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Contents

"EMJ closes the year in style with EMJ 3.4; this truly interdisciplinary journal brings together fascinating articles from a range of therapeutic areas..."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

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

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

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

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

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EMJ is supported by various levels of expertise:

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EMJ 3.4

EMJ Rheumatology 5.1

The European Medical Journal has published the latest edition of *EMJ Rheumatology*, which includes a full review of the European League Against Rheumatism (EULAR) congress...

VIEW ALL JOURNALS ←

Welcome

The end of December draws near and with it the end of a fantastic year for medical advances. As 2019 rapidly approaches, we celebrate this year's triumphs and consider its setbacks, to not only help push the boundaries of medical science but to also better ourselves. EMJ closes the year in style with *EMJ 3.4*; this truly interdisciplinary journal brings together fascinating articles from a range of therapeutic areas, including allergy and immunology, nephrology, and beyond. This a publication I am truly proud to present to you.

Immunotherapy is a technique of ever-growing importance as patients, scientists, nurses, and physicians work collaboratively to combat cancer. The Editor's Pick for this edition, by Ayers and Nasta, takes a haematological slant on this point and considers the most recent developments in the immunotherapeutic treatment of non-Hodgkin's lymphoma. Encompassing chimeric antigen receptor T cells, T cell engager antibodies, and immune checkpoint inhibition, this fascinating review highlights the successes and aims to guide future study.

For the rheumatologists among you, *EMJ 3.4* includes three captivating contributions. Firstly, Littlejohn and Guymer consider the key drivers behind the progression of fibromyalgia, a chronic pain disorder that affects 3–6% of the world's population, and discuss how these factors should be placed at the heart of treatment. Despite preconceptions to the contrary, osteoarthritis is more prevalent than rheumatoid arthritis in the global population and Sofat offers a fascinating discussion on evaluating bone marrow lesions. The medications used to treat osteoarthritis are broad and nonspecific and Sofat details how an improvement in the understanding of disease pathology is crucial to the advancement of therapeutics. Lastly, Yang and Li highlight the use of mesenchymal stem cell-based therapies in the treatment of autoimmune arthritis.

Also in this eJournal, Spensley and Tam debate the role of urinary MCP-1 as a biomarker for diabetic nephropathy and antineutrophil cytoplasmic antibody-associated vasculitis; Frossi et al. provide details of their study that resulted in the identification of chronic spontaneous urticaria in patients with common variable immunodeficiency for the first time; and there are many more articles of interest.

It is a pleasure to work on all the EMJ journals published this year, and I feel *EMJ 3.4* is the culmination of a fantastic year. I would like to pass on my thanks to all the contributors who have worked on this publication: the authors, peer reviewers, our brilliant Editorial Board, and, of course, the wonderful EMJ team.

Best wishes,

Spencer Gore

Chief Executive Officer, European Medical Group

Non-melanoma skin cancer (NMSC):

Getting to the root of the problem

NMSC is the most common cancer, and its incidence is rising[1]

Studies in Canada, the United States, Switzerland, and Australia have shown that the incidence of NMSC has been increasing at 2% to 8% per year since the 1970s.[2]

Advanced NMSC can be debilitating, with significant psychosocial and functional impacts on patients[3,4]

Typically, NMSC is curable with complete surgical excision. However, the cosmetic and functional results of treatment for advanced disease, such as scarring and disfigurement from surgery or radiation, can have a profound impact on patients.[1,3,4]

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REGENERON | SANOFI GENZYME

Sanofi Genzyme and Regeneron are committed to providing resources to better understand the incidence, identification, and treatment of NMSC and to research the unmet needs of patients with this disease.

Foreword

Dear colleagues,

A warm welcome to this year's final stimulating edition of the European Medical Journal, which reviews the latest cutting-edge scientific developments in understanding of the pathophysiology of diseases, ranging from infections to autoimmune-mediated diseases, urticaria, neoplasia, and osteoarthritis (OA). Stem cell therapeutic potential is also reviewed in detail.

Micellar polymer technology is rapidly becoming a powerful nanomedicine platform for therapeutic applications due to their small size (10–100 nm), *in vivo* stability, ability to solubilise water-insoluble drugs, and prolonged blood circulation times, and they can be targeted. Micelle formulations also have pharmacokinetic advantages with reduced systemic toxicity. In this issue, evidence for the potential therapeutic effects of liposomal glutathione in the management of HIV-1 infection and tuberculosis is reviewed.

Biomarkers are important diagnostic and disease monitoring tools. Diabetic nephropathy progression is difficult to monitor, while in ANCA vasculitis, there are no sensitive monitoring tools. A number of biomarkers in renal disease have been identified and these also now offer targeted therapeutic opportunities. This is a very exciting development in medicine.

OA causes significant disability, morbidity, and it is associated with the development of MRI defined bone marrow lesions. There are no targeted therapeutic approaches. The role of bone marrow lesions in OA is reviewed. An understanding of the mechanisms involved in their development and clinical associations may offer an avenue for specific targeting of the pathophysiologic pathways in OA to retard and/or inhibit the development of OA. There is, however, a need to develop and/or identify biomarkers for OA.

Finally, recent studies of surface cell markers in leukaemia and lymphoma have enabled the precise identification of human lymphocyte and granulocyte differentiation. The phenotypic features of lymphoma cells in non-Hodgkin's lymphoma have been characterised facilitating targeting with immunotherapeutic antibodies.

The Editorial Board and I, therefore, recommend to you this EMJ issue and sincerely hope that you will enjoy this latest edition and continue to find it a positive significant drive in your own thinking and work in the field of medicine.

With kind regards,

Prof Ian C. Chikanza

Barts and The Royal London Hospital, London, UK;
Catholic University of Zimbabwe, Harare, Zimbabwe

The Lancet Asthma Commission: Towards the Abolition of Asthma?

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Disclosure: Prof Bush is a National Institute for Health Research (NIHR) Senior Investigator and is supported by the NIHR Respiratory Disease Biomedical Research Unit, Royal Brompton and Harefield NHS Foundation Trust, London, UK and Imperial College London, London, UK. Prof Pavord is a NIHR Senior Investigator and is supported by the Oxford NIHR Respiratory Biomedical Research Centre.

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The more widespread use of inhaled corticosteroids (ICS) for the treatment of asthma following guideline recommendations 27 years ago[1,2] delivered huge benefits to many patients in terms of improved disease control and fewer of what were then called 'asthma exacerbations'. These benefits have been repeated many times as low-dose ICS have become freely available in low and middle-income settings;[3] however, what ICS have not done is ushered in a new golden age, wherein asthma is no longer a problem because of this new treatment. In fact, far from asthma being a thing of the past, it remains an ever-present threat of death and morbidity, with reduced quality of life during childhood, the loss of adult working life, and problems continuing into old age. There is also no sign of any improvement likely in the near future.[4] Why is this? We were previously charged with leading the Lancet Asthma Commission[5] addressing this question head on. We suggested that the lack of a new age of asthma therapeutic could be attributed to the following reasons:

- The success of ICS treatment has made the medical world complacent; we now believe that anyone can treat asthma and putting ICS in the tap water is the answer to all problems.
- We have come to accept that almost anyone who has any respiratory symptoms probably has asthma and no objective testing is needed; patients should just be prescribed ever higher doses of ICS until the symptoms disappear.
- We have complacently regarded asthma exacerbations as a trivial inconvenience, readily reversible with oral prednisone, and requiring no other special action.

Nothing could be further from the truth than these current and widely held beliefs. Taken point by point, the consequences have been:

- We have grossly over-treated many patients with asthma, having failed to heed the lessons of Dr Harry Morrow-Brown, who clearly demonstrated that only asthma with sputum eosinophilia responded to steroids.[6]
- Asthma has been grossly over-diagnosed and thus has become trivialised.
- Symptom relief has been regarded as the main goal of treatment with ICS, but complete symptom control is not attainable in many patients, despite good control of airway inflammation.

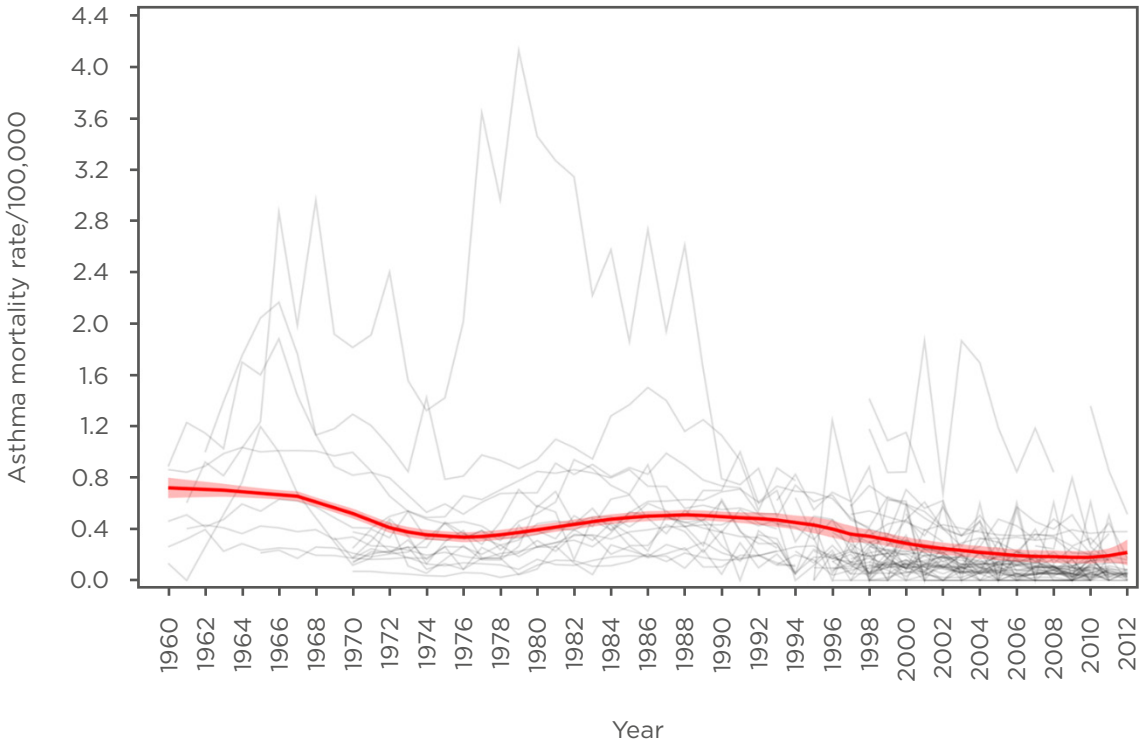

Figure 1: Crude asthma mortality rates from 1960–2012 for the 5–34 years age range in 46 countries and the two main eras of asthma management.

The locally weighted scatter plot smoother rates with 90% confidence intervals, weighted by country population, are shown in red. The association of the inflammation-based era with improved outcomes can readily be observed, as can the flat-line with regard to further improvements since 2005.

Adapted from Pavord et al.[5]

- The dose–response curve for ICS plateaus at relatively low dosage levels and the addition of further medications often fails to bring benefits, usually because the diagnosis is wrong or the originally prescribed medications are not being taken.
- The scientific community has failed to appreciate the fact that these acute deteriorations are asthma attacks not exacerbations. The acute deteriorations carry a huge risk of further attacks and death, as well as having long-term consequences,[7] and should thus be considered a red-flag never-event. This thinking has not entered the collective consciousness, despite repeated reports of asthma deaths.[8]

Against this background, with asthma outcomes stalled (Figure 1) and asthma mortality unchanged over many years, the Lancet Commission was conceived. There were seven major recommendations:

- Revolutionise airway disease and deliver precision medicine.
- Move beyond asthma control to prevention and cure.
- Emerge from age and discipline-associated silos.
- Test before treatment.
- Zero tolerance of asthma attacks.
- Maximise treatment opportunities in severe disease.
- Better research, especially basic and epidemiological.

The purpose of this article is to highlight some of the clinically important proposals of the Commission.

BEGONE, DULL ASTHMA!

The Commission aligns with previous suggestions that asthma is a term that has outlived its

usefulness.[9] Arthritis and anaemia were good umbrella terms for red, painful joints, and pale mucus membranes, respectively, but as clinically useful diagnostic terms they outgrew their usefulness decades ago and have been relegated from diagnosis to description. The same should be true of asthma; in the 21st century, it should be no more than a description of a symptom constellation (wheeze, breathlessness, chest tightness, and cough). The question 'Is it asthma?' should be replaced; instead, two questions should be asked: 'Does the patient have an asthma?' and, if so, 'What sort of asthma does the patient have?'. The Commission builds on the insights of the late Dr Freddy Hargreave,[10] who believed in deconstructing the airway, setting airway disease in the context of comorbidities and the environment (in the broadest terms), and especially in the concept of 'treatable traits'.

As such, if the patient has an airway disease, the next question is: 'What are the components of the airway disease?' (Table 1). Once the airway disease phenotype has been determined, appropriate treatment can be planned. This approach is particularly useful in the context of wheeze in children of pre-school age, in the elderly, and also when deciding whether asthma is present in the context of other airway or systemic diseases. Thus, in pre-school children with a wheeze, rather than engaging in debates about whether you can diagnose asthma at an arbitrary age, the right approach is to delineate those children who have eosinophilic airway inflammation and are thus likely to benefit from a long-term commitment to ICS treatment. The recent INFANT trial,[11] supported by other data,[12] suggests that the presence of aeroallergen sensitisation and a raised peripheral blood eosinophil count (≥300/μL) are predictive of a good response to ICS. In older children and adults, blood eosinophil count is a good predictor of response to mepolizumab[13,14] and is more useful than previously used descriptive terms, such as asthma, chronic obstructive pulmonary disease (COPD), and asthma–COPD overlap syndrome.[15] For patients with diseases like cystic fibrosis (CF), primary ciliary dyskinesia, and sickle cell anaemia, and for the survivors of premature delivery, rather than asking 'Do they also have asthma?', the questions that should be asked are: 'Do they have eosinophilic airway inflammation and are they thus likely to benefit from ICS?', 'Do they have variable airflow obstruction and are they thus likely to benefit from short-acting β_2 agonists?', and 'What are the treatable traits?'.

Ultimately, we need to move from phenotypes (the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment) to endotypes (a subtype of a condition, which is defined by a distinct functional or pathobiological mechanism). For example, one phenotype of airway disease is driven by persistent airway infection, characterised by neutrophilic inflammation, positive sputum culture, mucus hypersecretion, and secondary tissue damage. Phenotype-specific treatments are antibiotics, airway clearance, and mucolytics, all of which are nonspecific. CF is an endotype of chronic suppurative lung disease, caused by reduced or absent function of the CF transmembrane protein encoded by a gene on the long arm of chromosome 7. Understanding this endotype has resulted in moving from nonspecific, phenotype-driven therapies to an endotype-driven approach of pathway-targeted, specific therapies. An example of this is ivacaftor, a specific corrector of the molecular defect in Class III mutations that is increasingly being used in combination with other specific molecules to other gene classes. This should be the aim for asthmas, moving from airway eosinophilia to specific endotypes. Some progress has been made in this regard by the U-BIOPRED team,[16] who have shown that there are at least two endotypes of airway eosinophilia: the first is enriched by gene signatures for IL-13/T helper cell type 2 and innate lymphoid cell type 2 cells, while the second is characterised by upregulation of metabolic pathway, ubiquitination, and mitochondrial function genes. This is still futuristic for most of the asthmas, and indeed it could be argued that it is irrelevant to most patients with eosinophilic asthma because they are easily treated with ICS. However, as discussed in the following section, endotypes have relevance beyond immediate treatment.

Table 1: Components of airway disease that need to be considered before deciding the nature of the asthma experienced by an individual.

Component	Description
Fixed and variable airflow obstruction: not all variable obstruction is due to bronchoconstriction	› Bronchoconstriction (acute response to β2 agonist, also likely if changes in airflow occur over minutes or hours). › Airway anatomical instability: loss of alveolar guy ropes (suspect if airflow limitation seems fixed). › Intraluminal mucus (may be responsible for episodes of non-bronchodilator responsive airflow limitation, asthma attacks, and between clinic variability in airflow).
Airway inflammation	› Whether present or not and, if present, what phenotype (cell type). › Is inflammation a beneficial or harmful response? › Long-term aim is pathways (endotypes).
Airway infection and impaired host defences	› Especially if there is a chronic wet or productive cough.
Increased or reduced cough reflex sensitivity and efficacy	› A common clinical diagnosis in adults. Can be supported by objective assessment of cough reflex and cough counting.

BEGONE, PALLIATIVE CARE FOR THE ASTHMAS!

Another recommendation from the Commission is that we need to move towards the prevention and cure of the asthmas, rather than just palliating the symptoms with inhalers; research to make a better preventative brown inhaler, or a longer acting blue inhaler, is of little relevance when we know standard therapy works for most asthmas if properly and regularly administered. There is abundant evidence that the asthma soil is prepared, and the seed is planted antenatally and throughout the pre-school years. It is exactly here that we need to urgently know the endotypes leading to progression to established eosinophilic asthma in particular. Without biomarkers of which patients will progress; and knowledge of the pathway down which they are progressing, our hopes of finding a cure or establishing primary or secondary prevention is negligible. We know ICS do not modify this pathway,[17] so the initiating endotypes are likely to be different from those in established disease, and it is these early endotypes that hold the key to an asthma-free world.

The Commission has proposed a new approach to the evolution of asthma. There have been many birth cohort studies that have given us important insights into the evolution of asthma, although in many cases it is not immediately clear which asthma is being discussed. We now need to address the complexities of incorporating multiple small effects across the developmental trajectory into new models. New innovative approaches were proposed. Overall, the most important concept is that we must understand the progression of disease in order to stop it.

BEGONE, TALK OF 'ASTHMA EXACERBATIONS'!

We have long railed against the word 'exacerbation' as a feeble description, implying a trivial and readily reversible event.[18] In fact, the term 'lung attacks', which we prefer, carries an ominous prognosis in conditions as disparate as CF, primary ciliary dyskinesia, and interstitial lung disease. In the asthmas, lung attacks cause death, they are the single strongest predictor of further attacks and death, and they carry a longer-term risk of impaired airway development. We need to learn from the cardiologists and have a focussed response: why did the attack happen; what went wrong; was the asthma plan followed (if indeed it existed at all); does the plan need to be modified; was the patient adherent to treatment or were they over-using short-acting β_2 agonists, under-using ICS, not attending regular check-ups,

or having multiple emergency attendances. These are just a few of the issues that need to be considered. We should be aiming for asthma attacks to be seen as a catastrophic failure of management, not an issue that can be solved with a 3-day course of prednisolone.

The failure to recognise asthma attacks for what they are is inextricably linked to the trivialisation of the diagnosis and over-diagnosis of the condition. Letting inhalers become mere fashion accessories is of course expensive but has much worse consequences than being a waste of money. The lists of asthmatics in primary care become bloated by people with no or trivial airway disease, and the physicians despair of ever having time to do reviews of such huge numbers, and nothing happens. Would it be inappropriate to insist that having asthma can have consequences as serious as having cancer (i.e., dying)? As with cancer, the diagnosis should be objectively based and monitoring should be regular and taken seriously. An asthma attack is not dissimilar to the recurrence of cancer; it heralds a new and potentially fatal situation and requires a rapid response. An exaggerated view? Perhaps. But hopefully it provides a new view that offers correction to the affects of asthma on the modern world.

BEGONE, RESEARCH IMPRECISION!

Many aspects of improving research were discussed by the commissioners, but in this feature the problem of big asthma studies is discussed. The science of asthma research has never been more sophisticated, with genetic analyses and omics technologies integrated using novel systems biology. However, all too often the clinical inclusion criteria are superficial in the extreme, for example, 'Doctor-diagnosed asthma' or wheeze, despite the imprecision of this term. This is graphically illustrated by two genome-wide association studies. The Gabriel Consortium genotyped 10,365 patients with physician-diagnosed asthma and 16,110 unaffected people, and made very interesting discoveries, including the association of the *ORMDL3/GSDMB* locus on chromosome 17q21 with childhood onset asthma.[19] A much smaller and more focussed study, including 1,173 cases and 2,522 controls aged 2–6 years, identified *CDHR3* as an important gene.[20] Cases were defined by recurrent severe attacks of wheeze requiring hospitalisation, and thus patients were truly likely to have one of the asthmas. The *CDHR3* gene identification was an association missed by the much larger GABRIEL study.[19,20] The importance of *CDHR3* was further validated *in vitro* by the demonstration that the gene encodes for the receptor for rhinovirus-C.[21] Clearly, we need to find better ways of phenotyping large numbers of patients for big-data studies; the CAMP study, for example, measured bronchial responsiveness as an entry criterion in >1,000 children.[22] There is a clear need for biomarkers to diagnose and specify the type of asthma being studied for big genome-wide association studies and other studies if important findings are not to be missed.

CONCLUSION

The Lancet Commission poses significant challenges to the respiratory community. The core message is that asthma is an umbrella term, like anaemia and arthritis, describing a clinical syndrome while making no assumptions about underlying pathology. The next step is a greater commitment to making objective measurements to support a diagnosis, to deconstruct the airway and thus answer the question 'What sort of asthma does the patient have?', and to answer the same question in research subjects. We need to realise that we are badly letting down many people with an asthma. We propose a new age of personalised treatment of what should be appreciated as a killing disease. Above all, complacency needs to be swept aside and new thinking is needed if we are not to remain stuck in our present rut.

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Translating Knowledge of IL-23 Targeting into New Solutions for Psoriasis Treatment

This symposium took place on 12th October 2018, as part of the 2nd European Workshop on Skin Immune Mediated Inflammatory Diseases (SIMID) in Verona, Italy

Chairperson: Jo Lambert[1]

Speakers: Jo Lambert,[1] Stefano Piaserico,[2] Marc Radtke[3]

1. Ghent University, Ghent, Belgium
2. University of Padova, Padova, Italy
3. University Medical Centre of Hamburg, Hamburg, Germany

Disclosure: Prof Lambert has been a speaker, researcher, and advisor for AbbVie, Celgene, Janssen, LEO Pharma, Eli Lilly, Novartis, and UCB. Dr Piaserico has been a consultant and advisor for AbbVie, Almirall, Galderma, Janssen, Eli Lilly, Mundipharma, Novartis, and Pfizer; and has received lecture fees from AbbVie, Janssen, Eli Lilly, MSD, Novartis, and Pfizer as well as research grants from Pfizer and AbbVie. Prof Radtke has conducted clinical research for Abbott, AbbVie, Pfizer, MSD, Janssen, Biogen Idec, LEO Pharma, Basilea, Centocor, Celgene, Merck-Serono, Almirall, Stiefel, Mölnlycke Healthcare, Johnson & Johnson, Novartis, Eli Lilly, Sandoz, and PAREXEL; and has been involved in health services research for Abbott, AbbVie, Pfizer, MSD, Janssen, Biogen Idec, LEO Pharma, Basilea, Centocor, Celgene, Merck-Serono, Almirall, Stiefel, Mölnlycke Healthcare, Johnson & Johnson, Novartis, Eli Lilly, and Sandoz. Prof Radtke has also been an advisory board member for Almirall, Pfizer, Eli Lilly, AbbVie, Janssen, Hexal, MSD, Merck, Sandoz, Biogen Idec, Novartis, Sanofi, and LEO Pharma; and has received lecture fees from Medac, AbbVie, Pfizer, MSD, Janssen, Biogen Idec, Hexal, LEO Pharma, Eli Lilly, Basilea, Merck-Serono, Johnson & Johnson, Novartis, Sandoz, LaRoche-Posay, and Galderma.

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Meeting Summary

Following a brief introduction by Prof Lambert, the symposium started with an in-depth review of the current unmet needs in the clinical management of psoriasis, provided by Prof Radtke, who also reported on the multiple and cumulative negative effects of the condition on patients' health, activity engagement, family relationships, and overall quality of life (QoL). Prof Radtke went on to describe the factors contributing to the burden of psoriasis, other than disease severity, and highlighted the importance of taking a holistic approach to the management of the condition that takes into consideration the individual patient's expectations and needs. Prof Lambert continued the symposium with an overview of the core pathways involved in disease pathogenesis in relation to the development of novel targeted immunotherapies.

Prof Lambert reviewed the current clinical paradigms for the treatment of psoriasis, including targeted biological therapies, such as TNF-α inhibitors and newer agents acting on IL-17 and IL-23, which research shows may represent a more effective approach to the treatment of psoriasis and other autoimmune inflammatory disorders. The latest Phase III clinical trial data on therapies selectively targeting the upstream cytokine IL-23 were then presented by Dr Piaserico, with a focus on the monoclonal antibodies guselkumab, risankizumab, and tildrakizumab, and their potential to achieve consistent rates of skin clearance long-term, with the added benefit of prolonged dose intervals and intermittent treatment in some patients.

Burden of Psoriasis at Individual and Societal Levels

Professor Marc Radtke

The last decade has been one of the most exciting for dermatologists managing patients with psoriasis, and this has been largely due to the significant progress of targeted treatments. The key question is how to exploit these achievements to minimise the disease burden on individuals and society. Frequently underdiagnosed and undertreated,[1,2] psoriasis is a chronic, systemic inflammatory, autoimmune skin disease characterised by a relapsing-remitting course,[3,4] which is associated with multiple comorbidities.[2] Although psoriasis can occur at any age, the highest prevalence is among people aged 50–80 years, and the majority of patients face >40 years of life with the disease.[5] In children, prevalence rates increase from infancy to adulthood with each year of age.[5,6] The average lifetime period with psoriasis in this population is >60 years.[5]

Crucially, the effects of psoriasis on the skin are only the tip of the iceberg. Genetic and environmental factors can lead to systemic inflammation, an important contributor to the pathogenesis of cardiovascular and metabolic comorbidities;[5] therefore, psoriasis is often associated with atherosclerosis, psoriatic arthritis, obesity, diabetes, and hypertension.[5,7] The psychological component of psoriasis also needs to be considered; an estimated 77% of patients experience stigmatisation related to their condition and 30% suffer from depression.[5] Additionally, patients with psoriasis experience lower QoL as well as reduced work productivity and associated economic loss.[7]

As described by Kimball et al.,[8] the overall negative effects of psoriasis accumulate over time, resulting in impaired QoL, including a feeling of lost opportunities and unfulfilled potential. This concept is known as the Cumulative Life Course Impairment (CLCI) of psoriasis.[8] Understanding the patients' perspective is crucial to identifying those who are more vulnerable to cumulative impairment, improving CLCI prevention, and optimising treatment and allocation of healthcare resources.

Most psoriasis patients report the need to be healed of skin defects, free of itching, comfortable appearing in public, and able to lead a normal working life.[5] Patients also report a substantial burden in terms of the impact of psoriasis on health-related QoL (HRQoL). This is illustrated in a conceptual model developed by Narayanan et al.[9] using data from a study of patients with moderate-to-severe psoriasis in France, Germany, Italy, Spain, the UK, Brazil, Canada, and the USA.

The model shows how physical symptoms, such as pain and itching, impact the patient's HRQoL through negative effects on mental health (e.g., stress and poor self-image), activity engagement (e.g., avoidance of hobbies and changing careers), social life (e.g., avoidance of contact with other people), and family relationships (e.g., perceived lack of support).[9] Factors such as stress and infections can aggravate symptoms and further reduce QoL. However, the latter can improve with effective treatments and avoidance of symptom triggers.[9]

Clearly, disease severity is not the only determinant of the multifaceted clinical, individual, and economic burden of psoriasis. This view is further supported by the results of a survey of dermatologists (n=524) and their patients (n=3,821) that indicates that physical and psychological comorbidities as well as type of itch and lesion site all contribute to the overall burden of psoriasis.[7] The mean±standard

deviation Psoriasis Area Severity Index (PASI) of the surveyed patients was 6.4±7.0. However, having anxiety or depression significantly increased the likelihood of experiencing skin pain (adjusted odds ratio [OR]: 1.52; 95% confidence interval [CI]: 1.16–1.96) and itch (adjusted OR: 2.09; 95% CI: 1.68–2.61). The presence of itch increased the likelihood of experiencing skin pain (adjusted OR: 1.76; 95% CI: 1.43–2.17), worsening QoL (p<0.01), and increased percentage of overall work impairment (17%; 95% CI: 2%–34%).[7]

Furthermore, compared with patients with skin lesions affecting only nonvisible body areas, those with lesions in both visible and nonvisible body areas were more likely to have skin pain (p<0.0001), worsening QoL (p<0.01), and decreased adjusted mean EuroQol-5D (EQ-5D) utility weight.[7] Similarly, patients with lesions in both sensitive (the face, scalp, and genitals) and nonsensitive (including the torso, arms, and legs) areas were more likely to have skin pain (p<0.0001), worsening QoL (p<0.01), and decreased adjusted mean EuroQol-5D (EQ-5D) utility weight than patients with lesions in nonsensitive areas only.[7]

The correlation between site of psoriatic lesions and psychological comorbidities has been investigated in detail by Łakuta et al.[10] They reported the strongest association for experiences of stigmatisation and presence of skin lesions on the arms and hands (r=0.34; p<0.0001) and chest (r=0.33; p<0.0001), whereas depressive symptoms were found to be significantly related to lesions on the head and neck (r=0.28; p<0.0001), genitals (r=0.25; p<0.0001), and upper back (r=0.25; p<0.0001).[10]

Other research has identified a link between psoriasis and sexual dysfunction. This affects 22.6–71.3% of patients, according to a systematic review by Molina-Leyva et al.[11] The same review established that the risk of sexual dysfunction is higher in patients with genital lesions.[11] Therefore, evaluating the presence of the latter, as well as the presence of sexual dysfunction, is important in order to determine disease severity when making treatment decisions.

Understanding patients' expectations is also crucial, as these do not always necessarily align with those of doctors.[5] The psoriasis treatment landscape has evolved considerably over the last 15 years, and current therapies achieve improvements in PASI score of up to 90–100%, corresponding to near-complete or complete resolution.[12,13] It is important not to focus exclusively on these targets but to consider also what matters to patients in terms of their QoL goals.

Pathophysiology of Psoriasis and Implications for Treatment

Professor Jo Lambert

Etanercept, the TNF receptor protein, is a widely-used systemic treatment for chronic, moderate-to-severe plaque psoriasis. Its clinical efficacy has been linked of the downregulation of the IL-17 pathway, suggesting that downstream suppression of Th17 cells is an essential response to TNF inhibitors.[14]

The IL-17 pathway plays a prominent role in the pathophysiology of psoriasis, contributing to the clinical manifestations of the disease.[15] Among the new therapies that have been developed to target this pathway, the human monoclonal antibody, ustekinumab, inhibits both IL-12 and IL-23, and, unlike TNF inhibitors, does not increase the risk of malignancy.[16] Ustekinumab also has the highest drug survival period, a measure of the time a patient remains on a certain therapeutic agent. A prospective, observational cohort study using data from the British Association of Dermatologists Biologic Interventions Register (BADBIR), compared with patients on the TNF inhibitor adalimumab, those receiving ustekinumab had an increased likelihood of remaining on therapy (hazard ratio [HR]: 0.48; 95% CI: 0.37–0.62), whereas those receiving etanercept or infliximab were more likely to discontinue therapy (HR: 1.63; 95% CI: 1.45–1.84 and HR: 1.56; 95% CI: 1.16–2.09, respectively).[17]

However, ustekinumab is not effective in some patients. Langley et al.[18] demonstrated, in the Phase III NAVIGATE trial, that these patients may become responders when switched to a therapy exclusively targeting the IL-23 pathway. Patients (n=871) with moderate-to-severe psoriasis were administered a 45 or 90 mg

ustekinumab dose at Week 0 and 4. At Week 16, 268 subjects with inadequate response were randomised (double-blind) to receive 100 mg guselkumab (an anti-IL-23 monoclonal antibody) or to continue the ustekinumab regimen. Compared with the randomised ustekinumab group, more patients on guselkumab achieved PASI 90 (51.1% versus 24.1%; p<0.001, respectively) and PASI 100 (20.0% versus 7.5%; p=0.003, respectively) at Week 52.[18]

The findings suggest that IL-23 has a primary role in the regulation of inflammation and the pathophysiology of autoinflammatory diseases, including psoriasis.[19] Therefore, IL-23 may be a more appropriate target for the effective treatment of the condition than IL-17. Blocking IL-23 can lower the number of Th cells, resulting in the long-term reduction of cytokines, including IL-17. Conversely, when IL-17 is inhibited, the downstream production of cytokines such as IL-17A and IL-17F remains unaffected.[19]

Adequate targeting is important, because effective treatment can prevent negative outcomes, such as premature death. A prospective population-based cohort study by Noe et al.[20] found that untreated patients with physician-reported psoriasis body surface area (BSA) >10% (n=856) had increased mortality risk compared with the general population (adjusted HR: 1.68; 95% CI: 1.08–2.61).[20] This highlights the importance of clinicians being ambitious in treating psoriasis, by aiming to reduce BSA as much as possible: preferably to below 3%.

Inhibitors of the IL-17 pathway include guselkumab, risankizumab, tildrakizumab, secukinumab, ixekizumab, and brodalumab, all of which have a demonstrated good efficacy and safety profile, and are specific to psoriasis.[21] However, the aforementioned drugs are expensive and have limited long-term data. With the exception of secukinumab and ixekizumab, these newer biologics target IL-17F, which can halt the pathogenesis of psoriasis by preventing neutrophil influx and the expression of IL-8 and extracellular signal-regulated kinase (ERK)1/2 in keratinocytes.[22] As a downside, IL-17F inhibition can increase patient susceptibility to mucosal infections, particularly *Mycobacterium tuberculosis*.[23] In the AMAGINE-2 and AMAGINE-3 clinical trials, brodalumab demonstrated a faster onset of action compared with ustekinumab in patients with moderate-to-severe psoriasis.[24] However, brodalumab also inhibits IL-17E (otherwise known as IL-25),[25] which is thought to contribute to the establishment of physiological pregnancy[26] and to the prevention of fat accumulation in the liver.[27] Thus, potentially, brodalumab might not be appropriate for women of childbearing age with psoriasis and for patients with psoriasis in general, given the high prevalence of metabolic disease in this population.[28] Guselkumab, on the other hand, selectively targets the IL-23 subunit p19.[29] Specific IL-23 p19 blockade has been associated with improved and more sustained efficacy and safety as well as lower dosing frequency (every 8–12 weeks), compared with downstream targeting of cytokines of the IL-23/Th17 pathway,[19] resulting in long-term remissions that could potentially allow intermittent treatment.[30] In the clinical setting, PASI improvement from 28.8 to 1.8 has been observed with 6 months of therapy (two injections) of guselkumab.[31] Additionally, low dosing frequency may contribute to patient adherence.

Clearly, dermatologists have a wide range of more effective, newer biological therapies to choose from when it comes to managing psoriasis. But, since every patient is likely to have specific needs and expectations, it is important that they have an understanding of the mechanisms of action involved, in order to effectively personalise treatment. Active questioning of patients about comorbidities and lifestyle is also crucial.

Clinical Insights into Selective IL-23 Inhibitors

Doctor Stefano Piaserico

There is currently a need to raise the bar in psoriasis management. The focus of treating psoriasis should be on attaining long-term disease control to improve QoL and to reduce comorbidities and mortality. With regard to QoL specifically, a large body of evidence indicates that this improves as the patient's skin clears. This was the conclusion, for example, of a systematic review of randomised controlled trials evaluating biologics for moderate-to-severe psoriasis.[32] The analysis was based

on data from 22 treatment arms across 13 randomised controlled trials. It found a correlation between mean improvement in PASI and mean reduction in Dermatology Life Quality Index (DLQI) score (r^2=0.80, from baseline at Weeks 10–16), and the authors concluded that a ≥75% reduction in PASI can considerably improve QoL in patients treated with biologics.[32]

Against this backdrop, it is worth considering that upstream blockage of the IL-23/Th 17 axis, by agents like guselkumab, risankizumab, and tildrakizumab (all acting on the p19 subunit of IL-23), has several advantages over mid or downstream inhibition. In the Phase III VOYAGE study, by Blauvelt et al.,[29] compared with patients on adalimumab (n=334), significantly more subjects on guselkumab (n=329) achieved PASI 90 at Week 16 (73.3% versus 49.7%), 24 (80.2% versus 53.0%), and 48 (76.3% versus 47.9%) (p<0.001 for all comparisons). Similarly, in two Phase III studies by Gordon et al.,[33] significantly more patients treated with risankizumab achieved PASI 90 compared with patients treated with ustekinumab or placebo.[33] Tildrakizumab demonstrated superiority versus etanercept in the Phase III trial reSURFACE 1 by Reich et al.,[34] with 73% of patients on tildrakizumab achieving PASI 75 at Week 28, compared with 54% of those on etanercept.[34] Guselkumab, risankizumab, and tildrakizumab also showed superior efficacy versus placebo, in the above studies.[29,33,34] Furthermore, treatment with guselkumab has been found to be effective at increasing absolute PASI response consistently during 2 years of treatment,[35] and improving the appearance of body areas that are especially difficult to treat, such as the scalp, hands, feet, and nails.[29]

From a practical viewpoint, it is worth noting that PASI responses are maintained after withdrawal of guselkumab in a high percentage of patients. For example, in the study by Reich et al.,[36] guselkumab-treated patients with moderate-to-severe psoriasis achieving ≥90% improvement in PASI score from baseline were randomised at Week 28 to either guselkumab (maintenance group) or placebo (withdrawal group). Through Week 48, 36.8% of patients in the withdrawal group sustained a PASI 90 response versus 88.6% of those in the maintenance group.[36] Research has also found that >70% of adalimumab PASI 90 non-responders achieved and maintained PASI 90 or 100 after switching to guselkumab.[37] Both guselkumab and risankizumab combine a high degree of efficacy with a favourable safety profile, with no new safety concerns, including serious infections or malignancies, compared with other agents.[29,33] Of note, Zhu et al.[38] found minimal anti-drug antibody development with guselkumab. Among patients with psoriasis who were treated with this IL-23 inhibitor (n=943), 8.6% (n=81) developed anti-drug antibody by Week 100. Only 4 (4.9%) of these patients had antibodies that could neutralise the bioactivity of guselkumab *in vitro*, corresponding to an overall incidence of neutralising antibodies of 0.4%.

Open Discussion

The last part of the symposium was an open discussion on the topics presented by the speakers. The following is a summary of the main points.

- Asked whether human data are available on the role of IL-17E in the establishment of physiological pregnancy, Prof Lambert clarified that the evidence to date comes from animal studies.
- It was highlighted that using a cascade model to illustrate the superiority of IL-23 inhibitors is not appropriate. A cascade model implies causation, which is not the case in the pathogenesis of psoriasis. A circular model would be more appropriate.
- There was scepticism regarding the effectiveness of exclusively targeting the IL-23 pathway, given that the pathogenesis of psoriasis is fairly heterogeneous and some patients may develop paradoxical side effects. It was noted that the efficacy data are certainly positive, but it was acknowledged that additional long-term safety studies are needed.
- It was generally agreed that ustekinumab will continue to play a major role in the management of psoriasis, largely due to the good safety profile, the long life of the medication, and the high patient adherence. However, it was pointed out that some patients may be underdosed at 45 mg.

- Members of the audience highlighted the need to establish the cost-effectiveness of individual drugs, and to identify biomarkers of response. There was the perception that avoiding overtreatment, by lowering doses and prolonging dose intervals in the right patients, will contribute to reducing costs in the future.
- It was asked why guselkumab, in particular, would be the perfect candidate for intermittent therapy. Dr Piaserico explained that guselkumab will likely be one of several medications allowing this, and noted that prolonging the dose interval, rather than stopping treatment altogether, would probably be a better approach in super responders.

Conclusion

Clinical paradigms for psoriasis management have evolved substantially in recent years. They include targeted biologic therapies, such as TNF-α inhibitors and newer agents that can target the upstream cytokine IL-23 or the downstream IL-17. Several sub-targets exist for the latter, including IL-17A and IL-17F, the targeting of which has advantages and disadvantages that may impact on treatment decisions. Selective blockage of the upstream cytokine IL-23 may be a more appropriate approach, according to the latest clinical trial data, potentially improving the management of psoriasis and other autoimmune inflammatory disorders. Although long-term safety data are needed, there is evidence to suggest that targeting IL-23 may provide several advantages, including consistent skin clearance long term, prolonged dose intervals, and intermittent treatment, enabling dermatologists to meet patients' individual needs and expectations more effectively.

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Type 2 Inflammation and the Evolving Profile of Uncontrolled Persistent Asthma

This sponsored symposium took place on 17th September 2018, as part of the 28th European Respiratory Society (ERS) International Congress in Paris, France

Chairperson: G. Walter Canonica[1]

Speakers: Zuzana Diamant,[2] Sven-Erik Dahlén[3]

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Meeting Summary

The objective of this symposium was to provide an overview of Type 2 inflammation in asthma. The speakers covered the pathophysiology of Type 2 asthma, its heterogeneity, the associated economic burden, and methods for recognising Type 2 inflammation in severe asthma patients in clinical practice.

Asthma is a heterogenous disease and multiple phenotypes are common among patients. Type 2 asthma is so named because it is associated with Type 2 inflammation and typically includes allergic asthma and moderate-to-severe eosinophilic asthma, Prof Canonica explained. By contrast, non-Type 2 asthma commonly has an older age of onset and is often associated with obesity and neutrophilic inflammation.

Prof Diamant highlighted the scale and severity of uncontrolled persistent asthma. Globally, an estimated 420,000 people die of asthma every year, and many more have uncontrolled disease, putting them at risk of persistent airway inflammation and eventual lung decline. Patients may not recognise that their disease is uncontrolled, despite exacerbations and the impact of their asthma on daily activities. Prof Diamant described the impairments to health-related quality of life and the associated costs of uncontrolled asthma.

Prof Dahlén outlined how new predictive biomarkers will be needed to identify the type of asthma an individual patient has. No single biomarker will provide sufficient information, and as such, in the future, profiles of many markers will need to be integrated to produce subgroup-specific profiles for use in personalised medicine. He described ongoing research into protein arrays and lipid mediators in urine, and how cluster analysis and pattern recognition, with the aid of artificial intelligence, will form the basis of future diagnostic tools. Prof Canonica explained that an understanding of the mechanisms of asthma is important in achieving better symptom control. IL-4 and IL-13 are key players in the pathobiology of uncontrolled persistent asthma (IL-4 in inflammation and IL-13 in airway remodelling), but their roles overlap. The heterogenous nature of Type 2 asthma can make it difficult to diagnose; therefore, focussing on a single biomarker is likely to leave some patients sub-optimally controlled.

Introduction

This symposium aimed to establish the need for a greater understanding of the mechanisms of disease driving uncontrolled persistent asthma. The disease is heterogenous in nature and multiple phenotypes of Type 2 asthma are common among patients. The speakers reviewed scientific evidence supporting the role of key mediators of Type 2 inflammation in uncontrolled persistent asthma and discussed new and emerging methods for identifying patients with Type 2 asthma in clinical practice. The burden of uncontrolled persistent asthma is substantial for patients and society. An audience poll found that most participants thought that 45% of European asthma patients have uncontrolled persistent asthma, which Prof Canonica said was a reasonable estimate.

Defining Severe Asthma: Understanding the Evolving Profile of Uncontrolled Persistent Asthma

Professor Zuzana Diamant

Asthma is a prevalent disease that is not fully manageable. In 2016, the Global Burden of Disease study estimated that 300 million individuals worldwide have asthma,[1] which causes 420,000 deaths per year (Figure 1).[2] However, Prof Diamant stated that this number of fatalities may be an underestimate.

An understanding of the pathophysiology of asthma is essential for disease management. Airway inflammation is a key feature of uncontrolled asthma[3,4] and is associated with airway hyper-responsiveness and remodelling,[5] in which the lumen becomes narrowed and the airway wall thickened compared with controlled asthma.[3] These features (inflammation, fixed obstructive airway through hyper-responsiveness, and airway narrowing) interrelate and have been repeatedly shown to be more prominent in uncontrolled compared with controlled asthma.[6,7] Airway narrowing is mostly reversible, Prof Diamant said, but persistent airway inflammation is associated with eventual lung function decline.[6,7]

Over the past 20 years, attempts have been made to define severe asthma.[8] The latest recommendations on the identification, evaluation, and treatment of patients with severe refractory asthma were published in a 2014 joint consensus from the American Thoracic Society (ATS) and the European Thoracic Society (ETS).[9] The publication distinguishes between severe asthma, which requires treatment with high-dose inhaled corticosteroids (ICS), and asthma that is uncontrolled despite this therapy. Prof Diamant said it is important to identify and address factors such as adherence to therapy,

comorbidities, and chronic environmental exposures before making the diagnosis of uncontrolled asthma. This type of asthma remains uncontrolled despite intensive pharmacotherapy but includes asthma that is controlled for at least 6 months with high-dose oral corticosteroids (OCS).

According to the 2018 guidelines from the Global Initiative for Asthma (GINA),[10] asthma control should be assessed in terms of symptom control and future risk. Symptom control includes daytime symptoms, night-time awakenings due to asthma, the need for relief medications more than twice a week, and limitations on activity because of asthma. Future risks include the risk of exacerbations, medication side effects, and airway obstruction or lung function decline. Prof Diamant stressed that too many people (40–70%)[11] experience uncontrolled asthma and are exposed to these risks.

The GINA guidelines[10] are based on research that emphasises the importance of asthma control. One study found that poor asthma control almost doubles the risk of hospitalisation for asthma exacerbation;[12] another study found that exacerbations lead to increased symptoms and decreased lung function compared with controlled asthma.[7] A recent asthma exacerbation increased the risk of future exacerbations 6-fold compared with patients without a recent severe exacerbation (odds ratio [OR]: 6.33; 95% confidence interval: 4.57–8.76) in a 2007 study;[13] the OR was only slightly lower when corrected for physician-adjusted or GINA-adjusted disease severity.

OCS are associated with significant health problems. Patients who have received ≥4 OCS prescriptions are significantly more likely to experience adverse effects (AE), such as osteoporosis, hypertension, Type 2 diabetes mellitus, and gastrointestinal ulcers and bleeds (OR: 1.21–1.44, depending on the AE), compared with patients who have not taken OCS.[14] Therefore, the GINA stepped approach to treatment[10] reserves OCS for the last step of treatment, after the use of drugs such as ICS, beta-2 agonists, and theophylline.

The personalised approach to asthma control, recommended by GINA,[10] requires physicians to assess and diagnose the disease, adjust treatment, and review the patient's response. This includes addressing comorbidities and modifiable lifestyle factors and taking patient preferences into account. Treatment response should be reviewed on a regular basis.

Despite such guidelines for physicians, a substantial number of patients continue to live with uncontrolled disease and many do not realise that their asthma could be improved. The online survey REALISE (REcognise Asthma and Link to Symptoms and Experience)[15] included 8,000 patients from 11 European countries. Overall, only 20% of respondents had fully controlled disease, but 80% of patients who had experienced acute exacerbations in the past 12 months considered their asthma to be controlled. Similarly, in the Europe and Canada (EUCAN) Asthma Insight and Management (AIM) survey,[16] which included telephone interviews with 2,003 patients, over half of the group (51%) reported acute treatment of asthma in the past year, such as hospitalisation or unscheduled emergency visits. On the other hand, 77% of the group perceived their asthma to be well or completely controlled.

The LIAISON study[17] included 8,111 patients from 12 European countries. The study used the Asthma Control Questionnaire (ACQ) and the MiniAsthma Quality of Life Questionnaire (MiniAQLQ) and found that asthma control was suboptimal in 56.5% patients. Quality of life was related to asthma status and deteriorated as control of the disease lessened, Prof Diamant said.

Uncontrolled asthma impairs health-related quality of life. For example, sleep quality and quantity are decreased and sleep disturbances are more frequent and severe.[18] Many patients also experience impairment of outdoor and other daily activities,[19,20] and have increased symptoms of anxiety and depression that can complicate disease management.[21] Poor asthma control has a negative impact on an individual's wellbeing.

On a major scale, Prof Diamant said the health economic consequences of uncontrolled asthma are shocking. One report estimated that persistent asthma represented a €19.3 billion economic burden in Europe in 2010.[22] Most of these costs were due to uncontrolled asthma, with per-patient costs four-times higher than costs associated with controlled asthma.

Figure 1: Asthma: A global public health concern.[1,2]

Similarly, Demoly et al.[20] found poorly controlled asthma was associated with a substantial use of healthcare resources and a negative overall impact on work productivity and daily life.

Prof Diamant concluded by stating that a minority (5–10%) of patients with severe asthma account for around half of the total healthcare costs for asthma. She said a greater understanding of uncontrolled persistent asthma and a more personalised approach to asthma management are needed to achieve optimal disease control.

Type 2 Asthma: What is it? What Mediators Drive it?

Professor Sven-Erik Dahlén

An understanding of Type 2 asthma will underpin future developments in precision medicine, Prof Dahlén said. Asthma differs from other inflammatory diseases in that it involves not only chronic inflammation driven by immunological mechanisms but also the mechanisms leading to smooth muscle constriction and hyper-reactivity of the airways. Today, and in the future, combination therapy that addresses both components of the pathophysiology is necessary to treat both aspects of the disease.

There are four components of asthma: bronchoconstriction, airway inflammation, airway hyper-responsiveness, and chronic remodelling of airways. Inflammatory mechanisms act on all components with an interplay between several different cells and mediators, Prof Dahlén said.

Today's concept of Type 2 inflammation builds on immunological research from the 1980s into control mechanisms in the immune system. It was known that antigens interact with dendritic cells and, as a result, T cells are activated, causing them to differentiate and stimulate different immune pathways. Th2 cells were found to be prominently activated by allergic reactions, leading to allergy, eosinophilia, and the release of IgE and IgG1. Th1 cells, which release IFN-γ and TNF-α, were found to be more involved in cell-mediated immunity. Later, the autoimmunity pathway driven by IL-17 and IL-22 was recognised, along with the anti-inflammatory pathway driven by IL-10 and TGF-β.[23]

Most of this work was carried out in mice and 10–15 years ago its relevance in humans was doubted, Prof Dahlén explained. The breakthrough came 10 years ago when several groups started researching the mechanisms of human asthma. A seminal study carried out in San Francisco, California, USA, took brushings of airway epithelium in asthma patients and healthy controls and found that a Th2 profile

similar to that previously observed in mice was prevalent in asthma patients.[24] The Th2 profile was associated with expression of IL-5 and IL-13 and subsequent work showed that this profile was also associated with a good therapeutic response to inhaled steroids, which are still the mainstay of asthma treatment.

Allergic asthma is characterised by a Th2-dominated immune response. Inhaled allergens lead to increased serum IgE levels; IgE binds to mast cells and basophils, which secrete histamine and lipid mediators that cause bronchoconstriction and activate other inflammatory cells. Mast cells and basophils also secrete IL-4 and IL-13, causing plasma and cell extravasation and recruitment of eosinophils, the key cells involved in the inflammation.[25] An unmet need still present in asthma treatment is the need to limit mucus secretion driven by IL-13, Prof Dahlén said.

Eosinophils have a key role in Type 2 inflammation in asthma. IL-5 promotes eosinophil differentiation in the bone marrow; mature eosinophils migrate to the circulation; and Type 2 cytokines, including IL-4 and Il-13, attract the eosinophils to the airway. The inflammatory response is amplified by the recruited eosinophils via a positive feedback loop, driven by IL-4. The inflammatory mediators secreted by eosinophils and other inflammatory cells lead to tissue damage, creating a cycle of chronic inflammation (Figure 2).[26-28] Prof Dahlén said there is a suggestion that IL-4 and IL-13 may also drive airway hyper-responsiveness.

Prof Dahlén's group is currently studying samples of human lung tissue. When exposed to IL-13 *in vitro*, lung tissue has an increased contractor response to histamine and leukotrienes; IL-4 gives a similar response, suggesting that IL-4 and IL-13 upregulate airway sensitivity. Prof Dahlén noted that both IL-5 and IL-17 have been thought to impact airway responsiveness, but they were not shown to have this effect in his own research.

Asthma is now established as a heterogenous disease composed of many phenotypes (Figure 3).[29,30] It is classified as either Type 2 asthma, which includes allergic and eosinophilic asthma, or non-Type 2 asthma, which is associated with obesity and smoking and driven by neutrophils. However, there is overlap in the mechanisms driving these two types of asthma;[31] for example, IL-33 and IL-25, released in non-Type 2 inflammation, stimulate Type 2 innate lymphoid cells (ILC2) to release IL-13 and IL-4 and activate Type 2 inflammation.[3]

For this understanding to translate into precision medicine in the clinic, Prof Dahlén said effective predictive biomarkers will be needed to identify which type of asthma the patient has and what treatment is appropriate. To date, the key established biomarker of Type 2 inflammation is the number of eosinophils in blood and sputum samples. IgE levels may be a marker for allergic asthma. In experimental work, periostin appears to be a promising biomarker, but clinical measurements using current assays have so far been less convincing. Exhaled nitric oxide fraction (FeNO) is emerging as an alternative biomarker for Type 2 inflammation, he said.

In a 2009 study, patients with allergic asthma inhaled low doses of allergen on seven consecutive weekdays.[32] This allergen exposure caused significant increases in airway hyper-responsiveness, which were detected by measuring FeNO (mean±standard deviation: 46±31 ppb before versus 73±46 ppb after). This inflammation was blocked by ICS but not affected by beta-antagonists.[32] Other studies have also shown a relationship between increased FeNO and increased asthma morbidity,[33] and Prof Dahlén suggested that combining monitoring FeNO levels with eosinophil measurements in blood may be a better biomarker than eosinophil count alone.

However, more precise biomarkers are needed. Prof Dahlén suggested that no single biomarker will ever be sufficient and that, in the future, profiles of many markers will be integrated with artificial intelligence. The Human Protein Atlas[34] includes >25,000 antibodies targeting 17,000 proteins. Prof Dahlén and colleagues constructed an array of 177 proteins implicated in inflammation and asthma. They applied the array to plasma samples taken from 434 patients as part of the pan European U-BIOPRED study,[35] for which extensive clinical and physiological data were available. Measuring the expression of the array proteins in patients' blood samples identified six distinct patient clusters; the largest was a typical Type 2 inflammation cluster.

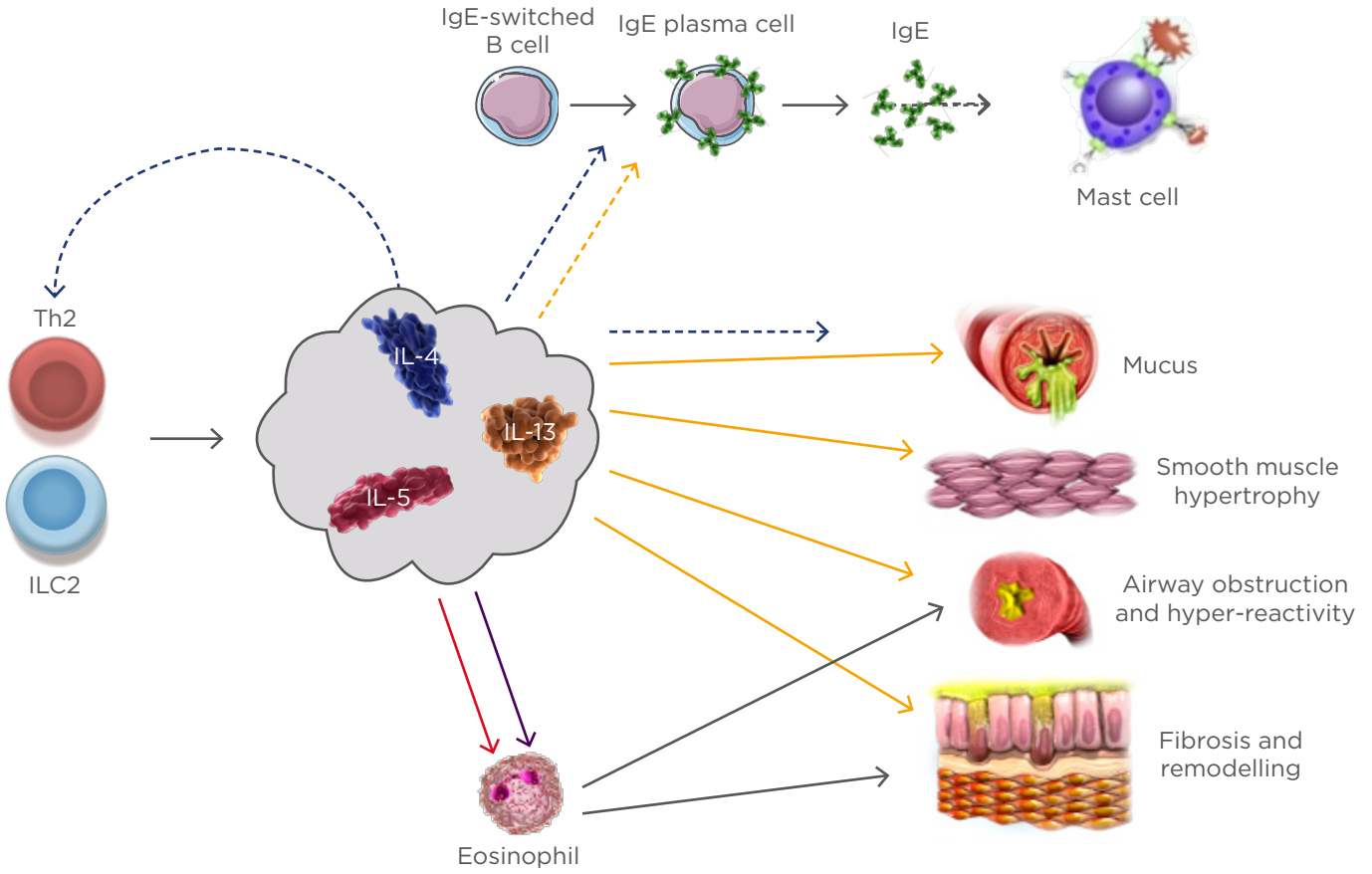

Figure 2: Key cells and mediators in Type 2 asthma.[26]

ILC2: Type 2 innate lymphoid cells .

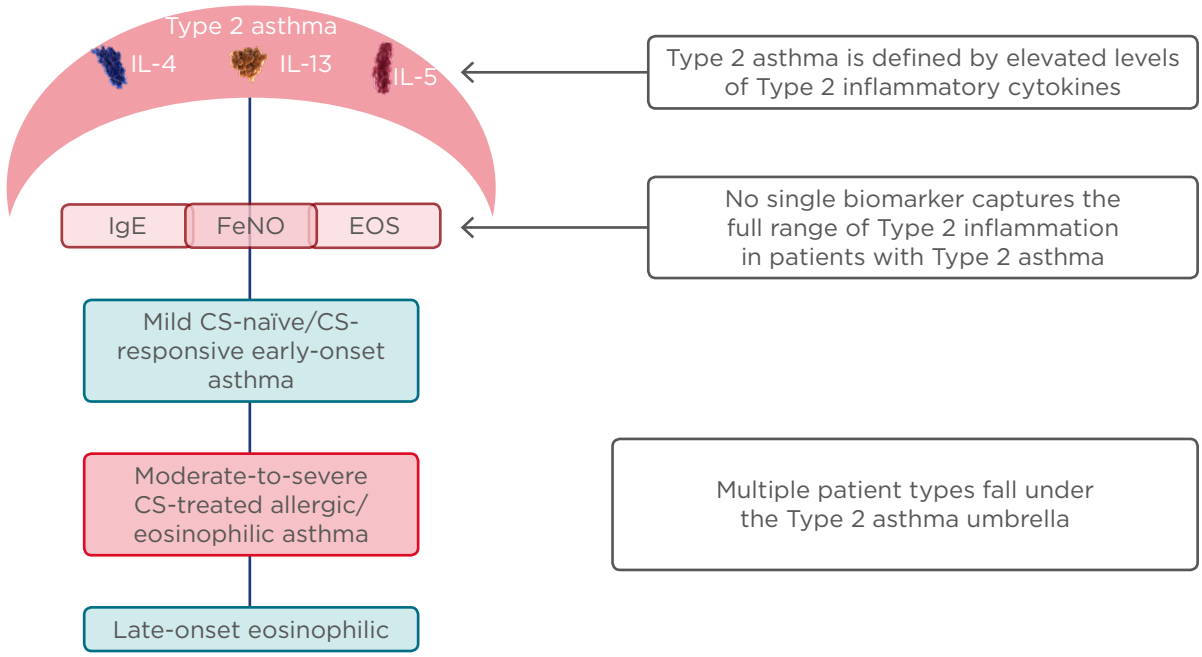

Figure 3: Type 2 asthma includes a wide range of patients with different phenotypes.[29,30]

CS: corticosteroid; EOS: eosinophil; FeNO: exhaled nitric oxide fraction.

Prof Dahlén said the work was at an early stage, but he predicted that this approach, looking for the protein 'fingerprints' in blood samples, will be used in the clinic in the future.

Another avenue of research is the measurement of lipid mediators in urine. Lipid mediators, such as prostaglandins and leukotrienes, are part of the inflammation response and Prof Dahlén's group has developed a method for quantifying 26 lipid mediators and their metabolites.[36] They have found that urinary leukotriene E_4 (LTE_4) levels are higher in severe compared with mild asthma, and higher in asthma patients than in healthy controls. There is an overlap in LTE_4 levels between groups, but extreme value analysis found that individuals with the highest levels of LTE_4 also had high eosinophil counts in the blood and sputum, suggesting that LTE_4 is a marker for Type 2 inflammation.[36]

Clustering analysis of the profiles of the main lipid mediator metabolites in urine found five subphenotypes, the biggest of which was Type 2-driven. Prof Dahlén said that, since this subgroup was identified solely on the basis of 11 metabolites in urine, it may be possible in the future to create a dipstick test for this subtype of asthma.

In summary, Prof Dahlén said that more needs to be discovered about Type 2 asthma, which is an umbrella term for asthma with inflammation driven by Type 2 cells and mediators; the list of involved mechanisms is increasing as research progresses. Type 2 asthma includes several subphenotypes with different clinical characteristics, which are likely to respond to different future treatments. Current biomarkers (blood and sputum eosinophil number, FeNO, and total IgE) are used to indicate Type 2 inflammation in asthma, but research is ongoing to find improved biomarkers for subphenotypes. Type 2 asthma classically responds to ICS, but this therapy is insufficient to provide optimal control in severe cases and OCS have significant side effects. Future goals include improved diagnosis of the subphenotypes of Type 2 asthma along with new precise treatments with a lower side effect profile than at present.

Identifying Type 2 Asthma in Clinical Practice

Professor G. Walter Canonica

Approximately 50–70% of asthma patients have Type 2 asthma,[37,38] which is characterised by Type 2 inflammation. This group typically includes allergic asthma, exercise-induced asthma, and late-onset eosinophilic asthma. By contrast, non-Type 2 asthma commonly has an older age of onset, is associated with obesity and smoking, and is neutrophilic, smooth muscle-mediated, and paucigranulocytic.[31]

An understanding of the mechanisms of asthma is important to achieve better disease control. In the clinic, patients are evaluated according to measures of control (exacerbations, forced expiratory volume in 1 second [FEV_1], and use of ICS/OCS), quantifiable biomarkers (eosinophils, FeNO, and IgE), and patient characteristics (allergies and comorbidities). Recently, the need to check for nasal polyps and allergic rhinitis was recognised; an Italian registry[39] found that 60% of severe asthma patients had a diagnosis of allergic rhinitis. The registry also found that 62% of patients have taken OCS, and that eosinophil counts were higher than desirable, Prof Canonica said.

The audience was asked whether they routinely use and rely on spirometry (FEV_1 and FEV_1/forced vital capacity ratio) in the clinical management of patients with uncontrolled persistent asthma. The poll found that 9% of the audience members use spirometry 25% of the time, and a further 9% use it 50% of the time. This is broadly in line with the results of an Italian study that found that only 69.5% of patients with doctor-diagnosed asthma had received spirometry testing as part of their assessment.[40] Prof Canonica said he routinely uses spirometry for patients with uncontrolled persistent asthma and he urged the audience to use this 'easy parameter' more frequently, especially considering that there is a greater chance of misdiagnosis when spirometry is not used to measure lung function.

When asked whether they use IgE measurements to manage this patient group, Prof Canonica was encouraged that >40% of audience members always relied on IgE measurements; however,

17% said they used it only 25% of the time. On the routine use of eosinophil measurements, around one-third (34%) of the audience said they used it either 25% or 50% of the time in this patient group.

Asthma was previously defined as being Th2 or non-Th2. The current distinction between Type 2 and non-Type 2 asthma acknowledges that cytokines associated with Th2 responses are broadly secreted by numerous cell types beyond the originally-described adaptive Th2 immune response.[26] These include ILC2, which are part of the innate immune system and do not require antigen interaction to secrete Type 2 cytokines. Adaptive and innate immune systems work independently, Prof Canonica said, but both produce Type 2 cytokines IL-4, IL-13, and IL-5.

IL-4 plays a central role in inflammatory cell activation (Th2 and ILC2), mast cell recruitment, and initiation of the signalling that drives asthma pathophysiology. IL-4 secretion mediates Th2 cell differentiation from naïve Th cells, can induce ILC2 proliferation, and is essential for the maintenance of the ILC2 phenotype. IL-4 promotes the downstream secretion of Type 2 cytokines via Th2 and ILC2; it also recruits inflammatory cells and induces IgE production.[26,41,42]

IL-13 is a key mediator of airway remodelling. It causes goblet cell hyperplasia and mucus overproduction, as well as an increase in subepithelial collagen deposition through the transformation of bronchial fibroblasts to myofibroblasts.[43] IL-13 promotes airway epithelial cell expression of inducible nitric oxide synthase, which increases the levels of FeNO.[43,44] The effects caused by IL-13 contribute to a notable increase in bronchial hyper-responsiveness;[43] IL-13 also contributes to asthma pathobiology through effects on the smooth muscle.[44-46] *In vitro* experiments suggest that IL-13 can increase cell contractility to acetylcholine in smooth muscle and reduce beta-agonist-mediated relaxation.[45]

The Type 2 cytokines IL-4, IL-13, and IL-5 are secreted by adaptive Th2 helper cells, innate ILC2 cells, mast cells, and eosinophils.[26] IL-5 promotes eosinophil differentiation and maturation,[26,27] while IL-4 and IL-13 promote trafficking of eosinophils to the airway.[26-28] Recruited eosinophils further amplify the Type 2 inflammatory response via a positive feedback loop by secreting more IL-4, and the inflammatory mediators secreted by eosinophils and other inflammatory cells lead to tissue damage, creating a cycle of chronic inflammation.[26]

The distinct and overlapping roles of IL-4 and IL-13 make them key players in the pathobiology of uncontrolled persistent asthma, Prof Canonica said. They induce inflammatory and structural changes that are characteristic features of this heterogeneous disease. The heterogenous nature of Type 2 asthma can make it difficult to diagnose and fully control. Prof Canonica said that no single biomarker could capture the full range of Type 2 inflammation and that focussing on a single biomarker is likely to leave some patients suboptimally controlled.

Prof Canonica concluded that achieving optimal asthma control in patients with uncontrolled persistent asthma can be challenging. The full spectrum of Type 2 asthma patients can only be captured using multiple biomarkers of airway inflammation. The key to achieving optimal control in patients with Type 2 asthma is understanding the underlying mechanisms driving this heterogeneity. The future will be more complicated but will allow proper selection of patients for different treatments.

Conclusion

A personalised approach to the diagnosis and management of asthma is called for. Asthma is a heterogenous disease and multiple phenotypes are common among patients. Type 2 asthma is characterised by Type 2 inflammation and typically includes allergic asthma, exercise-induced asthma, and late-onset eosinophilic asthma. Understanding of allergic, eosinophilic, and mixed allergic/eosinophilic phenotypes has greatly advanced and may underpin new approaches to improve asthma control.

Novel biomarkers may further improve personalisation. Biomarkers based on pattern recognition of metabolites in urine samples, for example, will be applicable at the point of care and will help in patient selection for new biologic agents, especially when patients

have atypical features of Type 2 asthma. New possibilities for patients will complicate the work of the physician. Prof Canonica concluded that professional education will be essential over the next few years if the potential of the new research is to be realised in the clinic.

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Management of Fluctuating Parkinson's Disease: From Science to Clinical Wisdom

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Meeting Summary

This symposium took place at the 4th Congress of the European Academy of Neurology (EAN) 2018 in Lisbon, Portugal, and focussed on the effective management of fluctuating Parkinson's disease (PD). Prof Poewe introduced the topic by explaining how response fluctuations, including wearing-off, remain a key priority in the effective management of PD. Wearing-off fluctuations are often categorised as motor or non-motor, but the reality is that patients are frequently affected by both, with a significant impact on daily activities and quality of life. Prof Stocchi went on to explain that management strategies include adjunct therapies with catechol-O-methyl transferase (COMT) inhibitors, monoamine oxidase (MAO)-B inhibitors, and dopamine agonists. Clinical experience shows that within a few years most patients will be receiving a cocktail of these drugs to manage PD symptoms. Although many antiparkinsonian drug classes have overlapping indications, they have distinct mechanisms of action that can complement each other. Opicapone is a third generation, highly potent and effective COMT inhibitor that received European Union (EU) market approval in

2016 as an adjunct to levodopa for PD patients experiencing response fluctuations. While the efficacy and safety of once-daily opicapone have been proven in clinical studies, Prof Ebersbach gave an overview of real-life data from his own clinics, which show that the benefits of opicapone can be observed within 3 days of treatment initiation. The final presentation from Dr Morgante considered the management of non-motor symptoms in PD. Classically, these non-motor symptoms have been managed as non-urgent symptoms but are now recognised as a significant source of disability. It is vital for clinicians to recognise that many of these symptoms respond to treatment.

Introduction

Professor Werner Poewe

PD is a progressive neurodegenerative disorder with a long course of evolution. It is now understood that there is a long prodromal period when the typical motor system symptoms of the disease are not yet sufficiently progressed to make a clinical diagnosis.[1] Most people with PD are treated as soon as they are diagnosed, often with levodopa or other monotherapies (such as MAO-B inhibitors or dopamine agonists). At this early stage, most patients enjoy stable, near-normal function with their first treatment. However, with continued disease progression, patients leave the so-called 'honeymoon' phase and enter a more complex treatment phase, during which they can experience a variety of response fluctuations (both motor and non-motor fluctuations) and dyskinesias.[1] This is a crucial stage for disease management, and this symposium reviewed current best practices for symptom management during this complex stage of the disease.

Current Treatment of Fluctuations

Professor Fabrizio Stocchi

The terminology of 'ON and OFF' effects and motor fluctuations was originally coined by Prof David Marsden >40 years ago.[2] While often considered as just one type of motor complication, the term 'motor fluctuations' actually covers a range of problems, including end of dose wearing-off, delays to ON, dose failures, and sudden OFFs.[3] End of dose wearing-off is often the first type of motor fluctuation to emerge and can be said to herald the start of the patients' transition to a more complex phase of the disease.[4] When patients develop signs of wearing-off, rather than experiencing the consistent and sustained response to levodopa that they had previously exhibited, they often start to notice subtle fluctuations in a variety of signs and symptoms. While they still show a good response to their levodopa dose, they typically notice a re-emergence of symptoms before their next scheduled dose.

Even today, there is no universally accepted definition of wearing-off to use in clinical trials, and this has led to a range of incidence estimates, from 40% within 4–6 years of starting levodopa treatment[5] to approximately 20–40% of patients within 2.5 years.[6] Ultimately, >90% of PD patients will experience levodopa motor complications,[5] and in young-onset PD the prevalence is even higher (>90% at 5 years).[7] In recent years, it has become clear that the risk of developing wearing-off (and dyskinesia) is closely linked to levodopa dosing.[8] Experts now advocate using the lowest dose of levodopa that provides satisfactory clinical control to prevent and delay the risk of both wearing-off and dyskinesia. Specifically, caution is advised when increasing the levodopa dose in large increments, and when considering doses >400 mg/day.[8] Once motor fluctuations develop, the symptoms quickly become a priority in disease management because they are disabling. Patients say motor fluctuations are the most problematic part of their disease,[9] and they have been shown to be a significant predictor for worse quality of life.[10]

Symptoms of Wearing-Off

It is important for clinicians to recognise that the first signs of wearing-off are neither well established nor the same for all patients. For example, while some patients may notice a return of their classic parkinsonian motor symptoms, (bradykinesia, tremor, and/or rigidity), others may first notice the return of non-motor

symptoms, such as pain, anxiety, fatigue, mood changes, difficulty in thinking, restlessness, sweating, or sialorrhea.[6,11] The subtleties of the symptoms mean that they may often go unrecognised by physicians and, therefore, remain untreated until they become prominent and disabling. Unless directly questioned, patients may not understand that the symptoms they are experiencing are because of their PD and instead attribute them to old age.[12]

Aetiology of Wearing-Off

In PD the loss of striatal dopaminergic neurons leads to a diminished ability to form, store, and regulate the release of dopamine. Accordingly, the brain becomes increasingly dependent on the availability of exogenous dopamine from levodopa.[13,14] With progressive neurodegeneration, it is believed that fluctuations in plasma levels of levodopa (with its short elimination half-life of 1.0–1.5 hours) are increasingly translated into fluctuations in striatal dopamine receptor activation and deactivation.[4,13,14]

It is believed that this pulsatile stimulation of dopamine receptors in the striatum causes further destabilisation of the basal ganglia network, leading to changes in gene and protein expression and alterations in intracellular gene and signalling pattern. Ultimately, these molecular abnormalities are believed to be translated into altered patterns of neuronal firing in basal ganglia output neurons, resulting in the development of motor complications.[4,13,14]

Management Approaches

There are many different approaches to the management of wearing-off. Most traditional treatment strategies, while effective in the short term, do not address the aetiology of wearing-off and therefore require frequent modifications, causing an unnecessary burden for patients and their caregivers.[15] Modification strategies of conventional levodopa formulations are the most commonly used approach because they are inexpensive. They include fractionation of the levodopa-dosing regimen, increasing the total dose of levodopa, changing the levodopa formulation (e.g., to controlled-release levodopa preparations), and the use of adjunctive therapy. While dose fractionation can reduce fluctuations in the short term, this approach does not eliminate troughs in levodopa plasma levels, which translate into a variable clinical effect[15] and may reduce treatment adherence due to the inconvenience of additional daily doses.[16] Continually increasing the size of individual doses of conventional levodopa usually leads to increased peak-dose dyskinesias and does not usually lead to any improvements in antiparkinsonian efficacy.[15]

Adjunctive therapy with MAO-B inhibitors (including selegiline, rasagiline, and safinamide) or dopamine agonists (e.g., pramipexole, ropinirole, and rotigotine) can also be used to reduce OFF time. These adjunct therapies may also allow for a reduction in levodopa dosage.[17,18] However, because these drugs do not change the pharmacokinetic profile of levodopa, they cannot address the underlying cause of wearing-off.[15] For this reason, COMT inhibitors (such as entacapone and opicapone) are often used as first-line therapy for wearing-off. COMT inhibitors act directly to reduce the metabolism of levodopa, thereby directly increasing the half-life of levodopa and decreasing the peak–trough variations in levodopa plasma levels that are associated with motor fluctuations.[19] The COMT inhibitor tolcapone is typically only used as a last-line adjunct therapy due to the risks of liver toxicity, which necessitate regular monitoring of liver function.[20]

As the patient's symptoms progress, most antiparkinsonian medications can be added to the patient's current therapy regimen in order to maximise motor efficacy and keep overall tolerability at a safe level. Clinical experience shows that within a few years most patients will be receiving a cocktail of therapies to manage their symptoms. Although many antiparkinsonian drug classes have overlapping indications, they have distinct mechanisms of action that can complement each other. While they are very efficacious, caution is advised with the dopamine agonists due to their propensity to cause problems, such as oedema and impulse control disorders;[21] patients should therefore be monitored frequently for any issues. When this adjunctive approach is no longer efficacious, advanced, surgical treatments (such as apomorphine infusion, levodopa infusion, or deep brain stimulation) should be considered.[18,22]

Managing Motor Fluctuations: A Case-Based Perspective

Professor Georg Ebersbach

Opicapone: A Once Daily Catechol-O-Methyl Transferase Inhibitor

Opicapone is a third generation, highly potent and effective COMT inhibitor that received EU market approval in 2016 as an adjunct to levodopa for PD patients experiencing response fluctuations. Based on research into the structure and function of the COMT enzyme, and using an analogue-based research approach, opicapone was specifically designed to reduce the risk of toxicity and improve peripheral tissue selectivity compared with other COMT inhibitors.[23]

In two pivotal studies, double-blind treatment with opicapone (25 and 50 mg) significantly reduced absolute daily OFF time by approximately 1 hour versus placebo.[24,25] Reductions in OFF time were mirrored by significant increases in ON time without troublesome dyskinesia. In the BIPARK-1 study,[24] the inclusion of entacapone as an active control helps understand the potential differences between opicapone and entacapone (with the caveat that the study was not set up for a head-to-head comparison). For example, responder rates for having at least 1 hour of reduced OFF time or at least 1 hour of increased ON time were significantly higher for the opicapone 50 mg dose (OFF time, p=0.0011; ON time, p=0.0028) compared with the placebo, but this was not the case for entacapone.[24] Moreover, in the BIPARK-1 open-label extension study, patients who switched from double-blind entacapone treatment to open-label opicapone treatment experienced an extra reduction in OFF time of -39.3 minutes (p<0.05), with a corresponding increase in ON time without dyskinesia of 45.7 minutes (p=0.0148).[26] For those patients who received double-blind opicapone 50 mg treatment, the magnitude of efficacy benefit was maintained throughout the 1 year open-label treatment phase.[26]

Real-life experience with opicapone has also been positive. A recent prospective observation of 88 in-patients with PD (mean age: 68.8 years, 73.5% male) treated at a single expert site between October 2016 and June 2017 found early benefits of opicapone within 3 days of treatment initiation. The study included 52 patients previously treated with entacapone who required a change due to lack of efficacy or intolerability (e.g., diarrhoea) and 8 patients previously treated with tolcapone.[27]

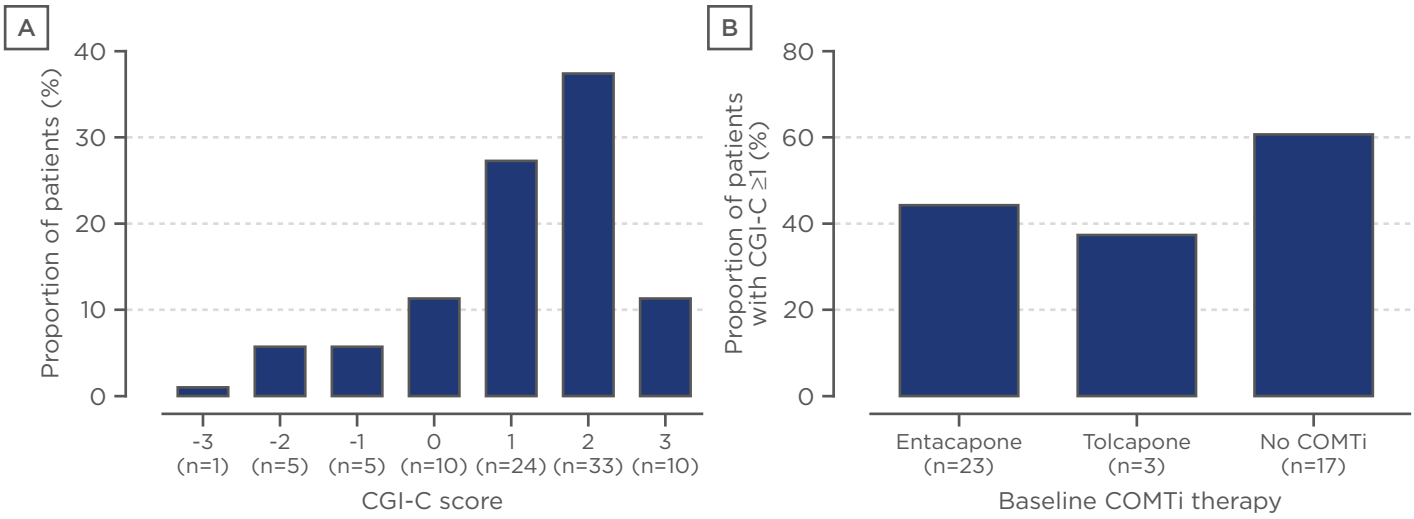

Figure 1: Clinical global impression of change at 3 days post initiation of opicapone adjunct therapy (A) overall and (B) according to previous catechol-O-methyl transferase therapy.

CGI-C: clinical global impression of change; COMTi: catechol-O-methyl transferase inhibitor.

Adapted from Gandor et al.[27]

Overall, the majority of patients reported improvement of clinical status (on clinical global impression of change) with opicapone initiation, with around 1 in 10 patients reporting no change after 3 days and the same proportion reporting worsening (Figure 1A). When analysed by previous COMT therapy, 44.2% of patients previously treated with entacapone and 37.5% of patients previously treated with tolcapone showed an improvement in clinical global impression of change scores (Figure 1B). However, 16 patients (18.2%) discontinued opicapone therapy: 4 due to side effects, 5 due to lack of efficacy, and 7 due to miscellaneous reasons. Overall, 6 of the 88 (7%) patients had hyperkinesias within the first 3 days of opicapone initiation, which were generally manageable through levodopa dose reductions. Thirteen patients reported hallucinations, which is more common than in the pivotal trials but reflective of the neuropsychiatric and other comorbidities in this real-world sample. These were not a common cause for discontinuation, and those patients who were known to be prone to hallucinations could be managed by reducing the levodopa dose before starting opicapone therapy. Levodopa dose reductions were easily conducted because patients only took one daily dose of opicapone, and clinicians could simply reduce the dose of levodopa according to individual patient needs without worrying about how to titrate the dose of COMT inhibitor.[27]

Facing the Challenges of Non-Motor Symptoms

Doctor Francesca Morgante

It is now accepted that non-motor symptoms are part of the PD 'syndrome'.[28,29] It is also now better understood that PD follows a clinical pattern, with a range of non-motor symptoms defining the premotor phase.[30] The development of many of these non-motor symptoms are consistent with the Braak pathology staging system, in which Lewy bodies appear to start from the dorsal motor nucleus of the vagus nerve, the olfactory bulb, and the enteric nervous system, later spreading to the substantia nigra, areas of the midbrain, and basal forebrain.[31] Indeed, it is now suggested that the vagus nerve and the gut–brain axis may act as the generator of the pathological process in PD.[32,33] Research is ongoing to evaluate whether clinical subtyping according to the types of non-motor symptoms experienced can help predict the longitudinal prognosis for people living with PD.[28,34,35] For example, researchers are now trying to identify those patients most at risk of developing impulse control disorders,[36] hallucinations,[37] and cognitive impairments or dementia.[38] Such information will help clinicians with decision-making, as the presence of these neuropsychiatric symptoms often modifies treatment decisions (e.g., avoid dopamine agonists or advise cognitive training strategies).

Classically, non-motor symptoms have been managed as non-urgent symptoms, but when present they are a significant source of disability and are associated with a worse quality of life.[39-41] It is vital to recognise that many of these symptoms respond to treatment. They may be related to the patient's OFF state (i.e., they are a non-motor fluctuation), in which case they may respond to optimisation of dopamine therapy, or they may be dopamine-independent and require separate management.

Conclusions

Despite all the many recent advances in the medical management of PD, the management of motor fluctuations remains a key issue for many patients. Wearing-off can emerge early in the patient journey, and clinicians should be proactive in asking the patient about any symptoms that begin to emerge when their levodopa dose is losing effect but disappear when they take their next dose. When weighing the risks and benefits of potential medications, treating clinicians should consider the full spectrum of motor and non-motor symptoms that the patient experiences. Combinations of therapies may make best use of the various modes of action available, but practical considerations, such as pill burden and ease of titration, should also be considered.

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Newest Approaches in Immunotherapy for Non-Hodgkin's Lymphoma

EDITOR'S PICK

Immunotherapy in patients with relapsed/refractory non-Hodgkin's lymphoma has shown promising progress. Research in the field of oncology has seen a growth in our ability to harness the host immune system to target malignant cells in a more sophisticated and effective way. This timely review from Ayers and Nasta discusses novel ways of engaging the immune system in non-Hodgkin's lymphoma, including checkpoint inhibition, T cell engager antibodies, and adoptive immunotherapy with chimeric antigen receptor T cell therapy. Longer-term follow-up data are warranted so we can identify when to use these therapies in patients.

Samantha Warne

Editor

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Abstract

Immunotherapy has revolutionised the treatment of haematologic malignancies. Patients with relapsed/refractory non-Hodgkin's lymphoma have poor response rates and short survival times when conventional cytotoxic chemotherapies are used. Immunotherapy offers a novel way to harness the host immune system to target malignant cells in patients whose disease may no longer respond to cytotoxic therapy. The increased and refined use of immunotherapy in this patient population has recently shown promise in a group with previously poor outcomes. In this paper, the authors describe the available data for immunotherapy use in non-Hodgkin's lymphoma, including checkpoint inhibition, T cell engager antibodies, and adoptive immunotherapy with chimeric antigen receptor T cell therapy.

INTRODUCTION

While the long-term overall survival (OS) rates in the front-line setting for non-Hodgkin's lymphoma (NHL) are upwards of 60–70%, a number of these patients will ultimately relapse and require further therapy.[1] Among this relapsed/refractory (r/r) population, outcomes remain poor with standard cytotoxic immunochemotherapy. According to the SCHOLAR[1] study, a large, patient-level pooled retrospective study of patients with refractory diffuse large B cell lymphoma (DLBCL), overall response rates (ORR) to the next line of therapy are 26%, with a median OS of 6.3 months. For this reason, earlier and more frequent consideration of novel therapies is warranted for these patients.

The last decade has shown a shift in the investigational and therapeutic arenas for NHL toward the use of immunotherapy. Under normal circumstances, the host immune system should recognise 'self' versus 'non-self' and aid in cancer prevention; however, tumours progress via circumvention of this safety mechanism with alterations in expression of surface antigens and evasion of the immune system via T cell exhaustion.[2,3] Some of the earliest evidence that manipulation of the immune system effectively combats cancer came in the form of allogeneic stem cell transplantation.[4] This treatment modality depends on the 'graft versus tumour' effect. The strength of a graft versus lymphoma effect is, however, unclear because data regarding the role for allogeneic transplant in lymphoma are inconsistent.[5-8]

In the 1990s and early 2000s, the benefit of antibody therapy became apparent with the resulting widespread adoption of rituximab for NHL.[9] The addition of rituximab to cytotoxic chemotherapy with cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) resulted in higher rates of complete response (76% versus 63%), as well as a statistically significant reduction in the risk of treatment failure (hazard ratio [HR]: 0.58) and death (HR: 0.64) in the elderly population. The MiNT study[10] then demonstrated the added benefit of rituximab to CHOP in younger patients, with an improvement in 6-year event-free survival of 74.3% versus 55.8% among patients with and without rituximab, respectively. The success with rituximab marked the beginning of the use of antibodies targeted against various extracellular targets, such as radioimmunoconjugates and chemoimmunoconjugates in lymphoid malignancies.

Research in the field of oncology has seen a growth in our ability to harness the host immune system to target malignant cells in a more sophisticated and effective way. This review will discuss the existing data for novel immunotherapy in NHL, including immune checkpoint inhibition (CPI), T cell engaging antibodies (TCE), and chimeric antigen receptor T cell (CAR-T) therapies.

IMMUNE CHECKPOINT INHIBITION

The ability to redirect the host immune system against tumour cells has advanced our ability to treat malignancy. Monoclonal antibodies that block T cell inhibitory signals can revive the existing natural antitumour response in the host. Currently, six different U.S. Food and Drug Administration (FDA)-approved therapies exist in this realm: ipilimumab (anti-CTLA4), nivolumab and pembrolizumab (anti-PD1), and atezolizumab, avelumab, and durvalumab (anti-PDL1). While more data exist in solid malignancies, concurrent investigations in lymphoid malignancies have shown promise as well.

Both ipilimumab and anti-PD1 antibodies have been tested among NHL patients.[11-14] A Phase I trial of ipilimumab in 18 patients with r/r NHL (follicular lymphoma [FL, n=14], DLBCL [n=3], and mantle cell lymphoma [MCL, n=1]) showed two objective responses.[11] One patient with DLBCL had an ongoing complete response (CR) at >31 months and one patient with FL had a partial response (PR) lasting 19 months following ipilimumab. The most common adverse events with CTLA-4 inhibition in this study included diarrhoea, fatigue, abdominal pain, thrombocytopenia, and anaemia.

Lymphoma response rates to anti-PD1 therapy are more encouraging than those seen with ipilimumab. The efficacy of CPI in classical Hodgkin's lymphoma (HL) is due to genetic alterations in 9p24 and subsequent

overexpression of PDL1 on >90% of Reed–Sternberg cells.[15] Although PDL1 is not as commonly expressed in NHL as in HL, there are subsets of patients who are specifically sensitive to CPI because of an overexpression of the PDL1 or PDL2 ligand. Primary mediastinal B cell lymphoma (PMBL) shares both histologic and genetic characteristics with classical HL, including genetic alterations in 9p24, which make it distinct from DLBCL. One group found that immunohistochemical stains showed PDL2 overexpression in 72% of PMBL biopsy specimens compared to only 3% of DLBCL specimens.[16] Additionally, copy number gains and translocations of 9p24 commonly appear in both primary central nervous system (CNS) lymphoma and testicular lymphoma.[17] Chronic infection with Epstein–Barr virus infection also leads to increased PDL1 expression on tumour cells.[18]

For these reasons, investigators have explored CPI in patients with PMBL, testicular lymphomas, and primary CNS lymphomas: subtypes of NHL known to have increased PDL1 expression. The KEYNOTE-013 trial[14] examined the antitumour activity of pembrolizumab in 18 patients with heavily pretreated r/r PMBL and found an ORR of 41%, with stable disease in an additional 35% of patients. Of note, median duration of response was not reached at the median follow-up of 11.3 months. This trial was followed by the extended, multicentre, Phase II study of pembrolizumab in 49 patients with r/r PMBL (KEYNOTE-170).[19] Intermediate follow-up data from KEYNOTE-170 presented in 2017 again showed an ORR of 41% with a 14% CR rate. Also in 2017, Nayak et al.[13] published their experience with four patients with r/r primary CNS lymphoma and one patient with CNS relapse of primary testicular lymphoma treated with nivolumab. All five patients in this group had clinical and radiographic response to PD1 blockade, and three patients had progression-free survival lasting 13–17 months. The Checkmate-139 study[20] is a Phase II study of nivolumab in patients with r/r DLBCL patients that is currently ongoing and should be very informative regarding the efficacy of PD1 blockade in relapsed DLBCL.

Anti-PD1 antibodies have also been investigated in NHL patients without 9p24 genetic alterations. In 2016, Lesokhin et al.[12] published a Phase Ib study of nivolumab in 81 patients with r/r lymphoid malignancies. This cohort included FL (n=10), DLBCL (n=11), and other B cell lymphomas (n=10) with a median of three prior systemic therapies. In patients with FL and DLBCL, ORR were 40% and 36%, respectively. Among all patients with B cell lymphoma, median progression-free survival was 23 weeks (range: 7–44 weeks).

Data suggest that CPI may also have an effect through its interaction with PD1-expressing tumour infiltrating lymphocytes in malignancies in which the tumour cells do not overexpress these ligands themselves. For example, in FL, PD1+ cells, both tumour infiltrating lymphocytes and follicular helper T cells, comprise the tumour microenvironment.[21] For this reason, FL is thought to be an extremely immunosensitive disease. Although results from single agent CPI in FL have not been as successful as the experience with HL, combination therapy with CPI and anti-CD20 antibodies seems to have a promising synergistic effect.

The MD Anderson Cancer Centre (MDACC) published their results of an open-label, non-randomised study of pidilizumab and rituximab in r/r FL.[22] This study included 32 patients with previously treated FL and found an ORR of 66%, with CR in 52% and PR in 14% of patients at a median follow-up time of 15.4 months. No Grade 3 or 4 autoimmune adverse events were seen with this combination therapy. The combination of atezolizumab and obinutuzumab also showed favourable results, with an ORR of 57%.[23] Pembrolizumab and rituximab combination therapy has shown an ORR of 80% among patients with r/r FL.[24]

Checkpoint Inhibitor Toxicity

In general, the side effect profile of immune checkpoint inhibitor therapy reflects the mechanism of action of these treatments via amplification of the patient's immune response and includes autoimmune pneumonitis, hepatitis, thyroiditis, colitis, and hypophysitis. While safety data for these therapies are more robust within the realm of solid malignancies, available data regarding the toxicities among NHL patients mimic that of patients with solid malignancies.

The Phase I trial of nivolumab in solid malignancies showed an adverse event rate of 41%, with 6% of these Grade 3 or higher.[25] Among the studies described above in NHL patients, similar results are seen. For example, in the Phase Ib study[12] of nivolumab in r/r NHL, 34% of patients experienced immune-mediated adverse effects. Eleven percent of patients experienced pneumonitis with 4% Grade 3 or higher, including one death. This study also showed a 9% rate of pruritus and rash, 7% rate of diarrhoea, and 17% rate of fatigue. Endocrine toxicities include thyroid abnormalities seen in up to 10% of patients with solid malignancies and, less commonly, hypophysitis in about 1%.[26] Given the wide range of immune-mediated toxicities, a comprehensive review of systems must be performed regularly along with basic laboratory and thyroid function testing with a low threshold to send chest imaging or additional laboratory tests to assess for autoimmune activity.

Of note, CTLA4 inhibition carries a higher risk of severe immune-mediated colitis not seen as commonly with PD1 inhibitors. The Phase I study[11] of ipilimumab in NHL patients cited a 28% incidence of Grade 3 or higher colitis. Signs or symptoms suggestive of autoimmune colitis require prompt intervention with corticosteroids, with rapid escalation to infliximab for persistent symptoms.

Pembrolizumab recently received FDA approval for r/r PMBL after two or more therapies. While data show that CPI is safe and effective in patients with r/r NHL, the published literature is composed of small sample sizes and, as such, should be interpreted with caution. Larger studies moving forward will be critical to identify the role for CPI in NHL treatment and to investigate whether combinations with other treatment modalities are of additional benefit.

T CELL ENGAGER ANTIBODIES

TCE are another treatment modality that manipulates and invigorates the host immune response to tumour cells. Immunoglobulin fragments that can recognise two distinct epitopes transiently bring the host T cell face-to-face with a tumour cell. As with CPI, the autologous T cells are subsequently stimulated to kill tumour cells that express a given antigen.

Blinatumomab is one such TCE that recognises both CD19 on B cells and CD3 on T cells. This therapy has shown promise in B cell acute lymphoblastic leukaemia (ALL) and has been approved based on a study showing improved efficacy compared to salvage chemotherapy among heavily pre-treated B cell ALL patients.[27] Concurrent studies have investigated the potential role for blinatumomab in NHL. In 2016, Goebeler et al.[28] published the results of a Phase I study of blinatumomab among patients with r/r NHL. As in ALL, the short half-life of blinatumomab necessitates that this drug be given via continuous infusion over 28 days with dose-limiting side effects of neurotoxicity. Seventy-six patients with heavily pretreated NHL (FL: n=28; MCL: n=24; and DLBCL: n=14) were included for analysis and, of patients treated at 60 µg/m^2/day, ORR was 69% across all subtypes and 55% among DLBCL patients with a median duration of response of 404 days. Neurologic events occurred in a dose-dependent manner, with 22% Grade 3 or higher events and 71% all Grade events among evaluable patients, with the most common events of headache (36%), tremor (18%), dizziness (15%), and aphasia (12%).

Viardot et al.[29] later published a Phase II study of 21 patients with r/r DLBCL after a median of three prior lines of therapy. Overall response rate after one cycle of blinatumomab was 43% with a CR rate of 19%. Again, Grade 3 neurologic events were seen in a significant portion of patients, with encephalopathy and aphasia each seen in 9% of the patients.

As is shown in the aforementioned studies, blinatumomab carries promising efficacy in a heavily pretreated population of patients with aggressive NHL, although the data are not robust. Blinatumomab poses a significant risk for serious neurologic toxicity, which necessitates slow uptitration of the drug in the inpatient setting when initiating therapy. Additional TCE therapies under investigation include REGN1979 and FBTA05, which target CD20 and CD3, with the hopes of mitigating the neurotoxicity associated with anti-CD19 antibodies.[30,31]

CHIMERIC ANTIGEN RECEPTOR T CELLS

In the last few years, the genetic engineering of autologous T cells has been refined and broadened to commercial use in both B cell ALL and NHL. CAR-T are autologous T cells that are genetically modified to express a chimeric antigen receptor that consists of a transmembrane protein with an extracellular antigen recognition domain, a transmembrane hinge, and an intracellular signalling domain for T cell activation. Improvement in therapeutic efficacy has resulted from enhancement of both the costimulatory signals and the host conditioning prior to T cell infusion.

CAR therapy in haematologic malignancies was first described by Till et al.[32] in 2008. However, without costimulatory molecules, first-generation CAR did not persist. It soon became evident that activation via costimulatory molecules was required for proliferation and persistence of the autologous T cells upon reinfusion.[33,34] In later generation CAR, both CD28 and 4-1BB are commonly used. Potentially prolonged T cell survival is associated with 4-1BB when compared with CD28. Other costimulatory molecules, including CD27, OX40, and ICOS, are used less frequently.[34,35]

Further improvement in CAR efficacy came with lymphodepletion prior to T cell infusion. While the mechanism of improved disease response is not entirely understood, Maus et al.,[34] among others, postulated that lymphodepletion with chemotherapy or radiation therapy may improve results by reducing regulatory T cells and decreasing disease burden prior to T cell engraftment. Additionally, the use of conditioning chemotherapy may increase the number of endogenous cytokines available for T cell engraftment by removing 'cytokine sinks' or other competing elements of the immune system.

Multiple centres have investigated the use of CD19-specific CAR-T cells in NHL, culminating in the first FDA-approved CAR-T products for r/r NHL patients. In 2015, Kochenderfer et al.[36] first published their experience with anti-CD19 CAR-T cells in patients with chemotherapy-refractory DLBCL. This group from the National Cancer Institute (NCI) treated 15 patients with r/r NHL (DLBCL: n=9; chronic lymphocytic leukaemia: n=4; and indolent lymphoma: n=2) with fludarabine and high-dose cyclophosphamide prior to T cell infusion. Results showed 8 CR with durations between 9 and 22 months. This group later published their results with lower-dose conditioning chemotherapy for 22 patients with r/r NHL (DLBCL: n=19; FL: n=2; and MCL: n=1) in which patients received fludarabine 30 mg/m^2 and cyclophosphamide 300 mg/m^2 or 500 mg/m^2 on Days -5 to -3 prior to infusion.[37] Results showed an ORR of 73% and 55% CR rate, with duration of response ranging from 7–24 months. While the median duration of CR was 12.5 months, no PR or stable disease were ongoing at the time of analysis. Interestingly, high peak IL-15 levels were statistically significantly associated with higher peak blood CAR cells and remission rates compared to patients who did not achieve high IL-15 levels.

In 2017, the results from the ZUMA-1 clinical trial[38] led to the approval of the first CAR-T product for r/r NHL. Neelapu et al.[38] published their results of a Phase II trial of axicabtagene ciloleucel (Axi-cel, marketed as Yescarta® [Kite Pharma Inc., Los Angeles, California, USA]) in 111 patients with r/r DLBCL, PMBL, and transformed FL. This product uses the CD28 intracellular costimulatory domain and had previously shown promising results in a Phase I study.[39] The ORR was 82% with a 54% CR rate and, at the median follow-up of 15.4 months, 42% of patients had an ongoing response, with 40% still in CR. Median duration of response among patients with CR was not reached in comparison to a median of 1.9 months among patients with a PR. OS at 18 months was 52%. These encouraging results led to the FDA approval of axicabtagene ciloleucel for the treatment of r/r DLBCL.

The University of Pennsylvania group published results with another anti-CD19 CAR-T product (CTL019) in 28 patients with r/r DLBCL or FL in 2017.[40] Unlike the axicabtagene ciloleucel product, CTL019 uses a 4-1BB costimulatory domain. Lymphodepleting regimens in this study were chosen by the investigator and included cyclophosphamide, radiation, bendamustine, and modified EPOCH

(doxorubicin, etoposide, cyclophosphamide without vincristine/prednisone). The ORR was 64% with a CR rate of 43% among patients with DLBCL and 71% in patients with FL, with responses maintained among 86% and 89% of patients with DLBCL and FL, respectively, at 28.6 months. Median duration of response was not reached. As a result, the FDA recently approved tisagenlecleucel (Kymriah® [Novartis, Basel, Switzerland]) for use in patients with r/r DLBCL.

Currently, axicabtagene ciloleucel and tisagenlecleucel are both approved for use in patients with r/r NHL, including DLBCL, DLBCL not otherwise specified, PMBL, high grade B cell lymphoma, and DBLCL arising from FL, after two or more lines of systemic therapy. Recommended dosage is $2x10^6$–$2x10^8$/kg of CAR-positive viable T cells for axicabtagene ciloleucel and 0.6–$6x10^8$ /kg for tisagenlecleucel. Cyclophosphamide and fludarabine for 3 days prior to infusion is recommended as lymphodepletion therapy. It should be noted that T cell therapy complicates the assessment of response within the first 30 days and, thus, evaluation is needed at 3 months and longer to accurately measure treatment response.

Chimeric Antigen Receptor T Cell Toxicities

Given the recent commercial availability of these products, it is important to recognise the common side effects and management of adverse events related to adoptive immunotherapy. Cytokine release syndrome (CRS) has proven to be the most common, severe, and potentially life-threatening toxicity associated with CAR-T therapies.[41] CRS typically presents within days of T cell infusion with median time to onset of 2 days (range: 1–12) for axicabtagene ciloleucel and slightly longer at 3 days (range: 1–51 days) for tisagenlecleucel, and consists of a clinical syndrome of fever (78%), hypotension (41%), hypoxia (22%), and tachycardia (28%).[38,42,43] High levels of inflammatory cytokines, including IL-6 and IFN-γ, and elevated laboratory markers of inflammation, including ferritin, C-reactive protein, and hypofibrinogenaemia, have been associated with severe CRS.[44,45]

Severity of CRS, based on a grading scale of 1–3, dictates the management approach (Table 1).[46] In mild CRS, expert opinion suggests supportive care with intravenous fluids, supplemental oxygen, and close monitoring for clinical decompensation. In cases of severe CRS with refractory shock or hypoxaemia, anti-IL-6 therapy with tocilizumab has proven efficacy, with benefit seen usually within hours of therapy.[47] The FDA label mandates that two doses of tocilizumab are available for each dose of CAR cells prior to infusion. Of note, different grading scales between the different CAR products makes comparing toxicities between these products difficult.

Table 1: Cytokine release syndrome: Penn Grading Scale.

Grade	Signs and symptoms
1	Mild reaction: Treated with supportive care, such as antipyretics and/or antiemetics.
2	Moderate reaction: Intravenous fluids required; some signs of organ dysfunction (Grade 2 creatinine clearance LFT).
3	More severe reaction: Prolonged reaction requiring hospitalisation for management of symptoms or organ dysfunction. Includes hypotension treated with intravenous fluids or low-dose vasopressors, and hypoxia requiring supplemental oxygen, including high-flow nasal cannula or BiPAP.
4	Life-threatening complications, such as hypotension requiring high-dose vasopressors and hypoxia requiring mechanical ventilation.
5	Death.

BiPAP: Bilevel positive airway pressure; CRS: cytokine release syndrome; LFT: liver function test.

Table 2: Chimeric antigen receptor T cells encephalopathy syndrome grading.

Sign or symptom	Grade 1	Grade 2	Grade 3	Grade 4
CARTOX-10 score*	7–9 (mild impairment)	3–6 (moderate impairment)	0–2 (severe impairment)	Critical condition and/or assessment cannot be performed
Raised intracranial pressure	N/A	N/A	Stage 1–2 papilloedema or CSF opening pressure <20 mmHg	Stage 3–5 papilloedema or CSF opening pressure ≥20 mmHg or cerebral oedema
Seizures	N/A	N/A	Partial seizure, or non-convulsive seizure on EEG that responds to benzodiazepines	Generalised seizure, or convulsive or non-convulsive status epilepticus, or new development of motor weakness

*CARTOX-10 score: Five points for being able to name the year, month, city, hospital, and president or prime minister of their home country (one point per answer). Three points for being able to name three nearby objects (one point per answer). One point for writing a standard sentence and one point for counting backwards from 100 in tens.

CSF: cerebrospinal fluid; EEG: electroencephalogram; N/A: not applicable.

In addition to CRS, neurologic toxicities appear in a significant portion of patients after T cell infusion (Table 2). Neurologic events, including encephalopathy (57%), headache (44%), tremor (31%), dizziness (21%), and aphasia (18%), occurred in 87% of patients, with a median time to onset of 4 days.[38] The FDA recommends treatment for Grade 2 or higher neurologic toxicity with tocilizumab, in the presence of concurrent CRS, or with intravenous corticosteroids in the absence of concurrent CRS. Providers should also consider prophylactic antiepileptics for any Grade ≥2 neurologic toxicities. Toxicity profiles and rates of events are similar between the two commercially available CAR products.

CONCLUSION

In summary, immunotherapy in the r/r NHL patient population has shown promising progress in the last several years. As this patient group has previously had an extremely poor prognosis with relapsed disease after failing front-line therapy, the published literature described above offers hope for patients with typically poor outcomes. CPI and TCE, along with adoptive immunotherapy and CAR-T cells, result in high remission rates and prolonged responses among this heavily pretreated population. Currently, the appropriate timing for these novel agents in the sequence of lymphoma treatments remains unknown. With longer-term follow-up data that enables us to compare outcomes of adoptive immunotherapy to those of more well established treatment modalities, such as autologous stem cell transplantation for relapsed lymphoma, we will perhaps be able to better identify when to use these novel therapies in the treatment of these patients.

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iBiopsy® for Precision Medicine

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Abstract

A high-throughput artificial intelligence-powered image-based phenotyping platform, iBiopsy® (Median Technologies, Valbonne, France), which aims to improve precision medicine, is discussed in the presented review. The article introduces novel concepts, including high-throughput, fully automated imaging biomarker extraction; unsupervised predictive learning; large-scale content-based image-based similarity search; the use of large-scale clinical data registries; and cloud-based big data analytics to the problems of disease subtyping and treatment planning. Unlike electronic health record-based approaches, which lack the detailed radiological, pathological, genomic, and molecular data necessary for accurate prediction, iBiopsy generates unique signatures as fingerprints of disease and tumour subtypes from target images. These signatures are then merged with any additional omics data and matched against a large-scale reference registry of deeply phenotyped patients. Initial applications targeted include hepatocellular carcinoma and other chronic liver diseases, such as nonalcoholic steatohepatitis. This new disruptive technology is expected to lead to the identification of appropriate therapies targeting specific molecular pathways involved in the detected phenotypes to bring personalised treatment to patients, taking into account individual biological variability, which is the principal aim of precision medicine.

INTRODUCTION

Over 52% of health system executives expect artificial intelligence (AI) tools for medical imaging to have a significant effect on their organisations in the next 5 years, according to a November 2018 report from the Center for Connected Medicine.[1] At this time, nowhere in medicine has the impact of AI been more disruptive than in medical imaging. Many medical image equipment manufacturers are already incorporating various AI tools in their product offerings. Early computer-aided diagnostic applications have been used to assist radiologists in image interpretation and the detection of abnormalities in CT and MRI scans since the 1980s, but the use of these techniques were limited by high rates of false positives.[2] In recent years, powerful AI algorithms using supervised learning based on neural networks, have vastly improved the accuracy of computer-aided detection. These new algorithms require vast amounts of manually annotated images for

training. To date, early efforts are limited and have been able to assemble fully curated registries of a few thousand images. The small scale of these registries has limited the predictive power of the AI tools for precision medicine.

The next frontier in AI is unsupervised learning, more akin to actual human learning, whereby the system learns hidden patterns in the data by itself without explicit prior labelling. Such unsupervised approaches will be critical to the widespread adoption of AI for precision medicine.[3]

iBiopsy® (Median Technologies, Valbonne, France) is a novel, image-based, high-throughput phenotyping platform, currently under validation, which combines unsupervised, or predictive, learning for the automated detection and characterisation of phenotypic abnormalities extracted from medical images with real-time similarity search across large libraries of previously classified disease signatures. This new disruptive technology is expected to lead to the identification of appropriate therapies targeting the specific molecular pathways involved in the detected phenotypes to bring personalised treatment to patients, considering their individual biological variability, the principal aim of precision medicine.

THE CHALLENGE OF PRECISION MEDICINE

The Precision Medicine Paradigm

The Precision Medicine Initiative launched by USA President Barack Obama in January 2015 signalled a paradigm shift in healthcare, with an evolution away from the 'one-size-fits-all' approach to disease prevention and treatment strategies designed to meet the needs of the average person, towards a personalised treatment approach based on individual biology. Dr Francis Collins, who led the Human Genome Project, defines precision medicine as involving prevention and treatment strategies that take individual variability into account; in this sense, precision medicine has been vastly improved by the development of large-scale biologic databases, powerful methods for characterising patients, and computational tools for analysing large sets of data.[4]

Precision medicine relies on the availability of useful biomarkers, defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.'[5]

There is a need to identify which patients are likely to derive the most benefit from targeted therapies. This may include merging information from imaging, pathology, blood biomarkers, clinical data, genomic, and proteomic data into a final treatment decision.

Limitations of Electronic Health Record Systems for Precision Medicine

To meet the goals of precision medicine, large collections of detailed patient data registries (CDR) will be necessary to assess which patients may respond to which treatment and to predict outcome. To assemble such real-world evidence in advance, directly from providers, a very popular approach has been to mine data already collected from various electronic health records (EHR).[6] The problem with EHR approaches is the reliability, quality, and validity of the data collected.[7] The main problem is, as stated by Prof John Quackenbush, Dana Farber Cancer Institute, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA: "EHR are not designed for treating patients, not designed for biomedical research, they're designed for getting paid."

In addition to quality and reliability issues, the current IT infrastructure is inadequate to store, process, and analyse the mass of omics data, such as genetic, pathology, and radiology data, produced by modern hospitals today, which are critical to the implementation of precision medicine approaches. The conclusion of a recent industry report by KLAS, an independent research group, was that precision medicine is too complex for EHR.[8]

The Promise of Medical Imaging for Precision Medicine

Medical images possess valuable diagnostic and prognostic information that can be mined through advanced image processing and data analytics methods. Computer-aided detection tools using imaging biomarkers were initially introduced in the 1980s[9] to assist in the

detection of abnormalities in medical images. While often quite sensitive, such methods lacked specificity and often required substantial manual preprocessing by skilled technicians.

The latest progress in computational power has given rise to a recently developed field of research called 'radiomics'. Radiomics, which was first formally introduced in 2012,[10] employs state-of-the-art machine learning techniques to extract and study large quantities of imaging data, such as features from radiological images; these data are used to decode underlying genetic and molecular traits for decision support. In radiomics, medical images that hold information on tumour pathophysiology are transformed into mineable high-dimensional data. This information can be harnessed through quantitative image analyses and leveraged via clinical-decision support systems. West et al.[11] recently showed that the radiomics analysis on CT images of treatment-naïve hepatocellular carcinoma can reliably identify the expression of specific genes that confer chemoresistance to doxorubicin.[11] This study may help tailor future treatment paradigms via the ability to categorise hepatocellular carcinoma tumours on the genetic level and identify tumours that may not have a favourable response to doxorubicin-based therapies.[11] Additionally, a recent study showed that radiomics signatures can also define the level of lymphocyte infiltration of a tumour and provide a predictive score for the efficacy of immunotherapy in the patient.[12]

Radiomics and the Curse of Dimensionality

Like any high-throughput data-mining field, radiomics is subject to the 'curse of dimensionality',[13] which was coined by mathematician Richard Bellman in 1961.[14] The curse of dimensionality refers to various phenomena that arise when analysing and organising data in high-dimensional spaces (often with hundreds or thousands of dimensions) that do not occur in low-dimensional settings. The common theme of these problems is that when the dimensionality increases, the volume of the space increases so fast that the available data become sparse. This sparsity is problematic for any method that requires statistical significance.[13] To obtain a statistically sound and reliable result, the amount of data needed to support the result often grows exponentially with the dimensionality (also known as the Hughes Phenomenon).[15] At the same time, the quality of a machine learning model is closely tied to the complexity of the model or the number of dimensions. As the number of dimensions increases, the performance first increases, then rapidly decreases as new features are added. The lack of scalability of radiomics models has resulted in the inability to generalise results from many of the early studies to large populations. The curse of dimensionality is a major problem for all omic data. The nature of microarray data and its acquisition means that it is subject to the curse of dimensionality, the situation where there are vastly more measurable features (e.g., genes) than there are samples. Reductionist analysis of high-dimensional microarray data can lead to feature selection approaches that are liable to extreme type I error (false positives) and will not identify enough features to provide for individual differences within the patient cohort.[16] Current radiomics studies suffer from the effects of similar reductionist approaches by seeking to select a few representative features from a large set of initial features. Unfortunately, the rapid growth in popularity of this immature scientific discipline has resulted in many early publications that miss key information or use underpowered patient datasets, without production of generalisable results.[17]

The predictive potential for systems biology, especially when applied to medicine, is the ability to capture not just the similarities between individuals in a cohort but the differences between them, enabling individualisation of patient treatments. It is widely predicted that novel deep learning methodologies derived from the latest developments in AI will help bring precision medicine into clinical practice.[18]

A fundamental problem in current translational radiomics research is the traditional paradigm of sifting through large numbers of features to identify a select group of markers to use prospectively for the diagnosis or prediction of therapies or outcome. If the potential of state-of-the-art imaging technologies is to be fully exploited for translational research and the individualisation of cancer therapies in particular, this paradigm needs to fundamentally change.

Rather than striving for simplification, the goal of radiomic analyses should be to develop analytic approaches that use multidimensional datasets and embrace the complexity of genomic data for personalised medicine.

THE ARTIFICIAL INTELLIGENCE REVOLUTION

AI is basically machine perception or the power to interpret sensory data. Humans have the innate ability to recognise certain patterns, for example, when humans see two pictures of two different but similar-looking individuals, they can recognise that they are mother and daughter. Also, humans can hear two voices and recognise that the people talking have the same accent. This is a much more complex problem for a computer, but it is made possible through supervised learning computer algorithms, in which the algorithm is trained by being given thousands of examples of what you would like it to recognise. This technology is useful in voice and facial recognition software and many other applications, including recognition of features, such as tumours, in medical images. With the use of deep learning, subtle changes can be detected with much greater accuracy as compared to human analysis and the new algorithms will greatly enhance our ability to accurately detect and interpret imaging biomarkers.

Deep learning is a subset of machine learning. Its foundation was laid by Geoffrey Hinton and Yan LeCun,[19] one of Hinton's PhD students in the mid-1980s, although the mathematical principles reach as far back as the 1960s. The full potential of those methods available could only be realised following the exponential increase in computational power and the introduction of powerful and inexpensive graphics processing units (GPU).

AI is rapidly transforming the field of medical imaging and leading organisations are incorporating machine learning with the objective of improving physician decision-making and supporting the aim of individualised diagnosis and treatment of precision medicine.[20] Deep learning has become extremely popular again in medical imaging since 2013, when new models based around convolutional neural networks substantially outperformed earlier systems for tasks, such as tumour classification and detection.[20] Derived from the field of computer vision, convolutional neural networks are ideally suited for the large-scale analysis of medical images.

The First Generation: Supervised Machine Learning

The first major advances in the integration of AI in medical imaging involved supervised deep learning systems. The deep learning systems selected a set of features based on training with prior labelled datasets. The performance of such systems for basic classification tasks, such as segmentation with so-called deep neural networks (DNN), often matched or even exceeded that of trained radiologists.[20]

Supervised machine learning can be used effectively for anatomic structure segmentation.[21] A deep learning model has also been used to train an algorithm to label individual voxels (pixels in three-dimensional space with added depth) within a liver CT scan, based on whether they are normal tissue or lesion. The accuracy of segmentation was reported at 94%, with each volume taking 100 seconds to process.[22] While the accuracy of supervised learning models may be impressive, classification performance is closely tied to the combined dimensional complexity of the model and the large labelled dataset required for effective training. In most real-life situations, such large labelled datasets are generally unavailable. The total number of datasets may be limited, and the prior labelling may be very labour intensive or simply not known.

Second Generation: Unsupervised Machine Learning

"The next AI revolution will not be supervised," argued the current Director of AI Research at Facebook, New York City, New York, USA, Dr Yan LeCun, at a recent AI Seminar.[23]

While fully unsupervised machine learning algorithms have been used in a variety of fields, the application of this technology to medical image analysis has been limited to fairly simple applications, such as image segmentation.[24] Most unsupervised methods use algorithms that are entirely different from their supervised

counterparts. Most of them fall in the category of clustering methods, the most common being the k-means clustering. Clustering methods are even older than deep learning methods, with their origin dating back to the 1960s with the publication of the monograph 'Principles of Numerical Taxonomy' by Sokal and Sneath in 1963.[25] Just like DNN, clustering methods have become increasingly popular since the advent of fast processors to handle the complex computational requirements. Recently, a variety of new clustering methods have been introduced, one the most popular being the density peaks clustering method,[26] introduced in 2014; this method is widely used for the clustering of gene expression microarray data.[27]

Another type of unsupervised machine learning network is referred as a restricted Boltzmann machine (RBM). The RBM unsupervised feature learning model has been applied to a number of tasks, including automatic detection of microcalcifications in digitised mammograms. It was used to automatically learn the specific features which distinguish microcalcifications from normal tissue, as well as their morphological variations. Within the RBM, low level image structures that are specific features of microcalcifications are automatically captured without any appropriate feature selection based on expert knowledge or time-consuming hand-tuning, which was required for previous methods.[28]

Transfer Learning

Training a DNN from scratch is difficult because it requires a large amount of labelled training data and a great deal of expertise to ensure proper convergence. A promising alternative is to fine-tune a DNN that has been pretrained using, for instance, a large set of labelled data from another field of medical imaging.[29] However, the substantial differences between medical images from different modalities may advise against such knowledge transfer; knowledge transfer from a certain type of CT image, such as chest CT to abdominal CT, is more likely to succeed in the retraining of a DNN.[30] However, this does not address the fact that large numbers of labelled images of any kind are hard to find in medical imaging. One of the largest collections available for research is the Lung Image Database Consortium image collection (LIDC-IDRI), which consists of diagnostic and lung cancer screening thoracic CT scans with annotated lesions. Seven academic centres and eight medical imaging companies collaborated to create this dataset, which contains 1,018 cases. The Radiological Society of North America (RSNA) has started to recruit volunteer radiologists at its main annual meeting to address the problem by manually labelling some random images for future research use. A main challenge with weakly supervised learning in medical imaging is that most works are proofs of concept on a single application, such as segmentation. This makes it difficult to generalise the results and gain insight into whether the method would work on a different problem. A review of >140 papers on transfer learning and weakly supervised learning concluded that, while the usefulness of the approach was shown, there was little evidence of successful results at this time.[31]

Generative Deep Learning Models

Generative DNN, more specifically Generative Adversarial Networks (GAN), can produce synthetic models of specific phenotypes against which target phenotypes can be compared. Such models have been applied to low-dose CT denoising, medical image synthesis, image segmentation, detection, reconstruction, and classification.[32] Relevant anomalies can also be identified by unsupervised learning on large-scale imaging data.[33] GAN can be used to increase the size of training sample sets by generating synthetic images of lesions.[34] Interestingly, such GAN generally outperform state-of-the-art discriminative deep learning networks for small to medium-size datasets, the typical situation in medical imaging.[35]

THE NEXT GENERATION: IMAGING PHENOMICS

Managing Complexity with Phenomics

The complexity of diseases such as cancer necessitates a paradigm shift in our approach to precision medicine using high-dimensional data derived from high-throughput next-generation technologies. Such a paradigm shift will impact researchers, clinicians, trialists, and pathologists alike. A deep phenotype-driven

approach, building models of integrated genetic, genomic, epigenetic, and clinical datasets, which represent individualised disease phenotypes, will allow physicians to predict a disease's clinical course.

In biology, the most widely accepted definition of the word phenotype is 'the observable traits of an organism.' The study of phenotype in medicine comprises the complete and detailed understanding of the spectrum of phenotypic abnormalities associated with each disease entity.[36] With this knowledge in hand, physicians can decide whether any given sign or symptom is related to an underlying disease or is an isolated feature, a decision that may help to stratify treatments and make the correct prognosis.

The phenome is the manifestation of all possible interacting influences, it contains information about all epigenetic and post-translational modifications above and beyond genes as well as environmental influences.[37] Phenotypes are often highly heterogeneous, with different subgroups having potentially very different causes; many are still based on diagnostic criteria defined a century or more ago, which potentially encompass similar phenotypes but have no similarity in molecular origin. This heterogeneity is one often-cited reason why molecular precision medicine approaches usually disappoint when applied to such diseases: if the phenotype used to select the subject includes multiple different diseases with different pathogenic mechanisms, the statistical power of the study disappears. Phenomics, through the analysis of detailed phenotypic data, provide an opportunity to elucidate the causal routes at play for specific genetic variants on a phenotype and may ultimately lead to better targets for the prevention of multiple conditions and complications.

In a phenomics approach, the data are collected in a 'hypothesis-free' mode; the only major consideration is having enough representatives of rarer phenotypes within a broadly unselected population. Accessing phenotypes with a population frequency >1% is a relatively easy reachable goal with a few thousand subjects enrolled.

In a phenomics-driven model, the depth of the phenotype can be extended to include any number of variables, such as immune response markers and epigenetic markers, such as methylation, immunochemistry markers, and molecular pathology to identify drugable molecular pathways. Already >80% of new drug candidates are identified through phenome-wide association studies (PheWAS) as opposed to genome-wide association studies (GWAS).

Molecular pathological epidemiology, which studies the effects of external and internal factors on the phenotypes of disease outcome, such as in cancer and non-neoplastic disorders, will also play a major role in precision medicine and phenomic approaches. With analyses of external and internal factors, bacteria, and immune cells in relation to the disease of interest, molecular pathological epidemiology analyses can provide new insights into environment-disease interactions.[38]

Imaging Phenomics

Imaging phenomics is the systematic, large scale extraction of imaging features for the characterisation and classification of tissue and disease phenotypes. Imaging phenotypes are highly effective tools for quantitatively assessing, classifying, and predicting disease processes and their severity. They can help define these precise subpopulations.

- Imaging phenomics may serve as a foundation for precision medicine surveillance of disease manifestation (occurrence, location, extent, and severity) and progression.
- Imaging phenomics can help identify molecular subtypes of tumours with specific outcomes. Tumour heterogeneity and other textural features extracted from medical images have been correlated with various tumour subtypes from histopathology and risk of recurrence.
- Imaging phenomics is a big data problem, requiring large parallel computing resources and advanced analytics. Imaging phenomics leverage the latest deep learning technologies that may be used to automatically identify which imaging features best predict a trait.

COMBINING IMAGING PHENOMICS AND ARTIFICIAL INTELLIGENCE: THE iBIOPSY PLATFORM

The iBiopsy platform is a tissue and disease high-throughput phenotyping platform specifically designed to acquire, index, and analyse thousands of individual image-based phenotypes to establish biological associations with high predictive accuracy. The key innovative features of the iBiopsy platform include an automated and fully unsupervised signature extraction and clustering engine and a real-time search and retrieval engine to match a target phenotype against a registry of reference phenotypes. When released in 2019, after receiving U.S. Food and Drug Administration (FDA) clearance, iBiopsy will deliver an easy-to-use solution, decoding image signatures from standard medical images, and is expected to revolutionise the way cancer and many other chronic diseases are diagnosed, treated, and monitored.

At its core, iBiopsy is designed to make holistic real-time interrogation of complex and voluminous data possible by using unsupervised predictive learning methodologies and big data analytic tools that process the data in high-dimensional space and capture all the biologic dimensions of a disease phenotype for an individual patient as a single fingerprint.

Content-Based Image Retrieval

Traditional medical image retrieval systems, such as picture archival systems, use structured data (metadata) or unstructured text annotations (physician reports) to retrieve the images. The content of the images cannot be completely described by words, and the understanding of images is different from person to person; therefore, a text-based image retrieval system cannot meet the requirements for the retrieval of massive images. In response to these limitations, content-based image retrieval (CBIR) systems using visual features extracted from the images in lieu of keywords have been developed and introduced in a number of fields, especially computer vision.[39] CBIR systems, with the advantages of high retrieval speed, have been widely applied for medical teaching, computer-aided diagnostics, and medical information management. Yet another important and useful outcome of a CBIR is the possibility to bridge the semantic gap, allowing users to search an image repository for high-level image features. In radiology, CBIR systems are an ongoing field of research.[40] Current CBIR systems perform global similarity searches (i.e., seeking to match an entire image to a set of reference images). In an image analysis environment, phenotypes may be understood as abnormal pathologies which are highly local in nature. From that perspective, an ideal CBIR performs in a manner analogous to a virus-checking software on a computer, detecting and indexing abnormal phenotypes.

Automated Image Processing

The CBIR system implemented by Median Technologies, uses patented algorithms and processes to decode the images by automatically extracting hundreds of imaging features as well as highly compact signatures from tens of thousands of three-dimensional image patches computed across the entire image. In addition to detailed phenotypic profiles, which can be correlated with histopathology, genomic, and plasmic profiles, the system generates a unique signature for each tile providing a fingerprint of the 'image-based phenotype' of the corresponding tissue (Figure 1). Using massively parallel computing methods, imaging biomarkers and phenotype signatures extracted from a target image are then organised into clusters of similar signatures and indexed for real-time search and retrieval into schema-less databases.

iBiopsy Functionality

The iBiopsy phenotyping platform is based on three main engines: a signature extraction and indexing engine, a real-time search engine, and a predictive analytics engine that translates the results of the similarity search into a phenotype or outcome probability.

While at its core, iBiopsy has focussed on the complex issue of dealing with unstructured imaging data, which is a source of highly accurate and noninvasive quantitative biomarkers of disease processes, the platform

is not limited to the handling and processing of medical images. iBiopsy is an ecosystem for big data analytics and can also accept pathology, molecular, genetic, and epigenetic data in the development of a comprehensive disease phenotype. iBiopsy is built on a highly scalable cloud-based framework capable of searching through petabytes of data in real time. It is designed to acquire, process, and cluster essentially any type of data, without the need to understand a priori the meaning of each data element.

Signature Extraction and Indexing

The image processing operations required for local content-based image feature extraction consist of two main tasks: firstly, tiling the images in a smaller volume-of-interest (VOI), typically a small cube, the size of which depends on the modality, the image resolution, and the purpose of the content-based query; secondly, performing feature extraction operations on the VOI.

As illustrated in Figure 2, the feature extraction engine performs totally unsupervised, automatic, and asynchronous extractions of features from the images, organises, and indexes them in a non-structured query language database based on a unique similarity metric.

The result of the first phase is a series of clusters of phenotype signatures. Since the clusters are self-organising, their pathophysiological meaning is not readily apparent and requires further analysis. The characterisation of each cluster is typically performed by analysing representative samples and their respective correlate with histopathology. Eventually, after a series of iterations, the clusters are organised to correlate with distinct tissue subtypes identified by their signature similarity. The final number of clusters is not known a priori and depends on the heterogeneity of the underlying imaging phenotypes.

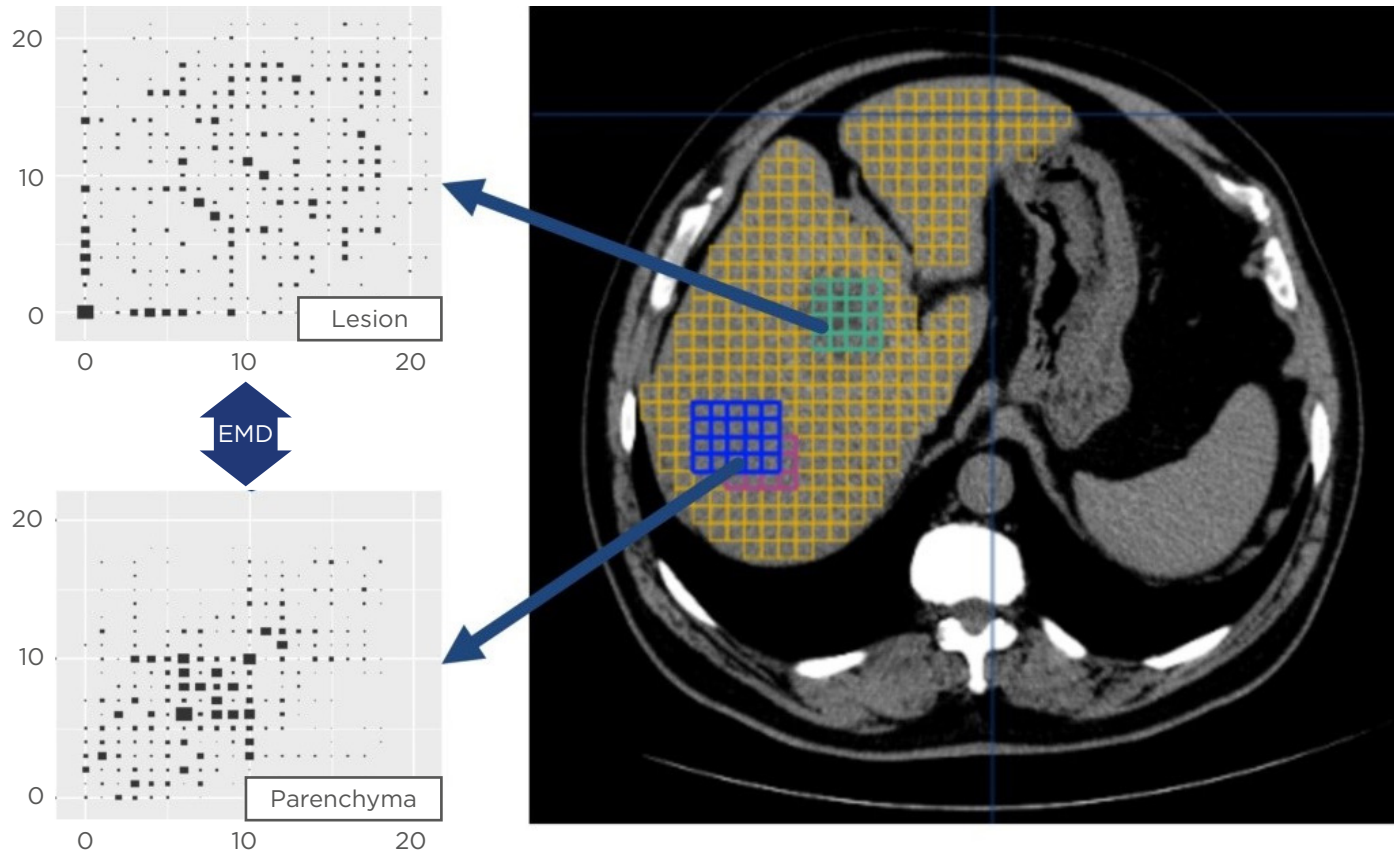

Figure 1: Unique image signature extraction for each tile provides a fingerprint of the matching tissue.

EMD: Earth mover's distance.

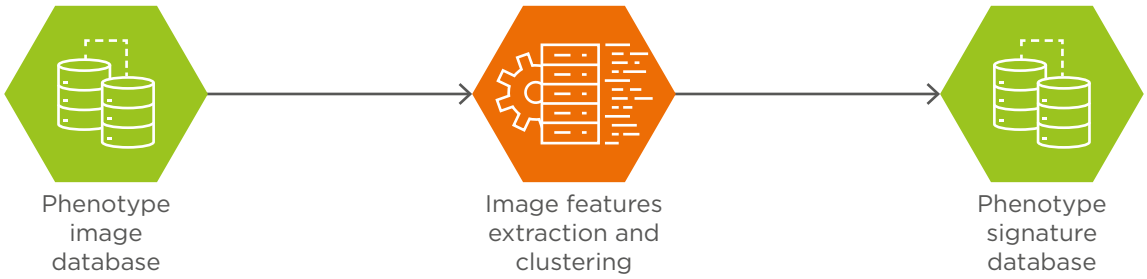

Figure 2: Feature extraction engine.

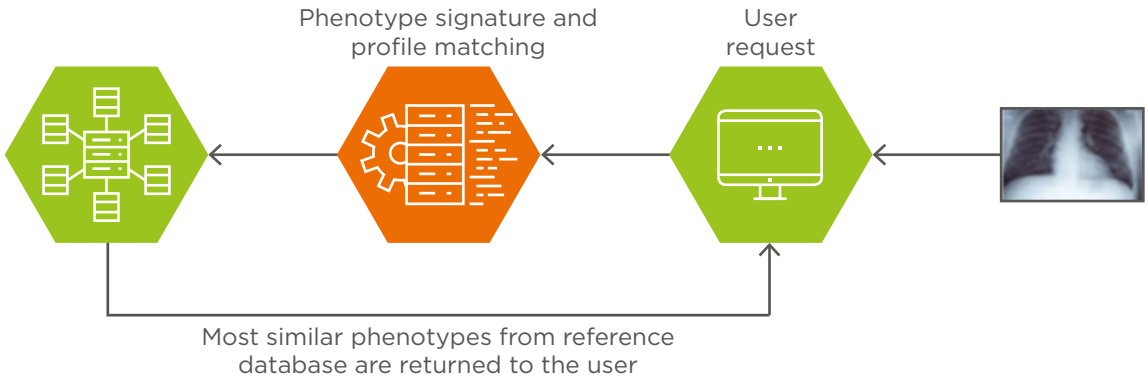

Figure 3: Real-time similarity search engine.

Real-Time Search Engine

The interactive search subsystem provides users with a query interface to the search engine where they can select or upload a query or example image tile and set metadata constraints for the search. The metadata may include the imaging modality (such as CT, MRI, or PET), voxel size, radiation dose in cases of CT imaging, or imaging protocol (e.g., T1 and T2) in cases of MRI. The presence of a contrast agent can also be included. A questionnaire may provide information related to patient data such as weight, size, prosthesis, previous local treatment, or prior disease conditions likely to affect biomarker evaluation. The search subsystem is then invoked to transform the user provided examples to a form suitable for query processing (i.e., performing any pre-processing and feature extraction operations on them) and then to determine the best matching signatures based on the content index. Finally, the interactive visualisation subsystem receives the identifiers of the best matching signatures and displays the corresponding VOI results. The analysis of the data is performed in real time with great flexibility using an optimised metric indexing image archiving system.

As illustrated in Figure 3, the Median Technologies, CBIR system performs real-time similarity searches of indexed phenotype signatures against one or more target patient signatures in the CDR. As a result of the search, a series of matching signatures are returned together with their associated metadata. In addition to returning actual images, the query returns a series of summary statistics, which may be used to predict diagnosis or prognosis based on CDR. The larger the CDR, the more accurate the prediction.

Predictive Analytics Engine

The third element of the iBiopsy platform is the predictive analytics or inferencing engine, which uses various AI algorithms to make a prediction to a clinical question asked by a user, such as identifying the specific tumour subtype, and make a prognosis about outcome based on patients in the CDR with similar subtypes.

A unique strength of the iBiopsy platform is the fact that is built entirely hypothesis-free as opposed to fit-for-purpose (i.e., it is not configured to answer a predefined set of questions, but rather it can answer any number of questions that may not have been considered at the time of design). In that sense, iBiopsy is very similar to an internet search engine, except that it operates both on textual metadata and on data automatically extracted and indexed from raw images. To make a final prediction, iBiopsy uses an ensembling strategy whereby independent algorithms reach a result and the final prediction is obtained by consensus between the methods for higher reliability.

Current Status and Future Challenges

The validation of the iBiopsy platform as a robust and reliable image-based high-throughput phenotyping platform for large-scale deployment in clinical research and routine practice is ongoing and following a multistage process.

The first stage involves the analytical validation of the platform for phenotype extraction and indexing. This mostly involves large scale tests of repeatability and reproducibility. Repeatability testing involves comparing results from image data generated within short intervals on the same patient in the same setting. Reproducibility testing involves testing the robustness of signatures under different acquisition conditions, with different equipment, technicians, and physicians. These tests have largely been completed and the results presented at major radiological and other medical conferences.[41]

The next phase involves qualifying the iBiopsy signatures against pathology and clinical outcome. For that purpose, large-scale data registries of images, pathology samples, genomic, and clinical data from patients with hepatocellular carcinoma are being assembled in the USA, Europe, and China with leading liver hospitals.

Initial results have also been presented on the challenging problem of noninvasively measuring the extent and severity of liver fibrosis in patients with nonalcoholic steatohepatitis (NASH).[42] Such measures currently involve invasive and costly biopsies. In partnership with major pharmaceutical companies, the platform is currently being tested for the discovery of various novel biomarkers for the staging and prognosis of NASH.

FUTURE OPPORTUNITIES AND CHALLENGES

Imaging phenomics offers the opportunity to leverage advances in AI in the field of medical imaging for precision medicine by identifying patients with specific disease subtypes, which may be the focus of targeted therapies, and by predicting the outcome from these therapies. Unsupervised learning has the potential to fundamentally change the treatment paradigm for many cancers and chronic diseases: rather than base treatment on empirically derived guidelines, it is guided by big data analytics to predict treatment response, leveraging vast biobanks and clinical data registries. There have been great advances in machine learning approaches, which can build predictive models from very complex datasets incorporating imaging, genetics and genomics, and molecular data, as well as environmental and behavioural data. AI approaches can be used to explore and discover new predictive biomarkers from these datasets. This information can then be used throughout the clinical care path to improve diagnosis and treatment planning, as well as assess treatment response.

There are limitations with current AI solutions. A case in point is the recent collapse of MD Anderson Cancer Center's ambitious venture to use the Watson cognitive computing system, developed by IBM, to expedite clinical decision-making around the globe and match patients to clinical trials. Launched in 2013, the project, while initially touted as revolutionising cancer care, never delivered actionable data to physicians and Watson was never used to treat patients at MD Anderson.[43]

To improve the predictive ability of AI-driven systems, models trained on vast and complex datasets are needed. AI can support the automated analysis of large and heterogeneous sources of data, increasing the diagnostic and prognostic value derived from image datasets alone. AI-based surveillance programmes can identify suspicious or positive cases for early radiologist review and extract information not discernible by visual inspection.

CONCLUSION

Several challenges still need to be addressed before fully unsupervised medical imaging can be taken into clinical practice, especially the constitution of large clinical data registries and the development of a new ecosystem for handling, storing, and analysing petabytes of omics data. While regulatory, legal, and ethical issues may constrain the scope of AI for precision medicine, imaging phenomics in particular is expected to play an increasingly important role in translational medicine for the development and validation of new therapies using phenome-wide approaches. The rapid adoption of AI-based approaches by the medical imaging community holds promise that imaging will continue its evolution, playing a pivotal role in enabling precision medicine to be widely realised in practice.

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Liposomal-Glutathione as a Potential Therapeutic Agent to Control HIV-1 Infection and Tuberculosis

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Abstract

This literature review provides insights into how glutathione (GSH) plays an important role in controlling HIV-1 and *Mycobacterium tuberculosis* infections. Since the discovery of HIV in 1981, >40 million affected individuals have died due to AIDS, and currently 40 million people are infected with HIV worldwide, which primarily infects CD4+ T cells. The natural pathogenesis of HIV consists of three stages: 1) the primary HIV infection phase, 2) the asymptomatic chronic phase, and 3) the late HIV symptomatic phase, which leads to an immunocompromised state resulting in increased susceptibility to opportunistic infections. It has been shown that HIV+ individuals have low levels of GSH; increased levels of proinflammatory cytokines, which correlate with increased production of reactive oxygen species and oxidative stress; and increased levels of TGF-β compared to healthy individuals. Consequently, increased reactive oxygen species levels lead to decreased levels of reduced GSH and increased levels of TGF-β, which has been demonstrated to inhibit the rate-limiting enzyme responsible for the *de novo* synthesis of GSH. In addition, the authors demonstrate that with supplementation of reduced GSH, there is improved intracellular control of an *M. tuberculosis* infection within macrophages. Therefore, decreased levels of GSH can leave HIV+ individuals prone to such opportunistic infections. The HIV transactivator of transcription (TAT) protein has also been shown to further increase oxidative stress and reduce GSH levels. Liposomal-GSH supplementation has the ability to bypass *de novo* GSH synthesis and provide protection against HIV and *M. tuberculosis* infections by increasing levels of GSH, improving redox homeostasis, and dampening the effects of TGF-β.

INTRODUCTION

Since the discovery of HIV in 1981, >40 million affected individuals have died due to AIDS. Currently, approximately 40 million people worldwide are living with HIV[1] and two-thirds of these reside in Africa, where disease prognosis is poor due to limited access to high-quality healthcare and drugs.[2] HIV infection leads to a deficiency in CD4+ T cells and eventual loss of immunity, causing increased susceptibility to opportunistic pathogens. *Mycobacterium tuberculosis* infection is the most common opportunistic infection in HIV-infected individuals, affecting approximately one-third of HIV patients and accounting for 26% of AIDS-related deaths.[3] HIV-1 primarily infects and destroys the Th1 subset of CD4+ T cells, resulting in compromised production of IL-2, IL-12, and IFN-γ, and increased susceptibility to opportunistic infections. Specifically, IFN-γ activates macrophages, neutrophils, and dendritic cells (DC) to destroy intracellular pathogens by producing reactive oxygen species (ROS), reactive nitrogen species, and antimicrobial peptides. IFN-γ can also activate CD8+ T cells and natural killer (NK) cells to produce perforin and granzymes, inducing programmed cell death of the infected target cells and destruction of the intracellular pathogen. Loss of IFN-γ can therefore dampen the ability of the host immune system to control intracellular infections. Glutathione (GSH) enhancement in a concentration-dependent manner (millimolar concentrations) in DC is directly associated with IL-12 production, which in turn favours a Th1 CD4+ T cell response, resulting in the production of IL-2, IL-12, and IFN-γ.

HIV PATHOGENESIS

HIV first comes in contact with and enters the body through a mucosal surface and infects the macrophages and DC present in the mucosal layer at this site of exposure. Here, the viral cell population increases, forming an important latent reservoir of viral cells.[4-6] During HIV infection, NK cells induce maturation of DC and initiate DC migration to the lymph nodes. HIV alters DC intracellular mechanisms to prevent NK cell-mediated apoptosis by upregulating cellular FLICE-like inhibitory protein (c-FLIP) and IL-10, thereby mediating protection; this allows for increased HIV viral replication. Concurrently, DC migrate to the lymph nodes where HIV migrates intracellularly towards the tips of the DC projections and is transferred to CD4+ T cells.[5] Viral glycoproteins gp120 and gp41 both play a critical role in HIV pathogenesis; when HIV-1 first comes into contact with the host cell, gp120 binds to the host via CD4 molecules expressed on CD4+ T cells and macrophages. Once gp120 binds to CD4, the chemokine receptor type 4 (CXCR4) is expressed, allowing gp120 to bind to it, which is followed by a fusion of gp41 with CXCR4. The viral RNA is then transferred into the CD4+ T cells (Figure 1).

HIV-1 is an RNA retrovirus, meaning that after entering the host cell the viral RNA is transcribed into complementary (c)DNA using reverse transcriptase. Viral cDNA is then incorporated into the host DNA. Preliminary evidence suggests that the binding of CD4 molecules to gp120 on the virus is dependent on the redox state of CD4 and monomeric reduced CD4 is the preferred state. Further studies are required to elucidate the role of GSH in this process.[7] A clear understanding of the underlying mechanism could lead to the development of therapeutics that can prevent HIV binding to CD4 molecules.

HIV infection causes a gradual decrease in CD4+ T cell count over time. Once the CD4+ T cell count drops below the threshold value of 200 cells/µL, opportunistic infections such as tuberculosis (TB) are increasingly likely to occur due to immunodeficiency. The natural history of HIV infection progresses in a three-stage process: 1) primary HIV-1 infection or the acute phase, 2) the chronic or latent phase of asymptomatic HIV infection, and 3) the late HIV disease phase.[8] In the acute stage, most patients experience symptoms of infectious mononucleosis, characterised by symptomatic fever, rash, and lymphadenopathy. There is also a transient increase in the viral load; however, eventually the HIV viral load is curtailed during this phase due to an HIV-specific CD8+ cytotoxic T cell response. CD8+ T cells produce perforin and granzyme to provide protection against viral infection, resulting in a decline in the viral load.[9] During the latent phase, the CD4+ cell count continues to decline while the viral count rises but the patient usually remains asymptomatic.

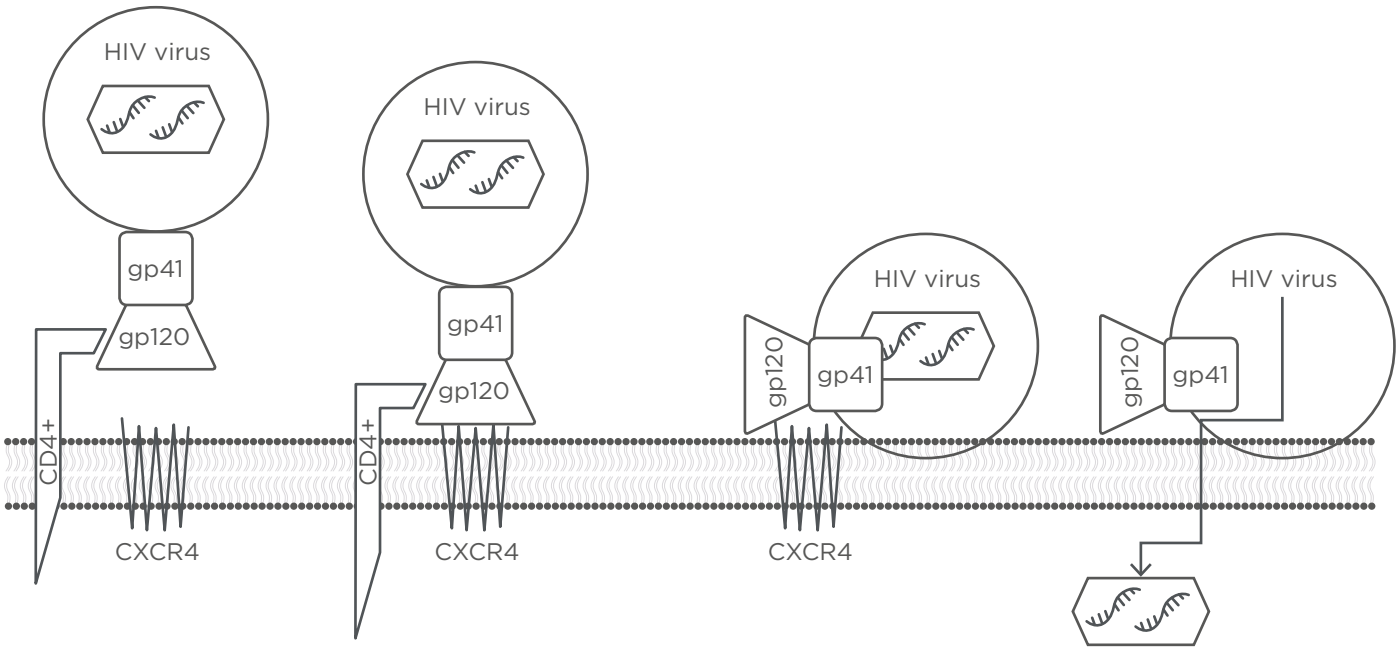

Figure 1: The three steps by which an HIV RNA-containing capsid enters a host CD4+ cell to be replicated.

The three steps include 1) attachment of the HIV envelope gp120 to the CD4+ T cell receptor ligand, 2) gp120 binding to CXCR4, and 3) gp41-mediated fusion of the HIV viral envelope with the host cell membrane via CXCR4, resulting in the transfer of HIV RNA into the CD4+ T cell.

CXCR4: chemokine receptor type 4.

This latent stage can last as long as 10–15 years among HIV patients and, while the patient is clinically asymptomatic, the virus is exceptionally versatile. Consequently, the viral cell count continues to increase inside the lymphoid organs with little or no immune response. During the late HIV disease stage, the CD4+ cell count drops to <200 cells/µL and patients are diagnosed with AIDS. This stage is characterised by the broadening of HIV tropism, resulting in an ever-greater rate of CD4+ T cell loss.[8]

GLUTATHIONE SYNTHESIS

GSH (γ-glutamylcysteinylglycine) is an important thiol tripeptide antioxidant that maintains cellular redox state and homeostasis in a variety of processes within mammalian cells, particularly those involved in the immune system (Figure 2).[10,11] GSH is present in the cytosol of cells in the range of 1–10 mM and 1–3 µm in the plasma.[11,12] GSH exists in two forms: the thiol-reduced (rGSH) form and the disulfide-oxidised (GSSG) form, with rGSH accounting for >98% of the total GSH.[13] The *de novo* synthesis of GSH uses glutamine, cysteine, and glycine, and takes place in two steps, both requiring ATP.[10] The first step in the biosynthesis of GSH is rate-limiting and is catalysed by glutamate-cysteine ligase (GCL).[10] GCL is composed of two subunits: a heavy, catalytic subunit (GCLC) and a light, modifier subunit.[10,14] The light, modifier subunit provides a regulatory function, while GCLC is responsible for the catalytic activity, which conjugates sulfur-containing cysteine with glutamate, forming γ-glutamylcysteine.[10] GSH synthetase catalyses the second and final step in the *de novo* synthesis of GSH through the conjugation of glycine with γ-glutamylcysteine.[5] GSH exerts a negative feedback inhibition on GCLC.[10,14] The regulation of GCL expression and activity is critical for the homeostasis of GSH.[10]

GLUTATHIONE AND OXIDATIVE STRESS

ROS contain partially reduced O_2 and are produced as biproducts of normal cellular respiration and during enzymatic reactions. ROS have beneficial effects on several physiological processes, including the destruction of

pathogens and tissue repair;[15] however, when there is a disproportionately high amount of ROS, it induces oxidative tissue damage. ROS are produced in response to a variety of stimuli, such as ultraviolet radiation, cigarette smoke, alcohol, nonsteroidal anti-inflammatory drugs, and infections.[15]

ROS include radical compounds, such as superoxide, hydroxyl radicals, and lipid hydroperoxides, and reactive nonradical compounds, such as singlet oxygen, hydrogen peroxide (H_2O_2), hypochlorous acid, chloramines, and ozone.[16] These molecules induce oxidative damage through unpaired valence-shell electrons that are highly unstable and reactive and have the ability to disrupt macromolecules, including proteins, lipids, carbohydrates, and nucleic acids.[15]

In the presence of ROS, rGSH is converted to GSSG by glutathione peroxidase (GPx) (Figure 3).[15] GPx links two GSH molecules through a disulfide bridge to form GSSG and, during this process, H_2O_2 is reduced to water and lipid hydroperoxides to their corresponding alcohols.[15] GSSG is unable to perform antioxidant functions but can be converted back to rGSH by glutathione reductase (GSR),[15,17,18] which requires the oxidation of NADPH to NADP+.[18] GPx and GSR play critical roles in protecting cells from the harmful effects of peroxide decomposition; however, the GSH antioxidant system can be overwhelmed if ROS are produced in excess, leading to increased levels of free radicals that can damage molecules that are essential to cellular homeostasis and metabolism.[19]

DECREASED GLUTATHIONE IN HIV+ INDIVIDUALS

It has been shown that HIV-infected individuals have diminished levels of GSH in their red blood cells, macrophages, T cells, and NK cells.[20,21]

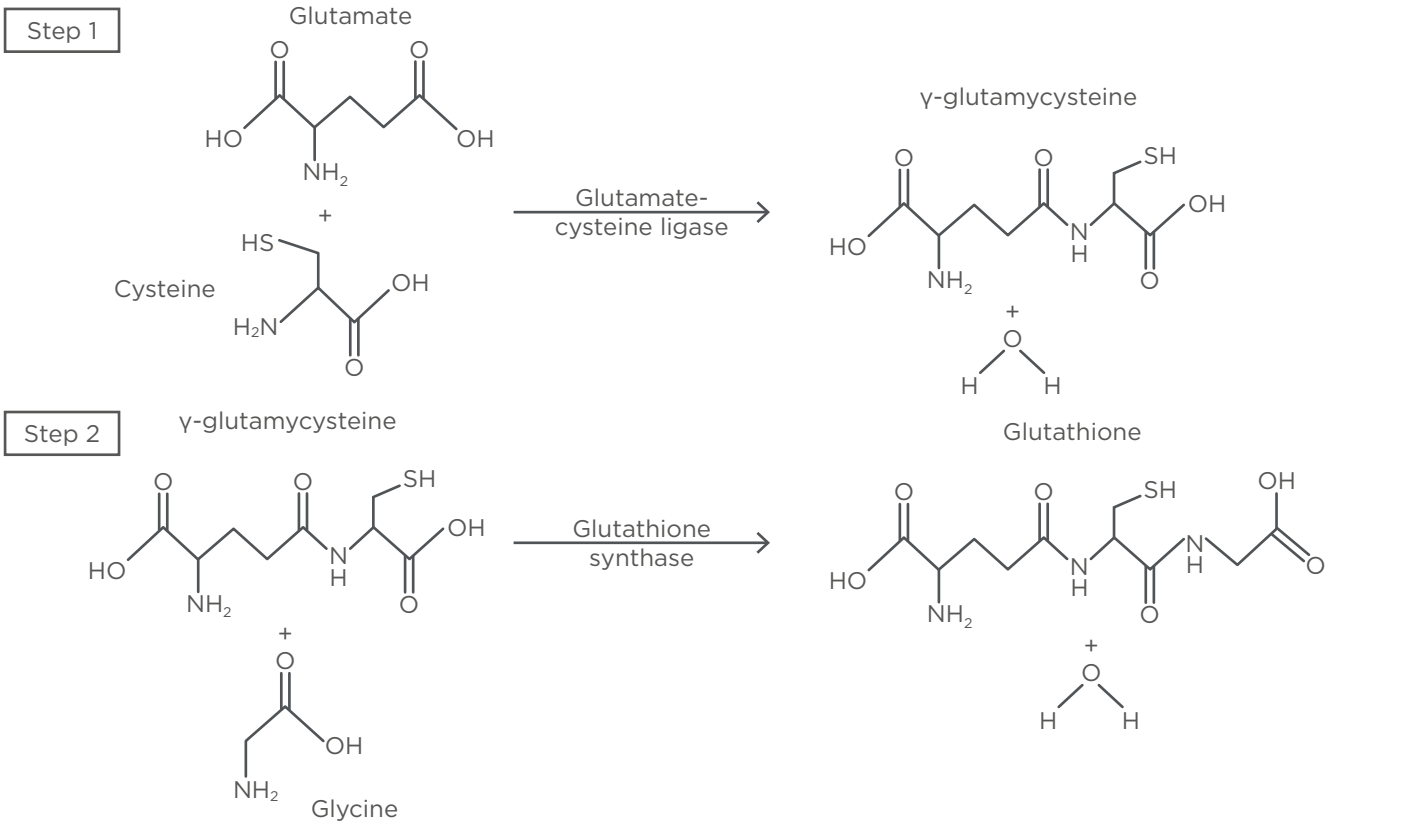

Figure 2: The *de novo* synthesis of glutathione.

The first and rate-limiting step of glutathione synthesis involves the conjugation of glutamate and cysteine by glutamate-cysteine ligase, forming γ-glutamylcysteine. Glycine and γ-glutamylcysteine are then conjugated by glutathione synthase in the second step, forming glutathione.

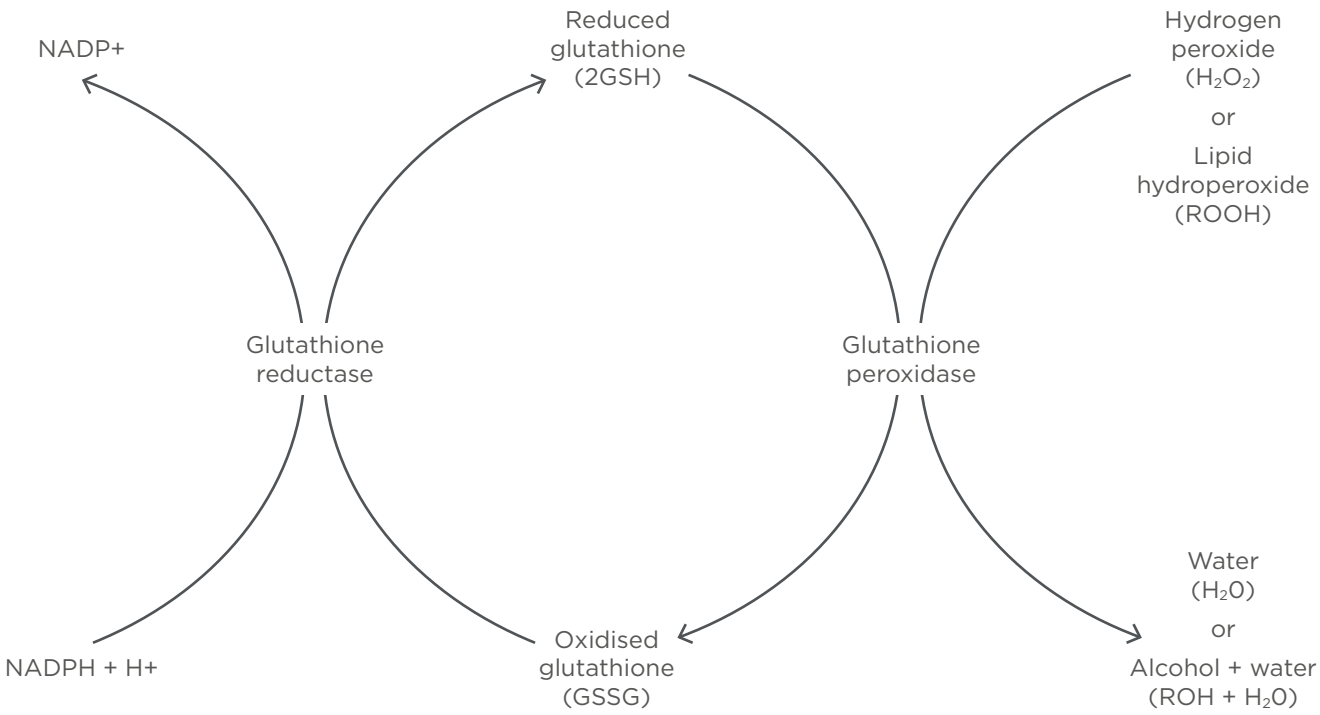

Figure 3: Glutathione redox reaction.

Reduced glutathione is converted to oxidised glutathione through a disulfide bridge by glutathione peroxidase in the presence of hydrogen peroxide or a lipid hydroperoxide, forming water or an alcohol and water, respectively. Oxidised glutathione is converted to reduced glutathione through glutathione reductase, which requires the oxidation of NADPH to NADP+.

The authors have demonstrated that within macrophages of HIV+ individuals, GSSG was more heavily favoured and decreased levels of rGSH correlated with a significant increase in the growth of *M. tuberculosis*.[21] In addition, there were also increased levels of proinflammatory cytokines, such as IL-1, TNF-α, and IL-17, which correlated with an increased production of free radicals. There was also a significant increase in the levels of TGF-β in these individuals. TGF-β is involved in regulating a variety of aspects of host defence to injury; however, when it is overexpressed it can contribute to pathogenic manifestations. Increased levels of TGF-β have been demonstrated to decrease expression of GCLC, essential for the rate-limiting step of the *de novo* synthesis of GSH.[21-24]

Furthermore, in previous studies it was demonstrated that GSH is essential for controlling the intracellular concentration of *M. tuberculosis* within macrophages.[25] In a later study, it was shown that *in vitro* treatment of whole blood cultures from HIV+ subjects with N-acetyl cysteine (NAC), a GSH precursor, resulted in improved control of intracellular *M. tuberculosis* infection, as well as decreased levels of proinflammatory cytokines IL-1, TNF-α, and IL-6, and increased levels of IFN-γ, promoting the host immune response to contain infection successfully.[20]

NK cells also play an important role in the innate immune defence and control of *M. tuberculosis* infection through cytolytic activity.[26] Like macrophages, NK cell functions are also impaired with low levels of GSH, allowing improved intracellular survival of *M. tuberculosis*.[26] The authors observed that treatment of NK cells with a combination of IL-2, IL-12, and NAC caused a significant increase in NK cell cytolytic activity and, therefore, control of *M. tuberculosis* infection. These growth inhibitory effects on *M. tuberculosis* correlated with an increased expression of the Fas ligand and CD40 ligand on the cell surface of NK cells.[22] With these findings, there is strong evidence that NK cells and macrophages work

together to combat intracellular *M. tuberculosis* infection, and GSH has been shown to enhance this.[26]

GSH does not only support the innate immune response in controlling *M. tuberculosis* infection but study results have revealed that it is also important to the function of T cells of the adaptive immune response. A study of T cells derived from HIV+ individuals showed that the cells were deficient in GSH, as well as having decreased levels of Th1 cytokines, leading to increased growth of *M. tuberculosis* inside the host's macrophages.[27] When GSH levels were enhanced, T cells were able to inhibit the growth of *M. tuberculosis* inside the macrophages and also produced increased levels of IL-2, IL-12, and IFN-γ, essential for the Th1 response and control of intracellular pathogens.[27]

In addition, the authors performed a double-blind study in which HIV+ individuals with CD4+ T cell counts <350 cells/mm^3 received either a placebo empty liposomal supplement or liposomal-GSH (L-GSH) (ReadiSorb™, Your Energy Systems, Palo Alto, California, USA) for 3 months. Prior to supplementation, baseline measurements of the HIV+ subjects demonstrated low levels of IL-2, IL-12, and IFN-γ, and high levels of IL-6, IL-10, TGF-β, and free radicals compared to the healthy subjects. Following the 3-month supplementation of L-GSH, there was an increase in GSH, as well as IL-2, IL-12, and IFN-γ, and a decrease in IL-6, IL-10, and free radicals, with stabilisation in the levels of IL-1, IL-17, and TGF-β compared to the placebo group, who showed no significant differences.[28] Overall, L-GSH supplementation provided restoration of redox homeostasis and cytokine balance, thereby aiding the immune system in the control of infections.

Furthermore, it has been demonstrated that higher concentrations of GSH help to keep HIV-1 in its latent state.[29] The GSH-synthesis inhibitor buthionine sulfoximine has been used in combination with histone deacetylase inhibitors as a complementary strategy to induce HIV-1 activation from quiescence by creating an oxidative environment that helps to stimulate HIV-1 transcription.[29,30] Due to the critical role that redox signalling plays in the pathogenesis of HIV-1 and HIV–TB coinfection, genetically encoded redox-sensitive green fluorescent proteins (roGFP), particularly a roGFP-based specific bioprobe of GSH redox potential (E_{GSH}; Grx1-roGFP2), have been created. This specific bioprobe allows for precise levels of E_{GSH} to be measured, particularly levels that would suggest reactivation of HIV.[31] In addition, it has been reported that bioactive lipids synthesised by drug-resistant strains of *M. tuberculosis* reactivate HIV-1 through increased intracellular E_{GSH}, thus providing a more oxidative environment and suggesting that the cohabitation of these two pathogens allows for an enhanced environment for growth and pathogenesis.[31]

TRANSACTIVATOR OF TRANSCRIPTION PROTEIN

The transactivator of transcription (TAT) protein is a 101 amino acid protein coded by the HIV viral genome.[32] It is a highly conserved regulatory protein of 14 kDa that operates within the infected host cell nucleus as an efficacious activator of HIV viral gene transcription.[33] TAT plays a major role in HIV replication because it upregulates transcription from the viral long terminal repeat promoter by binding to the TAR hairpin in the nascent RNA transcript, preventing the host cell from abruptly stalling the transcriptional process.[34] TAT functionality is vital for stable HIV replication due to the highly sensitive nature of the HIV promoter, which can be inhibited by various cellular regulators in the absence of TAT. The TAT-induced high rate of transcription of HIV allows the virus to outmatch the host immune response.

Furthermore, TAT directly participates in HIV infection due to its ability to act as an exotoxin, inducing cytotoxic and apoptotic effects on neighbouring T cells and resulting in high oxidative stress in the body.[34] This action results in the depletion of GSH levels; low GSH levels are associated with poor survival among HIV patients.[35] Moreover, TAT can increase TGF-β, causing a deficiency in the number of *de novo* synthesis enzymes, reducing GSH levels further.[36] Ultimately, TAT allows increased virulence of HIV, inadvertently decreasing serum GSH levels, and can be mitigated by ingesting L-GSH as a potential therapeutic agent.

SUMMARY AND FUTURE DIRECTIONS

Compromised CD4+ T cell counts in HIV-infected individuals and AIDS patients predispose them to a 26–31-fold greater risk of developing TB, as well as other potentially fatal opportunistic infections.[37] Currently, there is no cure for HIV and the present standard treatment for controlling HIV progression involves the use of antiretroviral therapy (ART) that targets different stages of the HIV lifecycle and enzymes essential for HIV replication and survival. Currently, 18.2 million people are receiving ART worldwide, but in the majority of patients, ART alone or in combination proves ineffective at preventing eventual progression of chronic HIV infection to AIDS or for the treatment of acute cases of AIDS.[28] This is partially due to the high mutation rate of HIV and the rapid development of mutant HIV strains that are resistant to antiviral drugs. Furthermore, studies suggest that various ART combinations increase oxidative stress in HIV patients, contributing to a further redox imbalance in HIV-infected individuals.[36,38] Modern-day HIV/AIDS research aims to formulate a vaccine or chemotherapeutic agent with the ability to completely ameliorate or circumvent HIV pathogenesis and infection. Until a cure is found, there is a need for other therapeutic agents and adjuncts to improve the prognosis of HIV patients and decrease complications due to opportunistic pathogens.

TB harbours a global burden, with one-third of the world's population latently infected with *M. tuberculosis*.[39] NAC is a GSH prodrug commonly used clinically for the treatment of pulmonary TB.[40] In HIV patients coinfected with TB, HIV causes upregulation of TGF-β, suppressing GCL in the *de novo* pathway of GSH synthesis. L-GSH supplementation has been shown to provide increased immunological protection against HIV progression and resistance to opportunistic infections by restoring levels of GSH, correcting redox homeostasis, and downregulating the levels of TGF-β. In contrast to NAC treatment, L-GSH is able to bypass the *de novo* pathway, potentially acting as a more effective therapeutic agent in the treatment of concurrent HIV and TB infections. Extrapulmonary TB has also become increasingly common since the emergence of the HIV-1 infection, seen in >50% of patients with concurrent AIDS and TB. The most common and severe form of extrapulmonary TB is TB meningitis, causing pronounced morbidity and mortality.[41] Recent research has reported decreased levels of GSH and increased levels of free radicals in brain tissue samples of HIV individuals, reducing neuroprotection and increasing the risk and occurrence of TB meningitis.[41] Additionally, studies have reported that individuals with Type 2 diabetes mellitus (T2DM) have decreased GSH levels due to compromised GSH synthesis and metabolism enzymes. T2DM individuals are at a 2–3-fold greater risk of developing TB compared to those without T2DM.[39]

Research indicates that L-GSH shows promise as an effective adjunctive treatment in patients with HIV or TB, and studies have reported L-GSH may also play a potential role in the treatment of other human pathologies that cause a decrease n the levels of GSH, a redox imbalance, and increased levels of TGF-β. Future studies are needed to optimise the dosage of L-GSH supplementation in HIV+ individuals with CD4+ T cells counts <200 cells/mm^3, as well as in individuals with CD4+ T cell counts between 200 and 350 cells/mm^3, in order to improve HIVprognosis and control TB progression.[28]

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From Renal Biomarkers to Therapeutic Targets: The Use of Monocyte Chemoattractant Protein 1, Transforming Growth Factor-Beta, and Connective Tissue Growth Factor in Diabetic Nephropathy and Antineutrophil Cytoplasmic Antibody-Associated Vasculitis

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Abstract

In an ideal world, every condition would have a sensitive and specific marker that could be measured in a noninvasive or minimally invasive way. Instead, the medical community depends on invasive biomarkers, which carry inherent risks, to make a diagnosis and plan treatment. In this review article, the current state of research into biomarkers for a range of kidney diseases is discussed, beginning with those biomarkers that are already in clinical use and then moving to conditions for which no validated biomarker yet exists. This review focusses on diabetic nephropathy at the proteinuric end of the spectrum and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis at the nephritic end. An interesting feature is that the same biomarker, monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), has been identified as a potential target in both conditions, which suggests a shared pathogenic process that results in two very distinct clinical presentations. One of the major limiting features of research into this area, particularly for ANCA-associated vasculitis, is the recruitment of a sufficient number of patients to generate strong enough evidence to justify the biomarker's routine use; this overlap in biomarkers may enable research in one

condition to be applied more generally. In addition to their role as biomarkers, these molecules are also therapeutic targets, and some early research has been carried out to investigate this. Overall, this review brings together research from diverse fields to focus attention on the outstanding areas and the future areas that warrant further investigation.

INTRODUCTION

The term 'biomarker' is commonly used, but a precise definition has not been agreed upon. One definition has been provided by the World Health Organization (WHO), which has defined biomarkers as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.'[1] In this review, the authors will focus more specifically on antibodies and cytokines that can be used as surrogate markers for the presence of renal diseases, assessing disease severity and monitoring progression.

There are a number of reasons why biomarkers are useful (Table 1). A major aim of the use of biomarkers is to prevent progression to end-stage renal failure and reduce the burden of organ replacement therapy. However, biopsy still has a crucial role to play in cases of diagnostic uncertainty or to assess the severity of a presentation. The use of noninvasive biomarkers in a way that complements, or avoids the need for, biopsy in certain cases, is a topic of much research.

In Goodpasture's syndrome, biomarkers have been clearly demonstrated to be useful. In the syndrome, a specific antibody to the glomerular basement membrane (anti-GBM) leads to rapidly progressive glomerulonephritis; this antibody can be detected in the serum, traditionally using enzyme immunoassays and immunofluorescence of renal biopsy specimens. While estimates vary, a recent study found a specificity and sensitivity of serum anti-GBM antibodies for Goodpasture's syndrome of 85.4% and 41.2%, respectively.[2] Therefore, detection of anti-GBM antibodies strongly supports the diagnosis; however, approximately 10% of patients do not have identifiable circulating antibodies, and thus renal biopsy is still recommended.[3] There is some evidence suggesting that higher titres of anti-GBM antibodies are associated with worse renal survival[4,5] and stronger evidence that antibodies against specific conformational epitopes may correlate with worse prognosis.[6] A fall in anti-GBM antibody titres usually correlates with clinical improvement, and in most cases recurrence does not occur. However, there is evidence from case reports that rising titres may associate with or precede clinical relapses.[7]

Table 1: The advantages of using biomarkers in the diagnostic and treatment settings.

Benefit	Explanation
Earlier diagnosis	Biomarkers may detect new disease and relapses earlier than relying on symptomatology (it may also help to select patients needing a confirmatory or staging biopsy).
Less invasive	A well-validated biomarker may achieve diagnosis before biopsy (important for patients who are not stable enough for an invasive procedure).
Prognostication	Biomarkers may give us as early indication of the severity of disease.
Minimise side effects	Based on levels of biomarkers, it may be possible to adjust treatment intensity and thus optimise the benefit to side effect ratio.
Directed therapy	Some biomarkers may predict response to a specific therapy and therefore allow targeted treatment options.

Table 2: Sensitivity and specificity of biomarkers in lupus nephritis.[11]

	Sensitivity	Specificity
Anti-double strand DNA	31%	67%
Complement C3	13%	89%
Complement C4	17%	72%

The archetypal example of the use of biomarkers in nephrology comes from the diagnosis of systemic lupus erythematosus (SLE). Antinuclear antibodies are the most sensitive biomarker for SLE, but they are the least specific, as 1 in 3 healthy individuals have detectable antinuclear antibodies, albeit at relatively low titres.[8] Anti-double stranded DNA (anti-dsDNA) antibodies are more specific for SLE, but there is a significant loss of sensitivity as assays may be negative early in the disease course. However, anti-dsDNA can be useful in disease monitoring because antibody levels typically increase prior to an exacerbation.[9] When anti-dsDNA levels are negative, the anti-extractable nuclear antigens become more useful, but they are not very specific, and there is little evidence that anti-extractable nuclear antigen titres reflect disease activity.[10] Reduction in complement C3 and C4 levels are also useful in monitoring of SLE, but play little role in diagnostics. These markers are all routinely used, though their sensitivities are relatively low; estimates vary, but quoted values are shown in Table 2.[11] William Egner produced an excellent table looking at when various markers are clinically useful.[12] There does not appear to have been research carried out into the sensitivity and specificities achieved through various combinations of these biomarkers that may improve the values. More recently, interest has been generated by research into the use of urinary proteomics in patients with lupus nephritis. Surface enhanced laser desorption/ionisation time-of-flight MS (SELDI-TOF MS) techniques have identified proteins in the urine that distinguish between active and inactive lupus nephritis.[11] While these have not been validated for the initial diagnosis, they provide a potential mechanism for early diagnosis of relapse. However, the technique has not become routine in current clinical practice.

During undergraduate medical education, renal diseases are often taught as being on a spectrum from pure proteinuria and nephrotic syndrome at one extreme to haematuria and nephritic syndrome on the other. To look at the current research into the translation of clinical markers from the lab to the clinic, this review focusses on two conditions at near opposite ends of the spectrum: diabetic nephropathy and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). By selecting these two conditions, the authors hope to highlight both the differences and surprising similarities in the use of biomarkers in these conditions.

DIABETIC NEPHROPATHY

Diabetic nephropathy may seem an odd choice; it is a secondary cause of nephropathy traditionally thought to be due to the deposition of extracellular matrix resulting in fibrosis. However, both experimental models and renal biopsies have shown an increased number of macrophages compared with controls.[13] A number of groups have identified signalling molecules that mediate these inflammatory markers. The best characterised are transforming growth factor-beta (TGF-β), connective tissue growth factor (CTGF), and monocyte chemoattractant protein-1 (MCP-1).

Transforming Growth Factor-Beta

TGF-β is a cytokine that is essential for normal organ development and function, yet it is associated with pathological fibrosis characterised by excessive extracellular matrix accumulation.[14] TGF-β expression is increased in the renal biopsies of patients with diabetic nephropathy mirroring its important role as a mediator in experimental models.[15] TGF-β production is stimulated at the transcriptional level by high glucose concentrations,[16] and expression levels are closely correlated with the degree of glycaemic control.[17] Increased TGF-β causes downstream profibrotic pathways, including synthesis of CTGF, resulting in progressive tubulointerstitial fibrosis and tubular atrophy.[18]

There have been no large studies looking at whether serum or urinary TGF-β levels are associated with the risk of diabetic nephropathy. However, a recent meta-analysis

found that TGF-β1 levels were significantly increased in patients with diabetic nephropathy compared to those with diabetes alone and healthy controls.[19] This is supported by studies that have found that a renin-angiotensin system blockade leads to a reduction in urinary TGF-β1 levels in diabetic nephropathy,[20] an effect shown to be enhanced by vitamin D replacement.[21] Overall, the evidence for the use of TGF-β as a biomarker is not available yet, but a potential problem arises from its lack of specificity to diabetic nephropathy.

Connective Tissue Growth Factor

CTGF is produced by mesangial and tubular epithelial cells and is present in the glomerulus of patients with diabetes. In mouse models, the amount of CTGF immunostaining correlates with the duration of diabetes.[22] There is good evidence that CTGF is a crucial mediator of TGF-β-stimulated matrix protein expression, which leads to scarring and inflammation. Specifically, CTGF mediates TGF-β-induced increases in fibronectin and collagen type I.[23,24] It has been demonstrated that there are increased amounts of CTGF in urine samples of patients with diabetic nephropathy[25] and that elevated levels of CTGF are predictive of an increase in microalbuminuria in the next year.[26]

Urinary CTGF has been demonstrated to correlate clinically with degree of albuminuria in patients with Type 1 diabetes mellitus. In the subgroup of patients with macroalbuminuria, urinary CTGF was lower in patients receiving angiotensin converting enzyme (ACE) inhibitor treatment than those not yet receiving ACE inhibitor treatment.[27] Plasma CTGF has been shown to be an independent predictor of end-stage renal failure and mortality in Type I diabetic nephropathy.[28] A Phase I trial of an anti-CTGF monoclonal antibody in patients with diabetes showed a reduction in microalbuminuria, although the trial was not designed to assess efficacy of treatment.[29] There is therefore good evidence for a role for CTGF as a marker of diabetic nephropathy and a potential treatment target.

However, while much of the focus has been on CTGF in diabetic nephropathy, there is evidence supporting its role in other conditions. CTGF expression has been shown to be increased in a range of inflammatory, glomerular, and tubulointerstitial lesions associated with cellular proliferation and matrix accumulation,[30] which included IgA nephropathy, chronic transplant rejection, crescentic glomerulonephritis, lupus nephritis, and membranoproliferative glomerulonephritis. In patients with non-diabetic chronic kidney disease, treatment with angiotensin receptor blockade leads to reduction of urinary CTGF levels, although plasma CTGF levels are unaffected.[31] Therefore, while the presence of CTGF in the urine may suggest a pathogenic process, it does not appear to be sufficiently specific as a diagnostic marker for diabetic nephropathy.

Monocyte Chemoattractant Protein-1

MCP-1 is a potent chemokine that activates monocytes and macrophages.[32] There is good evidence for its role in diabetic nephropathy. It has been demonstrated that renal mesangial cells produce MCP-1 in response to high concentrations of glucose.[33] MCP-1 knockout mice are protected from streptozotocin-induced diabetic nephropathy.[34] There is controversial evidence over the role of a genetic polymorphism in the MCP-1 gene leading to a higher frequency of diabetes in some studies,[35] or an increased risk of diabetic nephropathy in diabetic patients but not an overall increase in diabetes, as shown in other studies.[36]

MCP-1 is detectable in urine from patients with diabetic nephropathy.[37] Increased levels of MCP-1 correlate with the decrease in estimated glomerular filtration rate in diabetic patients over 6 years of follow-up, and this is an independent predictor from baseline proteinuria.[26] In patients with macroalbuminaemia, urinary MCP-1 is an independent risk factor for chronic kidney disease progression.[38] Intensive insulin therapy has been shown to lower urinary MCP-1 levels in Type 2 diabetics with microalbuminaemia,[39] but this was only over a short time period and did not correlate with any clinical improvement.

The most recent data comes from groups looking to validate the use of biomarkers in diabetic nephropathy. Verhave et al.[40] analysed seven urinary biomarkers in 83 patients followed over 2 years. They identified MCP-1 along with TGF-β as independent predictors of a fall in kidney function. The larger study from Nadkarni

et al.[41] used samples from 380 patients enrolled in the ACCORD trial and found only MCP-1 as a predictor of sustained decline in renal function over 5 years of follow-up, independent of baseline kidney function or degree of proteinuria. MCP-1, therefore, appears to provide some prognostic information in diabetic nephropathy. However, it is not specific to diabetic nephropathy, with some evidence for its relevance in lupus nephritis[42,43] and its potential use in AAV discussed below.

One additional angle to the MCP-1 story comes from a recent Phase II clinical trial studying the use of MCP-1 receptor inhibitors in the treatment of diabetic nephropathy.[44] Patients were recruited to receive either placebo or one of two doses of inhibitor for 1 year and the results demonstrated a significant reduction of albuminuria in both treatment arms. Therefore, MCP-1 appears to be not only a marker of diabetic nephropathy but also a potential therapeutic target.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

AAV are a group of conditions associated with the presence of ANCA, either perinuclear ANCA, targeted against myeloperoxidase, or cytoplasmic-ANCA, directed against proteinase 3. However, while these markers are sensitive and specific for diagnosis of an AAV, at 85.5% and 98.6%, respectively, for combined ANCA testing, the value of serial monitoring is less clear.[45] A meta-analysis has shown there is a modest association between relapse and a rise or persistence of ANCA during remission,[46] with some studies finding a stronger effect if patients without renal involvement were excluded.[47] Due to these limitations, there has been a broader search for alternative biomarkers. Some biomarkers that have been identified as potentially of interest include serum calprotectin, serum anti-pentraxin 3 antibodies, and urinary soluble CD163 (sCD163).[48] Of these, sCD163 shows the most promise; however, it is more useful for serial monitoring of disease activity in patients with an established diagnosis because it is not sufficiently specific for the initial diagnosis.[49] The best overall marker that has been identified so far is MCP-1.[50,51]

MCP-1 was first highlighted as a potential biomarker by Tam et al.,[50] who showed that urinary MCP-1 was significantly higher in patients with active renal vasculitis compared to those with inactive vasculitis, patients with active extra-renal vasculitis, healthy volunteers, or controls between whom there were no significant differences. The difference was not seen in serum MCP-1 levels. This study was supported by work from Lieberthal et al.[52] who also identified MCP-1 as the best discriminator for active and inactive vasculitis but found it nonspecific for renal flares. Both studies looked at MCP-1 as a diagnostic tool and were followed by a study by Ohlsson et al.,[53] which demonstrated that it is also a marker of poor prognosis. A recent systematic review looked at 161 marker molecules, and of these MCP-1 had the highest sensitivity (100%) and a reasonable specificity (75%).[51] The only other markers that achieved statistical significance were a C-reactive protein cut-off of 21.6, C3a in serum, and C5a in urine.[51]

The major limitation in the identification of biomarkers for AAV is the sample sizes. In the systematic review, the sample sizes ranged from 16–22 patients with active and inactive vasculitis.[51] Even large renal centres struggle to recruit sufficient numbers to detect small differences. More recently, interest has focussed on the cytokines APRIL (A proliferation-inducing ligand) and BAFF (B cell activating factor of the TNF family). There is some evidence that these associate with disease activity,[54,55] but there is currently insufficient data to draw conclusions.

DISCUSSION

The field of biomarkers is subject to extensive research, but very few markers have successfully transitioned from scientific interest to clinical usage. SLE provides the benchmark for the use of biomarkers in clinical practice, with a range of autoantibodies providing clinically useful information. However, there have been minimal further advances since the discovery of these autoantibodies, despite large volumes of work into the cytokines associated with renal diseases. Table 3 compares the biomarkers that have been discussed in this article.

Table 3: Comparison of the use of key biomarkers in Goodpasture's syndrome, lupus nephritis, diabetic nephropathy, and antineutrophil cytoplasmic antibody-associated vasculitis.

	Useful for diagnosis	Useful for predicting severity	Useful for detecting relapse
Goodpasture's syndrome			
Anti-GBM	✓	✓	✓ (Although relapse is uncommon)
Lupus nephritis			
ANA	✓	×	✓
Anti-double strand DNA	✓	×	✓
Complement	✓ (But need to combine with autoantibodies)	×	✓
Diabetic nephropathy			
Urinary TGF-β	×	?	?
Urinary CTGF	×	?	?
Urinary MCP-1	×	✓	Useful in assessing risk of progression
Antineutrophil cytoplasmic antibody-associated vasculitis			
Anti-MPO/PR3	✓	×	✓
Urinary MCP-1	×	✓ (For renal disease activity)	✓ (For final relapse)
APRIL/BAFF	×	×	?

✓: yes; ×: no; ?: unknown or uncertain; APRIL: a proliferation-inducing ligand; ANA: antinuclear antibodies; BAFF: B cell activating factor of the TNF family; CTFG: connective tissue growth factor; GBM: glomerular basement membrane; MCP-1: monocyte chemoattractant protein-1; MPO: myeloperoxidase; PR3: proteinase 3; TGF-β: transforming growth factor-beta.

Most of the biomarkers that have been discussed in this article have been identified using classical biomarker ELISA assays. The field is not only expanding in terms of the number of biomarkers identified but also the techniques used to identify them. Recently, urinary proteomics has been used to identify neprilysin and VCAM-1 as potential biomarkers for diabetic nephropathy,[56] although further studies are needed to validate these findings. There is also increasing interest in single-cell transcriptomics, which is a technique for the analysis of gene expression at the level of the individual cell and has been used to identify 21 distinct cell types within the kidney.[57] A deeper mechanistic understanding of diseases may help in identifying combinations of biomarkers that together provide the specificity which individual biomarkers currently lack.

One of the major challenges is the lack of large-scale trials in this area. The number of patients who present with AAV, and the acuteness of these presentations, make recruitment into prospective clinical studies more challenging, thus limiting the strength of the evidence obtained. Stronger data are seen in the diabetic cohorts who typically develop renal impairment more slowly and progressively, and in much larger numbers. Can the research community generate enough confidence in the data generated so far that will allow for their use in clinical trials? Or even application for individual patients?

An interesting finding is that urinary cytokines, particularly MCP-1, appear to be an important marker at both ends of the spectrum of renal diseases. While this limits their specificities, it may inform researchers of the general mechanism of kidney injury not unique to an

individual condition. Therefore, can it be inferred that such cytokines are markers of general inflammation and generalisable across a wide range of conditions? This may mitigate some of the challenges in AAV research by making available a wider cohort of patients, with a range of renal diseases.

To date, no biomarker has been identified that has the sensitivity, specificity, and prognostic value to replace a renal biopsy, so the biopsy remains the gold standard for diagnosis. The biomarkers may prove to be more useful not for initial diagnosis but in monitoring disease progression when the underlying disease is already known and therefore the lack of specificity is less problematic. However, biomarkers have not made the leap into the clinic, as currently the evidence is not sufficiently robust. Future research focussed more generally on their utility in renal disease rather than any specific condition may provide sufficient data to support their use.

The biomarkers discussed are not only increased in multiple kidney diseases (of both immune and nonimmune origins) but also in disorders of other organs; therefore, it is not surprising that urinary biomarkers currently show more promise in kidney disease as they localise the inflammation to the urogenital tract. Instead of attempting to replace the renal biopsy, the significance of these markers may be strengthened by the correlation of their concentration with the histopathological findings, an area which has not been fully investigated.

The biomarkers discussed have primarily been investigated for their use in identifying those with or without a condition. Another area that has been less well studied is their use in identifying individuals who will or will not respond to a particular treatment. This would potentially enhance the probability of therapeutic success.

However, these biomarkers may be mechanistic, providing prognostic information because they are directly involved in the renal damage themselves. The levels can be measured and clinical information inferred. Alternatively, the biomarkers could be important potential therapeutic targets; clinical trials are ongoing that are examining anti-CTGF antibody and MCP-1 receptor antagonists, and such therapies may indeed be the legacy of decades of research into these biomarkers.

CONCLUSION

This article reviewed the current state of biomarker knowledge for two important renal conditions. The role of urinary MCP-1 as a biomarker for diabetic nephropathy and AAV, as well as the potential of MCP-1 to become a therapeutic target, has been highlighted. However, MCP-1, along with other biomarkers, lacks the specificity for a particular disease and instead reflects a state of inflammation and fibrosis.

The field of biomarker research has huge potential that has not yet been fully realised. The authors have highlighted the small number of patients that can be enrolled into clinical trials as a key obstacle to the progression of the research into this field. However, the lack of specificity of these markers may allow us to assess their use across a group of conditions, thereby increasing the number of patients. Finally, avenues for future research, namely new techniques to identify biomarkers, using biomarkers in conjunction with histopathology rather than attempting to replace it, using biomarkers to identify likely responders, and biomarkers as potential therapeutic targets, have been discussed.

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Central Processes Underlying Fibromyalgia

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Abstract

Fibromyalgia is a common chronic pain disorder characterised by a robust clinical phenotype with principal features that include widespread pain and tenderness, as well as high levels of sleep disturbance, fatigue, cognitive dysfunction, and emotional distress. Fibromyalgia symptoms occur along a spectrum ranging from mild to severe. The impact on the patient can be very high, with significant effects on personal, recreational, and work activities. The pathophysiology of fibromyalgia is complex and involves abnormal processing of pain and other sensory inputs from the periphery to the brain. In turn, central processes, which modulate this input, are the critical elements that initiate the sequence of events that lead to the clinical phenotype. The functioning of the stress response through its links to pain and other sensory neural processing is a key upstream component of the fibromyalgia cascade. Furthermore, emotional distress appears as a common everyday driver of this process. The mechanisms contributing to the clinical phenotype of fibromyalgia are driven by a top-down process. The aim of this review is to discuss the key central processes that underlie the fibromyalgia clinical phenotype and discuss how these should be the focus of both current management strategies and future research.

INTRODUCTION

Fibromyalgia is a common disorder that affects 3–5% of most studied populations.[1] Fibromyalgia is characterised by the presence of widespread muscular and soft tissue pain and tenderness. These symptoms are accompanied by variable levels of poor-quality sleep, fatigue, and cognitive dysfunction, as well as a number of other symptoms that often include headache, abdominal pain, and mood change.[2] Generally, these symptoms persist over time but fluctuate in intensity from mild to severe. The resultant burden of disease is high, with a significant impact on personal, recreational, work, and study activities.[3-5]

While the pathophysiological mechanisms that lie behind the characteristic clinical features are complex, they are now better understood. In this review, the authors examine the evolving understanding of top-down modulatory mechanisms that contribute to the clinical features of fibromyalgia.

DIAGNOSTIC CONSIDERATIONS

Fibromyalgia is diagnosed using a compilation of common and characteristic clinical features.

For study purposes, the 1990 American College of Rheumatology (ACR) classification criteria[6] have been used for decades. These criteria require the patient to have generalised pain and widespread tenderness, reflecting the altered neurophysiology of the pain-related nervous system that lies behind these critical clinical features.

Over time, enhancements of these criteria have occurred through scoring methodologies; firstly, the number of painful or tender regions (widespread pain index) and, secondly, the degree of unrefreshed sleep, fatigue, and cognitive symptoms, as well as the recent presence of headaches, lower abdominal pain or cramps, and depression (symptom severity scale).[7-9] The summation of these two items gives a score ranging from 0–31, which has been given various names, including fibromyalgia research or survey score, polysymptomatic distress scale, or central sensitivity score.[10,11] The score allows any individual (i.e., those with and without fibromyalgia) to be placed on a spectrum. The cut-off score for fibromyalgia diagnosis was established as ≥12.[9] Patients with 'sub-diagnostic' scores may still manifest many features of fibromyalgia; this is termed 'fibromyalgianess'. As the score increases, it will reflect the presence of more of the clinical manifestations that characterise fibromyalgia. The total score can be used to assess severity or response to treatments.

Two important issues have been identified as a result of the current diagnostic criteria; the first reinforces the links of fibromyalgia to other central sensitivity syndromes. The criteria give most weight to the key presenting feature of fibromyalgia, namely widespread or generalised musculoskeletal pain and tenderness. Additionally, the scores highly rate other clinical features that link predominantly to central mechanisms, including sleep, fatigue, cognition, and mood. These features are common in a number of clinical conditions grouped under the banner of central sensitivity syndromes,[12] which are commonly comorbid with fibromyalgia. The sensitivity syndromes include migraine, regional pain syndromes, irritable bowel or bladder syndrome, restless legs syndrome, postural hypotension, and chronic fatigue, among others.

The second issue is the capacity of the criteria to define fibromyalgia as a spectrum disorder. It is important to appreciate that all individuals, both with and without fibromyalgia, will score somewhere along this scale and, therefore, have more or fewer variable musculoskeletal and central symptoms. Thus, variation in severity and fluctuations of key symptoms over time can be documented. The concept of fibromyalgia as a spectrum disorder is very useful in understanding the effect of psychobiological processes, such as stress, on fibromyalgia. Most biological systems are modulated by a number of inputs and give a range of responses along a spectrum rather than being an all-or-nothing response. Fibromyalgia fits with this type of biological response.

CENTRAL SENSITIVITY DIATHESIS AND FIBROMYALGIA

The natural history of fibromyalgia differs between individuals. In many, there is a history of central sensitivity syndrome-related conditions that began during childhood. Headaches, abdominal pain, restless legs, and 'growing pains' are not uncommon well before the patient experiences the widespread musculoskeletal symptoms that are required for the diagnosis of fibromyalgia. Thus, it is suggested that there is a central sensitivity diathesis imbedded into the pain-related neural systems of many people. This, in turn, likely relates to a mix of genetic and environmental factors. It is estimated that each factor contributes about 50% to the risk of developing fibromyalgia.[13]

The trajectory of fibromyalgia symptoms also varies between individuals. In some patients, there is a gradual accumulation of many symptoms over time occurring along the spectrum, captured by the central sensitivity score outlined previously. Eventually, the symptoms will aggregate to such a level that allows diagnosis. As the symptoms increase or decrease, the patient will be rated as worsening, flaring, improving, or going into remission. Other patients report a more rapid onset of symptoms; however, in these cases there is usually previous history of central sensitivity syndrome conditions. In these patients, there is a significant change in the level of any previous

pain symptoms or the onset of widespread pain within a short timeframe, measured over weeks or months. Upon the onset of fibromyalgia, the ongoing trajectory will also vary, with some patients continuing to have long-term symptoms while others do not. The symptoms in all patients may fluctuate by varying degrees over the course of time.

NEURAL SENSITIVITY IN FIBROMYALGIA

Patients with fibromyalgia exhibit a number of changes in their pain-related nervous system. In the periphery, there is evidence of neurogenic inflammation, likely contributing to peripheral dysaesthesia and local oedema and pain.[14,15] The peripheral neurogenic inflammation is caused by increased secretion of a variety of proinflammatory neuropeptides, cytokines, and chemokines primarily from the unmyelinated C fibres. This antidromic proinflammatory action occurs in fibres that, along with the myelinated Aδ fibres, otherwise transmit information related to actual or potential tissue damage to the pain-related neural pathways. The fibres do this through activation following threshold responses to certain chemical, temperature, or mechanical stimuli, relaying the signal neurons in the outer part of the dorsal horn and, more importantly, to those deep in the spinal cord.

In the spinal cord of patients with fibromyalgia, the neurons deep in the dorsal horn have an increased sensitivity to pain signals transmission;[15] this results in enhanced responsiveness to inputs from nociceptors leading to hyperalgesia. More importantly, the sensitisation of deeply placed dorsal horn neurons also changes the sensory response to the input from large fibre mechanoreceptors, in turn causing translation of otherwise innocuous touch and movement input into pain. This process, termed allodynia, is the essential process that causes the generalised pain and tenderness that characterises fibromyalgia. Mechanoreceptor input from deeply placed structures subject to significant biomechanical strains, such as the low cervical and lumbar spines, is likely to contribute to the segmental deep burning pain through the process of referred pain. The sensitisation process in the spinal cord is essential in the development of the widespread pain of fibromyalgia.

The sensitivity of the second order neurons in the spinal cord relates to changes in the function of modulating pathways originating in the brain. For instance, the aberrant function of the descending monoaminergic pathways that involve noradrenaline and serotonin in many patients.[16] Improvement of this dysfunction has the potential to decrease peripheral pain and other sensory symptoms in many patients.

There is also an abnormality in the function of brain networks that involve various regions that interact with the midbrain and other centres that relate to these sensory pathways in fibromyalgia.[2] These include links between the default-mode network, the pain-inhibitory centres, and other key sensory processing structures, such as the posterior insula.[17,18] Modification of the function of these regions has been shown to improve a number of characteristic symptoms of fibromyalgia.[19]

The Stress Response in Fibromyalgia

There are alterations in the function of the stress response (SR) in fibromyalgia, as manifested by modifications in the hypothalamic–pituitary–adrenal axis and sympathetic function.[20,21] The SR refers to the whole-body reaction to a triggering event that might include emotional, chemical, immunological, hormonal, biochemical, or homeostatic components. The SR has two main arms, namely the hypothalamic–pituitary–adrenal arm and the locus coeruleus–noradrenergic system arm. Both arms are activated by various stressors, particularly emotional stressors. Activation may be acute and therefore be part of the fight-or-flight response, or can be subacute and contribute to lesser but more prolonged symptoms. Recurring stimulation of these SR functional units can lead to increased sensitivity of their effector systems, resulting in smaller or alternate new stressors more easily activating the SR.

Thus, emotional distress can activate the traditional SR and lead to the modulation of sensory input to the brain. Each of these components can contribute to symptoms that characterise the clinical phenotype of fibromyalgia. The physiological effects of emotional distress are seen as an initiating factor in this top-down model of fibromyalgia. Emotional distress is generated in the context

of a range of psychological factors that influence the processing of life events. Although it is recognised that mind–body interactions are complex and bidirectional, a simple model of this not-fully-understood pathway in regard to fibromyalgia is presented.[22]

Life events are considered in the context that they occur and may result in thoughts. Under the influence of certain psychological factors, thoughts may be translated into emotions. These modulating factors may include an individual's personality and their ability to cope with and behavioural response to (e.g., a fear and avoidance response) a new event. For example, in fibromyalgia, symptoms are increased with certain personalities, the ability to cope, the proneness to catastrophise or ruminate, and the tendency to experience anxiety.[23] In general, negative thoughts and emotions are associated with fibromyalgia symptoms. The interaction between fibromyalgia, stress, and depression is complex. Depression may be associated with or result from fibromyalgia but is not thought, in the absence of the SR, to be a top-down cause of fibromyalgia.[21]

Finally, emotions that are generated have physiological effects, and these effects interact with elements of the SR and neural circuits that modulate sensory input to the brain.

Genetic factors also contribute to the risk of developing fibromyalgia.[24,25] They generally involve genes that are involved with monoamine and related molecules that are active in stress and pain pathways.[26,27] These factors interact with environmental factors to effect modulation of the pain-related neural systems to create a central sensitivity diathesis.

STRESSORS AS TRIGGERS IN FIBROMYALGIA

A trigger is defined as anything that serves as a stimulus and initiates or precipitates a reaction or series of reactions; this includes emotional and biological reactions. In the context of fibromyalgia, a trigger is defined as a specific event that is deemed to exacerbate or cause the onset of symptoms that delineate fibromyalgia. Reported triggers of fibromyalgia have included exposure to certain infectious diseases, chemicals, toxins, physical trauma, and various medical illnesses.[2,24] However, the most common trigger is the presence of significant emotional distress.[28] This may be present by itself or it may be associated with any of the aforementioned items.

Stressful situations are common throughout life and usually do not trigger any prolonged alteration in neurobiology that might cause longer-term symptoms. However, a range of symptoms may occur at the time of any stressful event. The symptoms will depend on characteristics of the stress, the duration, and the coping skills of the individual.[23,28] Eustress indicates a stressor that is perceived by the individual as positive, while distress defines a stressor that exceeds coping strategies and causes negative effects. Emotional distress defines negative stress effects that derive primarily from psychological processes.

PHYSICAL TRIGGERS OF FIBROMYALGIA

A number of physical triggers have been associated with the onset of fibromyalgia. These include infection, physical trauma, and vaccination, among others.[29-32] While the methodology, quality, and interpretation of these studies varies, they are in agreement with the clinical observations that there is a strong association between physical trauma and fibromyalgia onset or exacerbation.[32] However, the mechanism by which physical trauma triggers the development of fibromyalgia is not clear.[33]

Peripheral nociceptive input contributes to central sensitisation in animal models. It is also part of the response of the pain-related neural system to acute or persisting peripheral nociception in humans. The resultant increased sensitivity of the relevantly placed spinal cord neurons causes the typical clinical features of secondary hyperalgesia and allodynia, as well as spreading of non-neuroanatomical receptive fields. These peripheral inputs may also contribute to central sensitisation in fibromyalgia.[34] It is suggested that there is a continuum of influences from peripheral and central sources contributing to the increased central sensitivity in fibromyalgia.[34] For instance, reduction of nociceptive input from

symptomatic muscle or joints in patients with fibromyalgia, through active-versus-placebo treatment, caused improvement of background fibromyalgia features, including improvement of pain thresholds.[35] However, peripheral influences by themselves seem an unlikely explanation to account for the totality of fibromyalgia symptoms.[16] Fibromyalgia is more than amplification of responses in the pain-related nervous system. It involves increased response to a variety of other sensory inputs, such as light and noise, as well as background sleep, fatigue, cognition, and mood changes. These all relate to activities of the central nervous system.

Hence, to ascribe physical trauma as the key pathophysiological process causing fibromyalgia is too simplistic. Each of the studied and clinically observed cases of physical trauma have significant associated psychological aspects, often not considered in the term 'physical' trauma. Psychological response is inherent in any situation of physical trauma and, in the context of fibromyalgia, is deemed to be the major consideration for causation.

PSYCHOLOGICAL TRIGGERS AND FIBROMYALGIA

Defined Stressors

While there are no high-quality prospective studies that examine psychological stressors in fibromyalgia, there are a lot of cross-sectional and case control studies that indicate a strong association between emotional distress and fibromyalgia.[32]

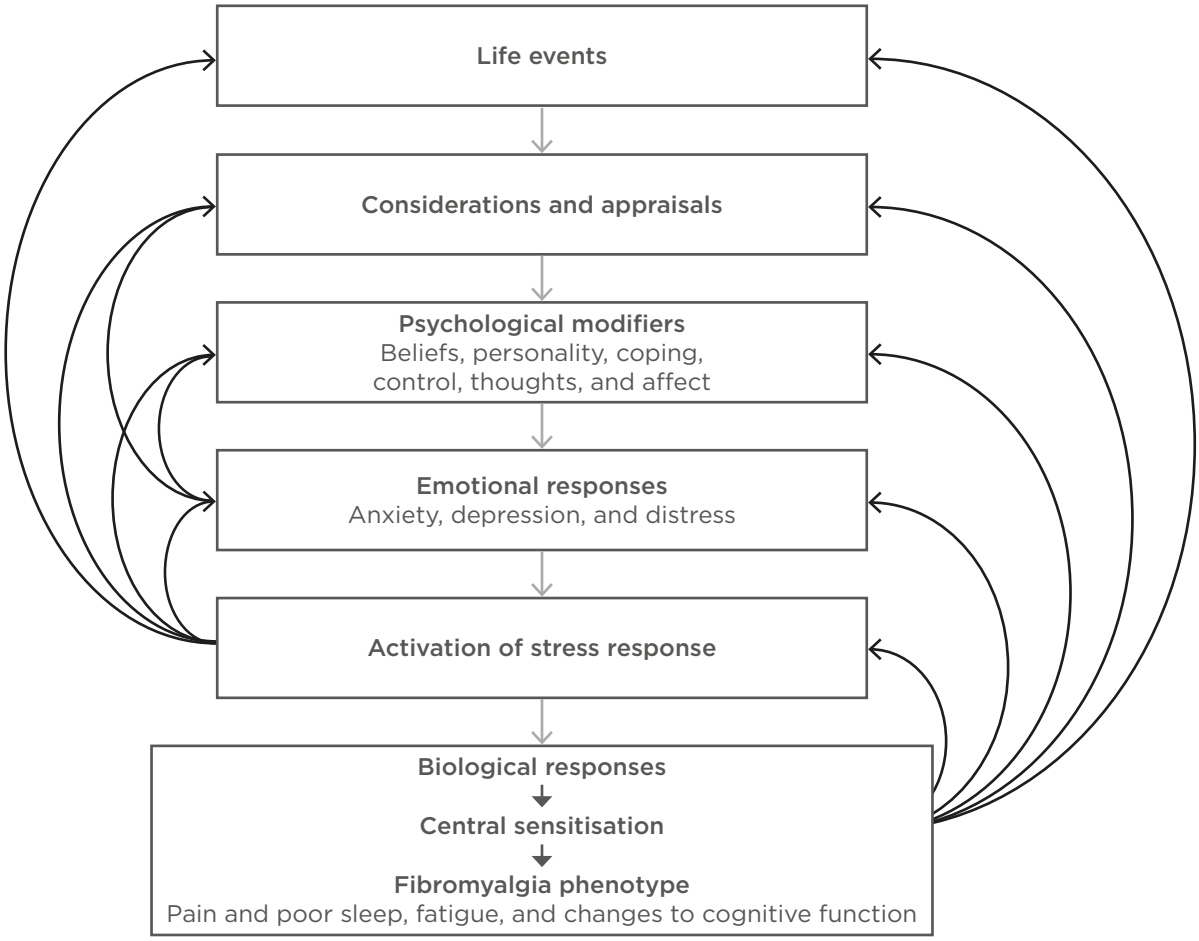

Figure 1: Key elements contributing to the top-down model of fibromyalgia.

Life events, such as psychological triggers, are appraised and translated into emotions under the influence of various psychobiological factors. Emotional distress activates biological responses, which include central sensitisation, the basis of fibromyalgia symptoms. Many feedback loops influence the phenotypic outcome.

Much attention has been given to psychological stressors that can be relatively easily identified. One common stressor is exposure to sexual or physical abuse.[36-52] These studies are subject to retrospective bias but generally indicate increased rates of fibromyalgia compared to various control groups in those exposed to this type of psychological trauma. A systematic review and meta-analysis of studies in this area concluded that there is an increased risk of fibromyalgia after psychological-related trauma.[53,54]

Post-traumatic stress disorder (PTSD) represents another well-defined situation in which emotional distress dominates the clinical picture.[32] There are high rates of PTSD in patients with fibromyalgia[55-58] and high rates of fibromyalgia in those with PTSD.[59-64] In a study of 395 patients with fibromyalgia, 45.3% had PTSD and 66.5% developed fibromyalgia after the onset of PTSD.[65] While both PTSD and fibromyalgia are defined for study purposes using strict validated criteria, both disorders exist on a spectrum. Further exploration of the associations between components of each disorder may show even stronger links between this type of emotional distress and fibromyalgia.

Background Stressors

Background psychological stressors are common and often not easily labelled as 'post-traumatic' because they are part of everyday life events. The development of emotional distress in response to these stressors and activation of the SR varies significantly in different individuals.

While genetic factors influence function at numerous levels in stress-related brain and spinal pathways, the majority of other contributing factors relate to psychological mechanisms. As indicated earlier, the response to everyday life events may trigger emotional distress in individuals when certain psychological buffers fail to work effectively. This situation may arise if the individual is prone to negative thoughts, or if there is excessive worry or rumination. Those who catastrophise easily or who lack control over an event are prone to develop emotional distress and activate the SR.

The responses of individuals to life stressors occur on a spectrum. Some only develop emotional distress with extreme provocation, while others will activate the SR with minimal stimuli. Most are somewhere in between these two extremes, with variable levels of emotional distress. This is akin to the symptoms of fibromyalgia, for which fluctuations in symptoms is characteristic. These two processes are linked through the process of central sensitisation; the SR is the initiator and the fibromyalgia clinical phenotype is the output. These processes are summarised in Figure 1.

IMPLICATIONS FOR MANAGEMENT OF FIBROMYALGIA

The top-down model allows for a better understanding of fibromyalgia management strategies. Strategies that focus on stress reduction, such as those that include relaxation, form the basis of management. These strategies are enhanced by those that are activity-based. Exercise and strength training benefit both physical and mental health and may have potent effects on fibromyalgia symptoms. Drugs that target sleep, stress, or the sensitisation processes are associated with better outcomes in fibromyalgia.[66] These so-called pain-modulators target the sensitisation mechanism of fibromyalgia and are contrasted to the action of potent analgesics, such as pure opioid analgesics, that are not effective in fibromyalgia.[66]

Future therapies that better modulate the process of central sensitisation will be dependent on increased understanding of the effects of the SR on neural sensitivity, particularly those involved in pain perception. Evidence-based strategies that modulate this response will likely range from those that are psychologically based to those that are pharmacologically based and may even include novel treatments such as transcranial magnetic stimulation.

CONCLUSION

Fibromyalgia results from a cascade of neurophysiological responses that are primarily driven by brain-related mechanisms. Among

these are links between thoughts, emotional responses, the SR, and sensory processing. Further research and management strategies for this common, high-impact clinical disorder need to start at the top.

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Customised Oocyte Donation Enhancement and New Findings Regarding the Role of Growth Hormone

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Abstract

In vitro fertilisation (IVF) with donated oocytes is the most effective assisted reproduction treatment currently available; however, repeated implantation failure (RIF) can occur with this treatment. The protocol of patient preparation for IVF with donated oocytes is relatively simple and works well in most cases; however, it can fail in a minority of women, which is what occurs in RIF patients. While the probability of RIF occuring is 20–35%, it is reasonable to take adequate measures in all patients in order to avoid procedural failure. The risk of oocyte donation failure can be minimised by applying a customised oocyte donation enhancement (CODE) protocol, in which pitfalls of the standard protocol are detected and corrected in the pretreatment phase, during the patient's uterus preparation for embryo transfer, and after the transfer. Growth hormone, recently reported to improve pregnancy outcomes in women with RIF after oocyte donation, is a possible component of the CODE protocol, but it cannot be considered a unique solution to RIF. This article reviews possible causes of RIF and places growth hormone treatment in the context of other important measures to be followed in the CODE protocol.

INTRODUCTION

Assisted reproduction with oocyte donation has very high success rates; if performed with fresh oocytes from donors <26 years of age, the probability of live childbirth is nearly 75%.[1] The probability of a live birth is slightly lower with older oocyte donors (within the age limits imposed by the law of each country in which oocyte donation is performed, usually around 35 years) or when cryopreserved oocytes are used.[2]

A question arises of what happens in those 25% of women who fail to become pregnant or who miscarry after oocyte donation. Sometimes it is unexplained and the patient becomes pregnant on the next attempt, which can be completed with spare cryopreserved embryos from the first attempt in most cases. However, there are cases of repeated failures of assisted reproduction with oocyte donation. The author has reported recently that growth hormone (GH) can be of help in some of these cases.[1] In the present paper, the most common causes of implantation failure in oocyte donation programmes are

discussed. A strategy of customised oocyte donation enhancement, aimed at avoiding all potential pitfalls responsible for these failures, is outlined, with particular attention to the indications for GH administration to donor oocyte recipients.

CURRENT STATUS OF OOCYTE DONATION

Since the first report of a term pregnancy using *in vitro* fertilisation (IVF) with donated oocytes in a patient with primary ovarian failure,[3] oocyte donation has gained traction in the treatment of infertility in many other conditions,[4] including young amenorrhoeic women with premature menopause[5,6] as well as naturally menopausal women,[7] repeated IVF failures,[8,9] and women with a genetic trait precluding the use of their own oocytes.[4,9] When performed with fresh oocytes from donors <26 years of age, the ongoing pregnancy rate can be as high as 75%,[1] the highest success rate among all other assisted reproduction treatment options available today. However, there remains an important question: what can be done for women who fail to become pregnant with this procedure? Is it merely a case of bad luck, or is there a factor that can be examined, determined, and treated? In the experience of the author, many of the women who fail to become pregnant with the first instance of oocyte donation succeed with the next attempt. However, some women experience repeated failures during subsequent attempts. This group of patients has a critical need for an individualised approach to achieve their goal.

Success Rate

The aforementioned 75% success rate can be considered optimal for the procedure, but it is not currently achieved in all oocyte donation programmes worldwide. In fact, real-world success rates reported by different IVF centres tend to be lower than this, especially when cryopreserved oocytes are used.[2] Oocyte donation attempt failure should not be automatically explained as 'bad luck'; in these cases, a systemic error is a more probable explanation. As with all systemic errors, the cause can be searched for, identified, and an appropriate solution can be devised. To make this possible, it is important to define the factors that can compromise the chances of success for couples seeking this kind of treatment.

Pitfalls

The cause of oocyte donation failure can be determined through careful examination in most cases; however, it is mandatory to follow the evolution of the patient's response to the treatment, both before and after embryo transfer, and to detect abnormalities compared with the normal course of events, in every case. Moreover, the possibility of implied male-borne factors should not be neglected. Consequently, possible causes of oocyte donation failure should be searched for, and corrected if necessary, even before the patient's inclusion in the programme.

CAUSES OF OOCYTE DONATION FAILURE

An oocyte donation attempt can fail because of female factors, male factors, or both. The female factor tends to be underestimated in the context of oocyte donation. The reason for this is that poor oocyte quality is the most common condition causing female factor infertility and IVF failure when using the patient's own oocytes; however, one problem could mask another, and the fact that the most probable cause of the patient's infertility has been taken into account does not exclude the presence of other, less frequent but no less important, problems. The probability of the male factor is the same as in IVF with the patient's own oocytes.

Female Origin

Problems of female origin can be detected before treatment initiation, during the treatment for endometrial preparation for embryo transfer, or in the period following embryo transfer.

Morphological Abnormalities of the Uterine Endometrial Cavity

Endometrial polyps, adhesions, and protruding leiomyomas are the most commonly found morphological abnormalities of the uterine cavity, and they can be found in women with a seemingly normal uterus. If not diagnosed and adequately treated, these conditions can lead to

repeated failures of assisted reproduction with donor oocytes. These pathologies can be easily detected by hysteroscopy; however, this is not always carried out before starting an assisted reproduction attempt because it may result in discomfort for the patient when conducted without anaesthesia, but using anaesthesia increases the cost of the procedure. The recently developed technique of virtual sonographic hysteroscopy,[10] which enables a more patient-friendly approach and avoids the need for anaesthesia, may be used in the future for all cases of IVF with donated oocytes to avoid unexpected failures due to uterine cavity abnormalities. Conventional operative hysteroscopy is, thus, only performed in women in whom abnormalities of the uterine cavity have been detected by this preliminary examination (Table 1).

Abnormal Blood Flow

Endometrial and subendometrial blood flow distribution pattern is correlated with the implantation and pregnancy rate after IVF treament.[11] This condition can be easily assessed by transvaginal colour Doppler ultrasound in the pretreatment phase and efficiently resolved by the administration of vasodilating drugs, such as pentoxifylline,[12] during the preparatory treatment of oocyte recipient women (Table 1).

Endocrine Disorders

Hyperthyroidism, hypothyroidism, hyperprolactinaemia, and insulin resistance can all negatively affect the outcome of IVF (Table 1). Here again, the possibility of one problem masking another must be discussed to avoid the temptation of oversimplifying procedural failure by blaming oocyte quality for all previous IVF failures, without taking into consideration other possible contributing factors.

Systemic Disorders

Systemic factors that can contibute to IVF failure in oocyte donation cycles include congenital or acquired thrombophilia, resistance to the activated protein C, lupus anticoagulant, and anticardiolipin antibodies.[13]

Abnormal Evolution of Endometrial Thickness and Morphology

It is generally assumed that successful implantation after IVF is less frequent in women who do not reach adequate endometrial thickness at the time of embryo transfer. The pregnancy rate in women with endometrial thickness <7 mm is lower compared to those with endometrial thickness ≥7 mm;[14,15] however, some patients prepared for IVF with donated oocytes do not achieve this value easily. There may be different factors causing this condition, among which an abnormal metabolism of exogenous steroid hormones is the most common.

Table 1: Customised protocol of endometrial preparation for oocyte recipient patients showing where and how pitfalls can be detected.

Procedural stage	Factors to consider	Factor correction
Pretreatment checkup	1. Abnormalities of the uterine cavity 2. Abnormal blood flow monitored by Doppler ultrasound 3. Endocrine abnormalities	1. Operative hysteroscopy 2. Antithrombotic and vasodilating drugs 3. Specific treatment
Treatment phase	1. Slow endometrial growth with low oestradiol values 2. Slow endometrial growth with normal or low oestradiol values 3. Premature endometrial transformation	1. Change in dose or route of administration 2. Use of a longer protocol 3. Use of a shorter protocol
Post embryo transfer phase	1. Low progesterone levels 2. Low oestradiol levels 3. Premature rise of prolactin	1. Change in dose or route of administration; repetition in an ovulatory cycle 2. Change in dose 3. Anti-prolactin therapy

Abnormal Metabolism of Exogenous Steroid Hormones

Patients receiving embryos originating from donated oocytes are usually treated with exogenous oestradiol and progesterone to ensure an adequate preparation of their endometrium for embryo implantation. If embryos are transferred during a natural ovulatory cycle, this treatment can, in theory, be avoided; however, this is not possible in cases of fresh oocyte donation, wherein the cycles of the oocyte donor and recipient have to be synchronised, in menopausal women, and in those with premature ovarial failure who lack ovulatory cycles.

There are different possible methods of oestradiol and progesterone administration. Oestradiol is most commonly administered orally or transdermally. When taken orally, most oestradiol is converted to less biologically active metabolites, oestrone and oestriol, in the liver during first-pass metabolism.[16] Transdermal application can avoid hepatic passage and prolong the active life of oestradiol;[17] however, vaginal administration of oestradiol has proved to be the most efficient method.[18] Progesterone can be administered orally, vaginally, or via intramuscular injections. Orally administered progesterone is rapidly metabolised in the gastrointestinal tract; oral administration has proved to be inferior to intramuscular and vaginal routes.[19] Significant differences in serum oestradiol and progesterone concentrations were observed between patients with the use of standardised protocols, leading to a need for repeated determinations during the treatment procedure.[20,21]

Male Origin

Oocyte donation cannot resolve problems of sperm quality,[20,21] although some consequences, especially those related to sperm DNA fragmentation, can be corrected by healthy oocyte cytoplasm.[22]

CUSTOMISATION OF PROTOCOLS FOR PATIENTS RECEIVING DONOR OOCYTES

Similar to ovarian stimulation protocols in women with poor ovarian reserve,[23] protocols for donated oocyte recipient can be customised before starting the treatment, during patient preparation for embryo transfer, and after embryo transfer.

Pretreatment Phase

Correction of Endometrial Pathologies

Different endometrial pathologies can be detected by virtual sonographic hysteroscopy, based on a simple vaginal ultrasound scan using Fly-Thru technology (Toshiba, Tokyo, Japan).[10] Owing to its patient-friendly nature compared to conventional hysteroscopy, virtual hysteroscopy can be performed in all potential oocyte donation patients to provide details of any problems that might contribute to treatment failure. Conventional operative hysteroscopy would then be performed only in women in whom virtual hysterosopy shows a relevant pathology that needs to be corrected.[10]

Abnormal Blood Flow

When subendometrial uterine blood flow determined by Doppler sonography is abnormal, treatment with vasodilating drugs, such as pentoxifylline, should be considered.[12,24]

Specific Treatment of Eventual Systemic Pathologies

When detected, systemic disorders, such as thrombophilia, and resistance to the activated protein C, lupus anticoagulant, and anticardiolipin antibodies should be treated accordingly using anticoagulants and corticosteroids.

Treatment of the Male Factor

Sperm DNA fragmenation, the most common male cause of IVF failure, can be alleviated in different ways.[22] Prolonged (2–3 months) oral antioxidant treatment (e.g., 1 g vitamin C and 1 g vitamin E daily) is sufficient in most cases. Melatonin has also been shown to protect sperm DNA against oxidative damage.[25] If no improvement in sperm DNA integrity occurs following oral antioxidant treatment, other solutions, such as modifications of the IVF technique or recourse to testicular sperm, can be considered.[22]

Between the Start of Endometrial Growth Stimulation and Embryo Transfer

Repeated Determinations of Endometrial Thickness and Serum Oestradiol

The protocol of endometrial growth stimulation in women prepared for IVF with oocyte donation is based on treatment with progressively increasing doses of oestradiol. In most cases, oestradiol is administered orally, either mimicking the normal cycle or at a fixed dose.[26] However, both kinds of protocol may result in an insufficient increase in blood oestradiol concentrations and/or the endometrial thickness. This problem can be avoided by repeated determinations of blood oestradiol and sonographic measurements of the endometrium.[20] If necessary, the planned dose or administration route of oestradiol can be modified at this stage, the vaginal route being the most robust to ensure blood oestradiol increase and endometrial growth. If the endometrium grows insufficiently in spite of elevated blood oestradiol levels, vasodilating agents, such as pentoxifylline (as mentioned earlier) or vaginal sildenafil,[27] can be of help. In addition to problems of oestradiol, prolactin can increase unexpectedly during patient preparation for oocyte donation, even if basal prolactin levels were normal (Tesarik J, unpublished observations). This can be easily corrected in the course of the treatment.

After Embryo Transfer

Assisted reproduction with oocyte donation is usually performed in anovulatory cycles. Consequently, there is no corpus luteum and the blood oestradiol and progesterone levels are entirely dependent on exogenous administration. In the author's experience, blood levels of oestradiol and progesterone may fall abruptly on days following embryo transfer, even when they were normal on the day of transfer. Prolactin can also increase prematurely, requiring appropriate medication. In the author's oocyte donation programme, oestradiol, progesterone, and prolactin levels are evaluated as early as 7 days after embryo transfer and then once a week during the first month. If no problems are detected during this period, subsequent controls are carried out every 2 weeks until the end of the first trimester of pregnancy. Vaginal progesterone supplementation is often insufficient during this period, leading to the need for intramuscular injections.

THE ROLE OF GROWTH HORMONE

In addition to its effects on oocyte quality,[28-30] GH has been recently shown to have a beneficial effect on the uterine receptivity for embryo implantation.[1] Experiments in a bovine model suggested an effect of GH at the uterine level; GH not only increased embryonic development in superovulated females, but it also improved post-transfer pregnancy rates when given to lactating recipient cows.[31]

Methods of Prescription and Dosage of Growth Hormone

GH was prescribed in oocyte recipient patients with unexplained implantation failure. Most of the patients showed a weak increase in endometrial thickness in response to oestradiol treatment during their preparation for oocyte donation. Subcutaneous injections of 1 mg recombinant human GH (Nutropin AQ®, Ipsen Pharma, Paris, France) were administed daily during 10 consecutive days of the endometrial proliferative phase induced by exogenous oral oestradiol. The treatment was adjusted individually for each patient so that the last of the 10 injections was administered 1 or 2 days before starting the treatment with vaginal progesterone to induce the secretory phase.[1]

Outcomes in Patients with Repeated Implantation Failure and Ensuing Questions

In a randomised controlled trial including 70 women with repeated implantation failure (RIF) after IVF with oocyte donation, patients treated with GH during endometrial growth stimulation showed a significantly thicker endometrium and higher pregnancy and live birth rates compared with RIF patients treated with placebo.[1] These recent observations raise several questions; for example, would RIF have been avoided if GH had been used during previous IVF attempts? If so, how could this patient's condition have been diagnosed? Secondly,

if diagnosis is not possible at the current stage of knowledge, should GH be used in all patients receiving donated oocytes to avoid unexpected failures? Finally, could GH improve the patient's chance of getting pregnant in other conditions, unrelated to oocyte donation?

Possible Mechanisms of Growth Hormone Action

GH is mainly secreted by the anterior pituitary gland, but it is also expressed in a variety of other tissues, including those involved in the reproductive system. GH can act via its own receptor or by means of the activation of insulin-like growth factor 1.[32] The mechanism of its effect on the uterine receptivity in IVF with donated oocytes is currently unknown, but a similar improvement has also been observed in women receiving their own frozen-thawed embryos.[33]

Towards a Better Definition of the Target Patient Group

In the absence of conclusive data for assessing which patients will benefit from GH treatment to improve their uterine receptivity, the decision about its use remains arbitrary. GH potentiates endometrial growth and its use can, thus, be considered in women whose endometrium does not grow sufficiently with standard treatments. In view of previous observations,[1] it might not be reasonable to wait for a patient to have RIF before making use of GH in the preparation of their uterus for oocyte donation. With the exception of pre-existing pathological conditions, such as certain types of tumours, postoperative and post-traumatic conditions, or acute respiratory distress, a short exposure to GH, such as that used in a recent study,[1] has not been reported to bring about any important negative health effect. Consequently, the relatively elevated price of the treatment (currently €230.00 in Spain) and administrative limitations of its prescription remain the most important obstacles for its wider use. Work is in progress to design diagnostics that can identify which patients could benefit from GH treatment to improve their uterine receptivity, not only in the context of oocyte donation but also in that of IVF and natural conception in general.

DISCUSSION

Oocyte donation is a highly efficient assisted reproduction technique if its main indication is the absence or a poor quality of oocytes in the recipient patient, without other associated contributing factors. However, the efficacy of oocyte donation decreases if the fertility problem is multifactorial, and other pathological conditions of male or female origin must be taken into account.

Possible additional female factors unrelated to the status of the patient's own oocytes, such as the presence of endometrial pathologies, abnormal subendometrial blood flow, different kinds of endocrine and other systemic disorders, and abnormalities of exogenously administered hormone metabolism, causing a deficient endometrial response in the proliferative or the luteal phase of development, are highlighted in this paper. The most common male factor causing RIF, in the current era of intracytoplasmic sperm injection as the preferred IVF technique, is excessive sperm DNA fragmentation. If detected, each of these factors can be treated adequately in the pretreatment phase, during the patient's preparation for embryo transfer, or in the period following the embryo transfer. RIF can also occur in cases in which all effort has been made to discover the cause, but everything appeared to be normal or, if applicable, adequately controlled. It has been shown that treatment with recombinant human GH during the proliferative phase of endometrium preparation is beneficial in some cases.[1] Nevertheless, there is no doubt that unexplored or poorly explored areas of research remain in this respect. Immunological incompatibility between the killer-cell immunoglobulin-like receptors (KIR) expressed in uterine natural killer cells, on the one hand, and the human leukocyte antigen-C (HLA-C), acting as KIR ligands, on the surface of the embryonic cells, on the other, are known to cause different types of pregnancy complications,[34] and preliminary data suggest that HLA-C/KIR may be implicated in RIF too. Therefore, in the author's oocyte donation programme, oocyte recipient patients are recommended to have their KIR haplotype tested. In cases of women with KIR-AA haplotype, known to be associated with an

increased risk of RIF or early embryo wastage, or when the result of KIR analysis is not available at the time of the treatment attempt, an oocyte donor is chosen who does not express HLA-C2 molecules, particularly prone to KIR-mediated complications of embryo implantation and pregnancy. This cannot be done if the HLA-C2 comes from the male partner of the patient treated by oocyte donation. Different therapeutic solutions to alleviate the KIR/HLA-C incompatibility are currently under development.

CONCLUSION

Oocyte donation is a highly efficient approach to the treatment of female infertility caused by the absence of oocytes or poor oocyte quality. Failures of assisted reproduction attempts using donor oocytes are mostly due to associated pathologies, of female or male origin, which are superimposed on this main pathology for which oocyte donation is indicated. Therefore, it is important to look for any possible pathological conditions that could hinder treatment outcomes and, if detected, to treat them adequately. However, unexplained repeated oocyte donation failures occur occasionally, in spite of the exclusion or adequate treatment of these oocyte-unrelated pathologies. Recent data[1] have shown that GH can be of help in some of these cases. Further research is needed to define the corresponding patient population and to explain the mechanism of GH action.

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Chronic Spontaneous Urticaria in Patients with Common Variable Immunodeficiency

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Abstract

Common variable immunodeficiency (CVID) is a predominantly antibody deficiency and is one of the most common primary immunodeficiencies in adulthood. Replacement therapy with Ig has significantly reduced infectious complications; however, malignant, autoimmune, and inflammatory diseases are still current major causes of morbidity and mortality. In recent years, interest has increased regarding allergic manifestations that may be associated with primary immunodeficiencies; however, no data are currently available on chronic spontaneous urticaria (CSU). In this report, the authors describe CSU in patients with CVID attending their centre. Three CVID patients were affected by CSU and were unresponsive to antihistamines. Patients were screened for the presence of serum autoreactivity by an autologous serum skin test; only one patient was positive for serum autoreactivity. The serum of this patient was found to induce CD63 upregulation on basophils and degranulation of LAD2 mast cells. All patients were treated with omalizumab therapy at the standard dose of 300 mg every 4 weeks. The patient with autoreactive serum was the best responder to omalizumab therapy, whereas the other two patients experienced urticaria flares related to intercurrent infections. In this article, the authors describe the presence of CSU in patients with CVID for the first time. Although autoimmunity is a feature of CVID, autoreactivity was documented in one patient only, thus showing that CSU in patients with CVID reflects the heterogeneity of this immune defect.

INTRODUCTION

Primary immunodeficiencies (PID) are a large group of rare diseases resulting from genetic errors of the immune system that affect particular immune responses, most of which are usually diagnosed in childhood.[1,2] However, some PID may develop in adulthood; for example, predominantly antibody deficiency and diseases such as common variable immunodeficiency (CVID) and IgA deficiency (IgAD).[2] CVID is the most common PID in adults and is a heterogeneous group of disorders characterised by the presence of low levels of at least two Ig isotypes (usually IgG and IgA) and a poor response to vaccinations in the absence of other demonstrated causes of hypogammaglobulinaemia.[3]

The defect predisposes the patient to infections and the development of neoplasia, and it is accompanied by immune dysregulation and autoinflammatory and autoimmune complications.[4] Autoimmune diseases occur in approximately 20–30% of patients with CVID, with the most frequent being immune thrombocytopenia and autoimmune cytopenia;[5] additional CVID-related systemic and organ-specific autoimmune diseases include systemic lupus erythematosus, pernicious anaemia, antiphospholipid syndrome, multiple sclerosis, Sjögren's syndrome, psoriasis, thyroiditis, uveitis, vasculitis, and primary biliary cirrhosis, while rheumatologic diseases may be detected in 10–30% of CVID patients.[4-7]

Among the autoimmune disorders, a peculiar disease is chronic spontaneous urticaria (CSU), which can be considered a disease that overlaps between allergy and autoimmunity.[8] CSU is a complex and heterogeneous disease in which autoreactivity is frequently observed. Indeed, one-quarter of patients with CSU display autoantibodies against thyroid autoantigens,[9] while 40% show antibodies against IgE or its high affinity receptor (FcεRI) that induces histamine release by mast cells (MC) and basophils.[10] An autologous serum skin test (ASST) is used to screen for the presence of autoreactivity in CSU,[11] even if its accuracy and reliability are still debated and controversial. An evaluation of the presence of autoantibodies against IgE or FcεRI, as well as the basophil activation test (BAT), can also be performed to confirm serum autoreactivity. However, no correlation between ASST positivity and the presence of autoantibodies has been demonstrated, suggesting that other unidentified factors are responsible for the development of wheals and angioedema.[12] Thus, CSU is not a single disease but a pattern of reactions that reflect cutaneous MC degranulation.

Previously, the authors have demonstrated that *in vitro* evaluation of MC reactivity to CSU serum could be a tool for CSU patient screening.[13] Moreover, they have recently described the co-occurrence of CSU in patients with IgAD. In these patients, CSU was associated with autoreactivity, as shown by a positive response to ASST and MC degranulation assays, as well as the presence of other autoimmune disorders.[14] Herein, the authors describe three patients with CSU who were also affected by CVID, another PID with predominantly antibody deficiency. In all patients, treatment with omalizumab was effective at controlling or improving symptoms such as wheals and pruritus. The ASST was positive in one patient and MC degranulation and BAT assays were also both positive. In the other two patients, flares of urticaria that were related to concomitant fungal infections were observed.

MATERIAL AND METHODS

Patient Selection

All patients were referred to the Tertiary Centre of Azienda Sanitaria Universitaria Integrata di Udine (ASUIUD), Udine, Italy. A diagnosis of CVID was established in all cases in accordance with the European Society for Immunodeficiencies (ESID) criteria.[15] CSU activity was estimated using the Urticaria Activity Score (UAS) according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines.[11] UAS over 7 days (UAS7) was assessed on the basis of symptoms evaluated the week before performing the ASST and again during therapy. The study subjects gave informed consent for participation in this study and publication of the data. At the time of the tests, the patients had stopped taking medication for at least 5 days.

Table 1: Clinical data of Patients 1–3.

Patient number	Sex	Age at diagnosis of CVID (years)	Age at onset of infections (years)	Age at diagnosis of urticaria (years)	IgG levels (mg/dL)	IgA levels (mg/dL)	IgM levels (mg/dL)	Comorbidities	Fungal infectious diseases	ASST	UAS7
1	F	49	47	50	540	65	69	Bronchiectasis Asthma	Pulmonary aspergillosis	Negative	33
2	F	54	54	54	696	92	36	Bronchiectasis CD4 deficit Autoimmune thyroiditis Chronic gastritis NASH Enteropathy GORD	Oesophageal and oral candidiasis	Negative	29
3	F	49	52	47	589	55	74	Chronic gastritis	None	Positive	40

Normal values: IgM: 65–210 mg/dL; IgG: 740–1,440 mg/dL; serum IgA: 140–400 mg/dL. ASST was considered positive when the red wheal had a diameter of at least 1.5 mm greater than the control saline solution.[17]

ASST: autologous serum skin test; CVID: common variable immunodeficiency; F: female; GORD: gastro-oesophageal reflux disease; NASH: nonalcoholic steatohepatitis; UAS7: urticaria activity score over 7 days.

Skin Prick Test

Skin prick tests (SPT) were carried out with commercial extracts as previously described.[16] Commercial extracts were provided by Lofarma Allergeni SpA (Milan, Italy) and Stallergenes SpA (Paris, France). A panel of common food allergens (Lofarma Allergeni SpA) was tested. Histamine phosphate and normal saline were used as positive and negative controls, respectively. The response was read 15 minutes after skin puncture and the results were expressed as the mean of the two largest orthogonal diameters in millimetres. A wheal diameter ≥3 mm with erythema compared with the saline control was defined as a positive reaction.

Autologous Serum Skin Test

ASST was performed by an intradermal injection of 0.05 mL of fresh autologous serum and the wheal and flare reaction was recorded after 30 minutes.[17]

Mast Cell Degranulation and Basophil Activation Test

Evaluation of MC degranulation was performed using the human MC line LAD2 as previously published.[13] Briefly, $2x10^5$ LAD2 cells were incubated with 2 µL of serum in Tyrode's buffer for 30 minutes at 37°C. The reaction was stopped by incubation on ice and the cell suspension was centrifuged. After solubilising the cell pellet with 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA), the enzymatic activity of the released β-hexosaminidase in the supernatants and cells was evaluated by measuring the amount of 4-p-nitrophenol produced. The extent of degranulation was calculated as the percentage of 4-p-nitrophenol absorbance at 405 nm in the supernatants out of the sum of absorbance in the supernatants and cell pellets solubilised in detergent.

Basophil activation was evaluated using the Fast Immune™ CD63/CD123/anti-HLA-DR kit (Becton Dickinson, San Jose, California, USA) following the manufacturer's indications. Briefly, peripheral heparinised blood was incubated with an equal volume of serum from patients or healthy donors at 37°C for 15 minutes. After red blood cell lysis, basophils were identified as CD63+/CD123+ cells. Sera from three sex and age-matched healthy subjects were used in both BAT and MC degranulation assays as controls. Data were compared by an analysis of variance (ANOVA) test using the post hoc analysis for paired multiple comparisons with Fisher's least significant difference test.

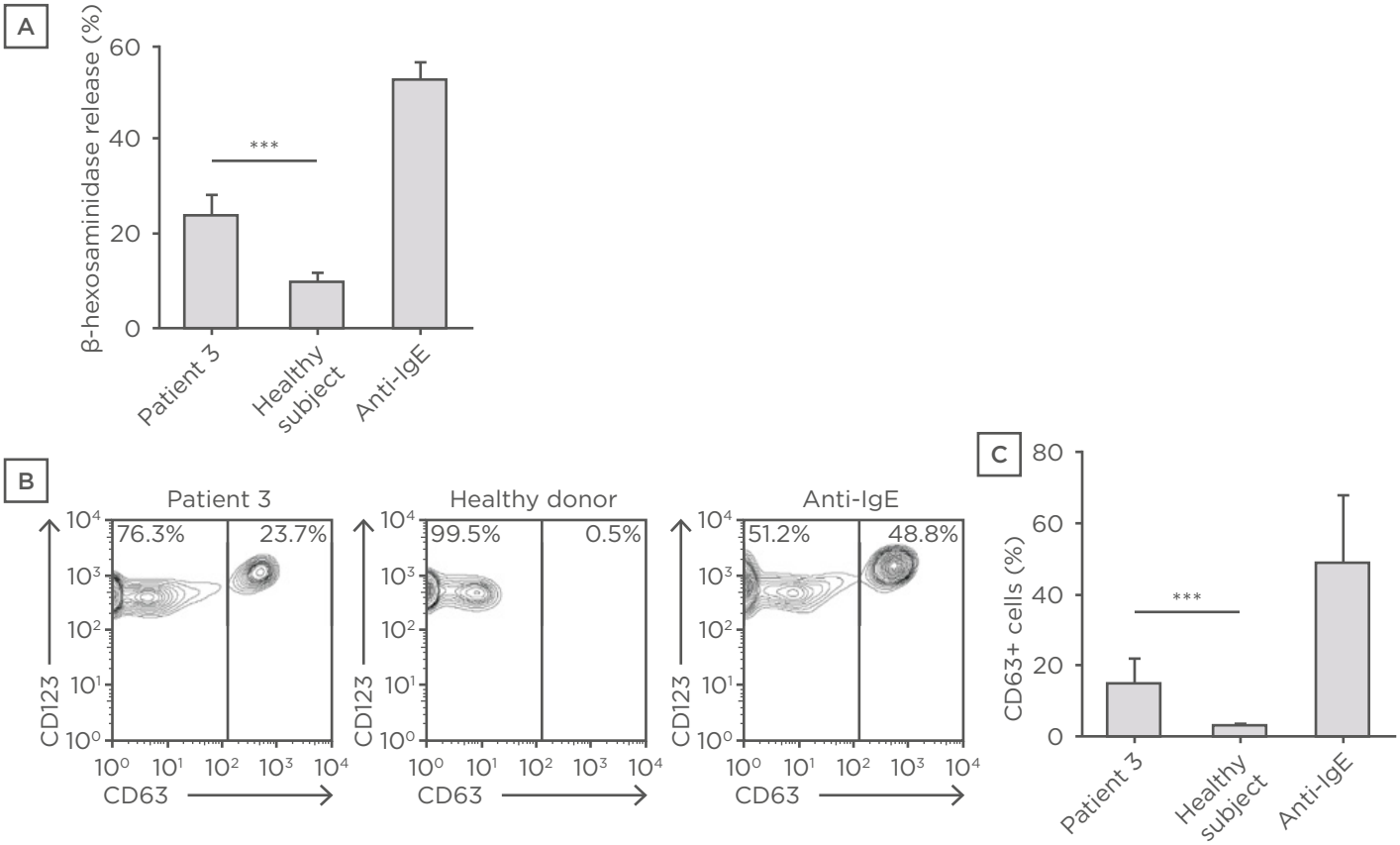

Figure 1: Mast cell degranulation and basophil activation induced by the serum of Patient 3.

A) LAD2 cells were incubated with serum from Patient 3 or a healthy subject. The degranulation response was measured as the amount of released β-hexosaminidase. The data shown are means of three independent experiments. The mean of β-hexosaminidase release obtained with three different healthy sera is shown. B) Peripheral blood was incubated with an equal volume of Patient 3 or healthy donor serum and a fluorescence-activated cell sorting analysis of degranulated basophils identified as CD63+/CD123+ was performed. C) Means ± standard deviations of CD63+/CD123+ cell percentages of two experiments performed in triplicate are shown. The mean of CD63+/CD123+ cell percentages obtained with three healthy sera is shown. Anti-human IgE were used as positive controls in all experiments.

***p<0.001

RESULTS

A computerised search was performed to identify patients with low Ig levels and a diagnosis of urticaria who were referred to the tertiary centre. Eleven patients were identified, five meeting the criteria of CSU and six with a history of acute spontaneous urticaria. Three out of five patients affected by CSU were found to be also affected by CVID.

Patient 1

A 49-year-old female with a history of allergic asthma caused by mites was evaluated for frequent severe exacerbations of asthma in the last 2 years. Asthma exacerbations were associated with airway infections with poor remittance between the acute phases. The diagnostic work-up showed a defect in IgG and IgA levels and bronchiectasis (Table 1). Replacement therapy with intravenous Ig was started with clinical improvement (25 g every 4 weeks). After 1 year, the patient began to complain of CSU that was unresponsive to up-dosed (four-fold) antihistamines (cetirizine). The UAS7 was estimated to be 33 and a SPT for food allergens and ASST were negative. Since asthma was not completely controlled by replacement therapy or high doses of long-acting beta-agonists (formoterol) and inhaled corticosteroids (budesonide), with still frequent

use of oral corticosteroids, omalizumab was initiated at a dose of 300 mg every 4 weeks, which improved asthma control and UAS7 (UAS7: 12, data not shown). Although still in therapy with both omalizumab and cetirizine, after 2 years the patient's symptoms of asthma and urticaria started worsening (UAS7: 38). A SPT for *Aspergillus fumigatus* was positive. Pulmonary high-resolution CT showed augmented bronchiectasis with a mucus plug and a tree-in-bud pattern, and the patient underwent bronchoscopy. The bronchoalveolar lavage fluid confirmed the presence of *Aspergillus*. Itraconazole therapy was added to the treatment regimen, resulting in the improvement of asthma and urticaria symptoms. However, as the therapy was interrupted, both asthma and urticaria then worsened. Therefore, vericonazole was prescribed, with a reduction seen in the extent of urticaria (UAS7: 12) and a better control of asthma. Although the patient is in therapy with both omalizumab and antihistamines, wheals and itching are still present. The UAS7 is now estimated to be 12.

Patient 2

A 47-year-old female was evaluated for the presence of urticaria in the last 3 months (UAS7: 29). Several days before the first evaluation, she underwent endoscopy, which showed the presence of oral and oesophageal candidiasis. Fluconazole therapy was initiated with remittance of the candidiasis. A SPT and ASST were negative, antithyroperoxidase antibodies were present, and low levels of thyroid-stimulating hormone were detected. As shown in Figure 1, IgA and IgG levels were reduced with a concomitant reduction in CD4+ T cells (394 cells/µL). HIV infection was ruled out and the patient was screened for the presence of CVID. Three weeks after the first evaluation, she complained of worsening of urticaria with severe itching and fever. Clinical evaluation, laboratory tests, and chest X-ray were suggestive of the presence of pneumonia, and therapy with amoxicillin/clavulanic acid in association with corticosteroids was prescribed. The diagnostic work-up for CVID showed the presence of bronchiectasis, enteropathy, and autoimmune disorders, such as autoimmune thyroiditis and chronic gastritis with intestinal metaplasia (Table 1). Other disorders not related to CVID were also detected, such as gastro-oesophageal reflux disease and nonalcoholic steatohepatitis. Replacement therapy with intravenous Ig was started (20 g every 4 weeks).

With resolution of the infections, CSU improved but there still was a significant number of wheals and complaints of pruritus not responsive to high doses of non-sedating second-generation anti-H1 antihistamines (cetirizine). CSU responded to steroids only and, therefore, as soon as replacement therapy was started, the patient underwent therapy with cyclosporine (3.0 mg/kg/day). Cyclosporine was effective at controlling wheals and pruritus (UAS7: 0) but frequent relapses of oral candidiasis occurred and required almost daily fluconazole. On the other hand, the daily dose of cyclosporine could not be reduced without flares of urticaria. Therefore, an off-label therapy with omalizumab at a dose of 300 mg every 4 weeks was started. On omalizumab and replacement therapy, the patient complained of flares of urticaria and oral candidiasis only occasionally. Interestingly, urticaria flares were associated with relapses of oral candidiasis and urticaria completely remitted when candidiasis was treated.

Patient 3

A 47-year-old female developed urticaria after a holiday abroad. She was treated with antihistamines (cetirizine) and steroids with initial response; however, even on therapy with high doses of antihistamines, she had >50 wheals and severe pruritus on a daily basis, which interfered with her sleep (UAS7: 40). She was admitted twice to hospital emergency departments for severe flares of urticaria with angioedema. Initial evaluation ruled out infections due to *Salmonella*, *Shigella*, and *Campylobacter*, and the presence of autoimmune thyroiditis. As shown in Table 1, Ig levels were reduced. Hypogammaglobulinaemia was not related to therapy since low levels of Ig existed before the prescription of corticosteroids. The patient was screened for comorbidities and chronic gastritis was diagnosed. SPT for airborne and food allergens were negative. To characterise the autoimmune nature of the urticaria, patient serum was used to perform an ASST and BAT and to measure MC reactivity; the ASST results were positive. To confirm the autoreactivity of patient serum, the ability to induce a degranulation response of

LAD2 cells was analysed; once activated, these cells release β-hexosaminidase.[13] As shown in Figure 1A, while sera from healthy controls did not induce a release of β-hexosaminidase, the serum of the patient induced a significant degranulation of LAD2 cells. To further confirm the autoreactivity of patient serum, it was used to stimulate donor basophils in an *in vitro* heterologous assay (Figure 1B). CD63 expression, detectable on cell membranes only after activation, increased in circulating basophils after incubation with patient serum, while no significant upregulation of CD63 expression was observed using sera from healthy controls (Figure 1C).

Since there was not a clear history of infections, cyclosporine (3.0 mg/kg) was started with slow and progressive improvement. After 1 month, the dose was increased to 4.0 mg/kg and, after 3 months of therapy, the UAS7 was 16. However, there was a slow increase of creatinine blood levels. Within 1 year, CSU was completely controlled (UAS7: 0) and cyclosporine continued to be tapered. Urticaria was completely controlled with a dose of 1.5 mg/kg but, after a few months, the patient decided to stop cyclosporine therapy. After 1 month, wheals and itching started again and could not be controlled by daily 1.5 mg/kg cyclosporine. The daily dose of cyclosporine was progressively increased; however, even at 4.0 mg/kg per day, the UAS7 was 24. Moreover, the patient began to complain of respiratory infections and her creatinine levels rose. At that time, omalizumab became available as a therapeutic option for urticaria and so treatment was shifted from cyclosporine to omalizumab (300 mg every 4 weeks). Within a few days after the first dose of omalizumab, CSU completely disappeared. Although cyclosporine was stopped, the patient still complained of respiratory infections and replacement therapy with subcutaneous Ig (6 g every week) was started. After 6 months of therapy followed by 5 months of therapy with a suspension of 2 months, omalizumab was stopped and urticaria was reported with an estimated UAS7 of 32. Therefore, omalizumab therapy was restarted.

DISCUSSION

CSU is characterised by the development of wheals, angioedema, or both for >6 weeks.[11] It is a disorder in which MC play a pivotal role and is thought to be associated with autoimmune mechanisms. Systematic reviews have shown that CSU is strongly linked to autoimmune thyroiditis and that CSU patients have an increased risk of autoimmune disorders, especially adult females and those with a positive family history and a genetic predisposition for autoimmunity.[18-20] Autoimmunity is a common feature of PID and autoimmune diseases can be detected in approximately 20–30% of CVID patients.[3,4] Many autoimmune disorders are known to be associated with CVID; however, to date, the occurrence of CSU has never been reported in patients with CVID. Here, the authors describe three patients affected by CVID and CSU attending their tertiary centre.

ASST was only positive in one of the patients; as shown in Figure 1, the serum of Patient 3 induced a significant degranulation of the LAD2 MC and a significant increase in CD63 expression on the cell membranes of heterologous basophils. Control sera from healthy donors did not induce either degranulation of LAD2 cells or increased expression of CD63 on basophils. In Patients 2 and 3, omalizumab was effective at controlling urticaria, whereas Patient 1 experienced significant improvements in wheals and pruritus but still reported daily wheals and/or pruritus during omalizumab therapy. Urticaria flares were also observed concomitantly to fungal infections. In Patient 2, flares were strictly related to the reappearance of oral candidiasis, whereas in Patient 1, omalizumab-related improvement was reduced by the onset of aspergillosis; moreover, in Patient 1, urticaria flares were associated with asthma exacerbation. Although an association of urticaria with aspergillosis and fungal infections has been reported,[21,22] fungi were not the cause of the CSU in any of the patients since CSU was present even when the patients were in remission for candidiasis and aspergillosis.

There are general concerns regarding treating PID patients with immunosuppressive therapy, but autoimmune and inflammatory diseases are frequently observed in PID and may require different immunosuppressive strategies.[23] For example, in CVID patients, cytopenia and some lung and gut diseases require treatment with immunosuppressive agents.[3,24] In all CVID

patients enrolled in this study, urticaria was resistant even at high doses of non-sedating second-generation anti-H1 antihistamines and patients used systemic steroids for controlling wheals and pruritus. Patients 2 and 3 were initially treated with low doses of cyclosporine since guidelines at the time considered cyclosporine as the next step of therapy, with a moderate level of evidence for efficacy and a moderate safety profile.[25] In Patient 2, cyclosporine therapy was started when infections were resolved and when replacement therapy was already established. In this patient, the low level of CD4+ cells was the likely cause of candidiasis and this prompted the authors to shift to a different therapy.

The authors have previously described the co-occurrence of CSU with autoimmune disease and IgAD,[12] a PID that shares some clinical features with CVID; these two predominantly antibody deficiencies may occur in members of the same family[26] and progression from IgAD to CVID has been reported in several cases.[27-30] However, pathogenesis of CSU in CVID seems to be more complex; ASST was positive in only one CVID patient with CSU, whereas serum autoreactivity was a common feature of IgAD patients.[14] Moreover, some outbreaks of urticaria were reported in CVID patients that were related to concomitant infections. No IgAD patients with CSU were symptomatic for infection and they were all affected by other autoimmune diseases. The data reported here suggest that CSU pathogenesis in CVID may be more heterogeneous, reflecting the immunological complexity of the underlying disease.

CONCLUSION

Here, the presence of CSU in patients with CVID is described for the first time. A limitation of this study is the small number of enrolled patients because of the rarity of CVID and, more generally, of PID. Therefore, it is speculative to estimate the real co-occurrence of CSU in CVID using a single centre. Although autoimmunity is a feature of CVID, autoreactivity was documented in only one patient. In the other two patients, the authors observed flares of urticaria that were associated with fungal infections. Taken together, these data show that CSU in CVID patients reflects the heterogeneity of immune defects and confirm that CSU is a pattern of MC activation induced by several mechanisms. Further research from different tertiary centres should be used to more effectively estimate the incidence of CSU in CVID patients and to evaluate the possible pathogenetic role of both autoreactivity and underlying infections.

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Drilling Down to the Bone: Evaluating Bone Marrow Lesions in Osteoarthritis

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Abstract

Osteoarthritis (OA) is the most prevalent form of arthritis worldwide and affects the whole joint. Changes in cartilage integrity, subchondral bone, and synovitis are recognised during OA progression. Although advances have been made in our understanding of OA pathophysiology, there are no current treatments that halt the progression of the disease. Treatments are largely based on physical therapies to improve joint function, anti-inflammatory agents to manage pain, and joint replacement surgery for late-stage disease in large weight-bearing joints. There is, therefore, an urgent need to better understand OA pathophysiology, which could help in the development of new treatments. The aim of this article is to review the evidence for structural correlates of pain and reduced joint function in OA; the data available for different joint compartments, including cartilage, bone, and the synovium, and their association with symptoms of OA are summarised and the use of imaging tools in assisting the understanding of OA pathophysiology is discussed. In recent years, more advanced imaging techniques, including MRI, have led to an improved understanding of changes at the bone–cartilage interface in OA, with a recognition that loss of integrity at this junction and development of bone marrow lesions (BML) in the subchondral bone are associated with OA pain in large epidemiological studies. One of the main challenges in OA BML research has been identifying the structural characteristics and patterns of gene and protein expression. Gene analyses of BML have demonstrated that they are highly metabolically active structures, providing evidence of angiogenesis, new bone and cartilage formation, and expression of neurotrophic factors. Findings from genomic and proteomic studies of BML, which are discussed in this review, have contributed to the identification of new molecular targets and an increase in our understanding of OA pathophysiology.

INTRODUCTION

Osteoarthritis (OA) is the most prevalent form of arthritis worldwide and is a leading cause of chronic pain and disability, resulting in a huge medical and socioeconomic burden.[1,2] The condition most commonly affects ageing populations worldwide, compounded by the rise in common risk factors, including obesity, previous injury, and joint overuse.[3] Current

concepts consider OA to be a spectrum of overlapping disorders, often with a multifactorial aetiopathogenesis and common biological, morphologic, and clinical endpoints, resulting in the structural and functional failure of the synovial joint.[4,5] OA can occur in all synovial joints but is most commonly seen in large weight-bearing joints, such as the hand, hip, knee, and spine, which have the greatest impact on disability.[6]

OA was previously considered to be a disease affecting articular cartilage; however, current theories suggest OA has a complex pathogenesis involving all structures of the joint, including the subchondral bone, ligaments, capsule, and synovial membrane.[5,6] Visualisation of changes in the bone and other structures in the OA joint has been aided by the advent of MRI-based assessments.[7] More recent studies have demonstrated that bone marrow lesions (BML) are an integral component of structural damage in OA and are likely to be a cause of pain.[8] Large cohort studies, such as MOST,[9] have supported observations that larger BML are associated with symptomatic OA and that synovitis, in particular detected by contrast-enhanced MRI, also contributes to OA symptoms of pain and impaired function. Other studies have also indicated increasing MRI-identified cartilage loss associated with OA progression; for example, in the large dataset of the OAI study.[10]

Joint degeneration often results in patients presenting with clinical symptoms, such as chronic pain, joint instability, and stiffness, leading these patients to seek care. As the condition worsens, structural changes become more apparent, including radiographic changes, such as joint space narrowing, osteophytosis, and subchondral sclerosis, often visible during disease progression. However, there is evidence to suggest a poor association between radiographic joint changes and symptoms, with pain sensitisation reported by many subjects in a study of knee OA.[11] Recent work has also shown OA structural changes present in asymptomatic groups.[12] There are data to suggest that certain stratified OA subsets can worsen over time, in comparison to other types of OA, for which symptoms can stabilise.[12]

There are currently few therapies that reverse OA progression or arrest its development. Recent studies have suggested that invasive ankle joint distraction may encourage cartilage regrowth and functional improvement.[13] Furthermore, recently, knee joint distraction has shown evidence of tissue repair[14] and knee osteotomy surgery has shown promise of cartilage regrowth and preservation of function.[15] Nonsurgical management options include implementing nonpharmacological treatments to improve joint function and reduce stiffness, e.g., weight loss, low-impact exercise, and orthotics; and pharmacological management to relieve pain, e.g., analgesics, nonsteroidal anti-inflammatory drugs (NSAID), and intra-articular injections. In the late stages of OA, if severe pain persists and patients fail to respond to other recommended treatments, then surgery, e.g., joint replacement, is considered a recourse to improve quality of life, reduce pain, and improve joint function.[16] The high unmet need to develop novel therapeutics for OA has led to the investigation of BML as a therapeutic target for OA, with recent studies showing improvement in knee OA pain, function, and BML size after zoledronate treatment.[17] Inflammatory changes in the OA joint, e.g., synovitis, have also been the focus of treatment in a recent trial of hydroxychloroquine in hand OA;[18] however, hydroxychloroquine was not found to be effective at improving pain or function in this type of OA.

IMAGING AND HISTOLOGICAL CHARACTERISATION OF BONE MARROW LESIONS

In recent years, the advancement of MRI techniques has improved methods of visualising bone and cartilage in OA, particularly at the bone–cartilage interface. Bone marrow signal changes were first described on MRI by Wilson et al.,[19] who used the term 'bone marrow oedema' to describe MRI findings in painful joints. BML are observed frequently in people with OA and are often described as diffuse areas of high-intensity signal on T2-weighted, fat-saturated MRI or in short tau inversion recovery sequences.[20] A large number of studies have delineated MRI BML changes in human subjects, particularly with large-joint OA.[21-28] The results of a study of individuals with severe hip OA undergoing joint replacement

established that the sum of BML measured via MRI corresponded to joint pain severity and the number of microfractures identified histologically.[29] Kornaat et al.[30] followed-up 182 OA subjects after 2 years and established that the total size of BML differed in 66% of the subjects, concluding that BML are involved in a dynamic process and, unlike cartilage loss, are not a static outcome. In addition, several longitudinal investigations have demonstrated that areas of BML are associated with subchondral cysts and could in fact be early precystic lesions.[31] BML have also been strongly associated with cartilage degeneration, predominantly within areas overlying the lesion.[5]

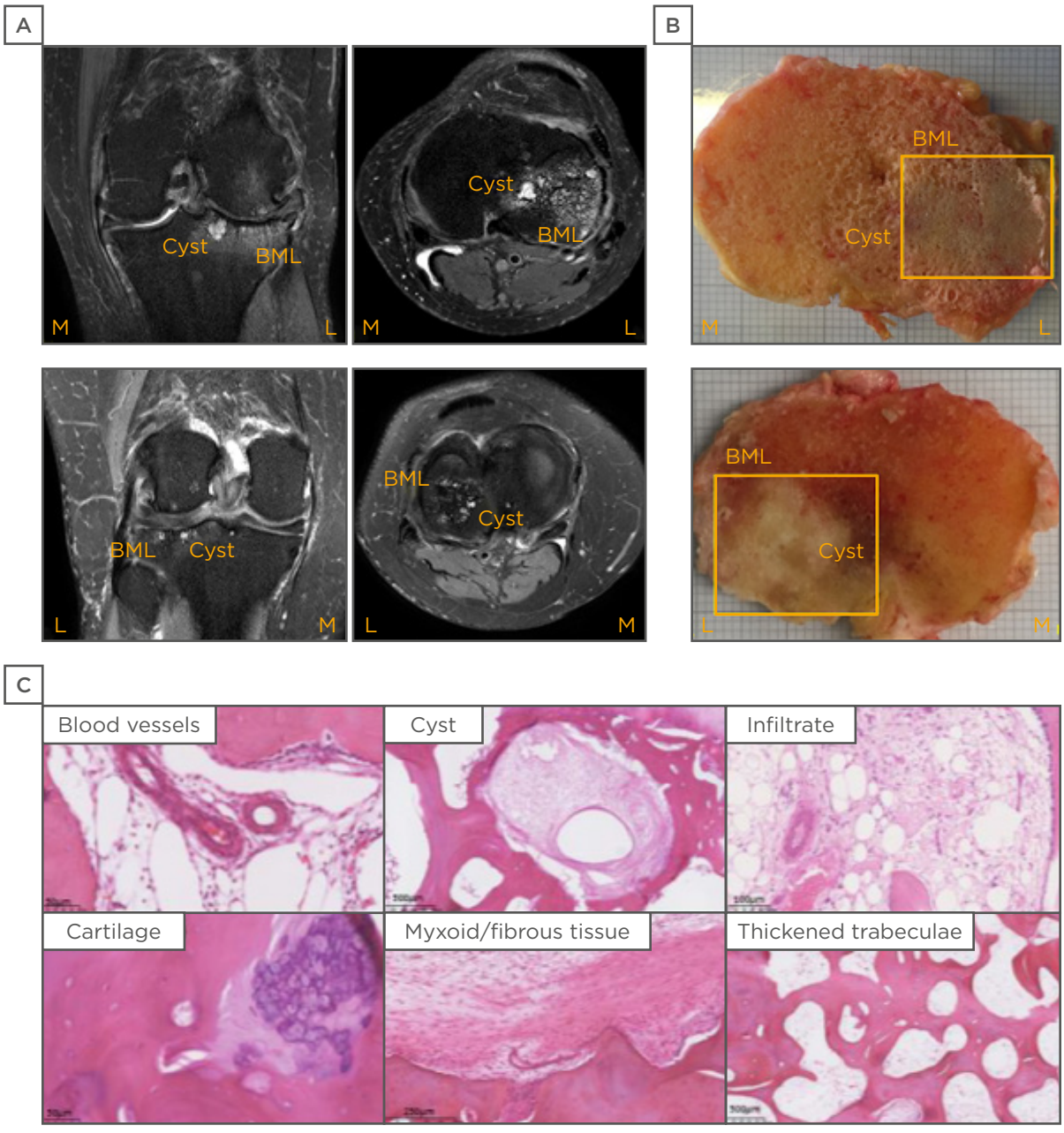

Figure 1: Imaging and histological analysis of a bone marrow lesion and the surrounding region.

A) Coronal and axial plane of a MRI scan visualising BML and an associated cyst. B) Macroscopic appearance of a BML identified by MRI at the time of tissue harvest during joint replacement surgery. C) Histology of a MRI-identified BML demonstrating typical pathological changes, including new blood vessels, cysts, cellular infiltrate, cartilage within the bone compartment, myxoid/fibrous tissue, and trabecular thickening.

BML: bone marrow lesion.

With regard to histological analyses, studies have so far focussed on acquiring data from patients undergoing joint replacement surgery of the knee and hip. Zanetti et al.[32] determined histologically that BML contain normal fatty marrow, with marrow necrosis, necrotic or remodelled trabeculae, oedema, and bone marrow bleeding. The same group matched MRI changes to BML abnormalities in a study of participants undergoing total knee replacement and found regions of normal tissue alongside bone marrow fibrosis, oedema, and bleeding.[32] In a study of hip and knee OA, Hunter et al.[33] reported increased bone volume fraction but decreased tissue mineral density within the BML using light microscopy. Samples from the lesion area showed increased trabecular thickness, with granulation, oedema, necrosis, fibrinoid deposition, and hyperplasia of the blood vessel walls. Taljanovic et al.[29] reported one of the largest histological studies of hip OA BML to date, in which regions of fibrosis and microfracture formation at different stages of healing were observed. Leydet-Quilici et al.[34] also described oedema, necrosis, and fibrosis within BML biopsies. Using MRI, Roemer et al. [35] previously demonstrated that progression of disease and the development of BML correlated with an increased risk of cartilage loss within the same subregion and that regions without BML were associated with a decreased risk of cartilage loss. Carrino et al.[31] also reported that 87% of subchondral cysts were associated with BML abnormalities on MRI.

Several pathologies are seen histologically within BML, including bone remodelling, oedema, fibrosis, osteonecrosis, and trabecular abnormalities.[32,36] Some theories propose that subchondral bone ischaemia may be an initiator for BML by impairing nutrient and oxygen exchange with articular cartilage, reducing cartilage integrity and increasing the risk of OA development.[37-40] Others have proposed that bony micro contusions resulting in necrosis may lead to BML. Kijowski et al.[41] suggested that increased intra-articular pressure leads to the permeation of synovial fluid and cellular infiltrate through defects in the articular cartilage into the subchondral bone, resulting in the proliferation of myxomatous tissue within the bone marrow.

PAIN AND BONE MARROW LESIONS

Recent work has shown that pain in OA arises from several structures within the arthritic joint, including the synovium (from which prostaglandins, leukotrienes, and inflammatory mediators are released), joint effusions, joint capsule involvement, and tendon and muscle weakness, which all contribute to reduced function.[42] Synovitis is often observed by MRI in OA and strongly correlates with pain.[35] Cartilage degradation is one of the hallmarks of OA disease[43] and exposes the structures from which pain is most likely arising, since cartilage is an avascular, aneural structure composed largely of extracellular matrix (ECM) embedded sparsely with chondrocytes. There has been growing evidence of BML correlating with recurrent pain and pain severity in several studies, particularly in large-joint OA.[44-46] A significant correlation was seen between the presence of larger BML in painful knees as opposed to non-painful knees.[30,44-46] A study evaluating BML changes over time in patients with painful knee OA found that, within 3 months, BML scores decreased in 37 patients and increased in 71 patients.[47] Epidemiological studies have shown a strong correlation between BML observed by MRI and OA-related knee pain in several large cohorts,[44,46] with an odds ratio of 3.2 for the association of BML with pain. These data demonstrate the multifactorial nature of OA and how pain mechanisms are supported by the biopsychosocial model of pain.

GENE AND PROTEIN EXPRESSION IN BONE MARROW LESIONS

Although a large body of data now exists describing how BML are implicated in OA disease onset and progression, with descriptions of their histology and histomorphometry, very few studies evaluating the genetic and protein expression of BML have been conducted. This study group recently conducted a transcriptomic study of BML in OA.[48] The novel findings demonstrated that BML have features of angiogenesis, fibrosis, new cartilage formation, and increased bone turnover, with disruption of the physiological osteochondral interface. In addition, a whole transcriptomic analysis of BML regions was used, finding upregulated

expression of genes involved in neurogenesis, pain sensitisation, chemokine and cytokine signalling, as well as cartilage remodelling pathways.[48] A multimodal approach with MRI to locate knee OA BML was also performed, followed by detailed histological analyses and whole transcriptomic techniques for a multivariate interrogation of the changes seen within BML. Detailed MRI matching with histological techniques allowed improved visualisation of BML, with direct observation of BML-associated cystic structures using MRI and transcriptomic expression analyses. Higher Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores with greater MRI Osteoarthritis Knee Score (MOAKS)-measured tricompartmental damage in advanced versus mild OA were found, as shown by previous studies.[46]

Cystic BML areas have been shown to be surrounded by regions of fibrosis, infiltration by inflammatory cells, and vascular proliferation. Previous hypotheses that BML could be precystic but that not all BML become cystic are also supported by the histological findings in this study and from other groups.[31] It has been observed that cystic structures found within the BML areas using MRI were cysts but were also adjacent to areas of fibrocartilage, vascular proliferation, chondrogenesis, and amorphous tissue deposition (Figure 1). The authors also observed new cartilage forming deep within the subchondral bone compartment (Figure 1); this new cartilage tissue within the BML could be arising from mesenchymal stem cells (MSC) in the marrow, which has been seen by other groups.[49] Campbell et al.[50] reported altered numbers and phenotypes of MSC in cultures isolated from hip OA BML, showing that BML-derived MSC undergo osteochondral angiogenesis and have lower proliferation and mineralisation capacities *in vitro*.

Microarray studies have shown that the highest upregulated gene from OA BML tissue was *STMN2*, encoding a phosphoprotein involved in regulating microtubule function, responsiveness to nerve growth factor, neuronal growth, and osteogenesis.[48] Upregulation of *STMN2* within BML may be involved in developing new neuronal structures and expansion of the BML in OA, thereby causing pain.[51] However, STMN2 protein expression was higher in physiological bone than BML bone, which could reflect increased STMN2 turnover in OA BML or loss to the circulation due to increased tissue damage. The authors' microarray study also identified neuronal markers, including THBS4, involved in the inflammatory response to central nervous system injury, presynaptic hypersensitivity, and neuropathic pain states.[52] In animal models of pain sensitisation, THBS4 is increased locally in dorsal root ganglion neurons and contributes to pain behaviour, which can be inhibited by the calcium channel modulator gabapentin.[53]

Genes implicated in neuronal morphogenesis have also been identified in BML, including *ATP6V0D2*, *PSIP1*, *NYAP2*, *FERM*, and *FRMPD4*, encoding proteins implicated in central nervous system development and pain states.[54,55] ECM genes have also been associated with BML, including *MMP13* and collagen genes, *COL16A1*, and those encoding fibronectins and growth factors that are known to be bound within the ECM.[56]

BML are regions of high metabolic activity with increased cell turnover, bone remodelling, and neuronal and inflammatory gene signatures, and gene ontological analyses have revealed the involvement of canonical pathways in chemokine, integrin, and cytokine signalling. There is evidence of neurodevelopment and pain pathway signalling represented by the Alzheimer's, Notch, catenin, and Wnt pathways, alongside vascular endothelial growth factor and angiogenic pathway expression. Studies by Hopwood[57] and Chou et al.[58] analysing the gene expression profiles of OA bone showed expression of bone remodelling signalling pathways, including Wnt, TGF-β, and bone morphogenic protein (BMP), and bone remodelling molecules, such as periostin and leptin. Additionally, Kusumbe et al.[59] described how the growth of blood vessels in bone and osteogenesis are coupled, proposing that Type H endothelial cells mediate local growth of the vasculature and provide specific signals for perivascular osteoprogenitors. The same group reported that endothelial Notch activity promotes angiogenesis and osteogenesis in bone.[60] The authors have also demonstrated *OMD* expression in BML tissue and Ninomiya et al.[61] showed that osteoclast activity induces *OMD* expression in bone, suggesting BML represent areas of active bone remodelling.

The expression of angiogenic and osteogenic genes, along with the tissue changes identified in BML, suggest that vascular proliferation and bone formation are likely to be coupled in BML development. Since blood vessels are formed within neurovascular bundles, it is likely that the increased neuronal pathway gene expression, including *STMN2*, *THBS4*, *PSIP1*, *NYAP2*, and genes encoding catenin, which are among some of the most highly expressed genes in BML analyses, are implicated in neural pathway development, nerve formation, and pain mediation in BML tissue.

Gene arrays of BML have also identified molecules within the Wnt signalling pathway, including catenin, and other studies have demonstrated a critical role of Wnt signalling in the production and persistence of neuropathic pain after nerve injury and bone cancer.[62] Studies using rodent models have suggested that in nerve injury and bone cancer pain models, Wnt signalling is activated, which may contribute to pain by regulating proinflammatory cytokines IL-18 and TNF-α, respectively, as well as NR2B and subsequent Ca^{2+}-dependent signals in the dorsal horn. This analysis also showed a high representation of inflammatory chemokines and cytokine signalling, while other groups have also identified chemokines involved in OA pain; for example, CCR2 was recently reported to mediate pain in a murine model of OA.[63] Microarray data suggest that chemokine pathway molecules could be pain sensitisers in BML.[48] Walsh et al.[64] highlighted that OA neurovascular changes at the osteochondral junction, including vessels and both sensory and sympathetic nerves breaching the tidemark, could be a source of joint pain. The genes identified in BML transcriptomic analyses support the hypothesis of neurovascular gene upregulation in BML tissue.

One of the most highly expressed genes in BML tissue is *MMP13*, traditionally thought to encode an enzyme expressed in cartilage that is involved in regulating ECM turnover and cartilage destruction in OA.[65] Increased bone matrix microdamage and altered vasculature in the subchondral bone of BML have also been demonstrated in a recent histological study of 73 subjects undergoing knee joint replacement surgery.[66] Supporting the importance of matrix turnover in OA, data have shown that Type II collagen degradation products are increased in the urine of subjects with advanced OA who had MRI-identified BML.[48] The *de novo* cartilage formation observed within BML, coupled with the increased transcriptomic expression of *MMP13* observed using microarray and the detection of MMP13 cleavage products, could suggest recapitulation of the embryonic bone development phenotype within OA BML regions.

Although much has been learned over the last few years with respect to gene expression and protein turnover at the osteochondral interface in OA, further validation of the molecules identified and their relative importance in distinct stages of OA pathophysiology is required. Validation of genes and proteins involved in matrix turnover, angiogenesis, and neurogenesis will not only assist in OA disease stratification but will also identify therapeutic targets.[67] It is conceivable that information on pertinent molecular markers in OA, coupled with MRI data, may assist in improved patient stratification that could help in developing more targeted treatments for people with OA.

SUMMARY

Recent work, supported by MRI studies and tissue analyses, has demonstrated that OA is a disease of the whole joint. Symptoms of pain and impaired function are influenced by joint synovitis, joint effusion, tendon and ligament involvement, and the presence of BML, which are most easily detected by MRI. BML in OA represent regions of high metabolic activity, with expression of genes involved in neuronal development, pain, ECM turnover, cartilage and bone formation, and angiogenesis. Recent work focussing on the bone–cartilage interface in OA and the deeper underlying bone has revealed that BML development is an important step in OA pathogenesis. The new findings from gene array and protein studies of BML suggest the possibility of developing new diagnostic tools; for example, identifying angiogenesis and bone turnover markers based on ECM turnover. However, further evaluation is required of the genes associated with specific markers of BML region, size, and severity. It is also possible that the enlargement of BML is associated with specific markers identified from gene array studies, which requires further work to evaluate

BML at different stages of development and formation. It is conceivable that in the future, combined testing of molecular markers identified from the synovia, cartilage, and BML with MRI of the target joint may be used to assess the degree and severity of damage of an OA joint. The findings from BML functional tissue studies also raise the possibility of developing future therapies targeting BML formation and turnover for OA, which is the most common arthritic disease worldwide.

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Asthma: Diagnosis and Treatment

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Abstract

Asthma is an obstructive lung disease affecting >230 million people worldwide and a significant cause of morbidity in patients of all ages. It is a heterogeneous disease with a complex pathophysiology and phenotype. Diagnosis is made with thorough history-taking and physical examination, and the condition is characterised by variable airflow obstruction and airway hyper-responsiveness. Understanding the severity of the disease is important, and treatment is aimed at symptom control and the prevention of future exacerbations. Pharmacologic treatment with beta-agonists for intermittent asthma and inhaled corticosteroids and a combination of inhaled corticosteroids and long-acting beta-2 agonists for persistent asthma are recommended. Additional and alternative treatments with leukotriene modifiers, anticholinergics, biologics, and bronchial thermoplasty are also available. However, understanding an individual's disease phenotype, endotype, and comorbidities is necessary for asthma treatment, with appropriate consultation with asthma specialists required for those with severe asthma.

BACKGROUND

Asthma is a heterogeneous disease that affects many individuals. There are approximately 235 million people worldwide who have asthma,[1] and in 2015 there were approximately 383,000 asthma-related deaths.[1] In the USA alone, the annual cost of asthma is approximately $56 billion, with a significant proportion of this figure comprising indirect costs, such as days lost from work or school.[2] For most patients, asthma can be controlled with appropriate inhaler-based therapy. For many of the more severe asthma patients, significant advances in medical care have reduced exacerbations and improved quality of life. Appropriate diagnosis, recognition of different phenotypes, and an understanding of the various treatment options are paramount in asthma management. This review will focus on the diagnosis and treatment of asthma.

DIAGNOSIS

Asthma is a disease of the lower respiratory tract that affects men and women of all ages. It is diagnosed clinically, but no single gold standard test is available; there is significant heterogeneity to asthma's pathophysiology and clinical presentation, and clinical overdiagnosis can occur, especially in those without spirometric confirmation.[3] Therefore, a thorough history and physical examination along with spirometry are important for the diagnosis of asthma.

Clinical Presentation

The symptoms of asthma can be nonspecific and varied, making the diagnosis difficult. Patients often present with wheezing, shortness of breath, and cough that occur more frequently during the night and early morning.[4] Symptoms are often episodic and can be caused by various triggers, such as irritants, specific allergens, and exercise. Wheezing and nocturnal dyspnoea have a strong correlation with diagnosis of asthma (relative risk: 26% and 29%, respectively), and wheezing is the single most sensitive and prevalent symptom for the diagnosis of asthma.[5,6] Respiratory symptoms that vary over time and in intensity, that are worse at night or in the morning, and that have specific triggers are associated with a higher likelihood of an asthma diagnosis.[7] On the other hand, the presence of chronic sputum production, chest pain, and isolated cough with no other respiratory symptoms decrease the probability of asthma.[7] Detailed history-taking is an important step in the diagnosis of asthma and evidence of variable airflow limitation confirmed by a physician is required to confirm the presence of the disease.[7]

Differential Diagnosis

Asthma can mimic other diseases and, therefore, it is important to consider various differential diagnoses in patients presenting with asthma-like symptoms. Differential diagnoses of asthma include diseases of the upper and lower respiratory tracks, pathologies of the cardiovascular and gastrointestinal systems, and psychiatric conditions (Box 1).[8] For example, congestive heart failure can cause wheezing and airflow obstruction from pulmonary oedema and pulmonary vascular congestion, mimicking asthma.[9] This condition has been termed 'cardiac asthma' and treatment of the underlying heart failure often leads to the improvement of the symptoms.[10]

Vocal cord dysfunction (VCD) is another common differential diagnosis of asthma. These patients often present with recurrent asthma exacerbations that are refractory to corticosteroids or bronchodilator treatment. VCD is caused by episodic extrinsic airway obstruction from paradoxical vocal cord motion and is closely associated with gastro-oesophageal reflux, laryngopharyngeal reflux, anxiety, and depression.[11] Recognition of VCD is important in limiting unnecessary corticosteroid exposure and healthcare utilisation.[11]

Chronic obstructive pulmonary disease (COPD) is a progressive, obstructive lung disease that presents similarly to asthma. Both diseases affect the small airways and have airflow obstruction seen on spirometry; however, COPD patients have limited airway hyper-responsiveness (<12% improvement in forced expiratory flow in 1 second [FEV_1] after bronchodilator inhalation on pulmonary function test [PFT]) and often have a significant smoking history. Asthma and COPD can exist as a spectrum of obstructive diseases and can sometimes be difficult to distinguish from one another, especially in patients with chronic, poorly controlled asthma that leads to fixed airflow obstruction due to chronic inflammation and airway remodelling, as this can make the distinction between the two diseases more difficult.[4,12] Some of these patients can have chronic persistent airflow obstruction with aspects of asthma and meet the diagnosis for asthma–COPD overlap syndrome.[13] Understanding and recognising these two disease processes are important.

Box 1: Differential diagnoses of asthma.

Upper airway
Vocal cord dysfunction Allergic rhinitis and sinusitis Tracheobronchomalacia Tracheal stenosis
Lower airway
Chronic obstructive pulmonary disease Allergic bronchopulmonary aspergillosis Endobronchial obstruction from mass or foreign body Churg–Strauss syndrome Obliterative bronchiolitis
Cardiovascular
Congestive heart failure Pulmonary embolism Pulmonary hypertension
Gastrointestinal
Gastro-oesophageal reflux disease
Psychiatric
Anxiety Panic attacks

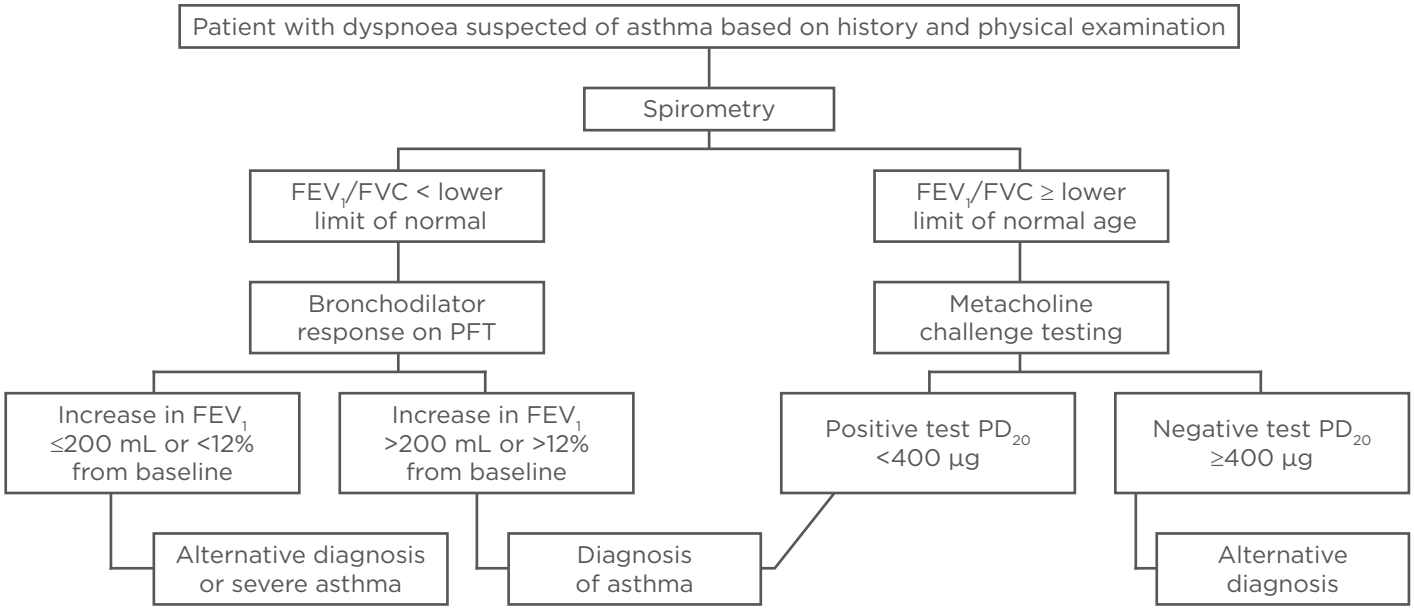

Figure 1: Asthma diagnosis algorithm.

FEV_1: forced expiratory volume in 1 second; FVC: functional vital capacity; PC_{20}: provocative concentration causing a 20% decline in FEV_1; PD_{20}: provocation dose causing a 20% decline in FEV_1; PFT: pulmonary function testing.

Spirometry and Bronchoprovocation Testing

Currently, the Global Initiative for Asthma (GINA) and National Asthma Education Prevention Program (NAEPP) recommend spirometry testing in patients suspected of having asthma.[7,14,15] Asthma is characterised by variable airway obstruction and hyper-responsiveness; airflow obstruction with a FEV_1/forced vital capacity ratio <0.7 or less than the lower limit of normal (LLN) and airflow reversibility after inhalation of a short-acting beta-2 agonist (SABA) defined as FEV_1 improvement by at least 12% and 200 mL indicates a diagnosis of asthma. However, given the variable nature of airflow obstruction asthma patients can present with normal spirometry results.

In such patients with normal spirometry results, bronchoprovocation with methacholine or mannitol can be useful in the asthma diagnosis. A >20% drop in FEV_1 provocation concentration (PC_{20} <16 mg/mL), and now recently a provocation dose (PD_{20}) <400 µg, are currently used and recommended for diagnosis. Methacholine is a direct stimulant of airway smooth muscle by binding to acetylcholine receptors, causing bronchoconstriction and airflow obstruction, and is a sensitive tool for diagnosing asthma. Patients with asthma will have a heightened response to methacholine inhalation, and this test can be used to help diagnose asthma, especially in those with active asthma.[16] Mannitol dry powder is an indirect stimulator of bronchoconstriction, which is a more specific than sensitive diagnostic tool. Several studies have shown that both methacholine and mannitol have similar sensitivities and specificities in diagnosing asthma, especially in patients without active symptoms.[17,18] Bronchoprovocation testing can therefore be useful in ruling out asthma, especially in patients not currently on inhaled corticosteroid treatment (Figure 1).

Fractional Excretion of Nitric Oxide

Nitric oxide (NO) produced by the airway epithelium is an indirect marker of elevated airway inflammation.[19,20] The level of NO in exhaled breath can easily be measured and has been used for detecting airway inflammation in patients suspected of and with the diagnosis of asthma. However, fractional excretion of NO (FeNO) is more sensitive to eosinophilic airway inflammation and is not as

useful in the diagnosis of non-eosinophilic asthma.[19] The American Thoracic Society (ATS) recommends the use of FeNO measurements <25 ppb in adults to indicate a lower likelihood of eosinophilic inflammation and corticosteroid responsiveness.[20]

There have been conflicting data regarding the use of FeNO in monitoring asthma. Studies have shown that an elevated FeNO level correlates closely with severity of asthma and that using FeNO and sputum eosinophil count to monitor asthma can help reduce the total exposure to inhaled corticosteroids (ICS).[21,22] However, a study by Shaw et al.[23] showed that there was no significant reduction in asthma exacerbations or the total amount of ICS use in those monitored using FeNO compared to those not monitored. In addition, a study by Szefler et al.[24] showed increased doses of ICS without improved symptoms in those monitored with FeNO testing than those without FeNO monitoring. Despite these findings, the ATS guidelines continue to recommend the use of FeNO measurements in monitoring of disease activity in asthma patients.[20,25]

Exercise Challenge Testing

For patients with exercise-induced bronchoconstriction (EIB), exercise challenge testing can be used for diagnosis.[26] During this test, patients rapidly increase their exercise intensity on a stationary bicycle or treadmill every 2–4 minutes to achieve a high level of ventilation of at least 17.5–21.0-fold their baseline FEV_1. A fall in FEV_1 ≥10% meets the diagnosis of EIB, with levels ≥25% and <50% indicating moderate EIB and ≥50% indicating severe EIB.[26] Exercise challenge testing should be considered in those with a suspicion of EIB with negative work-up.

SEVERITY OF ASTHMA

Understanding the severity of a disease is important for its management. The NAEPP defines severity as the intrinsic intensity of the disease prior to treatment with long-term control therapy;[15] an understanding of disease severity to initiate therapy and achieve control of the disease is emphasised in NAEPP guidelines. Asthma severity is divided into intermittent, mild, moderate, and severe and persistent. Frequency of daily symptoms, night-time awakenings, use of SABA, interference with normal life activities, and lung function are all components used to determine disease severity in treatment-naïve asthmatics.

GINA defines asthma severity based on the intensity of active treatment to achieve good control of the disease. Patients need to have been on controller medications for several months to establish severity, with the goal being to titrate treatment to the minimum effective level to maintain control.[7] Most patients can achieve good symptom control with long-acting controller medications; however, in patients with persistent symptoms, correct diagnosis, compliance, inhaler technique, comorbid conditions, and ongoing exposure to sensitising agents need to be assessed.

Since asthma is a clinical disease and spirometric measurements do not always reflect a patient's disease severity, symptom control questionnaires have been developed and validated as a quantitative assessment of patient symptoms. These include the Asthma Control Test (ACT), the Asthma Quality of Life Questionnaire (AQLQ), and the Asthma Control Questionnaire (ACQ), and these can be used at each visit to a doctor to better assess a patient's symptoms.[27-29]

In addition, measurements of peak expiratory flow rate (PEFR) are an important objective tool for monitoring a patient's disease process and intensifying the controller regimen. Studies by Ignacio-Garcia and Gonzalez-Santos[30] and Lahdensuo et al.[31] showed that subjects with daily PEFR home monitoring as part of an asthma self-management programme had improved healthcare utilisation, a reduction in additional medication use, and an increase in lung function. However, given the significant variation in PEFR measurements, which can be ≥20% diurnally, GINA currently recommends the use of PEFR monitoring only for patients with severe asthma and those with an impaired perception of significant airflow limitation.[7]

ASTHMA PATHOPHYSIOLOGY AND ITS PHENOTYPES

Asthma is a chronic airway disease of varying pathophysiology, which includes

eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic pathways. The classic pathway of asthma involves the release of thymic stromal lymphopoietin by epithelial cells when an allergen or infectious agent enters the airway. This then activates Th2 cells, which produce various cytokines, including IL-4, IL-5, and IL-13. These cytokines then lead to the IgE formation and eosinophil activation responsible for airway hyper-responsiveness (Figure 2).[25] Activation of mast cells via the attachment of IgE to high-affinity IgE receptors leads to the release of histamine, cysteinyl leukotrienes, and prostaglandins, which are also involved in bronchoconstriction.[32]

The non-eosinophilic pathway of asthma involves activation of airway epithelial cells and macrophages by TLR4 and CD14, which leads to the production of NFκB and IL-8, which further activate neutrophils.[33,34] There are many phenotypes and endotypes of asthma, each with a distinct clinical presentation and pathophysiology. Prior large studies have used clinical presentations such as sex, age of onset, allergic status, lung function, and asthma symptoms to categorise asthma patients into clusters. Many different phenotypes have been described but most can be distinguished by early versus late onset, the presence of atopy and significant allergic symptoms, severity of lung function reduction, and response to treatment.[32,35,36]

The early-onset, allergic phenotypes include those who present with symptoms early in childhood that last into the adulthood. These patients often have elevations in IgE along with associated allergic and atopic symptoms and respond well to treatments that target Th2 response and IgE downregulation. Patients with the late-onset eosinophilic phenotype, on the other hand, present with more severe, persistent symptoms that are less allergic in origin. These patients often do not respond to corticosteroids as well, and their disease process involves predominantly cysteinyl leukotriene pathway upregulation. Eosinophilic phenotype includes patients who exhibit significant sputum eosinophils (>2%) and have good response to corticosteroids. The exercise-induced asthma phenotype involves mast cell and Th2 cytokine activation, often with mild intermittent symptoms that occur during exercise.

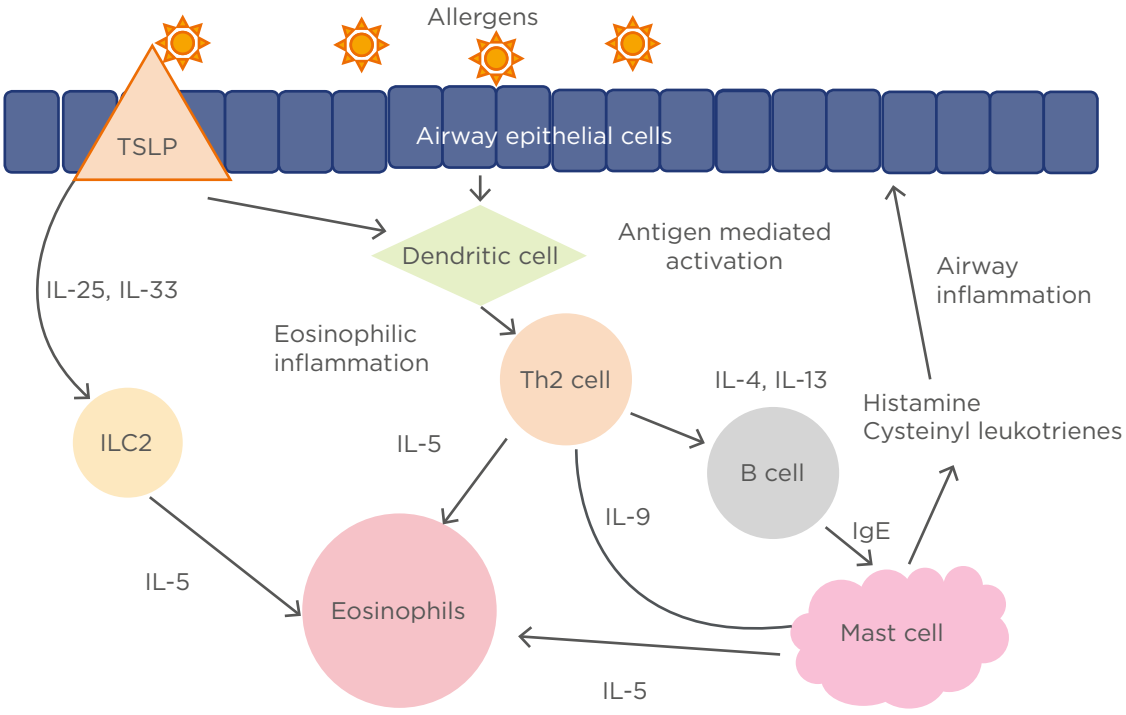

Figure 2: Th2 pathogenesis of asthma.

ILC2: Type 2 innate lymphoid cells; TSLP: thymic stromal lymphopoietin.

Patients with the obesity-related phenotype lack Th2 biomarkers and have a less clear pathway to airway hyper-responsiveness. The neutrophilic phenotype includes patients with persistent asthma who are less responsive to corticosteroids. These patients often have elevated neutrophils with exacerbations and tend to respond better to biologics and alternative treatments, including macrolide therapy. Patients with aspirin sensitivity, exercise-induced asthma, and bronchopulmonary mycosis will need additional treatment targeting each non-allergic cause. Therefore, understanding the different phenotypes and endotypes is important in determining one's treatment course.

As we better understand different asthma phenotypes and the biomarkers that identify them, we can target medical therapy more precisely and develop new agents that target specific pathological pathways of asthma.

TREATMENT

The goal of asthma treatment is symptom control and prevention of future exacerbations.[7,8] It involves an understanding of the heterogeneous pathophysiology and phenotypes of asthma and an individualised treatment plan. Patient education and a written asthma action plan can raise awareness of worsening symptoms, impending exacerbations, and the need for titration of therapy for better symptom control.[7,8,15] Self-management and a shared care approach have also been shown to improve asthma outcomes.[37,38] In addition, education about proper inhaler techniques, medication compliance, and avoidance of allergens and irritants is crucial to all asthma patients.

A stepwise approach to pharmacologic treatment is recommended. The initial choice of medication is determined by the aforementioned asthma severity classification by NAEPP (intermittent, mild, moderate, and severe persistent).[15] A step-up or step-down therapy is recommended depending on symptom control based on GINA guidelines.[7] Currently, it is recommended that all patients with asthma have SABA inhalers for rescue therapy. In those with persistent asthma, addition of low-dose ICS in titrating doses is recommended. For those with moderate-to-severe persistent asthma, long-acting beta-2 agonists (LABA) or leukotriene inhibitors are often added to the ICS regimen. Select use of biologic agents can be considered for those patients with more severe, difficult-to-control forms of asthma.

Beta-2 Agonists

Beta-2 agonists are bronchodilators that play an important role in asthma control and treatment of acute exacerbations. They bind to the beta-2 adrenergic receptors on the bronchial smooth muscle cells, causing smooth muscle relaxation and bronchodilation.[39,40] SABA are often used to treat mild intermittent asthma and acute exacerbations but should not be considered a controller medication; increased use of SABA has been associated with worse asthma control and ICS can sometimes be added to the treatment of those with mild intermittent asthma to limit SABA use.[41] SABA are most effective in treating acute bronchoconstriction and have a rapid onset of action of 1–5 minutes, with peak effects at 2 hours and median duration of action of 3 hours.[42-44] Examples of SABA include albuterol, levalbuterol, terbutaline, metaproterenol, and pirbuterol.

LABA include salmeterol and formoterol and can have bronchodilatory effects lasting >12 hours.[44] However, LABA should only be prescribed in conjunction with ICS in asthma patients. A large randomised control trial (SMART[45]) investigated >26,000 asthma patients and compared LABA (salmeterol) and placebo when added to usual asthma care. The researchers found that there were more respiratory and asthma-related deaths and life-threatening experiences in those treated with LABA than those receiving placebo.

The safety and benefits of the LABA/ICS combination, however, have been shown in multiple studies. Studies by Peters et al.[46] and O'Byrne et al.[47] showed that the use of a LABA/ICS combination was associated with a lower risk of asthma exacerbation and improved lung function compared to ICS alone. Therefore, the use of a LABA–ICS combination inhaler is safe and a potential step-up therapy for asthma patients.

Corticosteroids

Corticosteroids are integral to the management of acute asthma exacerbations and chronic disease control because a significant portion of asthmatic patients have an inflammatory phenotype. ICS are an important part of persistent asthma management, especially for those patients with an eosinophilic phenotype. The drugs decrease airway hyper-responsiveness and inflammatory response to allergens by downregulating eosinophil and mast cell activation.[48] Studies have shown that the use of ICS (budesonide) improved peak flow measurements in asthma patients compared to those on beta-agonist treatment only.[49,50] ICS have also been shown to reduce the rates of exacerbations and improve lung function.[51,52] In patients with moderate-to-severe persistent asthma, the addition of LABA to ICS has been found to be beneficial. Studies by Kavuru et al.[53] and Shapiro et al.[54] showed that a combination of salmeterol and fluticasone resulted in improvements in PEFR, reduced symptom scores, nocturnal symptoms, and albuterol use compared to fluticasone alone. A study by O'Byrne et al.[47] showed that ICS alone reduced the risk of severe exacerbations and poorly controlled symptom days, and that the addition of LABA to ICS further improved overall lung function. Examples of currently available ICS include beclomethasone, triamcinolone, flunisolide, ciclesonide, budesonide, fluticasone, and mometasone.

Systemic corticosteroids are especially important in the treatment of uncontrolled asthma and acute asthma exacerbations. Short-term use of systemic corticosteroids can be an effective tool in decreasing systemic inflammation and bronchial constriction. However, long-term use of systemic corticosteroids is discouraged due to their association with numerous long-term side effects, including weight gain, gastritis, osteoporosis, hypertension, adrenal suppression, and psychosis. There is no standard recommended duration or dosage of corticosteroids for acute asthma exacerbation treatment.[55] Patients who are unable to be weaned from systemic corticosteroids to maintain disease control should be assessed for treatment with biologic medications and for comorbid conditions and referred to an asthma specialist.

Leukotriene Receptor Antagonists and Synthesis Inhibitor

Leukotrienes are lipid mediators involved in bronchoconstriction and airway inflammation. Leukotriene-modifying drugs, including zafirlukast, montelukast, and zileuton, work by inhibiting leukotriene synthesis or as competitive antagonists of the leukotriene receptors.[45] Cysteinyl leukotrienes are released from mast cells and eosinophils and are involved in bronchial smooth muscle contraction and increased mucus secretion.[56] By working as receptor antagonists and inhibiting leukotriene synthesis, these drugs downregulate airway inflammation; they have also been shown to improve asthma symptoms and lung function and serve as an add-on therapy to ICS. Current guidelines recommend the use of leukotriene receptor antagonists only as an alternative treatment to ICS in those with moderate persistent asthma who cannot tolerate ICS and as an add-on therapy to those receiving combined LABA/ICS.

Antimuscarinics

The use of antimuscarinics for alleviating bronchoconstriction and dyspnoea dates back hundreds of years.[57] The parasympathetic system, controlled by acetylcholine and the activation of muscarinic receptors, contributes to airway smooth muscle constriction and mucous secretion.[58] Antimuscarinics are used to disrupt this vagally mediated muscarinic receptor activation, leading to subsequent bronchodilation. Currently available short-acting muscarinic antagonists (SAMA) include ipratropium and long-acting muscarinic antagonists (LAMA) include tiotropium, aclidinium, umeclidinium, and glycopyrronium.

Both SAMA and LAMA can be used to treat severe, poorly controlled asthma exacerbations and as an add-on maintenance therapy to LABA/ICS therapy.[59] Peters et al.[60] studied the effectiveness of the addition of tiotropium to beclomethasone compared to adding salmeterol to beclomethasone or doubling of the beclomethasone dose in 210 asthma patients. The results showed that the addition of tiotropium had greater improvements in PEFR, asthma control days, FEV_1, and daily symptoms

compared to the doubling of ICS or addition of salmeterol.[60] In addition, two replicate trials, PrimoTinA-asthma 1[61] and PrimoTinA-asthma 2,[62] investigated the effectiveness of tiotropium in patients with poorly controlled asthma on high-dose ICS/LABA treatment. This study showed that those who received additional tiotropium had an improved FEV_1 and time to first severe exacerbation, and a 21% reduction in exacerbation risk.[63] LAMA remain a potential treatment for those with poorly controlled asthma.

Biologic Therapy

For those with severe asthma, the use of biologic agents should be considered carefully. Targeted use of biologic therapy allows these patients to achieve control while limiting their oral corticosteroid exposure (Table 1).[64-72] Omalizumab is the first approved biologic for asthma and works by binding to IgE and downregulating activation of airway inflammation. In clinical trials, omalizumab has been shown to reduce overall asthma exacerbation rates by 25% and severe exacerbations by 50%, as well as improving asthma quality of life in those with uncontrolled moderate-to-severe asthma with perennial aeroallergen sensitivity.[64]

Newer biologic agents targeting IL-5 pathways are also available. IL-5 is a major cytokine responsible for the growth, differentiation, and survival of eosinophils, which play a large role in airway inflammation. Mepolizumab is a humanised monoclonal antibody against IL-5, hence it blocks the IL-5 pathway.

Table 1: Biologics for asthma treatment.

Biologic	Mechanism of action	Indication	Dose	Evidence
Omalizumab	Monoclonal antibody against IgE.	Poor control on ICS or LABA, positive perennial aeroallergen testing, total serum IgE level ≥30 IU/mL.	Subcutaneously once every 2–4 weeks based on IgE level and weight.	Reduced all exacerbations by 25% and severe exacerbations by 50%.[64]
Mepolizumab	Monoclonal antibody against IL-5.	Poor control on ICS or LABA, >2 exacerbations per year, eosinophilia >150 cells/µL.	100 mg subcutaneously once every 4 weeks.	>50% reduction in overall exacerbation rate and a >60% reduction in hospitalisation/ emergency department visits.[65,66]
Reslizumab	Monoclonal antibody against IL-5.	Poor control on ICS or LABA, multiple exacerbations, peripheral eosinophilia >400 cells/µL.	Intravenous infusion once every 4 weeks, based on weight.	>50% improvement in quality of life and an FEV_1 improvement by 90–160 mL.[67]
Benralizumab	Monoclonal antibody against Il-5 receptor.	Poor control on ICS or LABA, >2 exacerbations per year, eosinophilia >300 cells/µL.	Subcutaneous 30 mg once every 4 weeks (first three doses) then once every 8 weeks.	>50% reduction in exacerbations and a lung function improvement of 24%.[68,69]
Dupilumab	Monoclonal antibody against Il-4 receptor alpha subunit.	Eosinophilia >300 cells/ µL, FeNO ≥25 ppb.	Not approved in the USA or Europe for asthma (200–300 mg once every 2 weeks, subcutaneously).	Improved severe exacerbation rates by >47% and an improved FEV_1 by 320 mL.[70,71]
Tezepelumab	Monoclonal antibody against thymic stromal lymphopoietin.	Poor control on ICS/ LABA, >2 exacerbations per year.	Phase III testing (70 mg versus 210 mg once every 4 weeks or 280 mg once every 2 weeks).	Exacerbation lowered by >60% and FEV_1 improved by >110 mL in all groups.[72]

FEV_1: forced expiratory volume in 1 second; FeNO: fractional excretion of nitric oxide; ICS: inhaled corticosteroids; LABA: long-acting beta-2 agonists.

Mepolizumab trials have shown a >50% reduction in overall exacerbation rate, >60% reduction in hospitalisation or emergency room visitation rates, improvements in quality of life scores, and a 50% reduction of oral corticosteroid dose for those who are on chronic oral corticosteroids.[65,66]

Reslizumab is another monoclonal antibody against IL-5 that is approved for use in those with poorly controlled asthma and with IgE levels ≥400 cells/uL. Clinical trials have shown an improved exacerbation rate by >50%, increased asthma quality of life, and improved lung function by 90–160 mL over placebo, especially in those with higher levels of peripheral eosinophils.[67] Benralizumab is also a monoclonal antibody against IL-5 receptor that causes the body's own natural killer cells to target and eliminate eosinophils. It has been shown to reduce exacerbations by >50%, reduce the dose of chronic oral corticosteroids use by 75%, and improve lung function by 24%.[68,69]

Other biologics include dupilumab, a monoclonal antibody against IL-4 receptor that blocks IL-4 and IL-13. From Phase III trial data, dupilumab has been shown to reduce exacerbations, improve lung function, and reduce chronic oral corticosteroid use.[70,71] It is particularly more effective in patients with peripheral eosinophil levels >300 cells/µL and FeNO levels ≥25 ppb. Tezepelumab is a monoclonal antibody that blocks the action of the cell signalling protein thymic stromal lymphopoietin and downregulates the inflammatory pathway responsible for asthma. This drug is currently undergoing Phase III studies but has shown a significant decrease in asthma exacerbation rates in a Phase II study.[72] As more biologics become available, phenotyping and endotyping of each patient are necessary to provide insights into the most appropriate long-term therapy.

Bronchial Thermoplasty

Bronchial thermoplasty (BT) offers a non-pharmacologic therapy for those with asthma unresponsive to standard treatment with ICS and bronchodilators. BT uses thermal energy to bronchoscopically ablate airway smooth muscles to decrease bronchoconstriction and airway hyperplasia.[73] The effectiveness of this treatment was initially seen in the AIR trial in 2007, which randomised patients with moderate or severe asthma to BT or a control group. Those who received BT had significant improvements in morning PEFR, percentage of symptom-free days, and symptom score reduction.[74] In addition, the RISA trial randomised 32 poorly controlled asthma patients to BT or a control group and reported that the BT group had increased initial short-term morbidity but significantly improved pre-bronchodilator FEV_1 and asthma symptom scores.[75] These studies were followed by the AIR2 trial, which again demonstrated significant improvements in asthma symptoms and exacerbations in those randomised to BT.[74] BT may therefore be an effective non-pharmacologic treatment for asthma in those with severe disease resistant to pharmacotherapy; however, there are significant adverse reactions associated with BT, including life-threatening severe exacerbations and death.[74,75]

COMORBID CONDITIONS

Treatment of comorbid conditions and avoidance of environmental and allergic triggers are important in asthma management. For example, obesity, gastro-oesophageal reflux disease, anxiety and depression, rhinitis and sinusitis, and seasonal and perennial allergies have all been associated with worsening asthma symptoms.[8,15,76-79] Additional treatments targeting these comorbidities can significantly improve asthma control, especially in those with severe asthma.

CONCLUSION

Asthma is a heterogeneous disease affecting millions of people worldwide. It is characterised by airway hyper-responsiveness and airway inflammation with variable airflow obstruction. Understanding the various phenotypes and pathophysiologies and providing individualised treatment that is suited to the patient's comorbidities and lifestyle is important in the management of asthma.

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Genetic Associations and Environmental Exposures in the Aetiopathogenesis of Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis: An Updated Review

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Abstract

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) represents a heterogeneous group of rare diseases characterised by necrotising inflammation of the small blood vessels and the presence of ANCA with specificity for proteinase-3 or myeloperoxidase. Genetic susceptibility along with malignancy, drug exposure, and environmental exposures to infectious agents and silica are involved in disease progression. To date, growing evidence has revealed that ANCA specificity defines homogeneous groups of patients more effectively than clinical diagnosis, since proteinase-3 ANCA and myeloperoxidase-ANCA are linked with different genetic backgrounds and epidemiologies. This review presents current and updated knowledge on the central aetiopathogenic role of genetic associations and environmental exposures in AAV; discusses the main mechanisms of ANCA immunogenesis; and highlights the value of ANCA specificity for future classification criteria.

INTRODUCTION

The anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are rare, potentially life-threatening, multisystemic autoimmune diseases, identified by the nomenclature of the 2012 revised International Chapel Hill Consensus Conference (CHCC) as a specific group of small vessel vasculitides different from the immune complex-mediated vasculitides.[1] According to their clinical and pathological features, AAV have traditionally been subclassified into three clinicopathologic variants: granulomatosis with polyangiitis (GPA), formerly Wegener's granulomatosis; microscopic polyangiitis (MPA); and eosinophilic granulomatosis with polyangiitis (EGPA), formerly Churg–Strauss syndrome.[1] However, the traditional classification of AAV based on clinical phenotype has recently been challenged on epidemiological and genetic grounds. Accumulated evidence now suggests that ANCA specificity for the two major target antigens,

myeloperoxidase (MPO) and proteinase-3 (PR3), could better define homogeneous groups of patients compared to those identified by clinical diagnosis.[2,3]

As with most autoimmune diseases, the aetiopathogenic process of AAV is likely to be multifactorial, varies among individuals, and involves a complex interplay between innate and acquired characteristics of the immune system, as well as environmental exposures and genetic influences. In this review, the authors summarise the central role of genetic associations and environmental exposures in AAV aetiopathogenesis and immunogenesis of ANCA, and underline how current epidemiological and genetic data could guide an improved classification system based on ANCA specificity.

EPIDEMIOLOGY AND GENETIC ASSOCIATIONS IN ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

The annual incidence of AAV is approximately 13–20 cases per million individuals and the prevalence ranges from 46–184 cases per million individuals worldwide. EGPA is less common than GPA and MPA, with an annual incidence in the range of 0.5–2.0 cases per million individuals and a prevalence of 10–45 cases per million individuals.[4,5] The sex distribution is similar and the peak incidence occurs in the middle of the sixth decade of life.[4] Over the past few years, the increasingly well-documented epidemiology of AAV has highlighted geographical and ethnic differences according to clinical variants and their associations with ANCA subtypes. For example, GPA is more common in the north of Europe, while MPA is more common in southern Europe.[6] The populations studied in western USA, Australia, and New Zealand were predominantly white Caucasians of northern European descent, and the incidence of GPA in these areas has been reported as very similar to Europe.[5] A multiethnic population study conducted in Paris, France, reported that the prevalence of AAV in individuals of European descent (104.7 cases per million) was twice that of non-Europeans (52.5 cases per million). In the same study, GPA was less frequent in people of non-European ancestry than MPA, and none of the GPA patients came from Africa.[7] In a multiethnic series from Chapel Hill in the USA, GPA was also relatively uncommon in African-Americans compared to Caucasians.[8] Two geographical regions with a high incidence of MPA and much fewer GPA cases are Japan and Kuwait.[9,10] A large case series also reported that MPA is more common than GPA in China.[11]

In European studies, the ANCA antigen specificity of GPA is most frequently associated with PR3, while the specificity of MPA is more commonly associated with MPO. However, the overlap between ANCA specificity and the clinical syndrome is only partial. PR3-ANCA cases are present in approximately two-thirds of patients with GPA, but also in a quarter of patients with MPA, whereas MPO-ANCA cases are present in the majority of patients with MPA, but also in up to a quarter of patients with GPA.[12] In China, 60% of GPA patients have MPO-ANCA,[13] while in Japan, MPO-ANCA is present in 83% of cases of AAV, including a significant proportion of those with GPA.[9]

Recent developments in the understanding of genetics could now explain these geographical and ethnic differences and help us to achieve a better understanding of the disease aetiopathogenesis. Over recent years, several studies, based on candidate gene approaches, have been performed in the field of AAV genetics. However, many of these studies were small and not replicated. Knowledge of AAV dramatically increased after the publication of two genome-wide association studies (GWAS) conducted by the European Vasculitis Genetic Consortium (EVGC)[14] and the Vasculitis Clinical Research Consortium (VCRC).[15]

The EVGC GWAS was performed in a cohort of UK AAV patients and matched controls. This analysis reported both major histocompatibility complex and non-major histocompatibility complex associations with AAV. Subsequently, 158 single nucleotide polymorphisms (SNP) were genotyped in a replication cohort of northern European AAV patients and controls to confirm the GWAS data. Combined analysis of the two cohorts showed that the strongest genetic associations were with the antigenic specificity of ANCA (PR3 or MPO) rather than the clinical syndrome (GPA or MPA). *HLA-DP*; *SERPINA1*,

which encodes alpha-1 antitrypsin, a serine protease that represents the major inhibitor of PR3 activity; and *PRTN3*, which encodes PR3, were in association with GPA and stronger association with PR3-AAV.[14] Their specific loci and SNP were given as follows:

- *HLA-DP* (6p21.32, SNP rs3117242)
- *PRTN3* (19p13.3, SNP rs62132295)
- *SERPINA1* (14q32.13, SNP rs7151526)

A weaker association was found between MPO-AAV and *HLA-DQ* (6p21.32, SNP rs5000634).

The VCRC GWAS was conducted in a population of GPA patients and controls of European ancestry. The strongest association was with *HLA-DPB1* (6p21.32, SNP rs9277554) and *HLA-DPA1* (6p21.32, SNP rs9277341), and even more significant among the PR3-ANCA-positive subgroup. An independent SNP (rs26595) near *SEMA6A* on chromosome 5 was also associated with GPA, reaching genome-wide significance,[15] but this was not confirmed in an independent cohort of European patients.[16] The association between *SERPINA1* and GPA was also confirmed in this GWAS.[15]

Interestingly, the *HLA-DRB1**15 allele has been associated with PR3-AAV in African-Americans,[8] whereas *HLA-DRB1**1202 has been associated with PR3-AAV in a population of Han Chinese.[17] Furthermore, it is now possible to speculate that the uncommon appearance of PR3-AAV in China and Japan is due to the low frequency of the *HLA-DPB1**0401 and the PI*Z allele of *SERPINA1* genetic variants.[5]

A recent meta-analysis performed by EVGC using 62 studies, including the 2 GWAS, identified 33 genetic variants in or near 15 genes associated with AAV:[18] CD226 antigen, cytotoxic T lymphocyte-associated antigen-4, Fc fragment of IgG receptor IIa, *HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, hydroxysteroid 17-beta dehydrogenase 8, IFN regulatory factor 5, protein tyrosine phosphatase non-receptor type 22, ring finger protein 1, retinoid X receptor beta, *STAT4*, *SERPINA1*, and toll-like receptor 9. Moreover, genetic distinctions between GPA and MPO and between PR3-AAV and MPO-AAV were identified.[18] These data provide clear evidence that GPA and MPA have distinct genetic associations and support the concept that PR3-AAV and MPO-AAV are separate autoimmune syndromes.

ENVIRONMENTAL ASSOCIATIONS AND IMMUNOGENICITY OF ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES

Infections

The seasonal or temporal oscillations of AAV incidence reported in various epidemiological studies[19,20] could be suggestive of an infectious agent. Recently, Draibe et al.[21] confirmed, in Catalonia, Spain, the seasonal periodicity of AAV, showing it had a higher incidence in the winter.

Particular attention has been given to *Staphylococcus aureus* since chronic nasal carriage of *S. aureus* correlates with relapsing disease in patients with GPA.[22] Conversely, antimicrobial therapy with cotrimoxazole leads to a substantial reduction in disease relapse incidence.[23] These findings are supported by the fact that the immunisation of rats with bacterial proteins derived from *S. aureus* has been shown to cause AAV.[24] Other infectious agents have also been associated with the onset of AAV, especially in patients with subacute bacterial endocarditis. In an analysis of 50 AAV case reports, three species of bacteria (*Streptococci*, *Staphylococci*, and *Enterococci*) were associated with 63% of the reported episodes of infectious endocarditis and 42% of all infections reported.[25] Huan et al.[26] recently demonstrated that ANCA-positivity in patients with *Mycobacterium tuberculosis* infection is related to AAV, so the detection of ANCA in these patients must be excluded because GPA and MPA can present with clinical features that overlap with those of tuberculosis. Among the viruses suspected to play a pathogenic role in AAV, parvovirus B19,[27] Epstein–Barr virus,[28] and Ross River virus have been reported.[29]

The involvement of infectious agents in the pathogenesis of AAV could be explained through a process known as 'neutrophil priming'. Infections induce the production of proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-1, leaving neutrophils and monocytes in a pre-activated ('primed') state by the migration of autoantigen targets (PR3 and MPO) to the

cell surface to be presented to and activated by ANCA.[30]

Infectious agents can also be involved in the immunogenicity of ANCA. The so-called 'autoantigen complementarity theory' argues that the initial immune reaction is developed against a complementary peptide (c-peptide) to the self-antigen (original autoantigen). The c-peptide could be produced from the transcription of an endogenous antisense DNA strand or could be a mimic of an antisense peptide that is produced by a pathogen. Therefore, the initial immune reaction against the c-peptide results in the formation of anti-c-peptide antibodies, which in turn produce anti-idiotypic antibodies that identify not only the idiotope on the anti-c-peptide antibodies, but also the epitope on the sense peptide (original autoantigen).[31] Numerous microbial homologues of the complementary PR3, including antigens from microbes correlated with GPA and PR3-ANCA, such as *S. aureus*, Ross River virus, and *Entamoeba histolytica*, have been described.[32]

An alternative theory based on molecular mimicry between bacterial and self-antigens has been proposed as a mechanism for the immunogenesis of ANCA. Kain et al.[33] reported the presence of ANCA against lysosomal membrane protein-2 (LAMP-2) in approximately 90% of patients with active ANCA associated with necrotising crescentic glomerulonephritis. More importantly, the researchers showed that human LAMP-2 has 100% homology with the bacterial adhesin FimH, which is found in Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumonia*. Accordingly, rats immunised with FimH developed pauci-immune necrotising crescentic glomerulonephritis and antibodies to rat and human LAMP2.[34] However, this finding was not confirmed by Roth et al.[35] in another animal model using Wistar–Kyoto rats. Therefore, the pathogenicity of LAMP2 antibodies remains uncertain. Recently, in another experimental model of molecular mimicry, Kim et al.[36] showed that immunisation of C57BL/6 mice with a recombinant serine protease of *Saccharomonospora viridis*, which has >30% homology with human PR3, produced high levels of ANCA against mouse PR3.

Since stimulation of toll-like receptor 9 by bacterial DNA is known to promote B cell proliferation and antibody production,[37] Hurtado et al.[38] investigated and confirmed the possibility that unmethylated CpG oligodeoxynucleotides, found in bacterial and viral DNA, stimulate circulating autoreactive B cells to produce ANCA in patients with AAV.

Silica

Silica exposure has been associated with several autoimmune disorders,[39] and various case–control studies have documented that 22–60% of patients with AAV, principally those with MPO positivity, were previously exposed to silica.[40,41] Hogan et al.[41] showed that there is an enhanced risk for the disease with long lifetime silica exposure, but there is no evidence of a correlation between the length of time since last exposure and disease onset. It has been suggested that silica exposure could evoke accelerated apoptosis of polymorphonuclear leukocytes and macrophages. The released MPO could then be taken up by alveolar macrophages and presented to immunocompetent cells, resulting in the development of anti-MPO antibodies.[42] Furthermore, *in vitro* studies have showed that silica can activate apoptosis in human peripheral blood lymphocytes, with an increase in Fas-mediated cell death.[43] Fas expression was significantly higher in Treg for silicosis patients compared with healthy donors, and when cultivated with an agonistic anti-Fas antibody, Treg from silicosis patients also showed a higher degree of apoptosis.[44] Consequently, the reduction of Treg function makes these patients more sensitive to a disruption of self-tolerance.

Drugs

Drug-induced AAV has also been described (Box 1). The most common drug implicated with AAV is propylthiouracil (PTU), described in 80–90% cases of AAV cases induced by antithyroid drugs.[45] Evidence for an association with the development of drug-induced AAV has also been shown for several drugs: hydralazine, minocycline, levamisole-adulterated cocaine, TNF-α agents (adalimumab, etanercept, and infliximab), sulfasalazine, D-penicillamine, allopurinol, psychoactive agents (clozapine, thioridazine), cefotaxime, and phenytoin.[46]

However, in most cases, the evidence is restricted only to case reports and the causative link is much less convincing.

PTU is used to treat hyperthyroidism due to its ability to inactivate thyroid peroxidase. Human MPO and thyroid peroxidase nucleotide sequences display an average similarity of 46%, which becomes 58% within the sequence encoding the functional MPO protein, while the similarity of the amino acid sequences is 44%.[49] Thus, PTU treatment could reduce or competitively inactivate the oxidation activity of MPO in a dose-dependent manner, also altering its configuration.[50] Therefore, the altered MPO might serve as a neoantigen. Jiang et al.[51] proposed that drugs such as PTU and hydralazine in the presence of extracellular hydrogen peroxide could be transformed into cytotoxic metabolites by neutrophil-derived MPO. Under such conditions, the drugs and their metabolites are immunogenic for T cells, which in turn activate B cells to produce ANCA.[52]

Hydralazine, a DNA methylation inhibitor with suspected reverse epigenetic silencing properties, has been shown to increase the expression of MPO and PR3 autoantigens in neutrophils.[46] Some drugs, such as sulfasalazine, could activate neutrophil apoptosis, which, in the absence of priming, is related to translocation of ANCA antigens to the cell surface and a subsequent production of ANCA.[53]

ANCA with specificity to human neutrophil elastase are rare and infrequently detected in patients with AAV; however, they might be useful for the diagnosis of cocaine-induced midline destructive disease and AAV associated with other drugs, such as PTU and hydralazine.[46,54]

Malignancy

Malignancy could be a complication in AAV because immunosuppressive therapy decreases the immune system's ability to recognise and eliminate malignant cell clones and may have direct mutagenic properties.[55] However, AAV occasionally develops secondary to malignancy. Paraneoplastic AAV is uncommon and accounts for <5% of all paraneoplastic vasculitis.[56] Tatsis et al.[47] reported 14 patients diagnosed with GPA and simultaneously occurring cancer. In a retrospective review of 200 patients with GPA or MPA, 20 patients had concurrent malignancy (diagnosed within 6 months), and solid tumours were most common, notably colon cancer, breast cancer, and gynaecological cancer.[48] However, other studies have contradicted these results, reporting that the overall risk for malignancy prior to AAV diagnosis was similar to that of the general population.[57,58]

The exact pathophysiology of paraneoplastic-associated vasculitis is thought to be multifactorial.[59] Postulated mechanisms include increased cellular turnover leading to generation of autoantibodies with the formation of immune complexes of tumour-associated antigens/antibodies that cannot be appropriately cleared; release of tumour angiogenic factors and/or cytokines, which induce endothelial damage and increase vascular permeability, inflammation, and fibrosis; and direct effects of tumour cells on vascular walls. The detection of PR3 in a human renal cancer cell line,[60] in association with the demonstration that PR3 can act as a growth factor in non-haematopoietic cells, analogous to its role in haematopoietic cells,[61] and the increased risk for renal cancer in GPA,[47] suggest that a relationship could exist between PR3-positive renal cell cancer and the development of AAV.

Currently, further studies are needed to support the hypothesis that preceding malignancies and AAV have a causal relationship or shared pathogenic pathways. The microbial and non-microbial environmental factors, like silica and drugs, that have been linked to AAV are summarised in Box 1.

CONCLUSION

In this review, the authors document that, on the basis of recent genetic and epidemiological evidence, PR3-AAV and MPO-AAV are distinct autoimmune syndromes and, thus, new classification criteria for AAV are needed, which should include ANCA specificity as a major factor. The ongoing DCVAS study[62] is a multinational observational study designed to develop diagnostic criteria and improve classification criteria for six forms of primary systemic vasculitis, including AAV. Furthermore, like in most autoimmune conditions, considerable progress has been made in our knowledge of the immunogenesis of AAV.

Box 1: Environmental associations with anti-neutrophil cytoplasmic antibody formation and anti-neutrophil cytoplasmic antibody-associated vasculitis.

Silica[40-44]				
Infectious agents[22-29]				
Bacterial species	**Virus species**	**Protozoa**		
Staphylococci	Parvovirus B-19	*Entamoeba histolytica*		
Staphylococci	Epstein–Barr virus			
Enterococci	Ross River virus			
Escherichia coli				
Klebsiella pneumoniae				
Drugs[45,46]				
Antibiotics	**Antithyroid drugs**	**Anti-TNF agents**	**Psychoactive agents**	**Miscellaneous drugs**
Cefotaxime	Benzylthiouracil	Adalimumab	Clozapine	Allopurinol
Minocycline	Carbimazole	Etanercept	Thioridazine	D-penicillamine
	Methimazole	Infliximab		Hydralazine
	Propylthiouracil			Levamisole
				Phenytoin
				Sulfasalazine
Malignancy[47,48]				
Renal cell	Lung	Breast		
Colon	Uterus	Thyroid		
Gastric	Ovarian	Prostate		

The interaction between predisposing genes and triggering environmental factors is relevant also in AAV, leading to the loss of tolerance against self-antigens. These findings can now be tested in future clinical trials using new therapeutic strategies in well-defined homogenous patient populations according to their ANCA specificity.

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Effect of Mesenchymal Stem Cells in Autoimmune Arthritis

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Abstract

The most common autoimmune diseases that affect the joints are osteoarthritis (OA) and rheumatoid arthritis (RA). The pathogeneses of both OA and RA are complex: in both diseases, initiation and progression are dependent on multiple joint structures, including cartilage, bone, and synovium. Mesenchymal stem cell (MSC)-based therapies are the most popular new strategy in tissue repair and regeneration, due to their multipotent differentiation abilities. In addition, MSC have therapeutic potential for bone and joint diseases through the secretion of a variety of immune modulatory substances and cell-to-cell interactions that lead to the antifibrotic, anti-apoptotic, proangiogenic, and immunosuppressive properties of the treatment. Research using MSC in various joint diseases has gained attention and impetus. A significant amount of data has shown the efficacy of MSC treatment in OA and RA, in both animal models and human trials: however, the results are often diverse and clinical benefit varies between trials. The identification of successful therapy requires further research and development, both at the basic biology and translational study levels. In this review, the authors aim to emphasise the role of MSC-based therapies in the development of treatment and to define the mechanisms involved, alongside outlining the knowledge of the therapeutic mechanisms and the applications of MSC in OA and RA.

INTRODUCTION

Joint conditions have now become a major public health problem and cause pain, functional impairment, and physical disability. Joint conditions bring patients physical discomfort and high financial expenditure. In the past, there was no effective solution to joint conditions. Patients may require surgery or face an undiagnosed condition. Until recently, cell-based regenerative therapy appeared to be a promising approach to treat joint diseases; mesenchymal stem cells (MSC) have demonstrated a positive impact on tissue repair and regeneration.[1] MSC have the therapeutic potential to treat bone and joint diseases due to their multipotent differentiation abilities, the secretion of a variety of immune modulatory substances, and cell-to-cell interactions, which lead to its antifibrotic, anti-apoptotic, proangiogenic, and immunosuppressive properties.[2] MSC have been tested in several clinical trials for the treatment of different

joint conditions, such as osteogenesis imperfecta, osteoarthritis (OA), bone erosion, osteonecrosis, and bone fracture.[3] Here, the authors review the current knowledge of the therapeutic MSC mechanisms of action and the applications of these mechanisms in the field of autoimmune arthritis.

CHARACTERISTICS AND FUNCTIONS OF MESENCHYMAL STEM CELLS

MSC were first recognised as mononuclear and plastic cells in a monolayer culture of guinea pig bone marrow by Friedenstein et al. in 1968.[4] MSC are multipotent adult stem cells that are able to differentiate into mesengenic lineages such as cartilage, bone, fat, and muscle. MSC can be isolated from mesodermal tissues, such as bone marrow, synovium, cartilage, fat, and muscle, but also in endoderm (such as the thymus and the liver) and ectoderm (such as skin, dental pulp, and hair follicle) derived tissues.[5-7] Currently, the majority of research looks at the therapeutic effect of bone marrow-derived MSC.

MSC are nonphagocytic and have a fibroblast-like morphology with numbers being limited in human tissues. MSC lack specific markers; therefore, MSC are still characterised on the basis of morphology, plasticity, self-renewal, and immunophenotype, which is a combination of cell markers, including CD29, CD49, CD51, CD73, CD90, CD105, STRO1, and HLA Class I-positive and CD14, CD11b, CD34, CD31, and CD45-negative. The optimal markers for selection are debated frequently: leukaemia inhibitory factor, Wnt ligands, fibroblast growth factors (FGF), other growth factors, and a variety of other cytokines, including IFN-γ, have been involved in maintenance of the MSC phenotype.[8-10] MSC should meet a minimum criteria according to the International Society of Cellular Therapy (ISCT) recommendations, including surface markers CD73, CD90, and CD105-positive (≥95% expression); haematopoietic markers CD34, CD45, CD14 or CD11b, CD79a, or CD19-negative (≤2%); HLA Class II molecules absence; adhesion to plastic; and the potent of differentiation into three kinds of cells (chondrogency, osteogenic, and adipogenic phenotypes).[11]

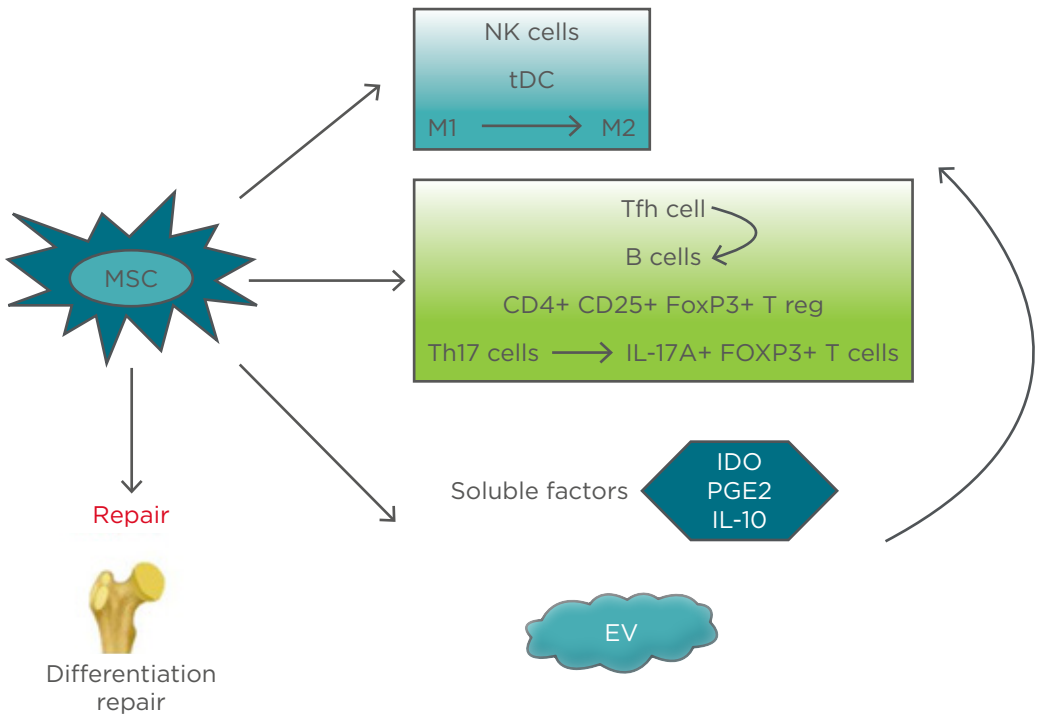

Figure 1: Mechanisms of mesenchymal stem cells therapy for bone repair and immune system.

EV: extracellular vesicles; IDO: indoleamine 2,3-dioxygenase; M1: proinflammatory macrophage; M2: anti-inflammatory macrophage; MSC: mesenchymal stem cells; NK: natural killer; PGE2: prostaglandin E2; tDC: tolerogenic dendritic cells; Tfh: follicular T helper cells; Th: T helper cell; T reg: regulatory T cells.

MSC could improve native cell viability, proliferation, and reduce apoptosis. These effects of the MSC population are achieved through the secretion of paracrine growth factors and cytokines; direct cell–cell interactions (tunnelling nanotubes); and release of extracellular vesicles, which contain peptides, mRNA, and microRNA. MSC are able to produce soluble anti-inflammatory mediators, such as indoleamine 2, 3-dioxygenase (IDO), prostaglandin E2 (PGE2), transforming growth factor (TGF)-β, and IL-6, which mediate MSC immunomodulatory effects.[12,13]

MESENCHYMAL STEM CELLS AND THE IMMUNE SYSTEM

It has been reported that MSC inhibit the innate immune response. The functions of natural killer (NK) cells and neutrophils could be suppressed by MSC.[14] MSC are able to interfere with dendritic cell (DC) maturation and generate tolerogenic (t)DC by inhibiting the toll-like receptor activation.[14,15] The production of cytokines was inhibited by lipopolysaccharide-activated DC co-cultured with MSC, relying on paracrine mediators acting on the NFκB pathway, which was upregulated by toll-like receptor-4 induced DC activation. On the other hand, the differentiation of macrophages toward a more anti-inflammatory phenotype (M2) could be induced by MSC.[16] IFN-γ secretion and the cytotoxic activities of NK cells were suppressed by MSC *in vitro*. In addition, suppression of NK cell function was mediated by MSC contacting with NK cells directly and via the releasing PGE2. Bone marrow (BM)-derived MSC promote IL-10-producing M2 macrophage generation that relay the cell–cell interactions and soluble mediator production, such as IDO and PGE2.[17-19] MSC could also produce survival factors (e.g., IL-6, IFN-β, and granulocyte macrophage colony-stimulating factor), which increase the lifespan of leukocytes.

In addition to actions on the innate immune system, MSC also influence the adaptive immune response. Several studies have shown that the survival of T cells can be promoted by human MSC, which maintain T cells in a resting state by suppressing cell proliferation and proinflammatory cytokines production, such as IFN-γ.[20] On the other hand, numerous studies have shown the ability of MSC to promote regulatory T cells polarisation as an important mechanism by which MSC inhibit autoimmune inflammation. It was also reported that MSC direct the conversion of Th17 cells into regulatory T (T reg) cells (IL-17A+, FOXP3+ T cells), which produce IL-10. *In vitro* co-culture of human MSC with peripheral blood mononuclear cells induces the expansion of the CD4+, CD25+, and FOXP3+ T reg population. The mechanisms by which MSC effect T cells occurs via the production of soluble mediators, such as PGE2, hepatocyte growth factor, TGF-β, IL-10, and IDO; MSC also act via cell–cell contact.[21-23] All of the effects MSC produce provide a favourable environment for tissue repair. Different results were observed following MSC regulation of B cell function and proliferation. Most of the studies reported that MSC inhibited B cells proliferation, differentiation, cytokines production, and antibody production;[24] however, some studies have shown that BM-MSC could promote naïve B cells proliferation, differentiation, and antibody secretion.

MSC secrete and function via extracellular vesicles (EV), including exosomes, microvesicles, and apoptotic bodies, alongside cell–cell signalling, which is a promising approach for immunomodulation therapy and tissue regeneration. The most common vesicles found in extracellular fluids are microvesicles and exosomes. The diameters of microvesicles range from 50 nm to 1 µm, which are shedding vesicles generated from plasma membranes. Exosomes are secreted by most cells as cell-derived vesicles of endocytic origin in culture systems. The mean size of exosomes ranges between 30 and 100 nm in diameter. Apoptotic bodies are released from apoptotic cells and range from 50–5,000 nm in diameter.[25,26] How the effects of these MSC-derived EV are mediated is still not clear, but it represents a novel strategy for future therapy. Microvesicles have been reported to include cytoskeletal components (such as actin, actin-binding proteins, tubulin, and myosin), enzymes (such as alpha-enolase and triosephosphate isomerase), membrane molecules (such as HLA-I and HLA-II antigens), proteins involved in vesicle generation and trafficking, and proteins involved in apoptotic bodies and cell debris.[25] Therefore, EV from MSC express their surface markers, such as CD29, CD73, CD19, and CD105, and cell adhesion

molecules. Inside EV, there are many kinds of molecules, including enzymes, nuclear receptors, cytokines, lipid subsets, miRNAs, and other RNA (e.g., viral RNA and fragments of tRNA). It was reported that EV from MSC could inhibit the proliferation of B cells and NK cells. The effect of EV-mediated immunomodulation relies on the ability of different immune cells to take up these microparticles. The role of EV on T cells has not been clearly demonstrated. The immunosuppressive effects of BM-MSC are enhanced by IFN-γ and TNF-α, as well as an increase in the production of ICAM-1 and EV.[27] EV from murine MSC were reported to inhibit the proliferation of CD8+ T cells and B cells. In addition, EV of MSC increased the T reg cell population. EV released by MSC can induce the transition and proliferation of the anti-inflammatory M2 phenotype from the proinflammatory macrophages.[28,29] The mechanisms of MSC therapy for bone repair and immune system are presented in Figure 1.

MESENCHYMAL STEM CELL THERAPY IN AUTOIMMUNE ARTHRITIS

Early studies have shown that MSC therapy suppressed graft versus host disease.[30] Currently, most trials focus on cardiac syndromes and spinal cord injury for the reparative capabilities of MSC.[31,32] In other respects, researchers studied the efficacy of MSC in autoimmune diseases due to their immunosuppressive effects. Among the autoimmune diseases causing joint conditions, OA and rheumatoid arthritis (RA) are the most common.

Mesenchymal Stem Cell for Treatment of Osteoarthritis

OA is the most common rheumatic disease, with progressive loss of normal joint function. OA is characterised by the degeneration of articular cartilage, leading to joint pain, stiffness, and loss of function. The most frequently affected joints are the knees, joints in the hands, and the spine. In addition, OA involves other joint problems, such as alterations of the meniscus, hyperosteogeny, and intermittent inflammation of synovium. Disability caused by OA is the fastest growing major health condition in the world and is expected to be the fourth greatest disease impacting on worldwide health by 2020.[33]

Current treatments for OA involve ameliorating pain and inflammation; physiotherapy and lifestyle regulations have been shown to slow the progression of disease, but has seen limited success.[34] When conservative forms of therapy are exhausted, patients may need surgery. MSC therapy acts via two main pathways, either by preventing the degradation of cartilage through the secretion of bioactive factors, or by differentiating to become chondrocytes, which may contribute to cartilage repair. The availability of a large number of MSC and their potential of differentiating to become chondrogenic *in vitro* has made MSC the most promising cell source for cartilage repair. It has been reported that MSC loaded on a 3D scaffold undergo chondrogenic differentiation in appropriate culture and could be used for cartilage repair in replacement tissue.[35] In an experimental model of OA, transplantation of a scaffold seeded with BM-MSC significantly improved the regenerated tissue. In addition, MSC as a cell therapy have been used directly for OA cartilage repair in the articular cavity.[36] It has been shown that MSC can migrate and engraft onto multiple musculoskeletal tissues and undergo environmental specific differentiation. It was reported that little loss of proteoglycans and osteophyte formation were observed in the animals treated with MSC. Co-culture of juvenile articular chondrocytes with MSC resulted in competent chondrogenic differentiation.[37-39]

OA is also associated with progressive and sometimes severe synovium inflammation. Therefore, the cell therapy approach to control such an inflammatory environment is needed. MSC have been shown to regulate the immune system, so are suitable for this purpose. MSC have either direct or indirect interactions with immune cells and may secrete soluble factors that can affect their local environment. Co-culture of MSC with chondrocytes significantly downregulate several proinflammatory cytokines production. MSC could inhibit the secretion of IL-1β, IL-6, and IL-8 by chondrocytes and synovial cells, which were isolated from the joints of OA patients, relying on the secretion of PGE2 by MSC. Other research reported similar results.[40-44] The expression of IL-1β, MMP-1, and MMP-13 was decreased by MSC in OA synoviocytes. In addition, a variety of factors were found in the secretome of MSC, such as TGF-β1,

thrombospondin-2, insulin growth factor-1, and stromal-derived factor-1, which could increase chondrogenesis *in vivo* and may have a therapeutic effect for cartilage regeneration.[40-44]

Many of the bioactive factors secreted by MSC are now being identified, which include cystokines and growth factors, as well as extracellular matrix molecules. MSC exosomes induce high levels of IL-10 and TGF-β1, and reduce levels of IL-1β, IL-6, TNF-α, and IL-12p40 in monocytes *in vitro*. It has been observed that MSC exosomes induce T reg production in mice, which indicated that the MSC exosome had an immunomodulatory effect.[45] On the other hand, miR-92a could mediate the effect of MSC exosomes in treating OA by targeting noggin-3 to induce proliferation of chondrocytes as well as matrix synthesis by the PI3K/AKT/mTOR pathway.[46]

Articular injection of MSC inhibits fibrosis and apoptosis of chondrocytes, stimulates the proliferation of chondrocytes, and increases extracellular matrix synthesis. The anti-fibrotic effect of MSC mostly relies on the secretion of hepatocyte growth factor.[47] Additionally, MSC decrease the apoptotic death of chondrocytes, which were induced by camptothecin.[48]

In humans, several trials have started to test the efficacy of MSC therapy for treatment of OA. A case report has demonstrated cartilage and meniscus repair, identified through MRI, as well as improved range of motion and reduced visual analogue scale score after injection of autologous BM-MSC into the knee of a OA patient.[49] In addition, in 18 patients with OA, the safety, function, and pain were measured at 12 months after BM-MSC transplantation. The safety is reliable, and function is improved, which continued at 30-month follow-up.[50] Currently, a Phase I/II trial is evaluating the effect of MSC compared with hyaluronan in patients undergoing meniscectomy to prevent subsequent OA.[51]

Mesenchymal Stem Cell for Treatment of Rheumatoid Arthritis

RA is a systemic autoimmune disorder characterised by aberrant leukocyte infiltration, persistent inflammation of synovium, and proteases within the joint, which ultimately leads to cartilage and bone destruction. Treatment strategies mainly aim to suppress autoimmune synovium inflammation by using disease-modifying antirheumatic drugs (DMARD) and new therapies, such as biological agents. Even in clinical remission, cartilage damage and bone erosion may already exist or continue to progress.

MSC-induced immunomodulation and regeneration of cells made it a potential therapy for the management of RA. Therefore, unlike in the treatment of OA, the use of MSC in RA has primarily focussed on immune modulation.[52] It has been shown that injection of human MSC in collagen-induced arthritis (CIA) mice decreased granulocyte macrophage colony-stimulating factor expressing CD4+ T cells in the blood and spleen, which is important in RA pathophysiology. In addition, MSC could induce a regulatory phenotype from Th17 cells by MSC in CIA mice and reduce the ratio of Th1:Th17 cells. The serum level of TNF-α was significantly decreased by MSC in CIA mice. MSC inhibited follicular Th cell differentiation in CIA mice, and suppressed their capacity of supporting B cells differentiation in a co-culture system. MSC inhibition B cells may be dependent on regulating T cells. The indirect effects of MSC on B cells may rely on suppressing follicular Th cells.[53-55]

MSC inhibit osteoclast-mediated bone resorption, which leads to bone erosion via the induction of T regs, and reduced the production of inflammatory cytokines, which promote osteoclastogenesis. It has been reported that MSC inhibits osteoclastogenesis via production of osteoprotegerin or by interactions with osteoclast precursors, through CD200 and CD200 receptor interactions.[56] A more recent study[57] suggested that injection of MSC to CIA mice prevented bone loss by decreasing osteoclast precursors in bone marrow, although the mechanisms remain unclear.

The EV produced by MSC have been shown to mediate tissue regeneration and immunomodulation as a new way to treat RA. EV secreted by MSC can mirror the effect of MSC. Studies have indicated that MSC-EV can reduce arthritis scores and pathological changes in CIA mice by decreasing plasmablast population and increasing IL-10 secretion of regulatory B cells.[27]

Environmental factors affect the function of MSC. Studies have shown the addition of TNF-α

can reverse the immune regulatory effect of MSC on T cell proliferation. The research suggests that environmental factors, particularly those proinflammatory cytokines, may influence the immunosuppressive capabilities of MSC.[58]

Several studies have obtained conflicting results in clinical trials. Intravenous injection of MSC in addition to DMARD in active RA patients who had deficient responses to DMARD induced a significant improvement of clinical signs compared with the control group.[59,60] In a recent multicentre, randomised, single-blind, clinical trial, intravenous injection of allogeneic MSC in active refractory RA patients showed significant clinical benefit, but these beneficial effects were not present 3 months later, which suggested that MSC treatment in RA may require repeated administration.[61] Some studies, however, reported a worse outcome. Conflicting results may be caused by determinants including MSC source, origin tissue, MSC culture conditions, timing of treatment, injection cell number, and vector of injection.[62] On the other hand, MSC may not be completely immune privileged. Donor-variability may also be a confounding factor. Many studies were unable to show whether these findings are reproducible using cells from different donors.[63] In summary, human trials indicate that MSC are beneficial in RA, but larger, multicentre clinical studies are needed for more evidence.

CONCLUSION

In conclusion, the medical community has made advances in understanding the mechanisms of MSC therapy in joint tissue repair and their anti-inflammatory and immunosuppressive properties. Research on MSC use in various joint diseases has gained attention and impetus. A lot of data have shown efficacy of MSC treatment in OA and RA, both in animal models and human trials although the results are often diverse and clinical benefit varies between trials. Development of a successful therapy needs more effort in research and development, both at the level of basic biology and in translational studies. The authors believe that MSC therapy has great potential in alleviating the disease burden of joint diseases through their ability of tissue repair as well as the property of immune regulation.

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