidiuretic hormone (ADH) secretion.

Following the conversion of angiotensinogen to angiotensin, it is then converted to angiotensin II in the pulmonary circulation by angiotensin-converting enzyme (ACE). Angiotensin II has three functions: i) potent vasoconstriction, increasing SVR and mean arterial pressure; ii) glomerular arteriolar vasoconstriction (efferent>afferent), preserving GFR in the face of falling renal plasma flow. Filtration fraction increases; the hydrostatic pressure within the peritubular capillaries drops further, osmotic pressure increases, and a higher proportion of filtered fluid is reabsorbed from the proximal convoluted tubule (PCT); iii) stimulation of aldosterone secretion. Aldosterone is a mineralocorticoid hormone produced in the zona glomerulosa of the adrenal cortex. Binding of aldosterone to mineralocorticoid receptors in the distal convoluted tubule and collecting ducts causes upregulation of basolateral sodium potassium pumps, producing sodium and water retention (and potassium loss).

Secondly, renin stimulates ADH (vasopressin [V]) secretion. ADH is a hypothalamic hormone that is stored in the posterior pituitary and has two principal mechanisms of action: i) widespread vasoconstriction via action on vascular smooth muscle V1 receptors; and ii) water retention via stimulation of V2 receptors on the basolateral membrane of the collecting duct leading to increased transcription of the aquaporin-2 gene and increased numbers of aquaporin-2 channels in the collecting ducts. The result is the production of low volume, concentrated urine.

As circulating volume decreases, renal cortical blood flow is diverted to the juxtamedullary glomeruli. This preserves perfusion of the most metabolically active and oxygen-dependent nephrons of the outer medulla. Furthermore, the reduction in solute delivery associated with a reduction in RBF lessens the oxygen requirement.

Humoral

Sympathetic activity in response to hypotension and hypovolaemia causes catecholamine release from the adrenal medulla. These neurohumoral agents act directly on the cardiovascular system, increasing HR, myocardial contractility, and SVR, and at the renal vasculature, causing vasoconstriction of the afferent and efferent arterioles in an attempt to maintain RPP. In addition, beta-adrenoceptor stimulation occurs at the PCT, causing sodium reabsorption, and at the juxtaglomerular apparatus, causing renin release.

Baroreceptors, located at the veno-atrial junction in the heart, are tonically active and act to inhibit ADH secretion. In haemorrhage, their rate of firing is reduced and ADH secretion increases as a result.

Osmoreceptors are located principally in the hypothalamus. They detect changes in serum osmolality and regulate ADH secretion and the sensation of thirst.

Hydrostatic

This is the fastest compensatory response. Hypovolaemia results in reduced capillary hydrostatic pressure, which promotes absorption of interstitial fluid into the intravascular space. Starling's[23] hypothesis states that fluid movement due to filtration across the wall of a capillary is dependent on the balance between the hydrostatic pressure gradient (Pc-Pi) and the oncotic pressure gradient (πc-πi) across the capillary.

$$\text{Net filtration pressure} = (Pc - Pi) - (\pi c - \pi i)$$

Factors favouring filtration

- Pc: capillary hydrostatic pressure
- πi: interstitial oncotic pressure

Factors opposing filtration

- Pi: interstitial hydrostatic pressure
- πc: capillary oncotic pressure

Importantly, the patient may be taking various drugs that impair the body's response to hypovolaemia and hypotension, e.g. ACE-inhibitors, beta-blockers, calcium channel blockers, non-steroidal anti-inflammatory drugs (NSAID).

Pneumoperitoneum

The insufflation of gas into the peritoneal cavity is considered essential for adequate exposure and visualisation in laparoscopic techniques. Carbon dioxide is used because it is non-combustible, economical, and highly soluble in blood, making significant gas embolism less problematic. However, it has the downside of causing respiratory acidosis.

Pneumoperitoneum has significant direct and indirect effects on renal physiology.[24] At insufflation pressures >10 mmHg, there is a reproducible, demonstrable reduction in GFR and RBF, and a transient oliguria. The reduction in RBF is directly related to the insufflation pressure and is affected by positioning (reverse Trendelenburg position being the worst), mitigated by fluid therapy, and independent of insufflation gas.[25] Renal function appears to return to pre-existing levels once the pneumoperitoneum is released and may not be of clinical significance; there is no demonstrable associated histological pathology or evidence of renal tubular damage in animal models.[26] Even in animal models with induced renal insufficiency, in whom prolonged pneumoperitoneum results in AKI lasting 1 week, renal function returns to pre-existing levels with no lasting impact on renal function.[27]

Direct effects

- Increased intra-abdominal pressure (IAP) causes increased RVR due to direct compression of the renal parenchyma and vasculature.[28]

$$RBF = \frac{RPP}{RVR}$$

- It follows that as RVR increases, RBF will proportionally decrease. In addition, direct compression of the nephrons reduces GFR.[28]
- Increased IAP compresses the inferior vena cava, producing a reduction in venous return from the lower body and a subsequent fall in stroke volume. Kirsch et al.[29] found that at insufflation pressures of 10 mmHg in rat models, caval flow diminishes by 93% and aortic flow by 46%. This leads to the conclusion that the renal dysfunction is caused mainly by renal vascular insufficiency from central venous compression.
- Importantly, compensatory sympathetic activity that serves to increase SVR and HR may be depressed in the anaesthetised patient. This may be further compromised in the elderly population due to adrenoceptor downregulation.[30]
- Direct ureteric compression does not appear to contribute to the renal effects of pneumoperitoneum, and ureteric stent placement does not mitigate the oliguria.[31]

Indirect effects

- Activation of the renin-angiotensin-aldosterone system (RAAS) in response to compression of the renal parenchyma (the Page effect).[32]
- Reperfusion injury following release of the pneumoperitoneum, as alterations in regional blood flow within the kidney normalise.[33] This oxidant-induced nephrotoxicity is attenuated by the use of anti-oxidants in animal models.[33,34]
- The respiratory acidosis induced by carbon dioxide insufflation can be offset by increasing ventilation. However, this may be difficult in prolonged surgery in the Trendelenburg position due to cephalad displacement of the diaphragm and ventilation-perfusion mismatching.[35] Hypercapnia has direct negatively inotropic effects on the myocardium and causes systemic vasodilation and tachycardia. Additionally, it sensitises the myocardium to arrhythmogenic effects of catecholamines. It is reasonable to conclude that these changes may have an adverse effect on renal perfusion.

Interestingly, insufflation with gas warmed to body temperature is associated with a significantly higher urine output compared to that at room temperature, probably due to local renal vasodilation.[36]

Direct Injury

Hypoperfusion experienced by the operative kidney during RAPN will result in postoperative renal dysfunction, but direct injury to the kidneys may occur during other robotic procedures.

Positioning and Compartment Syndrome

Positioning is key in robotic surgery. Direct pressure on a closed muscle compartment as a result of poor intraoperative positioning (particularly in combination with hypoperfusion due to hypotension) may cause compartment syndrome. The lower legs are at risk in RARP and RARC due to the steep Trendelenburg lithotomy positioning, and muscle breakdown in the back and gluteal

regions may also occur.[37] Some centres routinely release the legs part-way through operations to mitigate such complications.

Compartment syndrome occurs when the pressure within a closed osteofascial compartment exceeds the perfusion pressure, and results in muscle and nerve ischaemia.[38] Normal compartment pressure is 10–12 mmHg. Obstruction to venous outflow, increased capillary pressure, and reduced perfusion leads to ischaemia and infarction. The ischaemic injury causes an inflammatory response, causing capillary leak and worsening the compartmental hypertension.

Muscle ischaemia, damage, and eventual necrosis lead to rhabdomyolysis.[39] There is ATP depletion either due to increased muscle activity or failure of oxygen delivery to the myocyte. This then disrupts cellular transport mechanisms, namely the sodium potassium ATPase pump, the active transport of calcium into the sarcoplasmic reticulum, and electrolyte balance. A rise in intracellular calcium levels results in free radical production, which degrade the myofilaments and damage the cell membrane, resulting in leakage of cell contents (potassium, phosphate, urate, creatine kinase, and myoglobin) into the plasma.

There are four pathophysiological mechanisms by which rhabdomyolysis can cause AKI:[40]

- Myoglobin combines with Tamm–Horsfall protein, leading to the formation of insoluble casts, which cause mechanical tubular obstruction.
- Hyperuricaemia and urinary excretion of uric acid worsens tubular obstruction.
- Degradation of the haem moiety of precipitated myoglobin causes lipid peroxidation and free radical production, both of which are directly nephrotoxic.
- The scavenging effect of myoglobin and relative deficiency of nitric oxide results in inappropriate renal vasoconstriction in the presence of relative hypovolaemia due to third-space loss, which leads to ischaemic injury.

Creatine kinase levels should be monitored and the patient should be referred to critical care early if rhabdomyolysis is suspected. There should be a low threshold for measuring compartment pressures if compartment syndrome is suspected. The compartmental perfusion pressure should be calculated (the difference between diastolic blood pressure and compartment pressure) and a surgical fasciotomy of the affected muscular compartment should be performed without delay if the perfusion pressure is <30 mmHg.

POSTOPERATIVE INFLUENCES

The Physiology of Postoperative Oliguria

The stress response is a humoral response initiated by afferent neuronal impulses from the site of injury directed to the hypothalamus. Additionally, there is a cytokine-mediated inflammatory response.[41] A number of hormonal changes aimed at maintaining normovolaemia occur, which influence renal function. Increased ADH secretion (and oliguria) may continue for 3–5 days, depending on the severity of the surgical insult and whether complications develop. Additionally, renin secretion results in sodium and water retention.[42] The stress response is not significantly altered by the use of minimally invasive surgical techniques, in contrast to the inflammatory response.[41]

Post-Obstructive Diuresis

Between 0.5% and 52.0% of patients exhibit an inappropriate and exaggerated excretion of water and electrolytes following the relief of urinary tract obstruction.[43] Urine production of >200 mL/hour for 2 consecutive hours or >3,000 mL/24 hours is diagnostic of post-obstructive diuresis (POD).[44] Pathophysiologically, several mechanisms are responsible: decreased concentrating ability secondary to vascular washout and downregulation of sodium channels in the thick ascending limb of the loop of Henle, reduction in GFR, leading to ischaemia and juxtamedullary nephron loss, and NDI.

Physiological POD lasts for approximately 24 hours. Pathological POD persists after euvolaemia is attained, and risks hypovolaemia, hypotension, electrolyte disturbance, metabolic acidosis, and death.[44] The patient should be managed in a high-dependency environment with input from a nephrologist and treatment with intravenous fluids and electrolyte replacement. Excessive fluid administration will exacerbate the condition.[44]

Nephrotoxins

There are several classes of drug used perioperatively that have an adverse effect on renal function. Physiological renal autoregulation by the RAAS is prevented by the use of ACE-inhibitors and angiotensin II receptor-blockers. It is debatable whether these drugs should be

continued perioperatively, although a Cochrane review in 2008 failed to demonstrate a benefit.[45]

NSAID inhibit the autoregulatory prostaglandin-induced vasodilation seen in conditions that result in the systemic release of vasoconstrictors, and can result in reduced RBF.[46] This is especially important in those taking concomitant ACE-inhibitors, angiotensin II receptor-blockers, or diuretics. NSAID may also cause direct nephrotoxicity by producing an acute interstitial nephritis or, more rarely, acute papillary necrosis or renal vasculitis. Adverse renal effects are generally reversible on discontinuation of treatment; nonetheless, the Medicines and Healthcare products Regulatory Agency (MHRA) advocates caution when using these drugs in at-risk patients.[47]

Even low-dose aminoglycosides are directly nephrotoxic,[48] leading to acute tubular necrosis, and levels must be monitored carefully. Other antibiotics have been associated with the development of acute interstitial nephritis, e.g. penicillins, cephalosporins, and quinolones.

Contrast-induced (CI)-AKI is the development of AKI within 48 hours of a contrast load.[49] The exact underlying mechanisms are likely to be a combination of direct nephrotoxicity of reactive oxygen species, imbalance of renal vasoconstriction and vasodilation, increased oxygen consumption, contrast-induced diuresis, and increased urinary viscosity.[50] CI-AKI is more likely in elderly patients, those with underlying renal impairment, or those taking nephrotoxic drugs and is directly proportional to contrast load. Oral contrast is less likely to be associated with CI-AKI than intravenous. It is best to avoid contrast perioperatively if possible, and if imaging is warranted to use alternative modalities. If contrast is mandatory, the lowest dose of diluted, non-ionic contrast media should be used, all other nephrotoxic drugs should be stopped, and pre and post-contrast hydration with intravenous normal saline instituted.[50] The benefit of N-acetylcysteine in preventing CI-AKI is still the topic of much debate.

CONCLUSION

The perioperative influences on renal physiology of the most commonplace robotic urological procedures are broad and incorporate physiological, pathological, pharmacological, and surgical effects. Care of these patients necessitates early, effective, multi-disciplinary team input in experienced centres in order to minimise complications and achieve the best outcomes. Careful consideration should be given to hydration, nephrotoxic drugs, blood pressure maintenance, positioning, and surgical techniques to minimise adverse effects on renal function.

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THE ROLE OF INSULIN RESISTANCE ON FGF-21 AND INFLAMMATORY MARKERS IN OBESE ADOLESCENTS UNDERGOING MULTICOMPONENT LONG-TERM WEIGHT LOSS THERAPY

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ABSTRACT

Objective: The purpose of this study was to investigate the effects of a long-term weight loss therapy in two groups (insulin resistance [IR] and non-insulin resistance [non-IR]) of obese adolescents based on metabolic profile, biomarkers of inflammation, and fibroblast growth factor-21 (FGF-21) concentrations.

Methods: Obese adolescents (15–19 years) were randomised into two groups (IR=8 and non-IR=9) and monitored through clinical, exercise training, nutritional, and psychological counselling over 1 year. Measurements of inflammatory biomarkers and FGF-21 were performed. The effects of therapy were verified by two-way ANOVA and post hoc analyses were performed ($\alpha \leq 5\%$).

Results: A reduction in body mass, visceral fat, and an increase in adiponectin in both groups was found. Only the non-IR group demonstrated improved BMI, body fat mass, lean body mass, and waist circumference. Indeed, in the non-IR group, FGF-21 presence was positively correlated with high-density lipoprotein cholesterol and lean body mass and inversely correlated with plasminogen activator inhibitor-1 and triglycerides. In the IR group, there was a reduction in FGF-21 concentration, adiponectin/leptin ratio, insulin, total cholesterol, low-density lipoprotein cholesterol, and plasminogen activator inhibitor-1. FGF-21 was negatively correlated with delta-triglycerides, waist circumference, and low-density lipoprotein cholesterol. The IR prevalence reduced from 47% to 23.5% in the studied population.

Conclusions: Although the multicomponent clinical approach improves, in both analysed groups and in both metabolic and inflammatory states, the presence of IR resulted in a reduction in both FGF-21 concentration and adiponectin/leptin ratio. Additionally, in the IR group, FGF-21 was negatively correlated with

proinflammatory markers, and in the non-IR group it was positively associated with high-density lipoprotein, suggesting its role in the control of inflammation counteracting IR. In this way, we suggest that IR can impair the anti-inflammatory effects of FGF-21. It will be helpful if these results can be confirmed in a large cohort, underlying physiological mechanisms to explore how these results can help in setting up more prospective studies.

Keywords: Insulin resistance (IR), fibroblast growth factor 21 (FGF-21), adiponectin, leptin, obesity, plasminogen activator inhibitor-1 concentration (PAI-1).

INTRODUCTION

Obesity in adolescence urgently needs combatting, since, for the first time, the current generation will have a shorter life span than previous generations. Sedentary lifestyles and a high fat diet are the strongest predictive factors to the alarming increased prevalence of obesity.[1-4] The most prevalent risk factor related to obesity in adolescents, in our clinical practice, is a state of insulin resistance (IR), leading to metabolic syndrome, diabetes, and atherosclerosis.[5]

The IR state is defined by the deficiency of the action of insulin, which culminates in the decrease of the absorption of glucose by the tissues, and the increase of the endogenous production of glucose by the liver. These factors result in a state of hyperglycaemia in both fasted and postprandial conditions. The association between IR and obesity occurs due to several mechanisms that act together. Among the numerous inflammatory cytokines related to obesity, it has been verified that tumour necrosis factor-alpha is capable of promoting alteration in the insulin signalling pathway.[6]

In the investigation the authors are currently carrying out, we consider IR to be a divider of the study group, as this condition is one of the most common metabolic alterations seen in the obese population, which is associated with cardiovascular alterations. In accordance with this comorbidity, the proinflammatory state observed in obesity contributes to this condition, especially considering the hypoadiponectinaemia, hyperleptinaemia, and elevated plasminogen activator inhibitor-1 (PAI-1) concentration.[5,7-9] Moreover, previous experimental investigations have shown that fibroblast growth factor-21 (FGF-21) acts as an upstream regulator of adiponectin release, and exerts its effects on glucose metabolism and insulin sensitivity via adiponectin. It was revealed that FGF-21 increased adiponectin expression by activating peroxisome proliferator-activated receptor-gamma, and displayed various metabolic functions, mainly through adiponectin.[10-12] In human studies, analogues of FGF-21 induced increases in adiponectin levels, suggesting their cardiovascular protective factor.[13,14]

Additionally, leptin was considered a potent metabolic modulator of FGF-21, as seen in experimental studies and research performed *in vivo* with cell culture.[15,16] It suggested FGF-21 was an integrative hormone involved in multiple actions within mammalian organisms.[15,16] The high levels of PAI-1 are associated with an increased cardiovascular risk of thrombosis. As previously described, increases in PAI-1 are undoubtedly related to IR, and the mechanisms that could explain such an increase in the metabolic disorders, related to obesity and metabolic syndrome.[17-19]

FGF-21, a novel hormone-like protein expressed in many tissues, has recently been described as having a key role in the 'browning' of adipocytes, being originally associated with increased secretions by muscle tissues after exercise in both experimental and clinical studies. Thus, FGF-21 is referred to as the thermogenic protein, promoting energy expenditure by converting white adipose tissue to brown adipose tissue as well as activating brown adipose tissue.[20] In humans, the liver mostly secretes FGF-21; however, it is also released by adipocytes from both subcutaneous and visceral adipose tissue. Notably, FGF-21 has abundant adipose tissue receptors, which mediates the browning process.[21] Unfortunately, an excess of circulating FGF-21 may lead to a FGF-21 resistance in obese women, hindering its action.[22]

In addition, FGF-21 exerts beneficial effects on metabolic homoeostasis regulated by adipokines, such as adiponectin, leptin, and resistin. In particular, FGF-21 deficiency, rather than resistance, contributes to development of IR and hypoadiponectinaemia, independently of obesity in young people.[12] Moreover, the beneficial effects of FGF-21 in IR, lipid profile, and energy homeostasis have been demonstrated in clinical

and experimental studies,[13,14,23,24] suggesting a possible new therapeutic target for treating obesity and related comorbidities.[12] However, data on the role of FGF-21 in adolescents with obesity are limited.[25] In this way, we hypothesise that the IR condition present among obese adolescents could promote different metabolic responses in regard to FGF-21 concentrations and related metabolic and inflammatory effects.

Thus, the purpose of this study was firstly to investigate the effects of a long weight loss therapy in two different groups (with and without IR) of obese adolescents on FGF-21 concentration, metabolic profile, and pro/anti-inflammatory adipokines (including adiponectin and leptin) in visceral and subcutaneous adipose tissues. Secondly, this study aimed to verify if IR modulates the role of FGF-21 in obesity in a sample of obese adolescents undergoing long-term multicomponent therapy.

MATERIALS AND METHODS

Population

This preliminary study included 23 post-puberty Brazilian/Caucasian adolescents with obesity, aged 15–19 years (16.13±0.8) including both sexes (13 females and 10 males). Inclusion criteria were Tanner Stage 5,[26] primary obesity, BMI, >95th percentile of the CDC reference growth charts.[27] Non-inclusion criteria were the use of birth control pills, cortisone, anti-epileptic drugs, history of renal disease, alcohol intake, smoking, and secondary obesity due to endocrine disorders.

The adolescents were divided into two groups: presence of IR (n=8) and non-IR (n=9). The reasons for dropping out (n=6) of the study included financial and family problems along with school and job opportunities. No sex difference was observed in adherence rates. The study was conducted with the principles of the Declaration of Helsinki, approved by the ethics committee on research at the Universidade Federal de São Paulo (UNIFESP 152.281), Clinical Trial: NCT01358773. All procedures were clear to those responsible for the volunteers and informed consent forms were signed. All evaluations were performed at two different times (baseline: beginning, and after therapy: after 1 year of interdisciplinary weight loss therapy).

Anthropometric Measurements

Body composition and weight were measured by plethysmography scale (BODPOD equipment),[28] where patients wore minimum clothing where possible. Height was measured using a stadiometer (Sanny-model ES 2030) and BMI was calculated by dividing the weight by height squared (kg/m^2). Waist circumference was obtained at the midpoint between the last rib and iliac crest.

Serum Analysis

Blood samples were collected after an overnight fast in the outpatient clinic at approximately 08:00 am. After collection, the blood was centrifuged for 10 minutes at 5,000 rpm and stored at -70°C for future analyses. The materials used for collection were disposable and adequately labelled. Blood was collected by a skilled and qualified technician. A lipid profile was obtained from the blood. IR was assessed according to the Homeostasis Model Assessment of Insulin Resistance Index (HOMA-IR). The HOMA-IR was calculated as the product of the fasting blood glucose and the immunoreactive insulin levels (fasting blood glucose [µU/mL] × fasting blood glucose [M/22.5]). All of the variables were analysed using a commercial kit (CELM, Barueri, Brazil). Total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were analysed using a commercial kit (CELM). The reference values adopted were insulin (<20 µU/mL), HOMA-IR (>3.16), total cholesterol (<170 mg/dL), LDL cholesterol (<130 mg/dL) as previously described by Schwimmer et al.[29] The PAI-1, adiponectin and leptin were measured by enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, Minnesota, USA), and FGF-21 concentrations were measured using a commercially available MULTIPLEX assay (EMD Millipore, Burlington, Massachusets, USA; HMHMAG-34K).[30] For this study, leptin data were analysed according to reference values.[31]

Visceral and Subcutaneous Adiposity Measurements

Ultrasound measurements of the visceral and subcutaneous fat were taken. All abdominal ultrasonography procedures were performed by the same blinded diagnostic imaging specialist, who used a 3.5 MHz multifrequency transducer (broadband) before and after the intervention. Ultrasound-determined subcutaneous fat was defined as the distance between the skin and

external face of the rectus abdominis muscle; visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. The intra-examination coefficient of variation for ultrasound was 0.8%. The cut-off points for the definition of visceral obesity by ultrasonography were based on previous methodological descriptions produced by Ribeiro-Filho et al.[32]

Descriptive Methodology of Multicomponent Weight Loss Therapy

The study sample was completed by adolescents with obesity within both sexes (n=17). An interdisciplinary group of health professionals conducted all sessions. At three-times per week during 2 non-consecutive hours per day, the adolescents participated in supervised therapy for physical exercise practice, nutrition, and psychological attendances over 1 year (Figure 1).

Clinical Approach

The adolescents with obesity visited the endocrinologist with their parents once each month. The doctor monitored and evaluated all clinical examinations, including the initial medical history and a physical examination of blood pressure, cardiac frequency, and body mass. The adolescents were checked for their adherence to all interdisciplinary therapies (Figure 1).

Aerobic Plus Resistance Training Intervention

The adolescents followed a combined physical exercise training therapy programme. The protocol was performed three-times per week for 1 year and included 30 minutes of aerobic training plus 30 minutes of resistance training per session. The subjects were instructed to reverse the order of the physical exercises (aerobic and resistance) at each training session. The aerobic training consisted of running on a motor-driven treadmill (Life Fitness: model TR 9700HR) or bicycle at a cardiac frequency intensity representing the ventilatory threshold I (±4 bpm), which was determined by the results of an initial oxygen uptake test for aerobic exercises (ergospirometry). The physical exercise therapy and resistance training were based on the guidelines from the American College of Sports Medicine (ACSM) (Figure 1).[33,34]

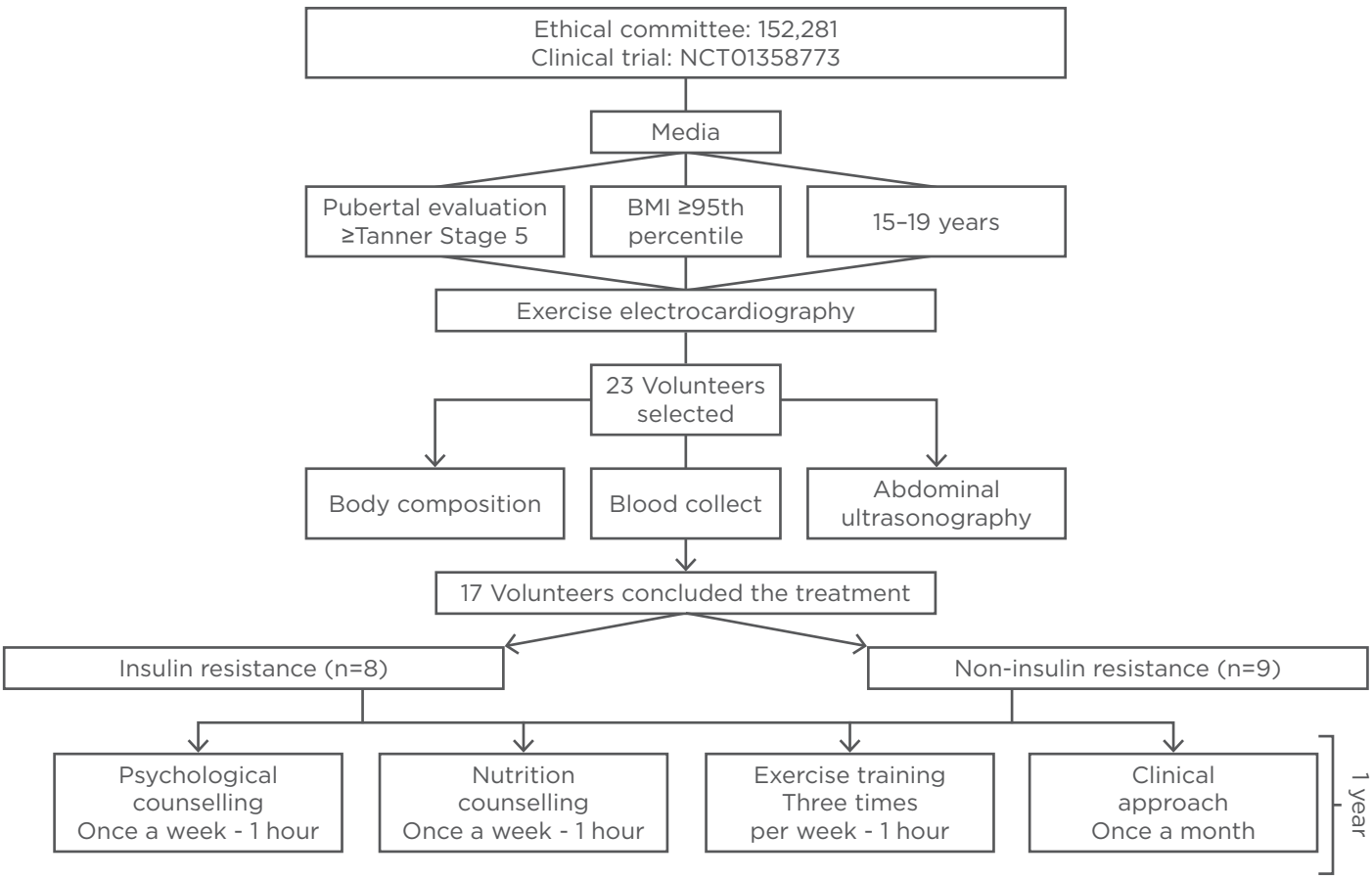

Figure 1: Diagram of interdisciplinary therapy.

Nutrition Counselling

Energy intake was set at levels recommended by the dietary reference intake for subjects with low levels of physical activity of the same age and sex.[35] Once a week, adolescents received dietetic lessons covering the topics related to a healthy eating pattern, including the food pyramid, diet record assessment, weight loss diets and fad diets, food labels, dietetics, fat-free and low-calorie foods, and other related topics. All participants had monthly, individual consultations (Figure 1).

Psychological Counselling

All adolescents participated in weekly psychological orientation group sessions based on the psychodynamic approach with a trained psychologist. Individualised psychological therapy was recommended when it was necessary according to their psychological assessment (Figure 1).

Statistical Analysis

Statistical analysis was performed using the program STATISTICA, version 7.0 for Windows Vista. The adopted significant value was $\alpha \leq 5\%$. Data normality was verified with the Shapiro Wilk test. Parametric data were expressed as mean ± standard deviation (SD), and non-parametric data were expressed as median, minimum, and maximum values. The effects of therapy were verified by two-way ANOVA, Tukey's post hoc analysis for parametric data, and the Wilcoxon test for non-parametric data. Correlation analyses were established through the Pearson's test for parametric data and Spearman's test for non-parametric data ($\alpha \leq 5\%$).

Table 1A: Long-term effects of multicomponent therapy in obese adolescents.

Variables	Insulin resistance group (n=8)			Non-insulin resistance group (n=9)		
	Baseline	After therapy	Δ Value	Baseline	After therapy	Δ Value
Body mass (kg)	97±21	91±19*	-6±5	93±9	88±11*	-6±6
Body mass index (kg/m^2)	36±3	34±2	-2±2	32±3	30±3*	-2±2
Body fat mass (kg)	44±9	37±7	-7±5#	34±8	29±9*	-5±6
Body fat mass (%)	45±5	41±5	-4±3	36±6	33±8*	-3±5
Lean body mass (kg)	54±14	55±14	1±1	60±8	59±8	-1±2
Lean body mass (%)	55±5	59±5	4±3	64±6	67±8*	3±5
Waist circumference (cm)	99±9	96±10	-3±4#	98±8	89±7*	-9±7
Visceral fat (cm)	5±1	4±1*	-1±1	4±1	3±1*	-1±1
Glucose (mg/dL)	91±3	93±8	3±8	91±6	92±7	1±8
Insulin (µU/mL)	18 [14/32]	13 [8/20]*	-6 [-24/4]	11 [8/14]	7 [6/26]	-2 [-6/15]
HOMA-IR	4 [3/8]	3 [2/5]	-1 [-6/2]	2 [2/3]	2 [1/6]	-1 [-1/4]
Total cholesterol (mg/dL)	188±43	166±28*	-21±20	141±41	130±33	-10±17
HDL-cholesterol (mg/dL)	43±7	44±6	1±4	49±10	50±8	0±3
LDL-cholesterol (mg/dL)	123±35	101±22*	-22±21	79±35	69±29	-9±17
Triglycerides (mg/dL)	75 [48/252]	94 [54/264]	9 [-39/29]	45 [35/165]	41 [29/133]	-2 [-32/14]
Adiponectin (ng/mL)	2±1	4±2*	2±2	2±1	4±4*	2±4
Leptin (ng/mL)	24±5	19±7	-5±8	19±14	15±10	-4±8
Plasminogen activator inhibitor-1 (ng/mL)	187±32	152±43*	-35±30	162±63	135±76	-27±61
Fibroblast growth factor 21 (pg/mL)	143±79	77±38*	-68±60	122±79	87±27	-35±80

Statistical significance: $p \leq 0.05$. Reference values: glucose (60–110 mg/dL); insulin (<20 µU/mL); HOMA-IR (>3.16); QUICKI (>0.339) as previously described by Schwimmer et al.[29]
*Statistical difference after therapy in the same group; #statistical difference after therapy between groups.
HDL: high-density lipoprotein; HOMA-IR: Homeostasis Model Assessment Insulin-Index Resistance; LDL: low-density lipoprotein.

Table 1B: Correlations analysis.

	Variables	r	p-value
All groups			
FGF-21 (pg/mL)	Lean body mass (kg)	0.88	0.0008
FGF-21/body lean mass ratio	Waist circumference (cm)	-0.89	0.007
Insulin resistance group			
FGF-21 (pg/mL)	Δ triglycerides (mg/dL)	-0.99	0.02
	Waist circumference (cm)	-0.99	0.01
	LDL-cholesterol (mg/dL)	-0.99	0.02
Non-insulin resistance group			
FGF-21 (pg/mL)	HDL-cholesterol (mg/dL)	0.96	0.03
	PAI-1 (ng/mL)	-0.98	0.01
	Δ triglycerides (mg/dL)	-0.95	0.04
	Lean body mass (kg)	0.99	0.008

Statistical significance: $p \leq 0.05$.
FGF-21: fibroblast growth factor 21; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PAI-1: plasminogen activator inhibitor-1.

RESULTS

All Groups

At baseline, there was no difference observed between groups for body mass and BMI (Table 1A). FGF-21 concentration positively correlated with lean body mass and negatively correlated with waist circumference (Table 1B).

Effects of Interdisciplinary Therapy in the Insulin Resistance Group

In the IR group, body mass, visceral fat, insulin concentration, total cholesterol, LDL cholesterol, PAI-1, FGF-21 (Figure 2A), FGF-21/lean body mass ratio, and adiponectin/leptin ratio (Figure 2B) were reduced. Adiponectin concentration was increased. In the correlation analyses, FGF-21 negatively correlated with delta-triglycerides, waist circumference, and LDL-cholesterol. The IR prevalence reduced from 47% to 23.5% in the study sample.

Effects of Interdisciplinary Therapy in the Non-Insulin Resistance Group

In the non-IR group, there was an improvement in body mass, BMI, body fat mass (kg and %), body lean mass (%), waist circumference, and visceral fat. In this group, adiponectin concentration was increased. In the non-IR group, FGF-21 positively correlated with HDL-cholesterol and lean body mass, and inversely correlated with PAI-1 and triglycerides.

DISCUSSION

The main purpose of the present investigation was to analyse the long-term effects of weight loss therapy on FGF-21 concentration, and whether this hormone can modulate the metabolic profile and pro/anti-inflammatory adipokines in obese adolescents with and without IR. Therefore, the most important finding in the present study was that in the IR group, FGF-21 negatively correlated with delta-triglycerides, waist circumference, and LDL-cholesterol. This is an important finding because, in recent years, it has been reported that FGF-21 may exert cardio-protective effects.[36] Only the non-IR group demonstrated an improvement in BMI, body fat mass, lean body mass, and waist circumference. Indeed, in the non-IR group, FGF-21 was positively correlated with HDL-cholesterol and with lean body mass. In accordance with our results, previously Li et al.[12] showed that reduced FGF-21 levels were negatively correlated with adiposity measures, such as BMI and waist circumference, in the obese paediatric population.

The present study, unfortunately, was not able to demonstrate a statistical dependence of changes in the biomarkers of inflammation with the body

weight change (a reduction of approximately 6 kg), according to the statistical analyses applied. It is relevant to note that improvements in adiponectin concentration occurred in both groups. Although, the PAI-1 was ameliorated only in the IR group, after weight loss therapy.

These data are in accordance with a previous study published by the same group, where we found that a low-to-moderate weight loss (>5.8–≤10.9 kg) induced metabolic changes, including improvement of HOMA-IR index, adiponectin/leptin ratio, and reductions in PAI-1 concentration. It was observed in this previous investigation that changes in body weight were considered an important determinant of changes on inflammatory profile in obese adolescents.[37]

It is important to note that, in these results, only the non-IR group was FGF-21 inversely correlated with PAI-1 and triglycerides. PAI-1 is expressed in many tissues, including visceral and subcutaneous adipose tissue depots, where adipose tissue-resident macrophages are the primary source of the cytokines in these depots. PAI-1 is the prime regulator of fibrinolysis, and subjects with increased levels of PAI-1 are predisposed to thrombotic events. Obese patients with and without Type 2 diabetes mellitus have elevated levels of PAI-1 as compared to lean individuals, and this is closely linked with IR,[38-40] supporting the data observed in the present study.

Another interesting result confirmed in the present investigation is that only in the IR group was FGF-21 concentration significantly reduced (Table 1A). Although it has been previously shown that in obese children and adolescents the serum FGF-21 level was higher in the IR group,[41] neither in the present study nor the study performed by Cheung and Deng[42] were these results confirmed. A strong association was shown between reduced FGF-21 levels, IR, and metabolic syndrome in obese paediatric patients.[12] In addition, FGF-21 has been demonstrated to alleviate IR, not only in adipose tissue but also in the liver and muscle tissues.[43,44] In fact, our data suggest a state of FGF-21 resistance in obese adolescents with IR, corroborating with previous research in obesity.[16] However, weight loss could reverse this resistance, a hypothesis that needs to be explored in a large cohort of obese adolescents.

The IR prevalence reduced from 47% to 23.5% in the present study, which partially explains the main effects of multicomponent therapy in enhancing the metabolic and anti-inflammatory effects in response to this approach. Moreover, both analysed groups present a reduction in body mass and visceral fat, showed a positive correlation between FGF-21 and lean body mass, and a negative correlation with waist circumference, contributing to our understanding of the role of FGF-21 in energy expenditure and 'browning'.[45,46]

FGF-21-like adiponectin has been considered to be metabolically protective against atherosclerosis. Additionally, it is important to note that in both groups, improvements in the hypoadiponectinaemia was shown in response to weight loss therapy.

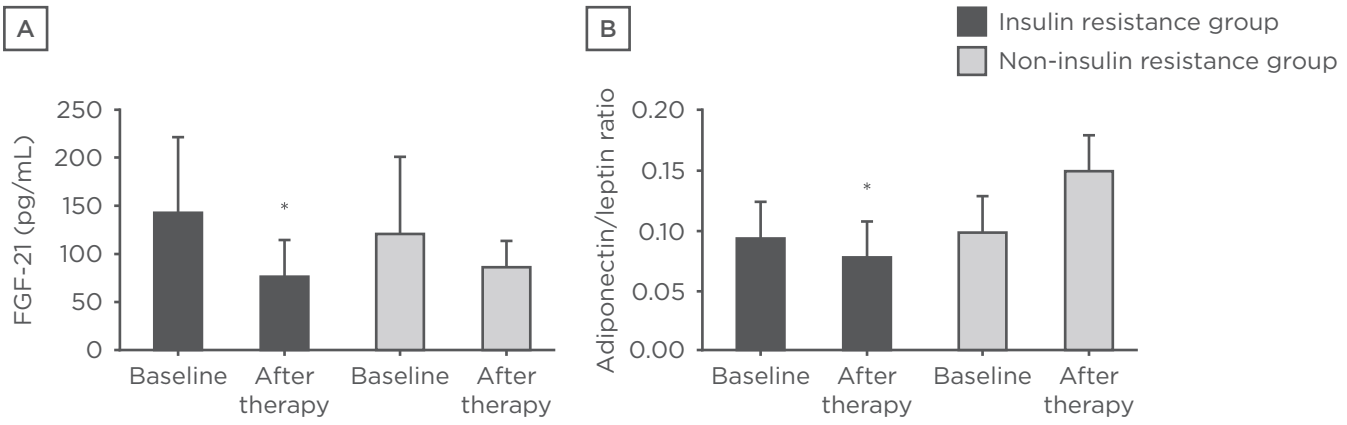

Figure 2: Effects of the interdisciplinary weight loss therapy on FGF-21 and adiponectin/leptin ratio.
A) Effects of the interdisciplinary weight loss therapy on FGF-21 in both analysed groups; B) Effects of the interdisciplinary weight loss therapy on adiponectin/leptin ratio in both analysed groups.
*Statistical significance.
FGF-21: fibroblast growth factor-21.

These data may contribute to a greater understanding on the FGF-21 effects in atherosclerosis, mediated by adiponectin secretion.[20,47,48]

Interestingly, the triglycerides, LDL-cholesterol, and waist circumference, were confirmed as a negative independent predictor of FGF-21 in the IR group. Inversely, HDL-cholesterol and lean body mass were positively correlated with FGF-21 in the non-IR group. In addition, PA1-1 and triglycerides negatively predict FGF-21 in this group.

Together, all these findings observed in the present investigation reinforces the link between IR and atherosclerosis, since reduced adiponectin/leptin ratio (a potent biomarker of anti-inflammatory state), was seen only in the IR group, leading to a hypothesis that IR can impair the anti-inflammatory effects of FGF-21. In the present study, an inverse correlation between FGF-21 and PAI-1, and positive correlation between FGF-21 and HDL-cholesterol, was observed when IR was not present in obese adolescents.

Although the multicomponent clinical approach has improved, in both analysed groups and in both metabolic and inflammatory states, the presence of IR resulted in a reduction in both FGF-21 concentration and adiponectin/leptin ratio. Additionally, in the IR group, FGF-21 was negatively correlated with proinflammatory markers, and in the non-IR group, this hormone was positively associated to HDL and negatively with pro-thrombotic and inflammatory risks, suggesting its role in the control of inflammation, counteracting the state of IR in obese adolescents. This is important to help health professionals consider that both early detection and treatment of obesity in adolescents could be the best tool to prevent future increases in comorbidities and healthcare costs in younger age.[49]

In this way, these results demonstrate interesting preliminary data that can be applied in clinical practice, considering the relevant activity of FGF-21 on the metabolic and inflammatory profile of obese adolescents in the presence of IR state. It is important to reinforce that, despite a small sample size in this study, the data presented promising results and was confirmed through statistical analyses (α=0.05), as considered and discussed in this article. Future investigations need to confirm these results as there are limitations to this study, including the small sample size and differences between sexes that were not investigated.

Additionally, future studies may explore the clinical evaluation of therapeutic action of exogenous FGF-21 administration; in particular, studies in treating diabetes, metabolic syndrome, atherosclerosis, and obesity are warranted. Finally, it will be helpful for further investigations to look into the underlying physiological mechanisms, to explore how this study's results can help in setting up more prospective studies.

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PROTEOMIC ANALYSIS OF ANTIPROTEASE IMMUNOGLOBULIN-E ANTI-SPERM AUTOANTIBODIES IN CHRONIC URTICARIA AFTER VASECTOMY

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ABSTRACT

Some types of chronic urticaria (CU) are associated with autoreactive immunoglobulin (Ig)E, as well as IgG. In the syndrome of autoimmune thyroid disease and CU, autoreactive IgE, as well as IgG against host thyroid tissue, is present. The author describes a patient with new onset of CU after vasectomy with evidence of both autoreactive IgE and IgG anti-sperm antibodies (ASA). Autoreactive sperm proteins are enzymes opposed to structural sperm antigens producing ASA in infertility and after anti-spermatocyte vaccines. The author suggests that autoreactive proteins with enzymatic activity either in host proteins, aeroallergens, or viral proteins may have increased propensity to generate autoreactive IgE. This model of autoimmune IgE ASA generation by sperm and other host enzymatic proteins in CU can be tested using proteomic technology.

Keywords: Proteome, proteasome, protease, anti-sperm antibodies (ASA), urticaria.

INTRODUCTION

Chronic urticaria (CU) is a syndrome in which symptoms persist for >6–8 weeks in the absence of an identified trigger.[1,2] Genetic and proteomic data, if available, as well as response to pharmacologic agents, are useful information that should be used to diagnose CU.[3] For example, response to targeted therapy, such as omalizumab, may provide unexpected insights into CU pathogenesis.[4-7] A response to omalizumab implicates immunoglobulin (Ig) E and the IgE receptor in many forms of CU. While the terms 'idiopathic' or 'spontaneous' are often used to describe CU, the author and others have suggested that these terms do not contribute any information to the diagnosis and should be eliminated.

In many cases CU is associated with autoimmune disease and autoreactive Ig, as shown by the ability of CU serum to activate donor basophils.[8-10] However, there is uncertainty regarding which proteins may serve as autoantigens in CU.[11] Regarding the autoreactive proteins that might serve as targets in proteomic studies and the mechanisms responsible, IgG against the IgE receptor and IgE have been described in many patients with CU. However, these IgG autoantibodies are not always present and do not correlate with disease severity or progression.[12] T lymphocyte proliferation has also been reported in response to the host IgE receptor protein in patients with CU although, as with autoreactive IgG, the prognostic significance of T cell proliferation in prospective studies of CU remains to be established.[8]

Another possible mechanism of CU is that autoreactive IgE, rather than autoreactive IgG, is responsible for priming mast cells to respond to host antigens. For example, patients with autoimmune thyroid disease are more likely to develop CU if they have autoreactive IgE, as well as IgG against thyroid tissue.[13,14] Autoreactive IgE could explain the intermittent nature of symptoms in CU, since mast cell activation would occur only with the combination of autoreactive IgE and flares of thyroiditis or other forms of tissue damage, such as viral replication in thyroid tissues releasing thyroid antigens into the circulation.[15]

In support of the autoreactive IgE hypothesis in CU, the author describes an interesting case of urticaria after vasectomy in which proteomic analysis with technology developed for studies of anti-sperm antibodies (ASA) was used to determine the presence of atypical sperm antigens after vasectomy and in infertility syndromes.[16-19] Inflammation and autoimmunity after vasectomy is common but usually self-limiting.[20,21] Similarly to antithyroid IgE, anti-sperm IgE, seminal fluid IgE, and IgG can occasionally persist due to a combination of human leukocyte antigen (HLA) presentation of antigens and inflammatory cytokines, arming mast cells to self-antigen as suggested by previous clinical reports.[22-25] If confirmed by additional proteomic studies, these observations could be useful in suggesting why some patients may generate autoreactive IgE and IgG against host tissues and viral proteins associated with urticaria.

CLINICAL PRESENTATION OF A PATIENT WITH NEW ONSET AUTOIMMUNE URTICARIA AFTER VASECTOMY

A previously healthy 39-year-old man presented with daily severe CU after an uneventful vasectomy in June 2002. The patient was previously fertile and had obtained a vasectomy for contraception. Urticaria started about 2 weeks after the vasectomy and continued every day with no specific triggers identified. The urticaria proved resistant to therapy with non-sedating antihistamines, oral corticosteroids, and cyclosporine A and levothyroxine. He had no urticaria or allergy previously. Skin and radioallergosorbent testing was negative to common aeroallergens and foods. He reported a flu-like illness immediately after the vasectomy that resolved after several days. Serum was obtained in 2003 after written informed consent for experimental diagnostic studies.

Due to the unusual presentation of the urticaria associated with a surgical procedure, some additional studies were obtained to clarify the diagnosis and assess whether an underlying systemic illness or infection was present. Stool for ova and parasites was negative, and serum total IgE was slightly elevated at 166 international units (IU)/mL (nl<114). Additional laboratory evaluation showed an anti-nuclear antibodies (ANA) positive 1:40 speckled pattern anti-DNA negative. Anti-thyroglobulin (7 IU/mL, nl<2) and anti-microsomal thyroid peroxidase antibodies (44 IU/mL, nl<2) were present, but thyroid stimulating hormone was normal as were liver function and complete blood count. IgG and IgM serology for hepatitis B and C were negative.

When tests were repeated after >1 year of daily CU, thyroid autoantibodies were present at unchanged levels, while previous borderline-positive ANA testing was no longer positive. A serum CD203c basophil activation assay demonstrated one of the highest values recorded in this assay, with approximately 50% activation of donor basophils by the patient's serum versus ≤5% for patients without CU and a mean value of >10% in patients with CU. The patient's HLA type was HLA B8 (BW6), B27 (BW4); HLA B27 is associated with increased risk of pustular psoriasis and other rheumatological conditions.

The basophil activation assay confirmed that the patient's urticaria was directly related to a circulating autoreactive Ig. However, the relationship between the epitope recognised on mast cells and basophils by the patient's serum was not established further in this study. Autoantibodies detected against sperm proteins could be identical, with the autoantibodies triggering mast cell and basophil release in the CD203c assay, or alternatively these could be distinct autoreactive antibodies triggered by epitope spreading or related mechanisms (see the 'Discussion' section). At the most recent follow-up, more than a decade after the onset of symptoms, the patient continued to have daily urticaria. He had also developed clinically significant thyroid autoimmune disease diagnosed after an episode of clinical depression and responding to replacement therapy with thyroid hormone.

As a result of the positive thyroid autoantibodies and transient positive ANA, as well as HLA type associated with autoimmune skin disease, it seemed possible that the patient's urticaria resulted from autoreactive Ig triggered by events associated

with the vasectomy and possible systemic exposures to autoreactive sperm proteins. Therefore, as described in the 'Materials and Methods section', additional studies were performed at no risk or cost to the patient using technology developed for studies of anti-sperm immune response in infertility research to characterise whether anti-sperm auto-IgG or IgE antibodies were present. The rationale for performing studies to identify urticaria associated autoreactive sperm antigens in this patient was that results could be compared to previous results from a large number of serum samples from healthy men after vasectomy and other conditions, such as infertility associated with ASA, which have previously been characterised using methods similar or identical to those described for this patient.[18,19] The findings of previous studies mean the normal immune response to vasectomy and other forms of ASA formation in patients without CU is well understood.

MATERIALS AND METHODS

Serum from the patient was obtained after >1 year of daily urticaria and was used to probe a sperm protein two-dimensional (2D) gel representing an array of human sperm proteins. Serum was used fresh within 48 hours and not frozen or otherwise processed, and was not available from the patient prior to the vasectomy. Conditions for the extraction of sperm proteins were optimised to enrich for cell surface expression, because proteins on the sperm surface would be expected to generate an autoimmune response as described.[18,19] Samples were focussed in the first dimension using immobilised (11 cm, 3–10 nl) pH gradient strips, and the second dimension was run on 8–16% Criterion gels. Both immobilised pH gradient strips and Criterion gels were obtained from Biorad, Hercules. California, USA.

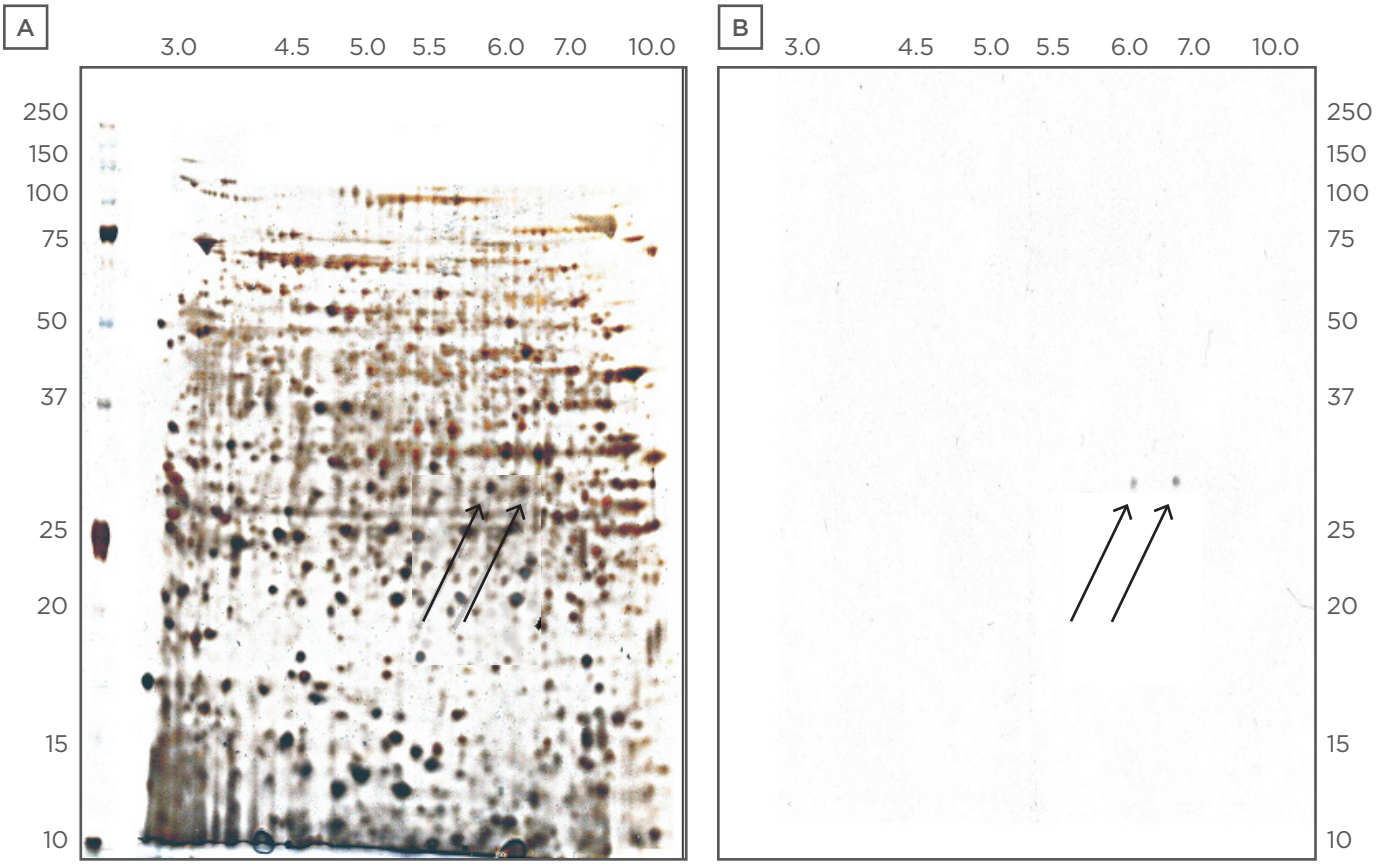

Figure 1: Electrophoretic two-dimensional gels.
A) Silver-stained for total proteins in the left panel, and B) Immunoblot using the patient's serum with secondary anti-human IgE antibody. Two proteins are evident that bind specifically with IgE from the patient's serum. These two proteins also bind to IgG from the patient's serum (IgG data not shown). Identification of these autoreactive proteins from proteomic analysis of peptides derived from extraction of these spots is shown in Table 1 and discussed in more detail in the text.
Ig: immunoglobulin.

Sample blots were compared against control blots of 13 pooled ASA negative male sera. None of the control serum donors were known to have CU, systemic illness, or autoimmune disease, and neither serum from other patients with CU were available for analysis in this study, nor have other proteomic studies of anti-sperm autoantibodies in CU been published in peer-reviewed form to the author's knowledge. Experiments were repeated to give n=3. Silver-stained gels were run on the same day in parallel with gels for blots. Positive spots were checked against controls and only those spots that were not present in control samples were cored from silver-stained gels and sent for tandem mass spectrometry microsequencing. Microsequencing results (Report number: H2D-040524-001) were obtained from the W.M Keck Biomedical Mass Spectrometry Laboratory, University of Virgina, Charlottesville, Virginia, USA.

Membranes containing blotted antigens, previously subjected either to 1D or 2D electrophoresis were incubated (1 hour, at room temperature) in the blocking solution (5% dry milk, 0.05% Tween-20 [vol/vol], phosphate buffered saline, pH 7.4). After washing, the blots were incubated (4°C, overnight) with the serum sample diluted 1:2,000 in the blocking solution. The horseradish peroxidase-conjugated secondary antibody (Donkey Anti-Human IgE, and IgG all subclasses [IgG data not shown]; Jackson ImmunoResearch, West Grove, Pennsylvania, USA) was then applied and the enzyme products were visualised by enhanced chemiluminescence using the manufacturer's protocol (Amersham, UK).

Levels of autoreactive Ig were not determined in the patient's tissues or seminal fluid in this study but may be of interest in future studies, and western blotting was preferred over other methods of autoantibody characterisation, such as enzyme-linked immunosorbent assay, to permit direct gel extraction and microsequencing of autoreactive proteins. Sera were not tested against the patient's own sperm, and as such the immunoreactive proteins are properly referred to as iso or alloantigens; however, for the purpose of this discussion they will be termed 'autoantibodies'.

IDENTIFICATION OF IgG AND IgE REACTIVE AUTOANTIGENS AS COMPONENTS OF CATALYTICALLY ACTIVE PROTEASOMES EXPRESSED IN SOMATIC HOST TISSUES

Of particular interest in the case of the present patient, was that the dominant immune response was directed to proteins that are components of proteasomes; furthermore, not only sperm-specific IgG, but IgE, was evident. Two approximately 28 kDA sperm proteins were highly reactive with both IgG and IgE in the patient's serum and were not detected in parallel tests with 13 pooled samples of serum from ASA negative men (IgE stained gels shown in Figure 1, IgG results not shown). Identity of these two proteins was determinedby microsequencing with tandem mass spectrometry of the 28 kDa spots cored from a silver-stained 2D gel that yielded specific peptides (Table 1).

Table 1: Microsequencing with tandem mass spectrometry of 28 kDa spots cored from a silver-stained two-dimensional gel, which yielded specific peptides.

Identified protein	APP MW (kDa)	pI	Peptides	Peptide mass
Testis specific protein 1	28	6.2	YYYVCQYCPAGNNMNR	2072.80
			CTLQHSDPEDR	1357.60
			ATCLCENKIY	1271.50
			KAVSPPASNMLK	1242.70
			EVTTNAQR	918.46
			AVSPPASNMLK	1114.60
Macropain subunit iota	28	5.8	AINQGGLTSVAVR	1285.70
			GKDCAVIVTQK	1218.60
			CDPAGYYCGFK	1337.50
			ARYEAANWK	1108.60
			ITENIGCVMTGMTADSR	1855.80
			YEAANWK	881.42

APP: amyloid precursor protein; MW: molecular weight.

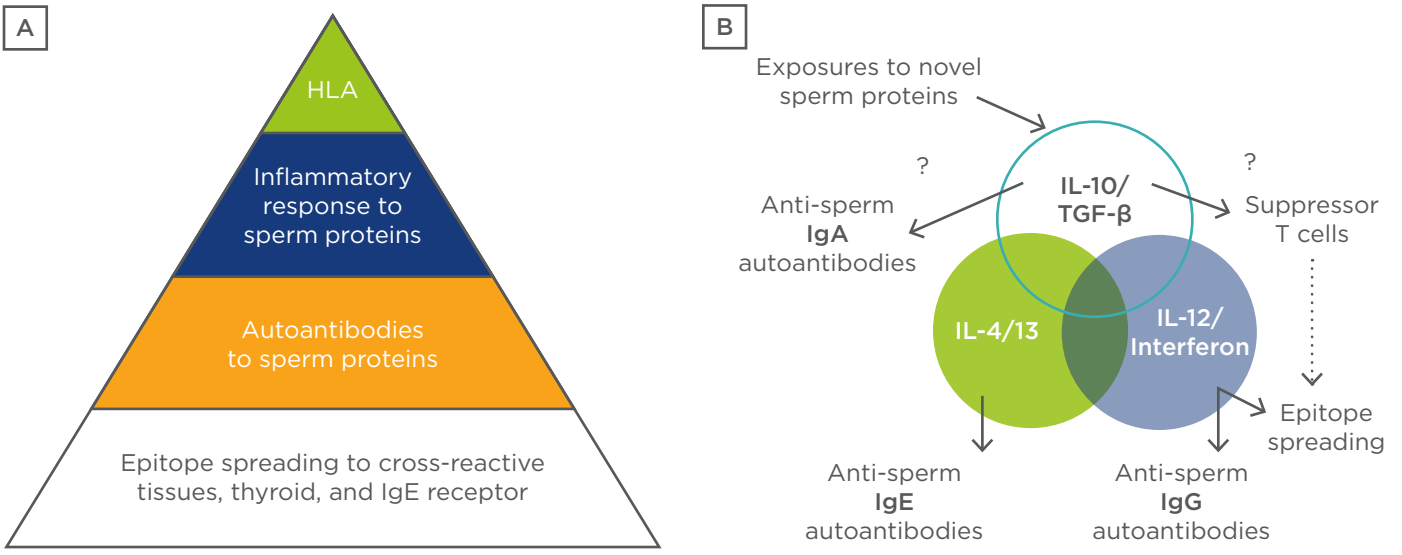

Figure 2: A model of autoimmunity after vasectomy.

A) Regulatory defects, including particular HLA antigens capable of presenting sperm proteins in altered configurations or co-incident inflammatory responses at the time of vasectomy, lead to epitope spreading with cross-reactive IgE and IgG antibodies binding to mast cells. Proteasome antigens contribute to inflammation and TH2 or TH1 cytokines, respectively, in a small minority of men after vasectomy as illustrated. B) TH3 cytokines, such as IL-10 and TGF-β, present on mucosal membranes should normally induce suppressor cells and IgA, which block or prevent further IgG and IgE autoantibody production, unless counteracting inflammatory stimuli, such as sperm proteasome antigens, shared with host tissues dominate the immune response. Strong innate inflammatory signals delivered by sperm or viral proteases could drive IL-4 and IL-13 polarised IgE responses, as discussed in more detail in the text.
HLA: human leukocyte antigen; Ig: immunoglobulin; IL: interleukin; TGF: transforming growth factor; TH: T helper cell.

A bioinformatic search of the National Center for Biotechnology Information (NCBI) protein databases revealed the proteins to be testis specific protein 1 (NCBI accession number: NP_003287), and similar to the alpha subunit, or the macropain subunit iota of the proteasome multicatalytic endopeptidase complex (NCBI accession numbers: CAA43964, AAH5552). Testis specific protein 1 is a member of the cysteine-rich secretory protein (CRISP) protein family.[26] The proteasome multicatalytic endopeptidase complex is a multicatalytic proteinase complex. Searching UniGene for tissue expression specificity information for testis specific protein 1 gave the following complementary DNA (cDNA) sources: testis, medulla, hippocampus, pooled germ cell tumours, prostate, human lung epithelial cells, and pooled metastatic prostate bone lesion, suggesting expression in the brain and lung in addition to the reproductive tract. CRISP-3 has been identified as a defence-associated molecule with predominant expression in the salivary gland, pancreas, and prostate.

The broad pattern of tissue distribution is striking for the alpha subunit, or the macropain iota subunit of the proteasome multicatalytic endopeptidase complex. The proteasome is a multicatalytic proteinase complex that is characterised by its ability to cleave peptides with broad specificity at Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. UniGene for tissue expression specificity information for alpha subunit of the proteasome multicatalytic endopeptidase complex yields an array of cDNA sources, including heart, kidney, brain, mixed tissue, chondrosarcoma, cartilage, pituitary, dorsal root ganglia, brain motor neuron, hypothalamus, prostate tissue, lung, spleen, and ovary. No primary sequence similarity was evident between these two proteins by standard sequence alignment algorithms, nor was any primary sequence similarity evident between either of the proteins and thyroid microsomal peroxidase, the thyroid autoantigen most closely linked with autoimmune urticaria.

PROTEASOME-ASSOCIATED IgE AND IgG AUTOREACTIVE SPERM ANTIBODIES ARE DISTINCT FROM AUTOREACTIVE SPERM ANTIBODIES DETECTED IN PATIENTS WITH OTHER FORMS OF ANTI-SPERM ANTIBODIES

The repertoire of immunodominant proteins recognised by infertile men with ASA is usually directed to testis-specific differentiation antigens unique to spermatocytes or spermatids.[16-19] Post-meiotic differentiation antigens that comprise the unique cyto-architectural features of the sperm, such as acrosome, outer dense fibres, and fibrous sheath, are frequently recognised. These post-meiotic antigens include the acrosomal proteins SP-10, SAMP14, SAMP32, and equatorial segment protein, and the fibrous sheath proteins AKAP3 (FSP95) and CABYR. Notably, these typical ASA are not associated with proteasomes.

In contrast, testis specific protein 1 is a member of the CRISP family. This protein contains SCP/Tpx-1/Ag5/PR-1/Sc7 family of extracellular domains. These domains are shared by the human glioma pathogenesis-related protein GliPR and a plant pathogenesis-related protein, and represent functional links between plant defence systems and the human immune system. These domains have no known function but appear to be highly antigenic. CRISP-3, from the same family as testis specific protein, plays a role in the pathophysiology of at least two other autoimmune diseases: Sjogren's syndrome and chronic pancreatitis. Similarly, the second ASA associated with onset of CU in this patient denoted 'similar to the alpha subunit, or the macropain iota subunit of the proteasome multicatalytic endopeptidase complex' is a proteasome associated protein not present in the normal ASA response.

DISCUSSION

As suggested in this paper, ASA-generated Ig due to novel antigenic exposures of sperm proteins to the immune system could provide a mechanism of autoimmune urticaria through molecular mimicry between sperm and somatic cell proteins, as well as the related phenomena of epitope spreading from tissue autoantigens to other tissue autoantigens. The autoreactive IgE detected in the patient studied in this paper represents a mechanistic link between autoimmunity and mast cell activation resistant to normal therapy, such as antihistamines and oral corticosteroids. Unfortunately, the patient was not interested in a trial of omalizumab, a humanised monoclonal antibody that specifically blocks IgE mediated mast cell activation; however, omalizumab and related therapy might be useful and informative in patients with this syndrome in the future.

Normally, suppressor cells induced at mucosal surfaces should prevent further progression of the immune response to generate autoreactive IgE or IgG (Figure 2). Current understanding of autoimmune mechanisms suggests that mucosal immune responses mediated by transforming growth factor (TGF)-β and interleukin-10 should suppress autoreactive IgE and IgG responses through the generation of IgA and suppressor T-lymphocytes, respectively. However, in the patient described, these protective mechanisms were apparently not functional, perhaps due to the proposed proteasome-medicated activation of IgE and IgG. In support of this hypothesis, the flu-like symptoms experienced by the patient after the vasectomy, but prior to onset of urticaria, could correspond to the release of T helper cell (Th)1 and Th2 inflammatory cytokines, rather than Th3 suppressive cytokines during primary ASA response to sperm proteins after vasectomy.

Based on these observations, the author suggests that this patient and previous reports of CU and/or anaphylaxis after vasectomy represent a putative new syndrome characterised by ASA proteasome-associated (ASAp). Remarkably, both atypical sperm proteins detected in this study are related to other inflammatory proteins. The CRISP proteins are not only related to members of the plant PR-1 proteins and tumour GliPR proteins, as noted above, but are also present in many snake venoms, where they are thought to trigger hypothermia through interference with membrane channel signalling. Human mast cells are highly sensitive to non-specific effects of snake venom proteases, such as hypothermia triggering mast cell release, and depletion of mast cells results in markedly increased mortality from venom.[27] These observations suggest that a primary focus of the mast cell and IgE system is co-ordinated with innate immune resistance to proteases, providing increased survival from snake and other venoms.[28,29] In direct support of this hypothesis, novel innate mechanisms of mast cell activation by a papain protease has been described in which a protease substrate on the mast cell surface appears

to undergo cleavage by papain causing non-IgE mediated mast cell activation and degranulation.[30]

Activation of the innate immune system by proteases, which are often also encoded by chronic viral pathogens as well as sperm proteins, is possibly a critical initial event in CU, thus accounting for those patients who lack IgE receptor autoantibodies and yet have antithyroid antibodies and CU and angioedema.[15] Protease-mediated activation of the innate immune system could, in turn, contribute to other findings in CU and related angioedema, such as complement activation and related innate mechanisms of inflammation.[31-33] Further studies of ASA and protease antibodies in CU and related syndromes, such as autoimmune thyroiditis, might help to clarify both the aetiology of these disorders, as well as their markedly increased prevalence in females compared to males.

In summary, an inflammatory response to sperm or viral proteases in this patient could both promote IgE and also break tolerance to other mast cell proteins, such as the mast cell and basophil IgE receptor present at the site of the inflammatory reaction, due to phenomena such as epitope spreading as previously described for IgG-mediated autoimmune disease.[34-37] An important prediction of this hypothesis is that both men and women with CU might, in some cases, have evidence of IgE-mediated autoimmunity to sperm proteases described in this work as well as to viral and host proteases.

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ROLE OF GENETIC VARIATIONS IN DETERMINING TREATMENT OUTCOME IN HEAD AND NECK CANCER

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ABSTRACT

Worldwide, head and neck squamous cell carcinoma (HNSCC) is responsible for >550,000 diagnoses and 380,000 deaths annually. It originates in the upper aerodigestive tract and has a multifactorial origin involving both genetic and lifestyle risk factors. The clinical management of HNSCC involves surgery, radiotherapy, and chemotherapy. Several studies point to the role of genetic variations in predicting drug efficacy and toxicity. Cancer pharmacogenomics has fast emerged as a new and promising field for the early identification of genetic markers that can predict drug response or toxicity, with the number of studies of genetic polymorphisms as prognostic factors of HNSCC treatment outcomes growing. The number of studies evaluating the association of candidate polymorphisms in drug-metabolising Phase I and II enzymes with treatment outcome far exceed the studies involving other candidate genes, such as those involved in drug metabolism, DNA repair, and cell cycle regulation. This review focusses on the relevance of genetic variations in genes, where the corresponding gene products play an important role in drug metabolism (*TPMT*, *DPD*), DNA repair (X-ray repair cross complementing 1), cell cycle (tumour protein P53), and carcinogenesis (matrix metalloproteinase 3 and 7), thereby contributing to the treatment outcome for HNSCC. This could greatly help clinicians in identifying genetic markers useful for the selection of optimal drugs, dose, and treatment duration on an individual basis, resulting in improved drug efficacy and decreased toxicity. However, further studies are needed in well characterised and larger HNSCC populations with proper validation of pharmacogenetic markers in experimental settings before application in clinical routine diagnostics.

Keywords: DNA repair, drug metabolism, genetic variations, matrix metalloproteinases (MMP), p53.

INTRODUCTION

It has generally been observed amongst patients that a medication proven efficacious for the majority of patients often fails to work in some other patients. In those cases where it works, it may cause serious side effects, even death, in a small number of patients. It is now well established that a large variability of drug efficacy and adverse drug reactions in patients decides the clinical use, regulation, and market withdrawal of clinical drugs.[1] Similar observations have also been made for cancer medication as significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents has been found to exist across the human population.[2]

Recent studies from our laboratory have shown that treatment response in head and neck squamous cell carcinoma (HNSCC) patients treated with a combination of chemotherapy and radiotherapy regimen of combination drugs, such as cisplatin and 5-fluorouracil (5-FU), was poor in patients with variant genotypes of *CYP2C9*, *CYP2C19*, and *CYP2D6*.[3,4] Furthermore, an association with poor treatment response to chemotherapy was also observed in HNSCC cases for genotypes of *CYP2A6*, *GSTM1*, *GSTT1*, and *GSTP1*.[5,6] Apart from the Phase I and Phase II drug metabolising enzymes, studies were also conducted to evaluate the role of genetic variants in other drug metabolising enzymes, such as thiopurine S-methyltransferase (TPMT) and dihydropyrimidine dehydrogenase (DPD), in determining the treatment outcome in HNSCC patients.[7] This review will focus on some of the major genetic variations in *TPMT*, *DPD*, and some of the other key target genes, such as those involved in DNA repair (X-ray repair cross complementing 1 [*XRCC1*]), cell cycle (tumour protein P53 [*TP53*]),

and carcinogenesis (matrix metalloproteinases, [*MMP*]), which might play an important role in determining the treatment outcome in HNSCC cases. The genomic structures of *TPMT*, *DPD*, and *XRCC1* are shown in Figure 1, whilst Table 1 lists the single nucleotide variants associated with drug response.[8]

DRUG METABOLISM POLYMORPHISMS: THIOPURINE-S-METHYLTRANSFERASE AND DIHYDROPYRIMIDINE DEHYDROGENASE

TPMT and DPD are enzymes involved in the metabolism and disposition of drugs, such as 6-mercaptopurine, azathioprine, cisplatin, 5-FU, and 6-thioguanine, that are used for the treatment of different cancers, autoimmune disorders, dermatological conditions, and as an immunosuppressant in graft transplantations.[9-12] TPMT functions by methylating mercaptopurine and thereby reducing its bioavailability for conversion into thioguanine nucleotides, which is the cytotoxic form of the drug. Approximately 10% of patients have an intermediate enzyme activity and have a greater incidence of thiopurine toxicity, while 0.3% are deficient of TPMT activity, having severe or fatal toxicity from mercaptopurine therapy.[13]

Genetic variants present in *TPMT* may alter the treatment response in cases receiving chemotherapeutic drugs. The four major variant alleles that lead to 80–95% intermediate and low enzyme activity of the *TPMT* gene are *TPMT*2* (238G>C), **3A* (460G>A and 719A>G), **3B* (460G>A), and **3C* (719A>G).[14] *TPMT*3A* mutant allele contains two nucleotide transitions, 460G>A and 719A>G in the open reading frame, leading to the substitution of amino acids Ala154Thr and Tyr240Cys, respectively. Our study has shown that the treatment response after receiving cisplatin and 5-FU was modified in patients who carried variant genotypes of *TPMT*3B* and **3C*.[7]

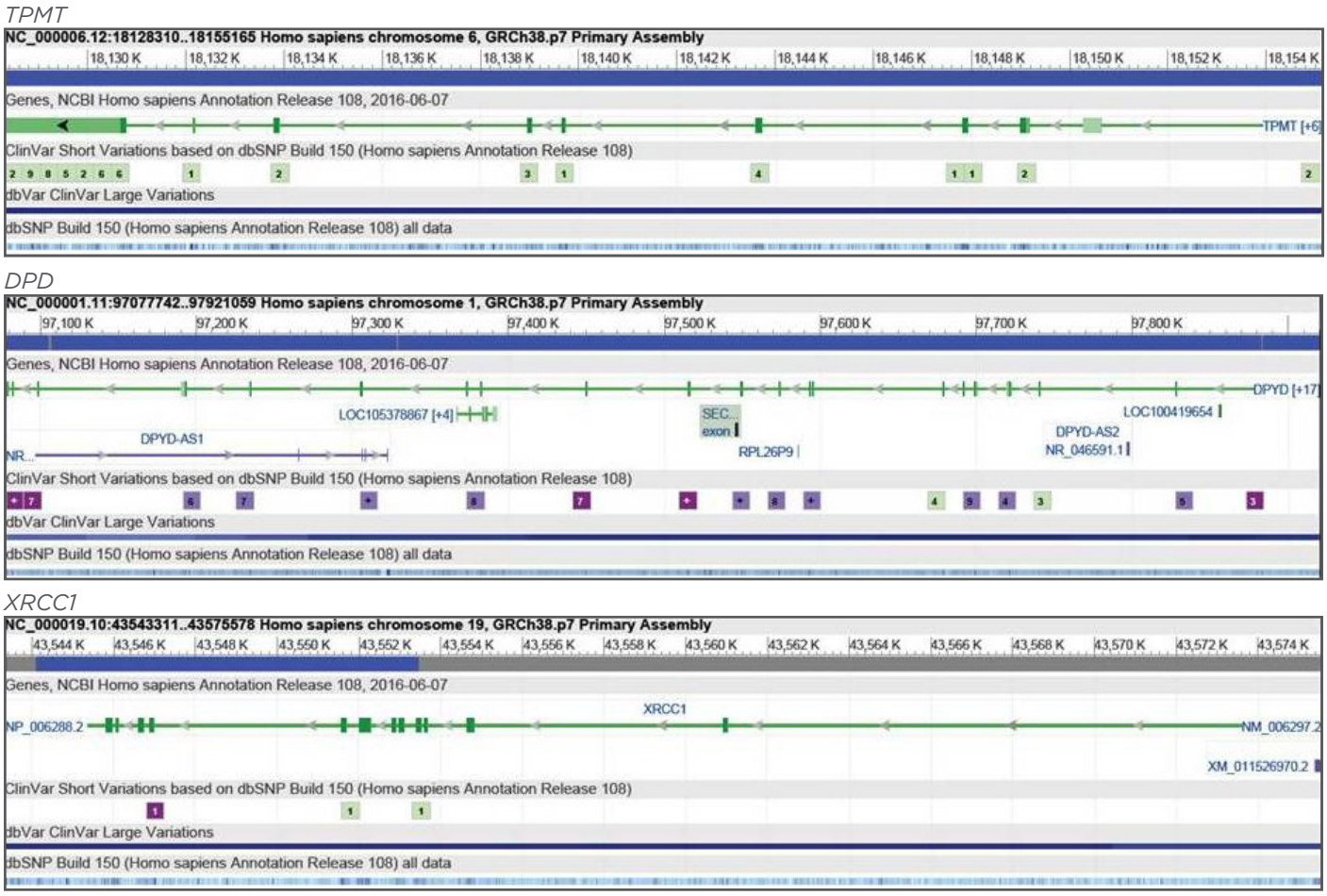

Figure 1: Genomic structures of *TPMT*, *DPD*, and *XRCC1* genes.
DPD: dihydropyrimidine dehydrogenase; *TPMT*: thiopurine 5-methyltransferase; *XRCC1*: X-ray repair cross complementing 1.
Adapted from National Center for Biotechnology Information (NCBI).[8]

Table 1: Table showing the single nucleotide variants associated with drug response.

Gene	Variant ID	Variant allele	Transcript change	Protein change	Molecular consequence
TPMT	rs1142345	C	c.719A>G	Tyr240Cys	Missense variant
		C	c.674A>G	Tyr225Cys	Missense variant
	rs56161402	T	c.644G>A	Arg215His	Missense variant
		T	c.599G>A	Arg200His	Missense variant
	rs1800584	T	c.626-1G>A	NA	Splice acceptor variant
		T	c.581-1G>A	NA	Splice acceptor variant
	rs74423290	C	c.500C>G	Ala167Gly	Missense variant
	rs1800460	T	c.460G>A	Ala154Thr	Missense variant
	rs56019966	T	c.420-4G>A	NA	Intron variant
	rs1800462	G	c.238G>C	Ala80Pro	Missense variant
DPD	rs2297595	C	c.496A>G	Met166Val	Missense variant
		C	c.385A>G	Met129Val	Missense variant
		C	c.1A>G	Met1Val	Missense variant
		C	n.735A>G	NA	Non-coding transcript variant
XRCC1	rs25487	C	c.1196A>G	Gln399Arg	Missense variant
TP53	rs1042522	C	c.215C>G	Pro72Arg	Missense variant
		C	c.-939C>G	NA	2KB upstream variant
		C	c.98C>G	Pro33Arg	Missense variant

DPD: dihydropyrimidine dehydrogenase; NA: not applicable; *TPMT*: thiopurine 5-methyltransferase; *TP53*: tumour protein 53; *XRCC1*: X-ray repair cross complementing 1.

Since individuals with *TPMT*2*, **3A*, **3B*, and **3C* variants are reported to be associated with lower or intermediate enzyme activity,[15,16] the poor response rate in patients could probably be a result of decreased TPMT activity, which may lead to relatively higher intracellular concentration of cisplatin and therefore higher incidence of cisplatin-related toxicity. Liu et al.[17] performed a genome-wide association study of primary erythrocyte TPMT activity in children with leukaemia (N=1,026) and found that *TPMT* was the only gene that reached genome-wide significance (top hit rs1142345 or 719A>G; $p=8.6\times10^{-61}$). Furthermore, TPMT activity in patients behaves as a monogenic trait, further corroborating the importance of *TPMT* genetic testing in clinical settings.

The *DPD* gene also has several reported polymorphisms, out of which consistent data are available for the *DPD*2A* allele, which is caused by a G>A transition at a GT splice donor site flanking exon 14 of the *DPD* gene (IVS14+1G>A).[18] This allele is the most common and results in the elimination of a 55-amino-acid-long fluorouracil binding site in the DPD protein, leading to the skipping of exon 14 and the production of an inactive protein in 0.9% of the Caucasian population.[19-21] Both anabolic and catabolic pathways are involved in the cytotoxic effects of 5-FU;[9] thus the activity of DPD plays a role both in determining the efficacy of the therapy with 5-FU and its toxicity.[22-24] Decreased DPD activity has been associated with >4-fold risk of severe or fatal toxicity from standard doses of 5-FU.[25] *DPD* has another mutation at codon 534, leading to a 1601G>A nucleotide change.[26] Our study has also shown that cases carrying the *DPD* genetic variant IVS14+1G>A also showed a poor treatment response.[7] Other studies also reported that a high prevalence of IVS14+1G>A was associated with increased toxicity and poor treatment response in patients of invasive ductal carcinoma and Grade 3 and 4 toxicity in HNSCC patients.[27,28] Amongst the reasons for the toxicity of 5-FU are its relatively narrow therapeutic index[29,30] and low DPD activity. Lowered DPD activity leads to less effective inactivation of 5-FU, causing an accumulation of excessive levels of fluorodeoxyuridine monophosphate, causing gastrointestinal, haematopoietic, and neurological toxicities that are potentially fatal.[21] Zhao et al.[31] investigated the association between germline genetic polymorphisms in *DPD* and outcome of acute lymphoblastic leukaemia following the treatment with 5-FU plus oxaliplatin, and found that the common *DPD* variant c.85T>C (rs1801265, *DPYD*9A*) was significantly associated with

complete response rate, event-free survival, and treatment-related toxicity.

DNA REPAIR GENE POLYMORPHISMS: X-RAY REPAIR CROSS-COMPLEMENTING GROUP 1

The DNA damage induced by anticancer agents is repaired by the DNA repair enzymes and, therefore, it can be hypothesised that polymorphisms in DNA repair genes may influence the outcome of treatment. Increased DNA damage may prove beneficial in treatment, as both chemotherapy and radiation rely on DNA damage as part of their mechanisms of tumour cell destruction though the same DNA damage that occurs within the tumour may also take place in normal tissues as a side effect of therapy. *XRCC1* is a major DNA repair gene in the base excision repair pathway and is involved with radiation-related DNA repair. The base excision repair pathway repairs single strand breaks through protein-protein interactions, by interaction of XRCC1 with PARP-1, PNK, Polb, and Lig3a.[32] XRCC1 interacts with PNK, leading to the stimulation of both the 5'-kinase and 3'-phospatase activities of PNK; the interaction with Lig3a increases the intracellular stability of the ligase.[32] Several single nucleotide polymorphisms (SNP) in *XRCC1* are reported, out of which the most extensively studied are Arg194Trp on exon 6 (dbSNP no. rs1799782), Arg280His on exon 9 (dbSNP no. rs25489), and Arg399Gln on exon 10 (dbSNP no. rs25487).[33]

A study conducted by Quintela-Fandino et al.[34] tested the ability of *XRCC1*-Arg399Gln gene polymorphism to predict response to cisplatin in peripheral blood mononuclear cells of Stage-IV HNSCC patients. *XRCC1* Gln/Gln was associated with 0% of cases with progressive cancer, 7.7% of cases with stable disease, 30.8% of cases with partial response, and 61.5% of cases with complete response. Duell et al.[35] reported that the rate of sister chromatid exchange after exposure to ionising radiation in human lymphocytes was greater in individuals with the *XRCC1*-Gln399Gln genotype than in those with only wild-type genotype. Alsbeih et al.[36] tested the association between *XRCC1* G28152A Arg399Gln (rs25487) SNP and late reaction to radiotherapy in 60 nasopharyngeal cancer patients. Significant association was observed between *XRCC1* G28152A genotype ($p \leq 0.05$) and a lower grade of fibrosis (odds ratio: 0.30; 95% confidence interval: 0.10–0.89; p=0.02), and therefore the wild-type was the risk allele. However, Mahimkar et al.[37] did not observe a significant association between polymorphisms of *XRCC1* (Arg194Trp, rs1799782 and Arg399Gln, rs25487) and clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. Zhai et al.[38] investigated the association of *XRCC1* Codon399 polymorphism with treatment efficacy and normal tissue toxicity in Chinese patients with locally advanced nasopharyngeal carcinoma after radiotherapy. It was observed that Codon399 Gln/Gln allele correlated with a higher medium-term tumour regression ratio after radiotherapy for primary nasopharyngeal neoplasm and metastatic lymph nodes (>80% versus 40–60%; $p<0.01$). Compared with the other two genotypes in this study, patients with *XRCC1* Codon399 Gln/Gln allele were more likely to obtain complete remission of the tumour (100% versus 76% and 67%; $p>0.05$). However, no correlation between *XRCC1* Codon399 polymorphisms and acute or late radiation-induced injury of normal tissues was observed. Ghazali et al.[39] conducted a systematic review of the association of SNP with the occurrence of HNSCC radiotoxicity and evaluated the association of 11 polymorphisms in 8 genes with acute radiotoxicity, and 6 polymorphisms in 4 genes for late radiotoxicity. The risk of severe acute mucositis was associated with the G allele of *XRCC1* (1196A>G) in patients treated with radiotherapy alone or chemotherapy.

CELL CYCLE AND MATRIX METALLOPROTEINASES POLYMORPHISMS: TUMOUR PROTEIN P53 (TP53) AND MMP3, MMP7

The p53 protein, rightly known as the 'guardian of the genome', or the 'cellular gatekeeper', acts by transmitting a variety of stress-inducing signals to different antiproliferative cellular responses. Some major events such as DNA damage, oncogene activation, or hypoxia can lead to p53 activation, thereby regulating processes such as apoptosis, cell-cycle arrest, senescence, or modulation of autophagy.[40] Somatic mutations of the tumour suppressor gene *p53* occur in around 50% of all human cancers; thus, *p53* is a major player in carcinogenesis.[41] In addition to its role in carcinogenesis, *p53* also plays a major role in deciding the clinical outcome of cancer treatment, because *p53* mutations render tumour cells resistant to chemotherapy.[42,43] The two *p53* homologues, namely *p63* and *p73*, are also activated by cytostatic

drugs.[44] p73 also functions in transduction of specific types of DNA damage, as activation of the p73/c-abl pathway by some chemotherapeutic agents causes apoptosis via target genes, such as *AIP1*.[45,46] *p73* exists as several splice variants at both N and C termini, with N-terminal variants lacking the transactivation domain and thereby acting as dominant-negative inhibitors of both *p53* and *p73*.[47] Out of the polymorphisms encoding either arginine (72R) or proline (72P) at codon 72 of *p53*, 72R is detected more commonly than 72P in squamous cell carcinomas.[48,49] There is an over-representation of 72R mutants in carcinomas, which can be explained by the fact that inhibition of *p73* is influenced by the polymorphism at codon 72 of *p53*.

Bergamaschi et al.[50] reported that the p53-p73 interaction and its regulation by *p53* polymorphism is an important determinant of response and survival in head and neck cancer. Patients with specific *p53* mutations in the 72R form are least likely to gain complete response to chemotherapy. Upon treatment with cisplatin-based combination therapy, HNSCC cases with the 72R SNP has a worse response than those with the 72P SNP.[50] Sullivan et al.[51] reported that the response of human H1299 p53 -/- cells to anticancer agents is strongly influenced *in vitro* by a SNP in wild-type p53. The clinical relevance of these observations was presented by the evidence that the superior activity of the 72R wild-type protein is reflected *in vivo* by significantly higher response rates and survival in patients receiving chemo-radiotherapy for advanced head and neck cancer, who express the 72R wild-type variant. Seventy patients with inoperable advanced HNSCC who received cisplatin-based chemo-radiotherapy were involved in the study. All the patients presented with locally advanced, unresectable, tumour, node, and metastasis Stage III/IV HNSCC. In the 43 patients who had a wild-type *p53* allele and had an evaluable response to treatment, complete response appeared significantly linked with *p53* status (wild-type 72R *p53* allele, wild-type 72P *p53* allele, or both). Wild-type 72R allele produced the highest complete response rate (27 out of 28 patients [96%]). Similarly, overall survival and progression-free survival were significantly longer in patients with wild-type *p53* allele (either 72R or 72P) than in those lacking a wild-type allele. Significant differences for overall survival and progression free survival were observed between cases retaining a wild-type 72R allele, cases retaining a wild-type 72P allele, and cases retaining both wild-type 72R and 72P alleles with the best prognosis in cases retaining a wild-type 72R allele. These clinical results corroborate the *in vitro* data wherein the expression of 72P wild-type *p53* resulted in G1 cell cycle arrest with minor induction of apoptosis after challenge with drug doses that cause extensive apoptosis in 72R-expressing variants.[50]

MMP are a family of proteins involved in extracellular matrix degradation, cell proliferation, and angiogenesis, the processes that are involved in carcinogenesis.[52] Fas ligand (FasL) is a member of the tumour necrosis factor superfamily that induces apoptosis in susceptible cells upon cross-linking of its own receptor, FasL (Apo-1/CD95), which contributes to immune homeostasis and cell-mediated cytotoxicity. FasL is upregulated in cells treated with genotoxic agents such as cisplatin and 5-FU, and thus it can be expected that the downregulation of FasL at the surface of HNSCC tumour cells decreases the activity of anticancer drugs through modulation of the apoptotic pathway. Studies suggest that MMP7 and MMP3 could be implicated in the shedding of FasL from the cell surface, resulting in the generation of soluble FasL with less effective activity for triggering apoptosis by crosslinking with Fas.[53-55] This was experimentally shown by the work of Mitsiades et al.[56] in Ewing sarcoma and colon carcinoma cells. Blockade of MMP7 activity by MMP inhibitor resulted in sensitisation to treatment with doxorubicin, providing evidence for the role of MMP and FasL in chemotherapy resistance.

Varying expression patterns of *MMP* could be due to the existence of functional genetic polymorphisms in *MMP* promoter regions. The two polymorphisms that result in increased promoter activity of *MMP7* are one at position -181A>G and the other at position -153C>T. For *MMP3*, two alleles have been defined: the deletion (5A) or the insertion (6A) of an adenine at position -1612 (-1612insA), with the 6A allele being associated with low transcription levels.[57] Amongst other *MMP3* polymorphisms, six are in linkage disequilibrium with -1612insA and one (-A709G) has not been assigned functional importance.[58] Blons et al.[59] reported a correlation between *MMP3* promoter polymorphism and response to chemotherapy in HNSCC. It was shown, for the first time, that patients carrying the low transcription level allele 6A had a better response to 5-FU-cisplatin combination therapy than patients carrying the 5A allele. Furthermore, multivariate

analysis, including *TP53* mutational status, showed that *MMP3* was an independent factor in response to treatment. The results were in accordance to a study which showed that the induction of FasL after DNA damage is *p53* independent.[60] However, *MMP7* promoter polymorphisms did not influence response to treatment.[59]

The MMP have been postulated to interact with the Fas/FasL pathways. Poulaki et al.[61] extensively investigated the involvement of the FasL/Fas pathway in doxorubicin induced apoptosis in a model of Ewing's sarcoma. Clones of the Fas-sensitive, doxorubicin-sensitive Ewing's sarcoma cell line SK-N-MC, which were either Fas-resistant or FasL-deficient, were significantly resistant to Dox. It was also observed that cleavage of FasL by MMP7 (matrilysin) protected the parental SK-N-MC cells from doxorubicin, whereas inhibition of MMP7 activity increased their sensitivity. Gastman et al.[62] provided a unique observation for the Fas/FasL pathway. The study showed that the pathway can be potentially immunosuppressive and may be involved in the escape of human carcinoma cells from immune destruction. The study found that the expression of FasL on HNSCC cell lines was upregulated by pretreatment of tumour cells with the metalloproteinase inhibitor, BB-94. When PCI-cell lines pretreated with BB-94 were co-incubated with Jurkat cells, they induced DNA fragmentation in a greater proportion of lymphocytes than did tumour cells not pretreated with the inhibitor.

In conclusion, genetic variations in genes encoding drug metabolising enzymes have explained a great deal of interindividual variation in response and toxicity of anticancer drugs. However, drug metabolism is just one aspect of the interaction of drugs and genes and more efforts should also be made to uncover the role of genetic variations in other important target genes, such as drug transporters, drug receptors, DNA repair, cell cycle, and other pharmacodynamic genes. Reduction of the toxicogenetic and toxicogenomic side effects has been one of the major goals in the search for new anticancer drugs and therapy protocols. Genome-wide linkage analyses may be a more systematic approach to discover genomic regions likely to carry genes, which determine chemotherapy cytotoxicity rather than the candidate gene-based approaches. Efforts should be made to implement SNP genotyping into clinical diagnostics, therefore allowing practitioners to make more accurate decisions for treatment and risk assessment. This will help in clinical decision making regarding treatment strategies, with the goal of avoiding adverse drug reactions while achieving the best drug response.

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PATHOGENESIS, DIAGNOSTICS, AND TREATMENT OF HEREDITARY HAEMOCHROMATOSIS: A 150-YEAR-LONG UNDERSTANDING OF AN IRON OVERLOAD DISORDER

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ABSTRACT

Haemochromatosis is an iron overload disorder that can be inherited or acquired and when diagnosis is delayed, disease progression and death can occur. Iron overload was first described by the French internist Armand Trousseau in 1865 in an article on diabetes in which alterations in skin pigmentations were reported. Some years later, the German pathologist Friedrich Daniel von Recklinghausen coined the term 'haemochromatosis' for a metabolic disorder characterised by excess deposition of iron in the tissue. This disorder affects 1 in 200 subjects of Caucasians of Northern European descent. The systemic excess iron build-up condition quickly gained an intense clinical interest. Haemochromatosis can lead to severe pathological symptoms in multiple organs, including the liver, bones, spleen, heart, pancreas, joints, and reproductive organs. With the progress of the disease, hepatic damage predominates. Polymorphisms in several independent genes can lead to haemochromatosis. However, the most widely known haemochromatosis-associated and studied ones are genetic variants in the *HFE* gene, located on the short arm of human chromosome 6. Early detection and phlebotomy prior to the onset of fibrosis/cirrhosis can reduce morbidity and normalise life expectancy. Consequently, phlebotomy has been accepted for decades as a standard treatment for the reduction of iron load. Nowadays, other methods, such as erythrocytapheresis, therapeutic application of iron chelators and proton pump inhibitors, or hepcidin-targeted therapy, are discussed as alternative personalised treatments of hereditary haemochromatosis. This review focusses on the pathogenesis, diagnosis, and therapy of haemochromatosis.

Keywords: *C282Y,* diagnostics, gene variants, *H63D,* hepcidin, hereditary haemochromatosis (HH), iron overload, liver, phlebotomy, therapy.

NORMAL METABOLISM OF IRON AND HEREDITARY HAEMOCHROMATOSIS

Iron is an essential micronutrient that exists in different ionic states. This ability makes it a critical component of oxygen transport proteins, numerous metabolic enzymes, and reaction centres of a large variety of redox enzymes. Most of the body's iron is found in two proteins; around 70% of all iron is bound to haemoglobin in the red blood cells, while 15% is stored in myoglobin in muscle cells. The rest is stored as ferritin or haemosiderin in the liver, the spleen, and the bone marrow. Dietary iron is absorbed in the duodenum by enterocytes, in which Fe^{2+} is oxidised to Fe^{3+} in a ferroxidase reaction and transferred in the blood to transferrin. Subsequently, the iron-loaded transferrin binds to the transferrin receptor (TfR) and is taken up by receptor-mediated endocytosis (Figure 1A). In the cell, iron is released from this complex, while transferrin and its receptor are recycled to the cell membrane.

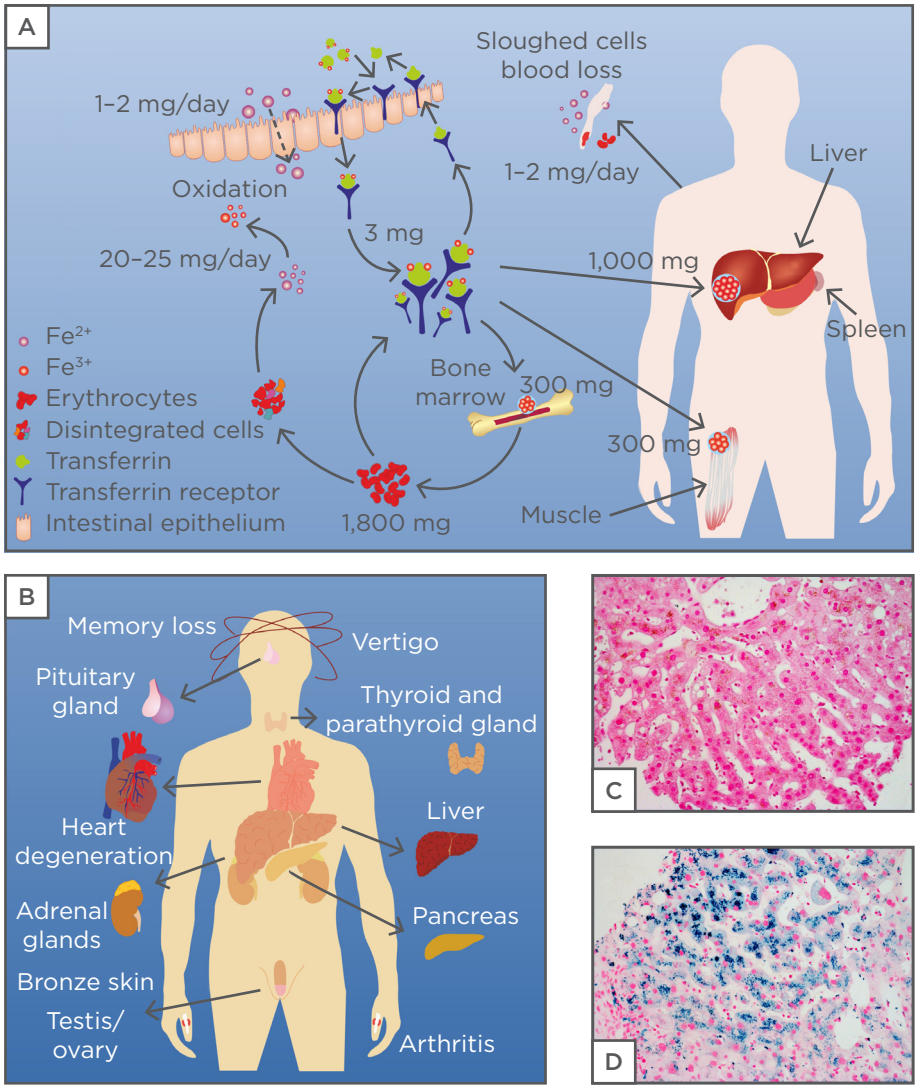

Figure 1: Body iron metabolism and iron overload.

A) Dietary iron is absorbed as part of a protein or in its ferrous Fe^{2+} form in the duodenum by enterocytes. In these cells, a ferroxidase reaction oxidises Fe^{2+} to Fe^{3+} and transfers it in the blood to transferrin, which has two specific high-affinity Fe^{3+} binding sites. Under physiologic conditions, there is approximately 3 mg iron bound to transferrin. The iron-loaded transferrin can bind to the transferrin receptor that is exposed to the cell surface and transported into the cell by receptor-mediated endocytosis. The main sites of iron storage are the liver (1,000 mg), muscles (300 mg), bone marrow (300 mg), and the erythrocytes (1,800 mg). Approximately 20–25 mg of iron per day is recycled in the reticuloendothelial system, which breaks down aged erythrocytes, while the total amount of iron through blood or sloughed cell loss is estimated at an average of 1–2 mg per day. B) Clinical manifestations of iron overload typically occur in the liver, pancreas, heart, adrenal glands, pituitary gland, and skin. Respective alterations provoke bronze skin pigmentation, hair loss, impotence, joint pain, lethargy, vertigo, and restriction of cognitive abilities.[95-98] Elevated concentrations of iron in cardiomyocytes results in heart cell degeneration reflected by arrhythmias and cardiomyopathy. In the liver, the elevated concentration of iron causes hepatomegaly and liver cell necrosis that is indicated by elevated liver enzymes and formation of fibrosis and cirrhosis. C, D) Nuclear fast red/Prussian blue stain of a liver specimen obtained from a healthy subject (C) and a patient with hereditary *HFE* (D). The deep blue stain in D) is due to tissue iron deposits that are not detectable in healthy subjects.

Elevated iron quantities can be extremely toxic because of iron's ability to donate and accept electrons, catalysing the conversion of hydrogen peroxide into free radicals, which can cause cell injury and organ damage.[1,2] Haemochromatosis is characterised by excess endogenous iron stores

in the body's tissues and organs, particularly in the skin, spleen, heart, liver, pancreas, and joints (Figure 1B). Because humans themselves cannot increase the excretion of iron, untreated haemochromatosis can lead to bone and joint disease, cirrhosis, liver cancer, diabetes, hypothyroidism, hypogonadism, infertility, impotence, depression, or premature death due to liver or heart failure.[3] The liver, as one of the main iron storage compartments and the place of hepcidin synthesis, is the central organ involved in maintaining iron homeostasis in the body. Overload of iron is a primary risk factor in the pathogenesis of liver disease.[4,5] In affected patients, the condition of iron overload can be impressively demonstrated by the prominent stainable iron in liver sections by common histopathology stains using Prussian blue detecting iron deposits in biopsy specimens (Figure 1C and 1D). There is currently a debate on the impact of haemochromatosis on the development of retinal degenerative changes. While experimental studies performed in mice showed that retinal pigment epithelium from iron-overloaded mice exhibited a hyperproliferative phenotype, recent studies in patients with hereditary haemochromatosis (HH) revealed no indication of a higher prevalence of retinal degenerative changes.[6,7]

ABSORPTION AND IRON RECYCLING

The daily dietary iron absorbed by the body is strictly balanced. In industrialised countries, the body iron store contains 4–5 g, which is predominantly stored as haemoglobin in erythrocytes, and in different complexes in the liver, bone marrow, and muscles (Figure 1A). Typically, the daily iron loss due to sloughing of epithelial cells or blood loss is about 1–2 mg, while the normal reference range for serum iron is 65–176 µg/dL for men, 50–170 µg/dL for women, and 50–120 µg/dL for children. In healthy subjects, these iron levels predict a transferrin saturation (indicating the ratio of serum iron and total iron-binding capacity) of 20–50%.[8,9] Red blood cell haemoglobin, liver, muscles, and macrophages of the mononuclear phagocytic system distribute the iron to locations in the body where iron is needed for proper cellular metabolism and respiration.[10] The uptake capacity is regulated to a large extent by two factors: the size of the stored iron and the rhythm of erythropoiesis.[11] The initial dietary iron is absorbed in the intestinal epithelium. There, the initial oxidised form of iron (Fe^{3+}) is reduced by the apical ferric reductase duodenal cytochrome b that is expressed in the duodenum. The reduced iron (Fe^{2+}) is then transported across the duodenal mucosa into the cytoplasm of the intestinal epithelium via the divalent metal transporter 1 (DMT1) by a proton-coupled mechanism.[12-14] A part of the absorbed iron might remain bound on ferritin in the enterocytes, from where it will be removed from the body with shed enterocytes, thereby eliminating iron by faecal excretion.[15] Iron transport from the enterocyte into the blood is mediated by ferroportin-1 (FPN1 or SLC40A1). This transmembrane protein is the only identified iron exporter from the basal membrane, macrophages, and hepatocytes.[16,17] In this process, SLC40A1 exports iron across the basal membrane, which is then oxidised by hephaestin representing a copper-dependent ferroxidase most highly expressed in the small intestine but also in other organs.[18,19]

In the circulation, Fe^{3+} is bound to plasma transferrin representing the major iron storage source for the production of new erythrocytes in the bone marrow.[20] It is utilised by erythroblasts (red blood cell precursors) for the synthesis of haemoglobin.[21] Erythrocytes also participate with the macrophages in iron recycling. When aged erythrocytes are recognised by macrophages and erythrophagocytosis in the phagolysosome occurs, iron is recovered by the degradation of haemoglobin and either stored in ferritin or routed back to the circulation by the SLC40A1 transporter across the plasma membrane.[22-24]

TRANSPORT

Daily iron losses in healthy individuals are mainly due to bleeding (menstruation, pregnancy) and the regular exfoliation of iron-bound epithelial cells in the mucosal line of the gastrointestinal and urinary tracts, as well as from skin.[25] There is no other effective way of iron removal, and existing iron has to be stored or used. A healthy individual typically contains 4–5 g of iron. More than half of this transition metal is bound in haemoglobin to transport oxygen, while the rest is bound in ferritin complexes. The liver represents the main store of iron and the hepatic stores of ferritin are the primary physiologic source of reserve iron in the body.[26] Furthermore, it participates in absorption, storage, and export of iron, indicating that it is a key organ for iron regulation. The TfR system is the major mechanism for iron uptake in the liver and other tissues. Under low iron

concentration levels, the expression of TfR1 is increased in the hepatocytes to promote cellular iron intake.[27] Thus, the TfR primarily maintains cellular iron homeostasis. Conversely, at high iron concentrations, iron-regulating proteins are inactivated and TfR1 expression is suppressed, thereby lowering iron uptake. Studies in mice and humans have shown that subjects lacking transferrin develop massive iron overload in non-haematopoietic tissues, including liver and pancreas.[28,29] Excess liver iron is known to promote steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.[30,31] If the iron concentration is too low, stored iron can be exported from the hepatocytes into the bloodstream by the transmembrane protein SLC40A1.[16]

REGULATION

The body's iron concentration is very tightly regulated. The intracellular supply and storage of iron is mediated principally by three proteins: transferrin, TfR, and ferritin. Transferrin is a serum glycoprotein responsible for transporting bound iron from absorption centres to tissues.[32] Furthermore, transferrin as a component of an iron-sensing system is crucial for the maintenance of iron homeostasis by modulating hepcidin expression.[33] Most cells modulate iron uptake by regulating the amount of TfR expression and activity. Regulation is mediated by intracellular iron levels and iron-responsive elements within cell mRNA that are recognised by special iron regulatory proteins.[32] The hepatic iron-regulatory hormone hepcidin, a major regulator of iron, is secreted into the blood and interacts with enterocytes to regulate the rate of iron absorption.

Hepatocytes have been proved to be the main source of the circulating hepcidin. Hepcidin is feedback regulated by iron concentration.[34] When iron is abundant, more hepcidin is produced by hepatocytes limiting further iron absorption and release from iron stores. In case of low iron levels or deficits, lower production of hepcidin allows more iron to enter the circulation. It is believed that the liver uses transferrin saturation as a critical iron sensor, since the response of the liver-derived hormone hepcidin to dietary iron is induced by increased iron amount bound to plasma transferrin.[35] There are two major cell iron uptake pathways: the transferrin-bound iron uptake and non-transferrin-bound iron uptake.[36,37] Physiologically, the transferrin-bound iron uptake mechanism takes place. In this pathway, iron is internalised after binding on TfR1. After endocytosis, a ferrireductase reduces the released ferric iron and the reduced form is transported by DMT1 to the cytosol and the labile iron pool from which it can be mobilised when needed.[37] The second, less characterised, non-transferrin-bound iron uptake mechanism is usually utilised in iron overload conditions when transferrin is saturated with iron. In this case, there is a necessity for the action of more cell surface ferrireductases or reductants (e.g. ascorbic acid) that will reduce ferric, non-transferrin bound, iron to its ferrous state, allowing its intracellular import by transporters such as DMT1.[13,37,38]

FIRST STEPS TO IDENTIFY HEREDITARY HAEMOCHROMATOSIS

The term haemochromatosis was coined in 1889 by the German pathologist von Recklinghausen who identified a patient with excess tissue iron deposits at autopsy.[39] In 1935, the English gerontologist Joseph H. Sheldon suggested that this disease was an inherited metabolic disorder associated with increased iron absorption.[40] Following these fundamental findings, it is now widely accepted that the iron overload is the outcome of increased intestinal iron absorption. The abnormal absorption by the gastrointestinal mucosa leads to excessive toxic quantities of iron in tissues that can provoke functional impairment of various organs, particularly in the heart and liver.[41] In the mid 1950s, the first encouraging results in treating patients with therapeutic phlebotomy were reported.[42] Since then, blood removal has been the method of choice for reducing excess quantities of iron. In 1975, HH was associated with the major histocompatibility complex (MHC) located on the short arm of human chromosome 6.[43] However, the precise genetic basis for the development of HH was unravelled in 1996 when Feder et al.[44] used linkage-disequilibrium and full haplotype analysis and identified a gene related to the MHC Class I family, initially termed human leukocyte antigen associated haemochromatosis (HLA-H), which was mutated in patients with HH.[44]

Nowadays, the *HLA-H* gene is referred to by its approved gene name *HFE* (high iron Fe-), which was given by the Gene Nomenclature Committee of the Human Genome Organisation (HUGO). It encodes a membrane protein critically involved in the regulation of circulating iron uptake. The HFE protein is primarily expressed in liver, intestinal, and, to a lesser extent, in immune cells.[45,46]

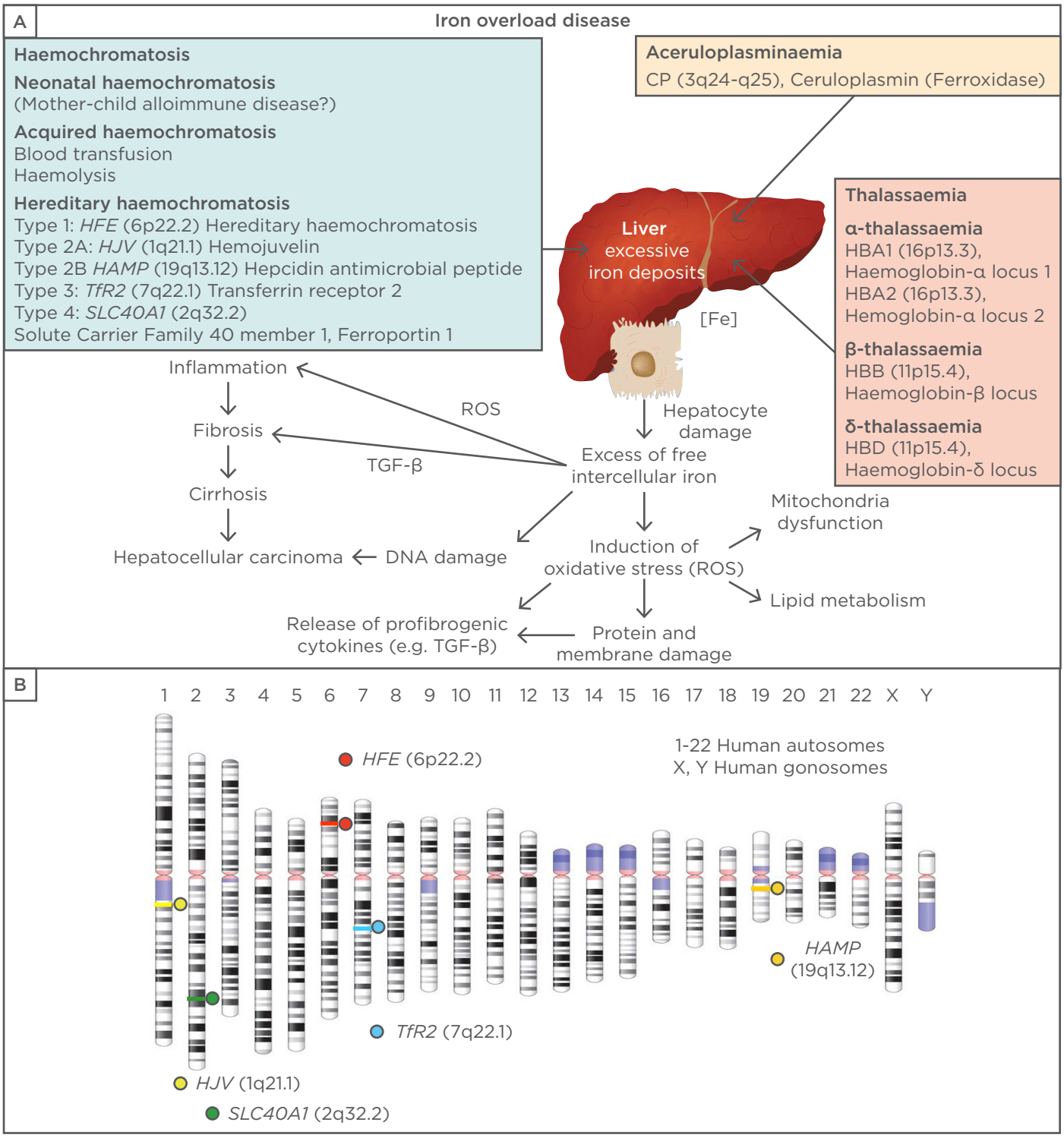

Figure 2: Genes involved in the pathogenesis of iron overload disease.

A) Excessive iron build-up may result from various mutations that impact iron homeostasis. Different types of hereditary haemochromatosis are induced by mutations affecting the genes *HFE*, *HJV* (hemojuvelin), *HAMP* (Hepcidin Antimicrobial Peptide), *TfR2* (Transferrin Receptor 2), and *SLC40A1* (Solute Carrier Family 40 member 1, Ferroportin 1). Other causes of Fe overload are neonatal haemochromatosis, acquired haemochromatosis, thalassaemia, or *CP* gene mutations encoding ceruloplasmin (also known as ferroxidase). In the liver, the elevated intracellular iron levels induce formation of reactive oxygen species (ROS), mitochondrial dysfunction, protein and membrane impairment, DNA damage, and lipid oxidation. These alterations lead to necrotic hepatocyte death and release of pro-fibrogenic cytokines, such as transforming growth factor-β (TGF-β), which induce inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma.[2] B) Chromosomal localisation of the human 'haemochromatosis genes'.

It interacts with the TfR required for iron import from transferrin into cells by endocytosis and is further critically involved in the regulation of the hepcidin gene (Hepcidin Antimicrobial Peptide [*HAMP*]), which reduces dietary iron absorption across the gut mucosa.

Studies on the *HFE* gene have revealed >20 mutations responsible for the majority of cases of Type 1 HH. However, the penetrance of these mutations is variable and a single mutation (i.e. Cys282Tyr or C282Y) in the *HFE* gene explains 80–90% of all diagnosed cases in populations of Northwestern European ancestry, while the penetrance of another variant (e.g. His63Asp or H63D) as well as their compound heterozygote forms (C282Y/H63D) are less frequently associated with the disease.[47] *HFE* mutations lead to inadequate production of hepcidin, which results in increased iron uptake by the enterocytes, the release of iron from macrophages into the circulation, and increased deposition of excess iron in numerous tissues, which promotes excessive damage.[48,49] HH and in particular the *HFE*-related type has been associated with liver damage, diabetes, insulin resistance, infertility problems, heart disease, stroke, neurodegenerative disorders, cancer, hormone imbalances, and artery disease.[49-56]

TYPES OF GENETIC HEREDITARY HAEMOCHROMATOSIS

A number of genetic alterations can result in the accumulation of excess iron and rise of haemochromatosis in the body. The most frequent are defects in genes encoding *HFE*, *TfR2*, ferroportin-1 (*SLC40A1*), *HAMP*, and hemojuvelin (*HJV*) (Figure 2A). All these defects are associated with elevated quantities of free intracellular Fe in the liver inducing formation of reactive oxygen species. These provoke oxidative damage of mitochondria, proteins, membrane structures, DNA, and lipids, thereby provoking cellular destruction. In the liver, the hepatocytes become necrotic and release large amounts of pro-fibrogenic cytokines, such as transforming growth factor-β, which induce inflammation, fibrosis, cirrhosis, and development of hepatocellular carcinoma.[2]

The different types of HH are classified by the underlying genetic mutations and mode of inheritance. Nowadays, five major genetic manifestations have been distinguished. Type 1 HH is by far the most intensively studied type and is linked to mutations in the *HFE* gene.[44] The HFE protein associates with Class I light chain β2-microglobulin (β2m) and interacts with TfR on the cell surface, thereby reducing their affinity for iron and stimulating hepcidin expression.[44,57,58] The Type 1 patients are most often homozygous for the C282Y mutated allele or carriers of compound heterozygote mutated alleles at amino acid position 282 and 63. In a cohort of >500 individuals, it was shown that homozygote C282Y carriers are genetically predisposed to accumulate iron and develop haemochromatosis with a prevalence of 34.3% for males and 29% for females to exhibit clinical symptoms.[59] According to Seckington and Powell,[60] the phenotypic spectrum of Type 1 haemochromatosis includes i) individuals with clinical *HFE*-HH who present organ damage as a result of harmful iron excess, ii) subjects with only biochemical *HFE*-HH where the iron accumulation is only reflected in increased transferrin-iron saturation and serum ferritin levels without impairment of organ physiology and function, and iii) non-expressing C282Y homozygotes who develop neither clinical symptoms related to *HFE*-HH nor iron overload.

Another much less common type of severe HH is juvenile Type 2 haemochromatosis, which in contrast to the Type 1 HH is a juvenile onset disorder. Juvenile forms of HH occur in two forms: Type 2A and 2B HH.[61] Haemochromatosis Type 2A is also an autosomal recessive disorder that results from a mutation of the *HJV* (or *HFE2*) gene that is located on chromosome 1 (Figure 2B) and encodes the protein hemojuvelin which is produced in the liver, heart, and muscles.[62] Hemojuvelin participates in the iron balance pathway by stimulating the expression of the iron regulating peptide hepcidin via the SMAD pathway.[63-65] The individuals carrying this mutation have excess iron load already early in life, and develop symptoms in childhood. Respective individuals, by the age of 30 years, are characterised by hypogonadotropic hypogonadism, which leads to delayed puberty and infertility and risk for heart failure due to the prominent cardiac iron deposition.[61] The juvenile haemochromatosis Type 2B is a phenotypically similar to Type 1 HH. It is caused by mutations within the hepcidin gene located on chromosome 19 and represents a key factor in the control of dietary iron absorption, storage, and tissue distribution.[61,66]

Type 3 HH develops typically before the age of 30 years, while Type 4 HH manifests similarly to Type 1 HH in adulthood, with clinical symptoms typically appearing between the ages of 40–60 years

in men and usually after menopause in women. Type 3 is caused by mutations in the *TfR2* gene, which is expressed on the basolateral surfaces of hepatocytes and a modulator of hepcidin expression in response to iron.[66,67] Type 4 haemochromatosis, or ferroportin disease, is caused by mutations in the *SLC40A1* gene encoding a cell membrane protein that has been suggested to be involved in iron export from duodenal epithelial cells and transfer of iron between maternal and fetal circulation.[66] The symptoms of ferroportin disease vary from one person to another. The variety of symptoms is related to the different mutations of the *SLC40A1* gene. Generally, ferroportin disease is divided into two forms: either a mild form or a more severe form resembling HH Type 1 in which liver damage and cirrhosis are more prevalent.[68,69] Types 2, 3, and 4 are also mentioned in the literature as non-*HFE* HH types.[66]

HFE-MUTATIONS AFFECT IRON REGULATION

Type 1 HH is an autosomal recessive disorder resulting in iron overload and variable multi-organ dysfunction. HFE is a MHC class 1-like protein that associates with β2m enabling efficient transport of the HFE protein to the cell surface where it interacts with TfR1. It is thought that this protein functions to regulate circulating transferrin-bound iron uptake by regulating the interaction of the TfR1 with transferrin. The C282Y mutation disrupts the signalling bridges in the α-2 and α-3 extracellular domains of the HFE protein preventing the association of HFE with β2-microglobulin and TfR1, while the mutant protein is retained in the Golgi apparatus.[70] The lack of the HFE/TfR1 interaction increases the affinity of TfR1 for the transferrin-bound iron, thereby modulating iron absorption by the duodenal crypt cells.[71] In contrast to the *C282Y* mutation, the *HFE*-H63D variant maintains its affinity for TfR1, thereby supporting clinical findings showing that the H63D mutation only rarely leads to haemochromatosis. The modified molecular signalling between the HFE protein and the TfR1 is being intensively studied as the main regulatory mechanism in the pathogenesis of HH.[72] Experimentation in mice deficient for the hepatic-*HFE* showed that iron homeostasis is predominantly controlled by regulating hepcidin that is produced in the liver and impacts the amount of iron absorbed from the diet and released from iron storage sites. Consistently, the over-expression of *HFE* is seen in mice lacking endogenous *HFE* upregulated hepcidin expression and normalised liver iron levels.[73] In the same line, the lack of *HFE* in mice decreased the concentration of hepcidin, which strengthened further iron overload.[74] Hepcidin expression can be modulated by mutations within *HFE*, *HJV*, and *SLC40A1* that lead to iron disorders. Hepcidin is capable of regulating SLC40A1, affecting the iron absorption by enterocytes, iron export from macrophages into circulation, and deposition to cells or tissues.[75]

HFE DIAGNOSIS

Although practice guidelines for the diagnosis and management of haemochromatosis have been reported,[76] new therapies are still in development with the aim to reduce discomfort and create quicker and permanent results. As discussed above, the majority of patients of Northern European descent with HH are homozygous for the *C282Y* mutation in the *HFE* gene. Although a significant proportion of patients with this genotype will develop iron overload (i.e. biochemical haemochromatosis), only a few will develop clinical symptoms and/or organ damage. The clinical penetrance depends on factors such as sex, age, genetic factors, and alcohol consumption.[77,78] For the diagnosis of the disorder, the clinicians can rely on a combination of biochemical evidence of iron excess in the body, clinical symptoms, non-invasive testing, and imaging data. Suitable biochemical parameters that indicate iron overload are elevated serum transferrin-iron saturation values and serum ferritin concentrations. Although transferrin-iron saturation is an early and reliable indicator of risk for iron overload Type 1 HH, there is no correlation between transferrin-iron saturation and clinical findings. Likewise, untreated *HFE*-HH leads to increased levels of serum ferritin concentration.[66] However, the serum ferritin level alone is not a specific marker of haemochromatosis because it belongs to the acute phase proteins that increase during inflammatory responses. Usually, elevated serum ferritin levels in association with elevated serum transferrin-iron saturation are more accurate for determining the disorder's diagnosis.[60] Additional testing could include a magnetic resonance imaging scan, which is a non-invasive method that allows measuring the degree of hepatic iron overload. If liver damage is suspected, quantitative iron measurements in a liver biopsy specimen might complete diagnosis.

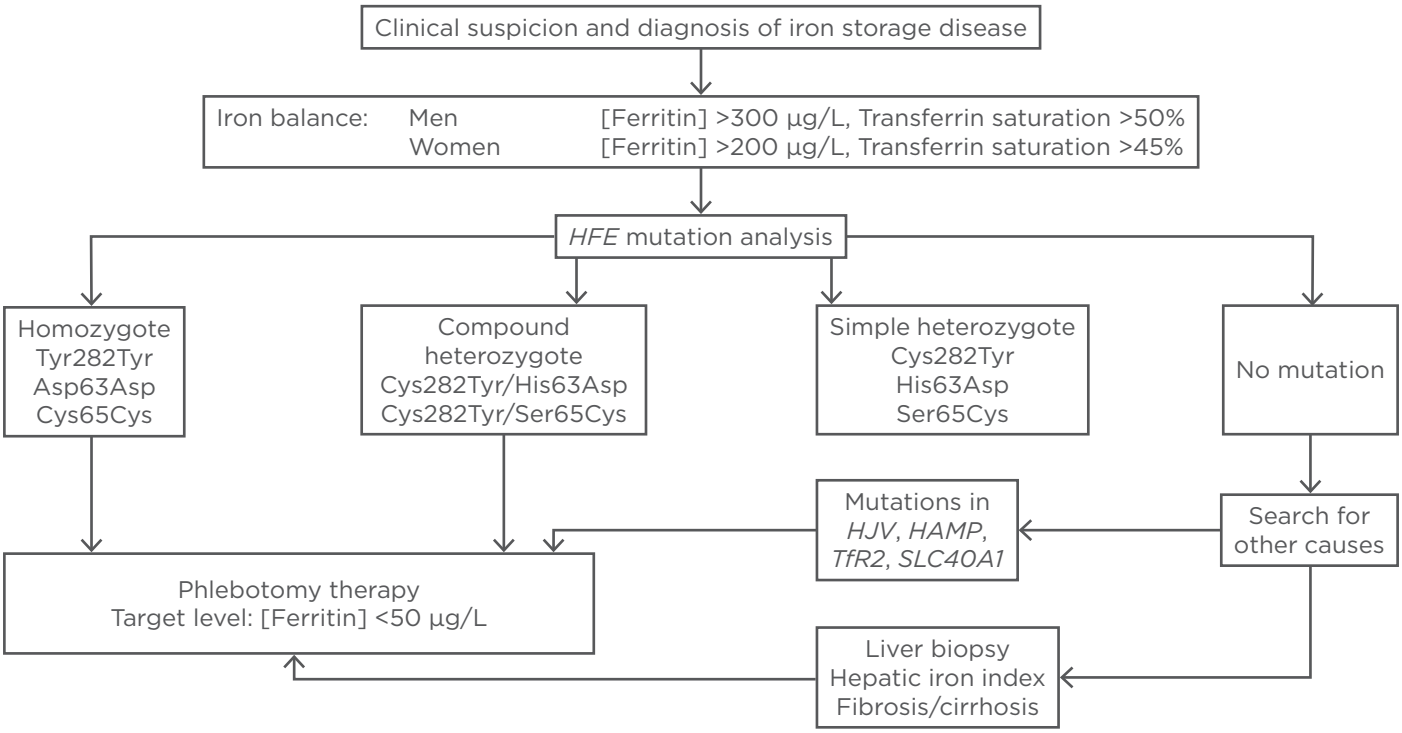

Figure 3: Diagnostic strategy in cases of suspected genetic haemochromatosis of Type 1.
A Type 1 HH can be diagnosed with certainty by detecting the mutations indicated. The detection of homozygous mutations in codons 282, 63, and 65, or the occurrence of a double heterozygous ('compound') mutation (Cys282Tyr/His63Asp or Cys282Tyr/Ser65Cys) is sufficient to diagnose HH. If no gene variants in the *HFE* gene are detectable, mutations in other genes (*HJV*, *HAMP*, *TfR2*, and *SLC40A1*) might be responsible for the iron overload condition. Therapy for iron overload in patients with proven mutations is done by regular bloodletting in which the target is a final ferritin blood level of <50 µg/L. The diagnostic strategy based on transferring saturation and genetic testing was first proposed by Eijkelkamp et al.[82]
HAMP: Hepcidin Antimicrobial Peptide; HH: hereditary haemochromatosis; *HJV*: hemojuvelin; *SLC40A1*: Solute Carrier Family 40 member 1.

The clinical symptoms indicating the progression of iron overload include hepatomegaly, hepatic cirrhosis, hepatocellular carcinoma, diabetes, cardiomyopathy, hypogonadism, arthritis, and progressive increase in skin pigmentation. Ultimately, genetic testing (C282Y, H63D, S65C) is suitable to confirm the diagnosis HH and to explain the occurrence of clinical symptoms and altered biochemical parameters. Subjects with abnormal iron tests should undergo genetic testing to confirm the diagnosis. In particular, several methods for genotyping of the C282Y, H63D, and S65C variant sites were established and are in daily routine use. These include restriction fragment length polymorphisms analysis, genomic sequencing, and real-time polymerase chain reaction analytics using fluorescence resonance energy transfer probes. The C282Y mutation disrupts a disulfide bridge that is located in the extracellular domain of the HFE protein, thereby preventing the association with β2m and the TfR and preventing the proper production of hepcidin.[79] Since transferrin and hepcidin are both considered key elements in the regulation of iron absorption from the diet, iron regulation is disrupted and too much dietary iron is absorbed in the duodenum. This increase of dietary iron leads to the iron overload characteristic of Type 1 HH.[73] The significance of the second most frequent *HFE* mutation, H63D, is still unclear.[80] In addition, the *HFE* S65C mutation may lead to mild hepatic iron overload, but does not encounter for clinical manifest haemochromatosis in most patients.[81] Likewise, a number of non-*HFE* mutations have been identified that are less commonly related to HH.

Aiming to identify individuals in a given population who are at higher risk for *HFE*-associated haemochromatosis, Eijkelkamp et al.[82] developed a screening algorithm (Figure 3) using liver function test and transferrin saturation. Based on the

general established guidelines, genetic testing is also recommended for all first-degree relatives of anyone diagnosed with HH, but general population screenings for *HFE* gene mutations remains a topic of debate.[83-85] In the present guidelines, in patients with clinical suspicion and diagnosis of iron storage disease, iron imbalances are identified by increased ferritin and transferrin saturation (Figure 3). Genetic testing of relevant variant sites (C282Y, H63D, and S65C) is the gold standard to diagnose HH. If in respective patients, none of the variants occur, mutations in other genes that are involved in the control of iron homeostasis are most likely. Concomitant measurements of the hepatic iron index, which is the iron content given in µmol per g of liver corrected for the age of the subject, and scoring of existing fibrosis/cirrhosis, are further diagnostic options to estimate the success of therapy.[86]

The classical Type 1 HH is sometimes considered as a multifactorial disease characterised by mutations within the principal gene (i.e. *HFE*) and modulated by minor genetic or environmental factors that critically contribute to the outcome of the clinical phenotype in haemochromatosis. Nowadays, there is an increasing list of gene polymorphisms and factors known to contribute the penetrance of *HFE* gene mutations. However, the impact of these modifiers, including gene variants in *HAMP*, *BMP2*, *FTL*, and *SLC40A1* genes, are not conclusive and are discussed controversially.[87]

THERAPY AND MANAGEMENT

HH is a disorder that causes an excess of iron deposition that can lead to toxic effects in tissues and multi-organ damage with clinical consequences that affect both quality of life and life expectancy. The aims in treating HH include diminishing of iron levels to the normal, preventing organ damage, treating possible complications such as liver disease, heart problems, or diabetes, and maintaining normal iron levels for the rest of life. Currently, the treatment of HH is based on phlebotomy therapy (venesection) and the elimination of iron by chelation therapy, depending on the aetiology.[88] To date, phlebotomy is preferred, as it is more effective and more pleasant for the patient. In the initial phase, 500 mL blood is withdrawn, by the same procedure as blood donation, which leads to a decrease in body iron. After phlebotomy sessions, excess stored iron is utilised by the body to produce new red blood cells. During treatment, the quantities of remaining stored iron and the effectiveness of treatment are monitored by serum ferritin measurements. In addition, determination of haemoglobin levels helps to prevent a sharp iron decrease. The treatment should be done on a regular weekly basis, usually for 2 years. After reaching the desired iron decrease that is indicated by levels of serum ferritin, the patient undergoes (for the rest of their lives) maintenance phlebotomy every few months that is controlled by monitoring of transferrin saturation (ideally <50%) and serum ferritin (desired level 50–100 µg/L).[89] Iron chelation is also a method to reduce iron pools in patients, particularly in those in which severe physical or medical difficulties in giving blood prevail. Therefore, this therapeutic regimen is to be classified as a more individualised treatment of HH. Iron chelating drugs presently used are: deferoxamine, which is administered by infusion, or deferiprone and deferasirox that are both given orally.[88,90] While deferoxamine must be administered mostly on a daily base, the two oral iron chelators have several favourable effects in regard to toxicity, metabolisation, distribution volume, frequency of side effects, and compliance.[90] These chelators successfully reduce hepatic and/or cardiac iron, but the application of these drugs is often associated with gastric discomfort, zinc depletion, musculoskeletal and joint pain, hearing impairment, visual disturbances, and skin irritations.[90] Although the effective elimination of iron in individuals treated prior to the development of cirrhosis results in a normal life expectancy, subjects diagnosed after development of cirrhosis have decreased life spans even with iron depletion therapy.[60] Also, nonhepatic manifestations, such as endocrine abnormalities and arthroplasty improve in only 20% of the patients that received iron depletion therapy.[60]

More recent findings suggest that the treatment with erythrocytapheresis is an alternative treatment. Erythrocytapheresis is the selective removal of erythrocytes by an extracorporeal apheresis procedure that leads to removal of more erythrocytes than during phlebotomy. As a consequence, this methodology requires drastically fewer treatment sessions necessary for the initial removal of overabundant iron.[91] Several independent studies have proven that elevating hepcidin concentration is another possible strategy to ameliorate iron overload. Hepcidin-targeted therapies in which novel therapeutics manipulate the mechanisms regulating hepcidin production (e.g. hepcidin-based agonists, hepcidin expression,

or activity stimulators) could provide a more fundamental future approach in HH treatment.[34,89]

In 2000, a clinical trial was initiated by the American National Institutes of Health (NIH) Clinical Center aiming to evaluate the effectiveness of a test using the mean corpuscular volume/haemoglobin monitoring guide for phlebotomy.[92] This ongoing study compares the usefulness of the standard ferritin test in phlebotomy therapy in HH patients with that of mean corpuscular volume indicative for red blood cell size. Moreover, the trial will also clarify whether keeping iron levels low during maintenance therapy can improve liver disease and arthritis in affected patients.

Last but not least, major advances in molecular biology and pharmacology will lead to improving diagnosis and HH disease management. During any of the above-mentioned treatments, the reduction of iron overload is also managed by dietary changes. These include reduction in alcohol intake to prevent liver damage, avoiding iron containing supplements or drugs, as well as the restricted uptake of nutrients such as vitamin C that improves absorbance or dietary iron.

CONCLUSION

Since the first description of HH by the French internist Armand Trousseau in 1865, the understanding of the pathogenesis of this inherited disease and the regulation of dietary iron absorption has increased dramatically. In particular, genotyping and measurement of blood parameters indicating elevated concentrations of iron have enriched the diagnostic tools. During recent decades, special emphasis has been given to the understanding of iron metabolism and effective treatment of HH. The most effective treatment of HH is still the conduction of serial phlebotomies. Improving therapies and designing well-targeted iron chelating agents could minimise the toxicity, discomfort, and duration of current treatments, and lead to a higher quality of living standards in patients suffering from iron overload diseases. However, there are still challenges in haemochromatosis research that need to be addressed. Recent work has unravelled several gene mutations, polymorphisms, confounding factors, and environmental determinants impacting the outcome or severity of iron overload disease. The precise mode of activity of several of these modulating compounds is still enigmatic. More investigative studies aiming to decipher how these factors influence the clinical prevalence of iron overload are urgently needed. In addition, the complex network of iron-modulating and iron-regulating factors, such as hepcidin, will offer in the future a large variety of alternate promising therapeutic strategies for HH. In this regard, small cyclic agonists or peptides (i.e. the 'minihepcidins') of the iron regulatory hormone hepcidin that lower iron levels in the blood have already shown some highly beneficial effects in animals.[93,94] In addition, the concomitant development of elementary guidelines for supervised application of reactive oxygen species scavengers would be helpful to prevent reactive oxygen species-associated cell damage and ongoing tissue damage in the setting of HH.

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ANKYLOSING SPONDYLITIS: A REVIEW

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ABSTRACT

Ankylosing spondylitis is a chronic autoimmune inflammatory condition belonging to the spondyloarthropathy category of rheumatic diseases. It typically affects the axial skeleton but may also present with peripheral arthritis and extra-articular features. Ankylosing spondylitis tends to occur in patients under the age of 45 years, has a higher incidence in males, and can lead to disability and reduced quality of life if not adequately treated. Management consists of a multidisciplinary team approach. Although traditional disease modifying anti-rheumatic drugs are less effective for the axial component of this disease, biologic therapies do seem effective. In severe cases, surgery may be warranted.

Keywords: Assessment of SpondyloArthritis international Society (ASAS), biologics, non-steroidal anti-inflammatory drugs (NSAID), spondyloarthropathy.

INTRODUCTION

Ankylosing spondylitis (AS) has been afflicting humankind as far back as ancient Egypt.[1,2] It was during the 1800s that the classical description of AS was made.[3] Throughout the 1900s, further understanding about the disease was established, including its hereditary nature.[3] The disease is recognised as part of the spondyloarthropathy group of rheumatic diseases. These include psoriatic arthritis, reactive arthritis, and arthritis associated with inflammatory bowel disease.[2] These conditions share similar clinical features and an association with human leukocyte antigen (HLA)-B27.

The primary sites of inflammation in AS are the sacroiliac joints. Males tend to be more commonly affected than females,[2] although studies over the years have shown this sex difference to be a lot more marginal than was initially thought.[3] It primarily affects young adults, with a higher incidence in patients younger than 45 years old. As the disease progresses it can result in total fusion of the axial skeleton, and can cause loss of physical function and spinal mobility.[4] Patients in which the disease has been inadequately treated or undiagnosed can develop a characteristic 'bamboo spine' where there is total spinal fusion. As well as chronic pain, this can also result in restrictive lung function, leading to respiratory failure.[2]

AS is not just limited to the spine; the peripheral joints can be affected, and organs such as the eyes, heart, and lungs can be involved. Patients can also complain of systemic symptoms such as fatigue or weight loss. There is a high risk of osteoporosis and vertebral fractures.[3] Chronic pain and immobility can lead to patients experiencing depression and anxiety. There is a socio-economic burden as patients may be unable to work, either due to their symptoms or a workplace that may not be adapted for people with arthritis. Thus, it is important to recognise that this is a multisystem disease and the clinician should be wary of focussing purely on spinal symptoms.

Definite diagnosis can be delayed as radiographic changes of sacroiliitis occur late in the disease process.[3] It can take up to a decade for radiographic changes to appear, with a proportion of patients never going on to develop radiographic changes at all. Patients who present without radiographic changes are described as having non-radiographic axial spondyloarthritis (nr-axSpA), with debate in the literature over whether this represents a separate disease entity altogether.[3,5] Classification criteria have been updated to

include the diagnosis of nr-axSpA. It should be noted that certain treatments, such as infliximab and interleukin (IL)-17 inhibitors, are not approved for nr-axSpA according to the Assessment of SpondyloArthritis international Society-European League Against Rheumatism (ASAS-EULAR) 2016 recommendations, but other biologic drugs are approved.[5] Overall, however, it is argued that only the single term of AS should be used to encompass the disease.

PATHOPHYSIOLOGY

Thanks to advances in imaging techniques, clinicians have a better understanding of how AS results in structural damage to the axial skeleton. While the underlying mechanism triggering the inflammatory process is unknown, study of the pathology of AS has revealed cells that are involved in the process.

HLA-B27, a Class 1 surface antigen, is found in ≤89% of AS patients,[3] and is strongly associated with the spondyloarthropathy group of diseases.[2] HLA-B27 binds antigenic peptides for presentation to cytotoxic T cells, thus enabling normal function of the immune response in targeting pathogens such as the influenza virus.[6] The exact mechanism by which HLA-B27 plays a role in AS is so far unexplained. Nevertheless, it is thought to involve abnormalities in antigen presenting cells, subsequently triggering the inflammatory cascade.[3]

There has been interest in the enzyme ERAP1 and how this may be linked to the pathophysiology of AS.[7] The enzyme's function is to trim peptides for binding to HLA Class 1 molecules.[7] It has been hypothesised that ERAP1 exerts a pathogenic effect by altering the interaction between HLA-B27 and immune receptors.[7] Further research is being conducted into this area of AS to ascertain whether ERAP1 could provide a therapeutic target.

Whatever the initial events, AS results in structural damage to the axial skeleton. Proinflammatory cytokines, such as IL-17 and tumour necrosis factor (TNF)-alpha, are released, which activate cells causing bony destruction. Another cytokine, IL-22, causes osteoproliferation. These processes can sometimes occur simultaneously.[3] As a consequence of new bone growth, syndesmophytes develop inside ligaments,[8] which are considered a hallmark radiological feature of AS. At its most severe, this process can lead to complete fusion of the axial skeleton.[3]

TNF-alpha has been found to be elevated in the serum and synovial tissue of patients with AS,[4] and has been an important therapeutic target. TNF inhibitors have been shown to be effective in reducing disease activity and stiffness;[4] thus suggesting an important role for this cytokine in the pathogenesis of AS. There is still debate, however, as to whether TNF inhibitors are truly disease modifying in AS.[8] The recognition of IL-17 and IL-23 in the pathogenesis of AS has enabled researchers to develop further therapeutic options.

CLINICAL FEATURES

Back pain is a common symptom that most people experience at one time or another during their lives. There are many different causes of back pain and, therefore, clinicians need to be aware of how to distinguish symptoms characteristic of inflammation from other causes. Inflammatory back pain characteristically improves upon activity and worsens with rest. It should be present for at least 3 months to warrant further investigation.[3] The pain is described as dull and insidious in onset and may be nocturnal, interfering with the patient's sleep pattern. Early morning stiffness lasting longer than 30 minutes is an important feature. Patients commonly complain of lower back or buttock pain.[3] This could be unilateral initially but become bilateral as the disease progresses.[2] The cervical and thoracic spine can also be affected but less commonly. Table 1 summarises the characteristic features of inflammatory back pain.[2,9]

AS typically presents in patients under the age of 45 years and is more prevalent in males, although it can occur in female patients too.[2] The pain may progressively worsen. Neurological symptoms can occur in AS secondary to spinal fractures. If a patient complains of new onset back or neck pain following injury with a background of severe AS, the patient should be investigated for fractures.[3] Clinicians should be vigilant of the possibility for osteoporosis in these patients and treat as appropriate.

Psoriasis and inflammatory bowel disease can occur secondary to AS.[3] Other extra-articular manifestations include anterior uveitis and peripheral arthritis.[2,3] The joints most commonly affected are the large joints of the knees, hips, and shoulders.[2] Hip disease in particular can be very disabling and some patients may require a total hip replacement if there is structural damage.[2] Enthesitis and dactylitis can be a presenting feature.[3] Aortic incompetence, upper lobe

pulmonary fibrosis, and renal disease secondary to amyloid deposition can occur in AS.[2] Some studies have also suggested AS patients are at an increased risk of nephrolithiasis when compared with the rest of the population.[10] Other cardiovascular abnormalities, such as conduction defects, can be present and can have important haemodynamic consequences.[11] Thus, patients should be screened for any cardiovascular and respiratory symptoms when seen in the clinic. Systemic features such as fatigue, weight loss, and low-grade fevers could be present, indicating an inflammatory process.

On examination, patients can display evidence of spinal deformity, including loss of normal lumbar lordosis and kyphosis. The sacroiliac joints may be tender to touch and there may be restriction of normal movement of the lumbar and cervical spine, along with reduced chest expansion. The Schober's test is used to measure lumbar flexion; <5 cm of flexion is considered an abnormal result. Patients may have swollen and tender peripheral joints in an asymmetrical distribution. There may also be tender enthesial points, such as the Achilles tendon insertion.

INVESTIGATIONS

Laboratory abnormalities, such as elevated inflammatory markers and normocytic anaemia, are present in most cases, but in some cases patient's blood tests can be normal.[12] An elevated C-reactive protein may only be found in 50% of cases. However, it is useful to request a C-reactive protein test as, if elevated, it can indicate a favourable response to biologic therapy and can help to differentiate from mechanical causes of back pain. HLA-B27 is sometimes requested to help aid diagnosis, although in clinical practice some clinicians do not routinely ask for this test as it can be present in up to 7% of the Caucasian population. Renal function should also be monitored.[2]

Table 1: Characteristic features of inflammatory back pain.

Age of onset	<45 years
Duration	>3 months
Onset	Insidious
Morning stiffness	>30 minutes
Improvement with exercise?	Yes
Improvement with rest?	No
Nocturnal pain?	Yes
Alternating buttock pain?	Yes

Table 2: Sacroiliitis grading on plain film.

Grade 0	Sacroiliac joints normal
Grade 1	Blurring of joint margins
Grade 2	Solitary erosions and juxta-articular sclerosis in small sacral or iliac areas
Grade 3	Manifested juxta-articular sclerosis, numerous erosions with widening of joint space, and possible partial ankyloses
Grade 4	Complete ankylosis

Table 3: Types of magnetic resonance imaging lesions in ankylosing spondylitis.

Active inflammatory lesions	**Chronic inflammatory lesions**
Bone marrow oedema	Sclerosis
Capsulitis	Erosions
Synovitis	Fat deposition
Enthesitis	Bony bridges/ankylosis

Plain radiographs of the sacroiliac joints can show characteristic changes of sacroiliitis. The joint margins can look blurred, with erosions and loss of joint space (Figure 1).[12] In advanced cases, the joints may be completely fused. Sacroiliitis can be graded radiographically from 1–4, according to the modified New York criteria (Table 2).[13] Plain films of the spine may show vertebral body squaring along with syndesmophytes.[12] Erosions may also be present. The bones may demonstrate radiological osteopenia, similar to other inflammatory conditions. Radiographs may also pick up vertebral fractures.

In the early stages of the disease, plain films may be normal.[3] There is also variation in interpreting plain films, which can cause uncertainty around reaching a diagnosis.[3] Therefore, magnetic resonance imaging (MRI) may be required, as it has been shown to be more sensitive in detecting inflammation than plain films or computed tomography (CT) scans.[3,12] Indeed, in the early stages of the disease, MRI will show inflammation whilst plain films can be normal.[5] An MRI should be requested in those patients who complain of inflammatory back pain but have normal plain radiographs. As well as showing spondylitis, erosions, and arthritis, MRI can also demonstrate enthesitis, which is not evident on plain films. Bone marrow oedema indicative of localised inflammation can also be revealed by MRI.[14] It can be used to objectively monitor disease activity beyond patient and physician reporting. Table 3 highlights active and chronic inflammatory lesions as seen on MRI according to the ASAS criteria.[15]

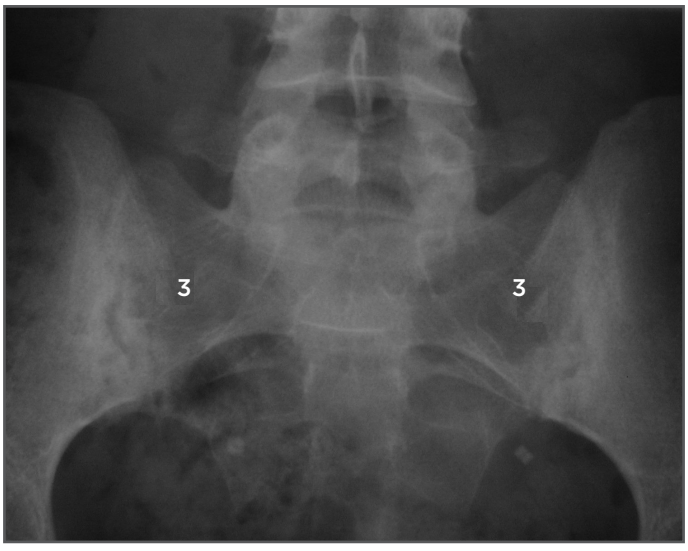

Figure 1: Bilateral sacroiliitis on X-ray.
3: Grade 3 sacroiliitis-erosions and sclerosis at the sacroiliac joints.

Active changes are best visualised on short tau inversion recovery sequence. Chronic lesions are best seen by a T1-weighted sequence.[15]

TREATMENT

Management of AS can be challenging. Conventional treatments for inflammatory arthritis lack evidence for efficacy in AS.[5] Most patients are young and are in work; AS can be debilitating and cause considerable socio-economic burden because patients have to take time off work and, in some cases, may have to leave their job altogether. Having a chronic illness can also be associated with depression and anxiety. Counselling and psychological support may be needed if the patient is suffering from depression or low mood secondary to their illness. Patient education is an important part of management.[2] Patients should be given leaflets on their condition and be directed to support groups, which can provide them with help and further information if needed. Patients with a greater understanding of their disease are more likely to be compliant with their management.[2]

The ASAS-EULAR group has recently updated its recommendations for the management of AS.[5] It recommends that AS be managed in a multidisciplinary setting, with due attention to both pharmacological and non-pharmacological treatments that take into account the societal and psychological costs of AS. Patients should complete questionnaires at every appointment to assess their pain levels, functional abilities, and quality of life. Inflammatory markers can be useful to help assess levels of disease activity and act as a useful guide to the likelihood of improvement with TNF inhibitors. Repeat imaging of the spine should only be conducted if necessary, as the information gleaned may be limited due to the slow rate of radiographic progression. If they are to be repeated then the ASAS-EULAR group recommends an interval of at least 2 years between radiographs.[5]

Non-pharmacological management includes advising the patient on lifestyle measures. Prompt referral to physiotherapy is essential, and patients should be encouraged to attend therapy appointments because exercise programmes are beneficial in maintaining patients' mobility and posture. They should keep up with exercise even when established on drug treatment.[2] Hydrotherapy can be recommended for patients who suffer from widespread body pain and stiffness. Stopping smoking may also be advisable, as there is a

possible association between disease activity and smoking.[16] If available, occupational therapy should be offered so patients can receive support with activities of daily living and any workplace modifications that may be needed.

Non-steroidal anti-inflammatory drugs (NSAID) are the first-line recommended agents for use in AS. One study examined the proportion of AS patients responding to NSAID after 4 weeks of treatment at the maximal tolerated dose. The study found that the majority of active AS patients benefitted from NSAID.[17] There was no difference in response between patients labelled as having non-radiographic AS when compared with radiographic AS. However, 44% of patients at the end of the study still had high disease scores and thus qualified for treatment escalation. Long term use of NSAID also has adverse effects, which include gastrointestinal upset, hypertension, and renal disease, thus limiting their use. In addition to NSAID, other analgesics, such as opioids, can be prescribed depending on patient and clinician preference.

If needed, local intra-articular steroid injections into the sacroiliac and peripheral joints can offer relief. However, systemic glucocorticoid treatment has not been proven to be as efficacious for AS when compared to other inflammatory conditions. Long term steroid use can also contribute to osteoporosis.[2] CT-guided sacroiliac joint injections have been shown to provide effective pain relief for up to 6 months provided correct positioning of the needle tip is used.[18] Clinicians should be aware of the risk of tendon rupture with local steroid injections for enthesitis and these should never be used around the Achilles tendon insertion.

Disease-modifying anti-rheumatic drugs, such as methotrexate and leflunomide, are not routinely used in AS due to lack of evidence for efficacy.[5,19] Methotrexate, at a high dose of 20 mg subcutaneously, was not shown to improve patients with axial symptoms in one study.[19] Evidence for its use in patients with peripheral arthritis due to AS still requires further study. However, there is evidence to suggest that sulfasalazine can be beneficial if patients are suffering from peripheral arthritis and early morning stiffness.[20] Patients should be counselled about the potential side effects, such as neutropenia and drug-induced lupus. Family planning also needs to be taken into consideration, as treatment can cause low sperm counts, although this is reversible upon drug cessation.

If patients have persistently high disease activity despite the above therapeutic options, then the next step would be treatment with biologic agents which include TNF inhibitors. The agents currently recommended are adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab, and their biosimilars. There is good evidence to support the use of biologics in AS.[3] Spinal inflammation, as detected by MRI, has been shown to reduce after anti-TNF treatment.[12] Up to 60% of patients have a good response to these agents. Patients report reduced pain scores and better physical activity with biologic treatment and are generally well tolerated.[4] Patients should be screened for hepatitis, HIV, and tuberculosis prior to starting treatment. Once started, a patient should be assessed after 12 weeks for treatment efficacy. If there has been no improvement or the patient is unable to tolerate the drug, then they can be switched to a second biologic agent. Side effects such as neutropenia and nausea can occur, along with drug-induced lupus, although uncommon. Congestive cardiac failure is a relative contraindication to these drugs. Occasionally, treatment may have to be interrupted if a patient suffers from an infection or undergoes surgery, and once recommenced may not be as effective. While there is no particularly preferred biologic agent to use in first-line therapy, it should be noted that etanercept is not effective in treating patients with inflammatory bowel disease or uveitis.[3]

IL-17 inhibitors, such as secukinumab, have been shown to be effective in AS[21-23] and can be considered an alternative in radiographic AS if anti-TNF agents fail; however, there are limited data for their use in nr-axSpA. Secukinumab has been shown to be generally well tolerated[22] and to have good efficacy in patients suffering from skin psoriasis. Clinicians should be aware that IL-17 inhibitors may exacerbate inflammatory bowel disease.

Targeting other cytokines in the inflammatory pathway, such as IL-23, is also currently being studied.[23] Some of the alternative agents with good efficacy in rheumatoid arthritis, such as rituximab and tocilizumab, have unfortunately shown little benefit in AS patients.[23] It is encouraging that at least clinicians can now consider IL-17 inhibitors following failure of TNF inhibitors. However, it is recommended that clinicians try an alternative TNF inhibitor first, before commencing treating with IL-17 inhibitors.

Patients with severe deformity may need referral for surgery. Spinal correction procedures and total hip arthroplasty may be needed in patients with refractory pain and structural damage. Anaesthesia in these patients can be a significant undertaking if they suffer from a rigid cervical spine[2] and therefore surgical options need careful consideration in terms of risks and benefits to the patient.

CONCLUSION

AS is an autoimmune inflammatory disease that causes severe spinal pain and deformity, and can be associated with extra-articular and systemic features. Untreated, it can result in chronic pain, immobility, and reduced quality of life. Patients complain of inflammatory-sounding back pain and may have other spondyloarthritis features. Investigations can include testing for HLA-B27 and inflammatory markers. Plain radiographs can be normal in early disease; thus, MRI is required to help make a definitive diagnosis. Treatment consists of a multidisciplinary approach and encouraging patients to exercise is essential to maintain mobility. Drug treatment includes anti-inflammatories as first-line and escalation to biologic therapy. Further therapeutic options that target specific IL are currently being developed.

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