EMJ EUROPEAN MEDICAL JOURNAL

ALLERGY & IMMUNOLOGY

ISSN 2398-9130 Vol 1.1 • August 2016 • emjreviews.com

INSIDE
Review of
EAACI 2016
Vienna, Austria

CONTENTS

ALLERGY & IMMUNOLOGY

Editorial Board

Allergy & Immunology

ALLERGY & IMMUNOLOGY 1.1 | AUGUST 2016 Published by the European Medical Group

The MedBIC, Anglia Ruskin University, Chelmsford, CM1 1SQ

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

Welcome

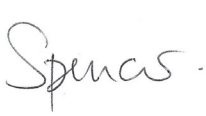
Welcome to *EMJ Allergy & Immunology*, the inaugural edition of a journal that offers comprehensive coverage of the latest and most important developments in allergy and immunology research and care. This includes a selection of peer-review articles exploring the current issues and challenges in the field and an in-depth review of the European Academy of Allergy & Clinical Immunology (EAACI) congress from earlier this year.

Thousands of participants descended upon the EAACI 2016 congress, held in the Austrian city of Vienna earlier this June. Its programme was designed to appeal to a diverse audience with an emphasis on marrying together scientific research and clinical practice. This journal provides a review of just some of the many insightful and informative research presentations at the congress, including a selection of those recognised and awarded by the EAACI as the best presentations overall. The topics of research featured in the abstracts include the impact of climate change on pollen in the air and exploring the role of the allergist in eosinophilic oesophagitis.

There is also a round-up of the most important news and announcements to come from the congress, which put a particular emphasis on the increasing importance of precision medicine to the field. The announcement made by the President of the EAACI stressing this importance will serve to promote a greater awareness of this novel therapeutic approach. Meanwhile, the organisation has also called for co-ordinated effort across the European Union by policy makers to improve the care and prevention of allergic diseases, which has been characterised by the EAACI as a health concern on a pandemic scale.

An array of peer-reviewed articles is also presented here, which engage with important issues in the field while offering invaluable insight into how they are to be addressed. There is a discussion of the need for biomarkers as an alternative approach towards effectively monitoring the complex pathophysiology of eosinophilic oesophagitis. The emerging understanding of the role of respiratory microbiota in asthma has also been discussed here and how it may direct future research and treatment.

We hope you enjoy the first *EMJ Allergy & Immunology* journal and find its content to be informative and useful to your own practice and research in the field. We look forward to creating and sharing many more editions and hope that many readers will be enthused to contribute to future issues.



Spencer Gore

Director, European Medical Journal

European Medical Journal Allergy & Immunology is published annually.
For subscription details please visit www.emjreviews.com

Front cover and contents photograph: Vienna, Austria, home of EAACI 2016.

Introducing Nucala, the first targeted anti-IL-5 add-on therapy for adult patients with severe refractory eosinophilic asthma

Nucala is the first targeted anti-IL-5 add-on therapy for adult patients with severe refractory eosinophilic asthma on high dose ICS and additional maintenance treatment(s)[1]

Licensed for use in the UK.
To find out more visit nucala.co.uk

For illustration purposes only.
Vial not shown to actual size.

1. Nucala SmPC, 2015.
Date of preparation: June 2016 RF/NLA/0070/16

Nucala® (mepolizumab) powder for solution for injection. Each vial contains 100 mg mepolizumab. After reconstitution, each ml of solution contains 100 mg mepolizumab. **Indications:** Indicated as an add-on treatment for severe refractory eosinophilic asthma in adult patients. **Dosage and administration:** Nucala should be prescribed by physicians experienced in the diagnosis and treatment of severe refractory eosinophilic asthma. *Adults:* Recommended dose of mepolizumab is 100 mg administered subcutaneously once every 4 weeks. Treatment is intended long-term and the need for continued therapy should be considered at least on an annual basis. Method of administration is subcutaneous only, administered by a healthcare professional. It may be injected into the upper arm, thigh, or abdomen. The powder should be reconstituted under aseptic conditions and administered within 8 hours, protected from sun light, at <30 degrees Celsius. **Contraindications:** Hypersensitivity to the active substances or to any of the excipients (sucrose, sodium phosphate dibasic heptahydrate and polysorbate 80). **Precautions:** Not to be used to treat acute asthma exacerbations. Asthma related adverse events or exacerbations may occur during treatment. Abrupt discontinuation of corticosteroid after initiation of therapy is not recommended. Hypersensitivity and administration-related reactions may occur within hours of administration, but some instances may have a delayed onset. Pre-existing helminth infections should be treated before commencing Nucala. **Special populations:** No dose adjustment is required for elderly, hepatic impaired and renal impaired patients with a CrCl 50-80ml/min (limited data for patients with CrCl <50ml/min). **Interactions with other medicinal products:** No interaction studies have been performed. The potential for interactions is considered low. **Pregnancy and breast-feeding:** Experience limited. Balance risks against benefits. **Side effects:** Very Common (≥1/10): Headache. Common (≥1/100 to <1/10): Lower respiratory tract infection, urinary tract infection, pharyngitis, hypersensitivity reactions, nasal congestion, abdominal pain upper, eczema, back pain, administration related reactions (rash, flushing, myalgia), local injection site reactions, pyrexia. *Immunogenicity:* In trials 15/260 patients (6%) developed anti-mepolizumab antibodies. Neutralising antibodies were detected in just 1 subject. See SmPC for other side effects. **Legal category:** POM. **Presentation and Basic NHS cost:** Nucala®. 1 vial - £840.00. **Marketing authorisation (MA) no. [EU/1/15/1043/001]; MA holder:** GlaxoSmithKline Trading Services Limited, Currabinny, Carrigaline, County Cork, Ireland. **Last date of revision:** *November 2015.* UK/NLA/0013/15. Nucala® is a registered trademark of the GlaxoSmithKline group of companies.

Adverse events should be reported. Reporting forms and information can be found at http://www.mhra.gov.uk/yellowcard. Adverse events should also be reported to GlaxoSmithKline on 0800 221 441

Nucala®
mepolizumab
TARGETED ANTI-IL-5 THERAPY

Foreword

Prof Dr Jacques Bouchard

Associate Professor of Clinical Medicine, Laval University; Head of the Medicine Department, La Malbaie Hospital, Québec City, Québec, Canada.

Dear Colleagues,

It is with great honour that I have accepted the opportunity to work with the European Medical Journal as the new Editor-in-Chief for the Allergy and Immunology journal. This represents a great challenge and a continuation of everything I have been involved in since the beginning of my career: knowledge transfer to my colleagues and health professionals.

In 2016, the world is changing in every aspect with new scientific evidence emerging every day. The incidence of allergic diseases seems to be increasing in many industrialised countries. Our knowledge is continuously challenged by all the new data reaching us.

We should exchange what we know, whether this comes from evidence-based medicine or clinical experience. This information should be transmitted to peers and translated to satisfy patients' needs; this is the ultimate goal of our journal. Our mission is also to be in a constant mode of bidirectional communication, so that readers have the opportunity to be involved in a discussion.

In this inaugural edition, we have selected some very interesting articles for you to catch up on all the latest research in the field of allergy and immunology. A review included comes from Arvind Bamanikar which covers all aspects of drug allergy, alongside Rose Hamm's review of the physiopathology of allergic drugs reactions. We also have two articles on eosinophilic oesophagitis; one by Neeti Bhardwaj and Gisoo Ghaffari with the basic science and what we should look out for in terms of diagnosis, and the second considering a variety of treatment options by Anna M. Lipowska et al.

As you are no doubt aware, the European Academy of Allergy and Clinical Immunology (EAACI) congress was held in June this year in the beautiful city of Vienna, Austria. It was a great success with a lot of very interesting topics covering all aspects of the practice of allergy. Naturally, the European Medical Journal team were in attendance to be able to bring you the most up-to-date and exciting of research updates from the meeting.

EMJ Allergy and Immunology strives to be the number one place for clinicians and researchers seeking unbiased and up-to-date information on the most important topics in the field of allergy and immunology; it is with great excitement that I will be involved in this goal.

Best wishes,

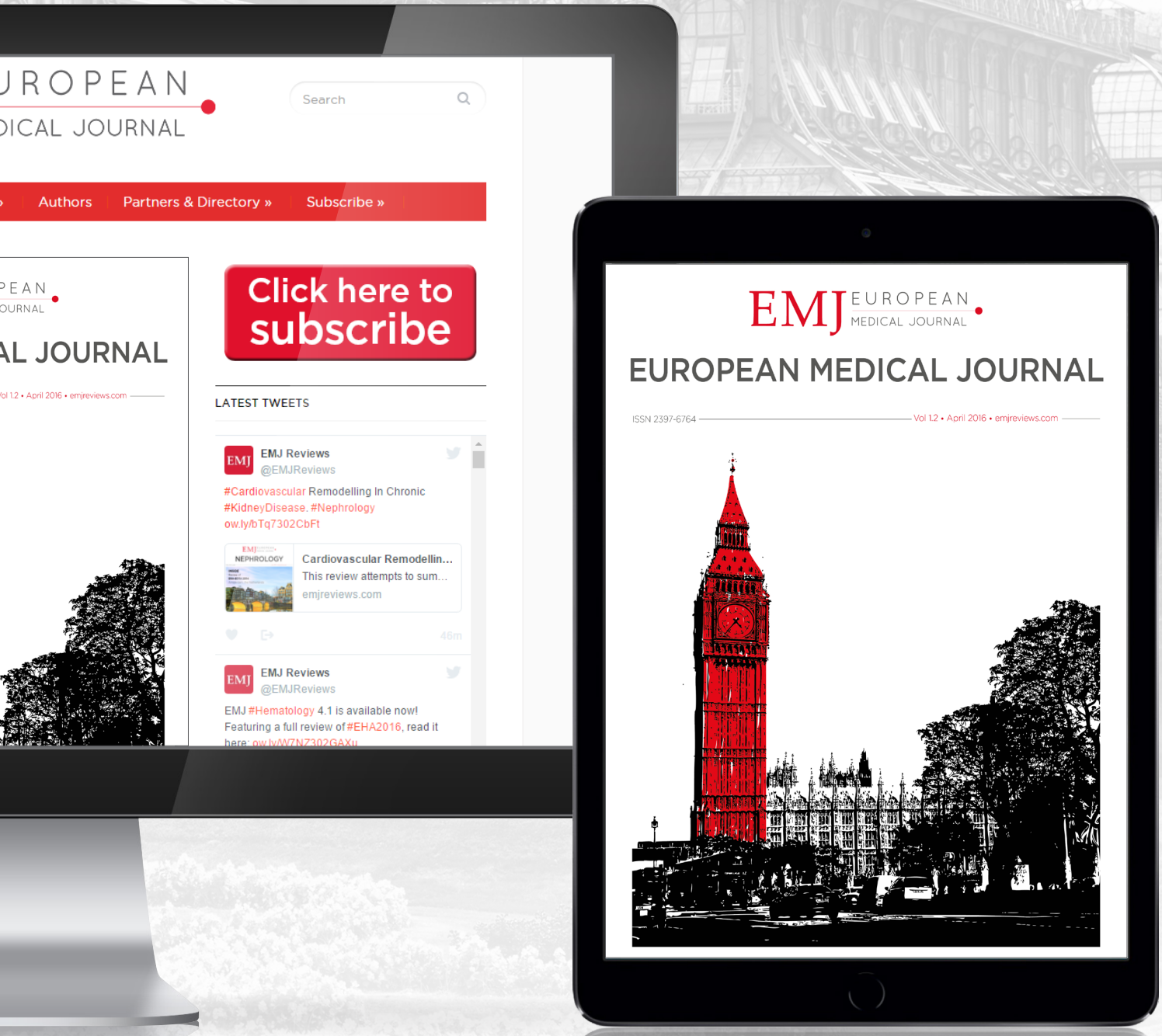
Jacques Bouchard

Associate Professor of Clinical Medicine, Laval University; Head of the Medicine Department, La Malbaie Hospital, Québec City, Québec, Canada.

Have you read the
European Medical Journal?

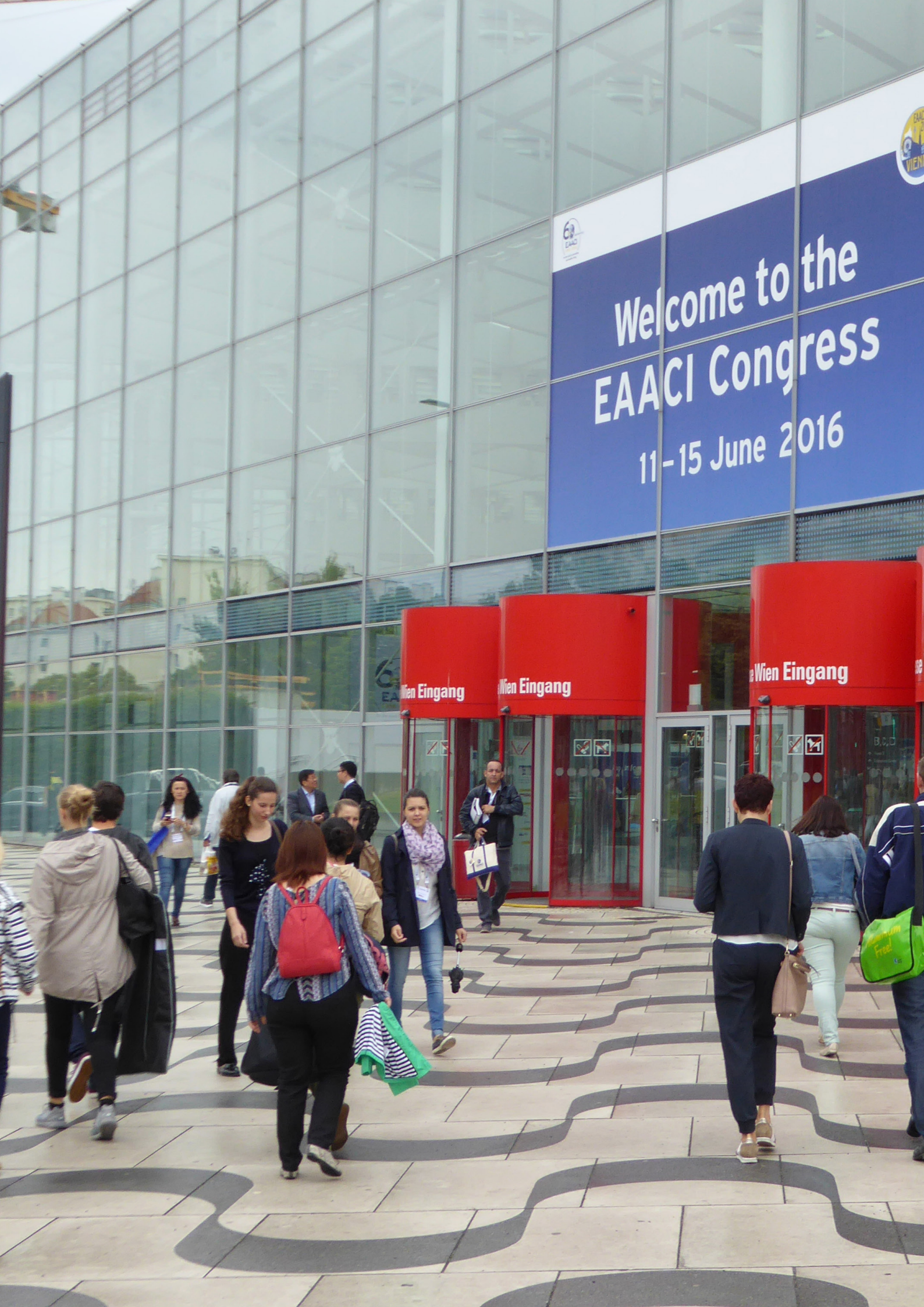
The first edition of the *European Medical Journal* is avai
our therapeutic areas. Topics cover urinary incontine
guidelines for oncology. Articles on the application of g
treatment amongst others are

www.emjreviews.com



lable now on our website, featuring articles from across ence, human sperm cryopreservation, and treatment enomic sequencing, as well as developments in dialysis featured in the second edition.

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13


Welcome to the
EAACI Congress
11 – 15 June 2016
Wien Eingang
Wien Eingang
Wien Eingang

EAACI ANNUAL CONGRESS 2016

IMESSE WIEN EXHIBITION AND CONGRESS CENTER,
VIENNA, AUSTRIA
11TH-15TH JUNE 2016

Welcome to the *European Medical Journal* review of the 60th Annual Meeting of the European Academy of Allergy and Clinical Immunology Congress

The largest and most populated city in Austria, Vienna has for centuries been the musical capital of Europe and this year hosts the annual European Academy of Allergy and Clinical Immunology (EAACI) Congress with the ideal location for its 'Waltzing with Allergens' theme. The melodious theme of this event was given the ideal impetus for orchestrating its attendees in a Congress Ball in a city that remains internationally renowned for its music and culture.

Last year, the EAACI 2015 Congress in the Spanish city of Barcelona attracted close to 8,000 attendees from more than 100 different countries. EAACI 2016 remained equally attractive this year by providing over 1,800 abstracts for discussion and presentation in oral presentations, poster discussions, and guided poster presentations. The congress was also an opportunity for the EAACI to celebrate its 60th year of dedication to improving the health of all people affected by allergic diseases and asthma. An exhibition was featured at the event detailing the history of the EAACI and highlighting the advances made in the field of allergy and immunology over the past decades.

Over 100 authors at the congress were the recipients of awards for abstract presentations deemed the best overall in the oral abstract, poster discussion, and thematic poster sessions. Each received a certificate of recognition and a €200 prize. The EAACI also continued its annual tradition of honouring European clinicians and researchers who have made significant contributions to the field of allergology, bestowed this year upon the following four individuals.

Presented at the EAACI 2016 opening ceremony, the Daniel Bovet Award for improving treatment and prevention of allergic disease went to Prof Erkka Valovirta, Department of Clinical Medicine, University of Turku, Turku, Finland. The EAACI recognises the work of Prof Valovirta in advocating the treatment of allergies in Europe, including his time spent as President of the European Federation of Allergy and Airways Disease Patients' Associations (EFA) from 1997–2003. Prof Valovirta is also known for his work in allergology societies such as the EAACI and the World Allergy Organization (WAO), and for the last 8 years has been dedicated to practical field work as part of the Finnish Allergy Programme.

The 2016 Clemens von Pirquet Award for Clinical Research was awarded to Prof Magnus Wickman, Institute of Environmental Medicine, Karolinska Institutet, Solna, Sweden. Prof Wickman was one of the first scientists to study the natural history of allergic disorders, atopic dermatitis, and asthma in childhood and adolescents in birth cohorts. His work is recognised for uniting population-based medicine and epidemiology with clinical medicine, molecular allergology, and genetics and epigenetics. From 1994–2014 he was the principal investigator of the Swedish population-based cohort (BAMSE). Investigating the influence of environmental and other factors for the development of atopy and asthma, Prof Wickman was a leader of the project which followed 4,000 newborn individuals until early adulthood.

Prof Jan de Monchy, Department of Internal Medicine, University Medical Center Groningen, Groningen, Netherlands, was the recipient of the 2016 Charles Blackley Award for Promotion of the Speciality. Prof de Monchy has long been working in the field, registering as an allergologist in 1980 and has been recognised for his strong advocacy of the speciality throughout his career. In 2007, he became the President of the European Union of Medical Specialists (UEMS) for the allergology board where he remained for 8 years, whilst also serving the EAACI as an adjunct member of the Executive Committee. Prof de Monchy has also been recognised for his involvement in the publication of an important paper, 'Allergology in Europe: The Blueprint'. It provided a description of the rationale for the medical speciality of allergology, underlining the need for quality in allergy healthcare and the importance of allergy centres.

Finally, Prof Cezmi Akdis, Director of the Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland, has been the recipient of several international awards, including the Ferdinand Wortman Prize in 1996, the Swiss Society Immunology Award in 1996 and 1998, and the WAO Award in 2013. Prof Akdis has been recognised for a long and distinguished career in allergy and immunology research. He has published more than 450 papers and the focus of his research incorporates the areas of developing novel vaccines and treatment modalities, the epithelial barrier in asthma and allergies, and disease endotypes.

This year's EAACI 2016 was an informative event that provided updates on the latest developments and emerging trends in the allergy and immunology field. In particular, it highlighted the increasing importance of improving allergy prevention and care, whilst also stressing the pressing need for all of Europe to tackle allergic diseases together. These are issues requiring close attention and substantial effort from everyone involved in the field to tackle. We look forward to checking in next year at EAACI 2017 in Helsinki, Finland, to hear about the progress made.

President of EAACI Draws Attention to Novel Precision Medicine

THE PRESIDENT of the EAACI has highlighted the importance of precision medicine for the management of allergic diseases.

Precision medicine is an innovative therapeutic approach, which takes into account individual variability in genes, environment, and lifestyle. It differs from personalised medicine by focussing on which current approaches will be effective with patients based on particular factors, rather than developing unique treatments for them. It is considered widely relevant to the management of asthma, rhinitis, food allergy, and atopic dermatitis. Its approach consists of treatments based on molecular, immunologic, and functional endotyping of the disease, patient participation in the decision-making process of therapeutic actions, and the consideration of predictive and preventative aspects of the treatment.

"Wide consensus between academia, governmental regulators, and industry for further development and application of precision medicine in management of allergic diseases is of utmost importance," explained Dr Antonella Muraro, President of the EAACI, Zurich, Switzerland. "Improved knowledge of disease pathogenesis together with defining validated and qualified biomarkers are key approaches to precision medicine," she added.

> "Wide consensus between academia, governmental regulators, and industry for further development and application of precision medicine in management of allergic diseases is of utmost importance."

60th anniversary

According to a press release from the 60th EAACI congress dated the 13th of June 2016, bringing precision medicine into clinical practice could help reduce the epidemic of allergies and chronic airways diseases. It mentions progress having already been made in profiling Type II immune response-driven asthma. However, for precision medicine to be effective within healthcare centres substantial medical advances first need to be made. This includes improved disease taxonomy, complete patient monitoring using novel digital technology, and an improved understanding and common usage of disease phenotypes, endotypes, and biomarkers preferentially at the point of care.

Biomarkers Recognised as an Effective Approach for Allergy Diagnosis and Treatment

BIOMARKERS are an important discovery for the implementation of precision medicine approaches aimed towards improving allergy diagnosis and treatment.

This is according to an EAACI press release dated 13th June 2016. Also mentioned was that biomarkers were a central topic of research presented at EAACI 2016, emphasising its importance as a building block towards precision medicine in regards to allergic diseases.

The value of biomarkers in the paediatric diagnosis and treatment of allergies has also been identified in research. Researchers from London and Manchester universities in the UK presented a study at EAACI 2016 which revealed how the identification of specific allergens could assist clinicians in tackling the symptoms of allergic diseases. The research team discovered a group of allergens unique to children with a persistent controlled wheeze. This would allow the children with the allergens present to be identified during their early years of life as susceptible to the symptom of wheezing, which could inform early interventions for treatment.

> ‘miRNAs signature’ that could be used in a similar approach as a biomarker towards identifying asthma pathology.

Additionally, the potential for future therapeutic approaches was identified as the result of profiling microRNAs (miRNAs) on eosinophils, specialised immune system cells. The researchers found that the profile could discriminate between asthma and a healthy status. This was described as a ‘miRNAs signature’ that could be used in a similar approach as a biomarker towards identifying asthma pathology. This could offer new insights into the disease and inform the development of effective treatments in the future.

EAACI Publishes a User's Guide to Molecular Allergology

A WORLD DEBUT has been made in a recent announcement by EAACI regarding the launch of their new user guide in molecular allergology. This is according to an EAACI press release dated the 13th June 2016, which also explains that the guide is the result of contributions from over 50 key opinion leaders. The new developments in molecular allergology have been brought together into the impressive guide allowing clinicians to obtain detailed information on sensitisation patterns. It will also facilitate more accurate interpretation of allergic symptoms.

The new developments in molecular allergology have been brought together into the impressive guide allowing clinicians to obtain detailed information on sensitisation patterns. It will also facilitate more accurate interpretation of allergic symptoms.

The information provided in the book is seen as an example of how molecular allergology is linked to precision medicine and its advancement of the therapeutic benefits of this approach. It is claimed to provide the basis for a more refined and earlier diagnosis of allergic reactions, allowing an individualised management of the patient and approaches to prevention.

More than 7,400 delegates

The book can be found on the EAACI website and its contents are split into three parts, beginning with the general concepts of molecular allergology. It then moves on to its various applications in clinical practice, offering information on various allergies such as soy allergy, peanut allergy, and cockroach allergy. Finally, the book explores the clinical relevance of cross-reactive molecules such as those making up the prolifin protein; which between 10% and 60% of pollen allergic patients are sensitised to according to this. In the preface, the EAACI President, Dr Antonella Muraro, explains the huge significance of the new guide at a time when patients of all ages are suffering from the steady rise in incidence of allergic disease, anaphylaxis, and comorbidities with allergy. She also calls for better education of healthcare professionals to address this important issue, and thus the reasoning behind the EAACI's timely publication of the user guide on molecularallergology describing its components, the clinical benefits of testing for components, as well as how to interpret results including understanding cross-reactions.

Over 1,800 abstracts for discussion

EAACI Calls for National Efforts in Tackling Allergies Across Europe

THE CO-ORDINATED efforts of policy makers across Europe is required in order to tackle the burden of allergies on public health issues according to EAACI.

The EAACI has called upon national and European Union (EU) policy makers to co-ordinate their actions towards improving allergy prevention and care. According to an EAACI press release dated 13th June 2016, allergies are a public health concern on a pandemic scale which require immediate and concerted action. It is believed that by 2025 more than 50% of all Europeans will suffer from an allergy. Therefore, disease prevention is considered vital to controlling the burden to public health that is likely to skyrocket in the coming years. This includes not only the burden on patients' quality of life, but also healthcare costs, economic loss due to missed workdays, and reduced productivity from the impact of the symptoms of allergy.

During the EAACI 2016 congress, meetings were held to progress EU policies and discuss concerted actions to bring about the necessary changes to tackle the issues of allergies in Europe. Among those in attendance at the meetings were EU stakeholders, representatives of EAACI leadership, EU allergy patient advocates, and members of industry.

> Allergies are a public health concern on a pandemic scale which require immediate and concerted action. It is believed that by 2025 more than 50% of all Europeans will suffer from an allergy.

In April 2016, the European Parliament Interest Group on Allergy and Asthma highlighted the health concern of allergies through an event which involved over 400 members of the public and included members of the European Parliament. The 'Test, Inform, Prevent' event was held in the European Parliament in Brussels which performed skin-prick allergy tests. It found that 53% of the participants tested positive for an allergy who were then provided with further information about how allergies can be diagnosed, managed, and prevented. Sixteen MEPs took the test and three of them subsequently joined the interest group.

Exclusive Interviews from the German Cancer Congress 2016

(Click on video clip to view)

Understanding the Unique Aspects of Immunotherapy for Lung Cancer

Martin Reck

Immunotherapy for Head and Neck Cancer

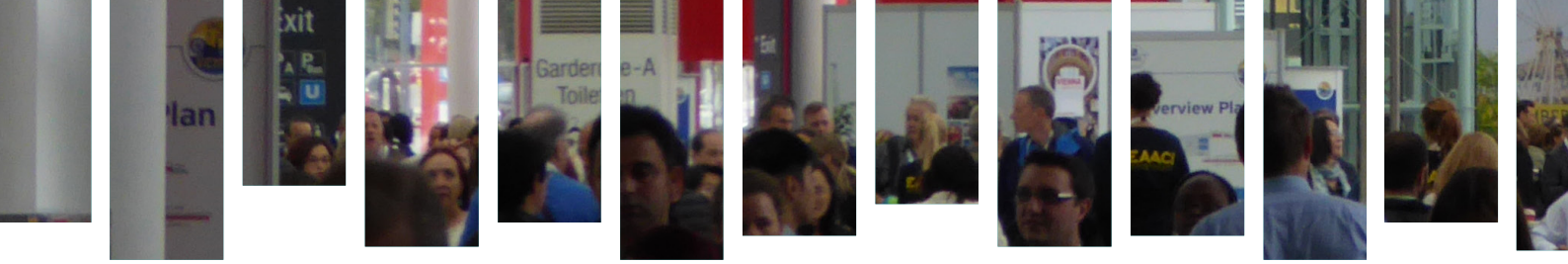
Viktor Grünwald

Predictive Biomarkers for Immunotherapy in Non-Small Cell Lung Cancer

Martin Reck

www.emjreviews.com

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13



Frans Timmermans

Vice President of the Netherlands Anaphylaxis/European Anaphylaxis Taskforce; Chair, European Academy of Allergy and Clinical Immunology's Patient Organisations Committee; Co-Chair, International Food Allergy and Anaphylaxis Alliance.

Q: Amongst your many roles, you are a founder and current vice president of the European Anaphylaxis Taskforce and as such you have a unique insight into the international issues and efforts to increase awareness of anaphylaxis. What changes to the healthcare system in the Netherlands, and the rest of Europe, do you think would be beneficial to these efforts?

A: For some reason the impact of the rising prevalence of allergic diseases and thus also the risk of anaphylaxis in society, from a quality of health and economic perspective, is still underestimated. This might be one of the reasons why, on a national and international health policy level, the priorities are low in comparison with other chronic diseases. It might also be related to the general outlook of allergies as "well it's just sniffing and coughing", and "it's not so severe, you can cope with it". When you realise however, that all these small disturbances from the outside world on your daily life are linked to an economic figure, these inconveniences accumulate into a very large amount of lost income. Just think of lost work days due to inability to work or inability to perform well due to loss of concentration.

I think policy makers must realise that allergies, particularly the ones with a risk of anaphylaxis, are a very serious problem in society which can be more or less easily dealt with. We need to be prioritising allergies and implementing better registration systems to monitor and assess the impact of allergies on and in society; on a societal as well as an individual level. As engineers we believe that to measure is to know, and if this information is available, one can see where to address the problem and how to solve it.

Q: What first interested you about the field of allergy and immunology?

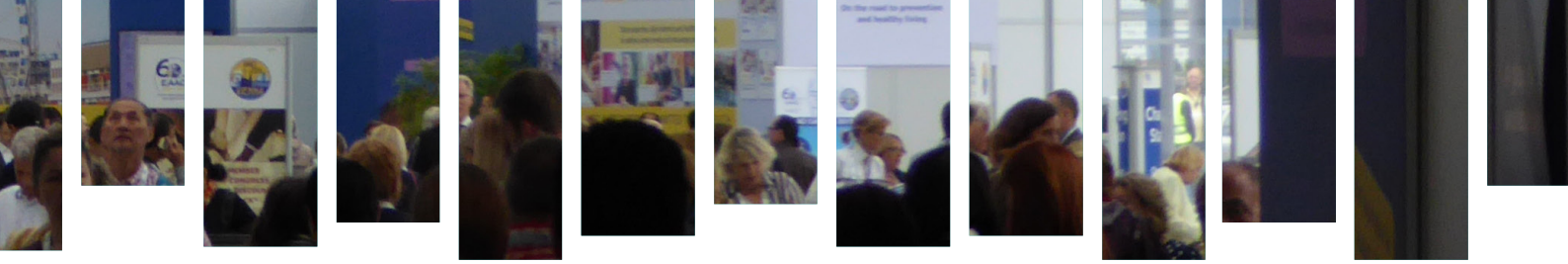
A: I got into the field of allergy and later immunology solely due to the fact that our youngest daughter, Beatrijs, had an anaphylaxis incident after accidently consuming a peanut. We rushed her to the hospital and fortunately she did survive. After this incident we were referred to an allergist nearby and she was diagnosed with several food allergies of which milk, peanut, and tree nuts could give her a severe reaction.

As there was little general public knowledge about this extreme variant of allergic reaction we have been the first to set up an information point which evolved into the Dutch information, knowledge, and training centre, the Nederlands Anafylaxis Netwerk. Due to the extensive international contacts, I also began the European Anaphylaxis Taskforce, as a platform for international and interdisciplinary interaction to raise awareness on the issue.

Q: How important is research into prevention strategies, and how does this research translate to public health?

A: Research is a very important part of raising awareness and advocacy. Without research, one cannot proceed.

However, research has a broad definition and when I look at this area of research, one sees that the area of research is mainly focussed on the medical discipline. Societal studies in the field of allergies are rare and although it is important to find out how allergies are prevented and why some individuals become allergic and others not, it will take a long time to find these answers. Societal studies may give an insight into how



people with allergies cope in their daily lives including how they communicate with their inner and outer circles. More generally, what is the public's perspective of allergies and anaphylaxis, how much do they know, do they realise what impact the disease has? All of this information will help in preparing for public health strategies and measurements to raise the quality of life of people with allergies and those with a risk of anaphylaxis.

Q: How do meetings such as the European Academy of Allergy and Clinical Immunology (EAACI) congress facilitate scientific discourse? How have such meetings changed since you first began attending them?

A: These meetings enhance the scientific discourse in a great way as the congresses (the annual congress is not the only one EAACI organises) have several allergy-related platforms where the scientific results are presented and discussed to an ever increasing audience. The congresses also facilitate young clinicians and researchers to present their own ideas and findings with poster sessions and oral abstract presentations. Also, due to the openness of EAACI, they are unique in having set up a patient organisations committee platform thus facilitating the collaboration, discourse, and discussion between representatives of patients, clinicians, and researchers.

Q: What exciting research presented at last year's EAACI has had the greatest impact on current work?

A: Actually there were several exciting research presentations last year. I will mention two that have impacted our current work most. Firstly, the ongoing effort in structuring and harmonising allergy guidelines across Europe which I hope from there can develop across the world for food allergy and anaphylaxis. Secondly, the results of the introduction of peanuts in the early life of children with a high-risk of developing a peanut allergy.

> "Research is a very important part of raising awareness and advocacy. Without research, one cannot proceed."

Q: What risk factors are dominating current research into food allergies?

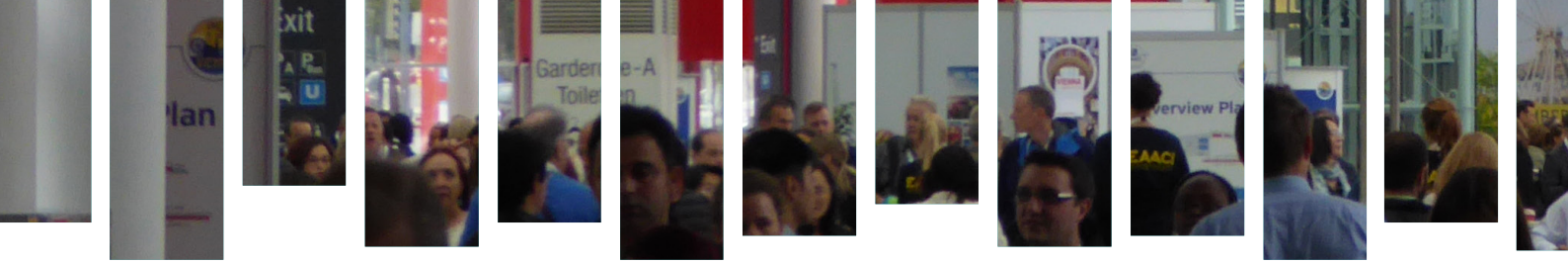
A: A major risk factor is the continuation of available funding at a national and international level. Although the European Union (EU) has provided profound financial support into researching the different aspects of food allergy, another risk factor is that research into anaphylaxis occurring can, ethically, only go on until a certain point. Thus, results in this field will always be on the safe side because the trials have to stop prematurely to reduce the risk of severe adverse events, meaning the results are limited because the study has to stop.

Q: How strong is our current understanding of the basic immunological mechanisms underscoring allergy?

A: Well there are very different levels of understanding of the basic immunological mechanisms. Within the EAACI target group I think the basics are fairly understood. If you look at the general public, I have to say that even a basic understanding of what the immune system is, is very low, and that is if they even know. Let me give you an example: during my training sessions where the symptoms were being shown and explained, the term 'organ system' was introduced for the purpose of explaining anaphylaxis. When the audience was asked if they knew the meaning of the term and how many organ systems a human had, most of the time the vast majority did not know the answer. So we still have a lot of work to do in teaching the general public, even the most basic, immunological mechanisms underscoring allergy.

Q: What are the most important strategies for the management and prevention of allergic reactions and anaphylaxis in your view?

A: First, one needs to have a proper diagnosis and guidance by clinicians. It is important that clinical colleagues from other medical specialities are educated in allergy and anaphylaxis, thus preventing adverse advice or wrong diagnosis. We need more allergy specialists. When an individual has had the proper diagnosis it is important he or



she knows what they react to and if the risk is constructed of a severe allergic reaction or anaphylaxis. Once that is explained they know what to avoid, and more importantly how to avoid! Only avoidance will prevent allergic reactions and anaphylaxis occurring!

Q: What are the fundamental motivations behind your work?

A: As said previously, the first and strongest motivator is my daughter Beatrijs as she is at risk of peanut and tree nut induced anaphylaxis. So from this perspective, making the world a safer place for her is my fundamental motivation.

Q: If you could offer one piece of advice to a budding immunologist, what would it be?

A: Talk to patients, their representatives, and collaborate with the EAACI Patient Organisations Committee and spread the word of your interesting, inspiring, and explorative work. There are so many new things to discover in the field of immunology that you will feel like an ancient explorer discovering new worlds.

Ingebjørg Skrindo

ENT Specialist, Department of Otorhinolaryngology, Akershus University Hospital, Lørenskog, Norway.

Q: Tell us about your role as Chairman of the Norwegian Society of Allergology and Immunopathology. In what ways does this body help improve the quality of patient care in this area of medicine in Norway?

A: As the Chairman of the Norwegian Society of Allergology and Immunopathology in Norway, my main focus is to ensure that doctors of different specialities with interests in allergology have a common meeting platform for discussion and education. We think that if we work together, our patients will benefit from a more complete treatment.

Q: To what extent has our understanding of ear, nose, and throat (ENT) conditions developed since you began working in this area?

A: This question is a little bit difficult to answer. Of course, both the understanding and treatment of cancer is changing rapidly, also in ENT! In allergy, I think the main shift has been from 'avoidance of allergens' to 'tolerance of allergens', especially regarding the increase in immunotherapy.

> "...the understanding and treatment of cancer is changing rapidly..."

Q: In your view, what more could governments and healthcare providers do to ensure greater awareness of the causes of ENT conditions amongst the general population in Europe?

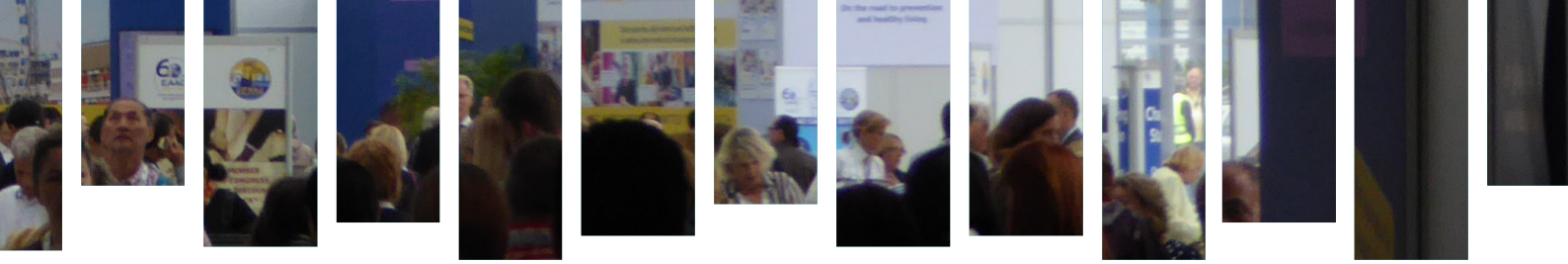
A: I think governments and healthcare providers are doing a great job, and need to keep up the good work when it comes to reduction of smoking and alcohol consumption, etc. Additionally, I think knowledge of allergy and asthma, including prevention and treatment, will benefit from a high focus in the next few years.

Q: How would you describe the quality of medical treatment in Norway? What lessons could other countries learn from the Norwegian experience?

A: We have a very good healthcare system in Norway for everybody, independent of where you live, your income, or insurance. I think this idea of equality is something I really appreciate with our healthcare system.

Q: Which of your achievements are you most proud of?

A: I am not sure. Maybe just being a good clinical ENT doctor!



Q: What are likely to be the biggest challenges facing medical practitioners in the field of allergy and immunology over the next decade?

A: Predicting the future is never easy! However, I do think we will still see an increase in the prevalence of allergic diseases and maybe also in the variety of allergies, leading to a higher demand for treatment. I am especially curious about what will happen in the field of immunotherapy!

Q: What is the main focus of your academic research? Is this focus likely to shift in the coming years?

A: My main focus is immunoglobulin E sensitisation among children and the development of allergic rhinitis in children. I am hoping that I will be able to work on this for a long time!

Q: What advice do you have for medical students who are thinking of beginning a career in allergy and immunology?

A: I would advise them to go for it! Allergy and immunology is a really interesting field with huge progress both in basic research and in clinical treatment. I think we will be able to cure more allergies with immunotherapy in the future and I think it will be fun to be a part of the progress!

Q: How far do we have to go before we fully understand the mechanisms and biology of allergic conditions?

A: Well, will we ever know everything? I am not quite sure...

Nikolaos G. Papadopoulos

Professor of Allergy and Paediatric Allergy, Centre for Paediatrics and Child Health, Institute of Human Development, University of Manchester; Royal Manchester Children's Hospital, Manchester, UK.

Q: What first inspired you to enter the field of allergy and immunology?

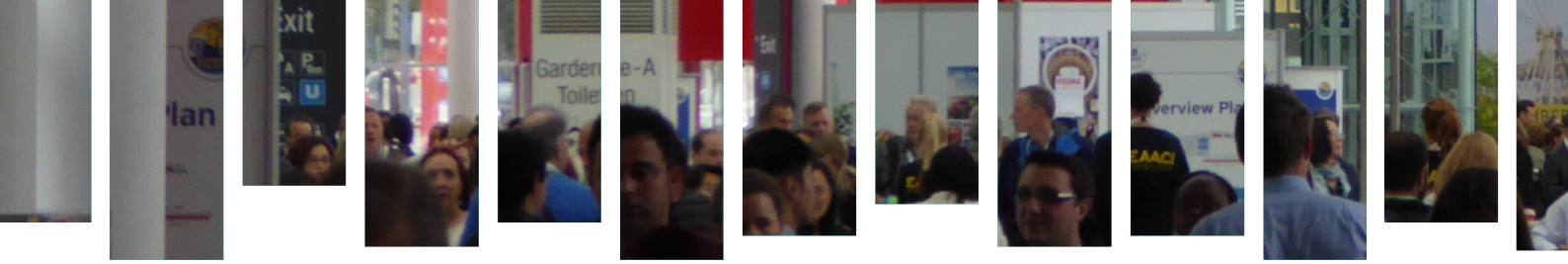
A: That was quite a while ago and the process was not completely straightforward. I was attracted by the complexity of interaction with the environment and more specifically, in tropical infectious diseases which I had come across during my early travels. Exploration of infectious diseases led me to studying immunology, as the key interphase with bugs. At the next stage, while I was amazed from the different aspects of immunology, I had to make a choice on a professional activity that could help patients in parallel to doing research. Among the various options, allergy came up as the most promising and attractive one.

Q: What areas of research do you believe would be most beneficial for creating a better understanding of how respiratory allergies develop and how they can be prevented?

A: Any research which is genuinely open-minded and methodologically sound cannot be anything but beneficial. It is not always necessarily cost-effective though and in our times we need to prioritise research that is most promising in terms of translational potential. Current breakthroughs in -omics, and personalised and systems approaches should be able to deliver bedside-relevant results soon.

Q: From your previous experiences with European Union (EU) funded projects, do you feel there is adequate funding available for research into respiratory allergic disorders and diseases at national and international levels?

A: If one takes into account the huge burden and cost of these conditions, or compares it to other domains, funding in the area of respiratory allergies is quite low. It appears that part of the decision for funding prioritisation is affected by



'impressions' and therefore respiratory allergies are frequently considered low priority. It would not be the case if objective criteria were used. International funding could also improve in order to stimulate transnational collaborations. Science is, and should be global, but most funding agencies are local or regional.

Q: Do you think there is enough public awareness across Europe concerning the risk factors involved in the development of various allergies and in knowing how to decrease the levels of exposure to these risks?

A: There is some public awareness on risk factors such as tobacco smoke or pollution and an increased sensitivity, although not necessarily always backed by evidence in relation to diet. Everybody would agree that our Western lifestyles and stress are to blame for much of the current disease burden. Unfortunately, we cannot claim that there are major interventions that may reduce allergies at a public health level. Recently, food introduction practices have been scrutinised, but we still need to incorporate these new findings into different cultural environments. So, I think we need more awareness about the problem, but take care on coming up with solutions that are not completely proven.

Q: You were involved in the co-ordination of the European Commission funded PreDicta project which came to an end in March earlier this year after more than 5 years of research. Can you offer any insights into some of the research findings of the project?

A: PreDicta was an exciting project and has already resulted in a few dozen scientific publications. The most interesting findings are only now being analysed and prepared for publication. Among these, I can identify a couple of my favourites such as the ability of human rhinoviruses to escape antibody recognition; the capacity of the virus to drive allergic inflammation at the epithelium level as well as the potential to block the virus with antisense technology.

Q: Do you anticipate a significant increase in the development of biotechnological products intended to cure allergic disorders such as food allergies, in the near future?

A: We are living at the epoch of biologicals; when I was starting my career we were producing 'magic bullets' suggesting that they would be able to cure everything. Now is the time that such promises are yielding results. In addition, modified allergens and other biotechnological advances are giving us enormous potential. However, greater flexibility on the part of the regulators would be an enormous help in this arena.

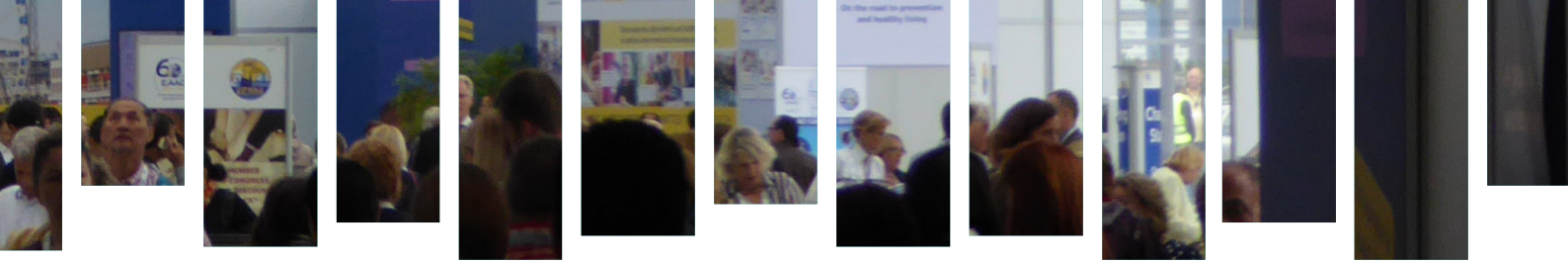
Q: What are some of the risk factors involved in the development of food allergy disorders and how important is an increased understanding of these factors?

A: Understanding the natural history of a disease and recognising the factors that affect it is clearly crucial. In relation to food allergies, we know that the type of food, rate of introduction and/or consumption, and the environmental context of exposure are important. What we still need to understand is the precise quantitative aspects of the above: how often, how soon, how much, under what level of 'tolerising' or 'sensitising' factors.

Q: What are the current bottlenecks in research which if resolved, could significantly contribute to developing approaches and tools for the effective management of food allergies?

A: Funding, regulation, and fragmentation are the major bottlenecks for research in allergy. We have argued for higher prioritisation from funding agencies, more reasonable regulation (e.g. use of allergen extracts or recombinant proteins for skin testing, requirements for study design in relatively rare allergies, etc), and European and international research integration.

> "Understanding the natural history of a disease and recognising the factors that affect it is clearly crucial."



Q: What do you feel to be the greatest achievement thus far in your career? What goals have you set for yourself to be achieved in the future?

A: I feel that my career has been smoothly building up; we have contributed in the understanding on how a viral infection interferes with allergy at several levels. Also, and this has been a great satisfaction, we have taken steps towards 'curing' children with food allergies using oral desensitisation. In the future, I would like to see effective antiviral and/or vaccine approaches influencing the incidence of asthma attacks. On the food allergy front, I expect real bedside treatments for most foods.

Q: What advice would you offer to someone considering a career in the field of allergy and immunology?

A: If it is their passion, it will not let them down! There is huge scope for understanding mechanisms through research, educating others, and improving the lives of patients. What more could a scientist or a physician hope for?

Cem Akin

Associate Professor, Harvard Medical School; Director, Mastocytosis Center, Brigham and Women's Hospital, Boston, Massachusetts, USA

Q: What first motivated you to work in the field of allergy and immunology and then to direct your research towards the mast cell disorder mastocytosis?

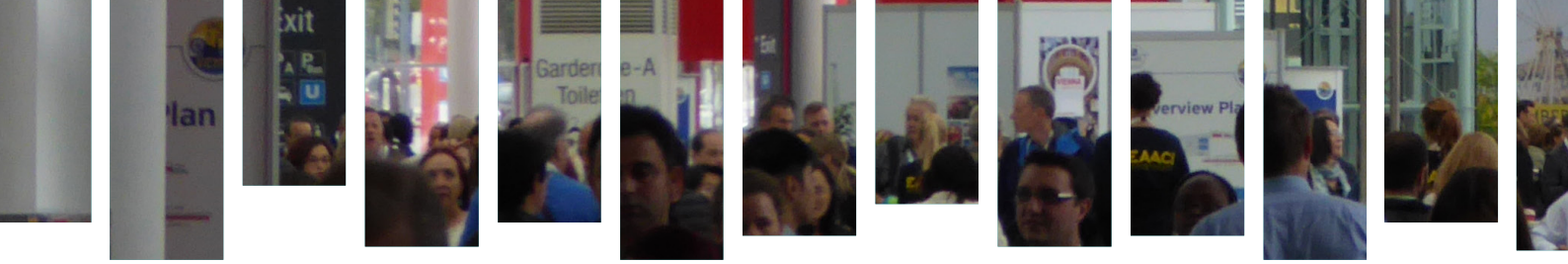
A: When I was little, I was determined to find a cure for cancer to help humanity. That childish enthusiasm followed me to medical school. I started reading about the immune system and thinking about using it to kill tumour cells. As I found out more about the immune system, my interest shifted to disorders of immune regulation and allergy. I was introduced to mastocytosis during my allergy/immunology fellowship at the National Institutes of Health (NIH). I was attracted to researching mastocytosis as a unique neoplastic disorder of an innate immune system cell (mast cell) mostly presenting with allergy-like symptoms where the traditional boundaries of haematology-oncology and allergy overlapped.

Q: What specific areas of the disorder are you currently exploring and what developments do you hope to achieve from doing so?

A: I am not sure if we will ever be able to find a way to completely eliminate the mast cells in the human body but I am always interested in finding new ways to inhibit activation of mast cells or limiting their survival. These treatments would not only help patients suffering from mastocytosis but also those with very common diseases such as allergic rhinitis, asthma, food allergies, and chronic urticaria.

Q: How far has our understanding and treatment of mastocytosis improved since you first began working in this area?

A: When I started working on mastocytosis, the *KIT D816V* tyrosine kinase mutation was just identified in some patients with advanced disease. By improving the detection methods, we and others have shown that the mutation occurred in virtually all patients with adult onset mastocytosis and that it was not only limited to mast cells but also involved other haematologic lineages in some patients. These findings highlighted the importance of targeting the mutation by kinase inhibitors as a potential therapeutic mechanism. We also realised that it may not be as rare of a disease as once thought. By using the same sensitive detection methods, we have found clonal mast cells as the underlying cause of anaphylaxis



in some patients. Recent research indicated that a substantial number of patients with bee sting allergies may also have mastocytosis as an underlying aetiology.

Q: Do you believe that in the near future treatment for the disorder could be curative or preventative, as opposed to current approaches which aim to relieve the symptoms?

A: We currently do not have a curative drug for mastocytosis, however good progress has been made towards identifying new drugs and treatment modalities that would slow down the progression of aggressive disease variants such as mast cell leukaemia. It is important to realise that mastocytosis is not a single clinical entity and its spectrum ranges from a benign indolent disease to very aggressive variants associated with decreased life expectancy. Most clinical trials with new drugs are conducted on patients with aggressive mastocytosis. It is possible that what slows down the progression of aggressive mastocytosis may be curative for indolent variants.

Q: Please tell us about your leading role as a director of the Mastocytosis Center at Brigham and Women's Hospital in the USA. What do you feel have been the greatest achievements made by the centre in the treatment and evaluation of the disorder?

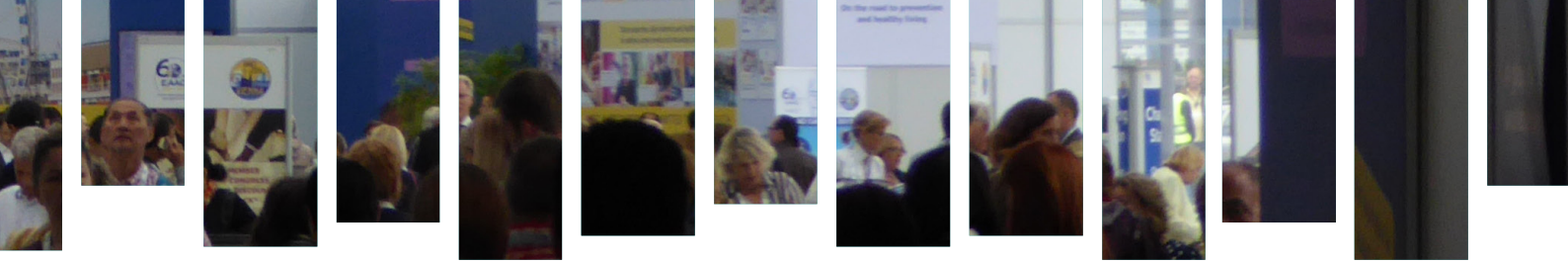
A: The centre was established approximately 5 years ago and acts as a platform to bring together the clinicians and researchers interested in mastocytosis and to facilitate cross-disciplinary interactions for the ultimate goal of improving patient care. It is completely funded by patient donations and has a modest budget. We have launched a patient registry database project and also have plans to create a tissue repository, which ideally would be accessed by anybody with a good idea on a collaborative basis after appropriate institutional review board approvals. We have identified new forms of mast cell disease including syndromes presenting with mast cell activation and helped establish diagnostic criteria and identify a pathologic basis of these syndromes. We are also working with industry to guide their efforts in new drug development and select the patient population that would most likely benefit from a given therapeutic approach.

Q: What new developments and research can be expected from the Mastocytosis Center in the years to come?

A: Our ultimate goal is to obtain external funding or a 'centre grant' that would protect the investigators' time for research. We need to understand better how mast cells function in physiologic processes in order to find ways to inhibit their growth or activation products. We are hoping to be at the forefront of drug discovery research and clinical trials.

Q: How would you describe the quality of care given to patients with mastocytosis and other related mast cell disorders in the USA? How does this compare with Europe in your opinion?

A: There are only a few centres of expertise for mastocytosis in the USA. Our referral numbers continue to grow each year and I would like to think that it is a sign of appreciation by the physician and patient community. Since it is impossible for these few centres to see all patients in the country, it is important to raise the awareness of the disease and educate the physician community in general to the point that they know when to suspect the disease and conduct an appropriate workup close to where the patient lives. I see education efforts as a crucial mission of our centre. I have seen many referrals from the community who have already gone through an excellent diagnostic workup which might be an indication that the education efforts are paying off. Here, I would also like to thank the patient support organisations for their partnership with us in advancing the education on the disease. In Europe, mastocytosis research is much better organised in comparison to the USA, owing to the European Competence Network on Mastocytosis (an initiative spearheaded by Prof Peter Valent at the University



of Vienna, Vienna, Austria), which brings together researchers and clinicians from many centres, from different countries, around their collaborative projects, and in their annual meetings. I have been privileged to attend these meetings as a member of their medical advisory board.

Q: What role do you expect biotechnology companies to play in the continuing research and development of new treatments for mastocytosis, mast cell disorders, and more widely in the allergy and immunology field?

A: No man is an island in medical research in this day and age. I think the partnership between the biotechnology companies and academics is the key to finding new treatments for the disease. The academicians have the knowledge on the disease, and biotech has the drugs and funding. While mastocytosis itself may still be regarded as a rare disease, some of the new drugs targeting mast cell survival and activation for patients with mastocytosis may also potentially benefit millions of patients with more common diseases such as allergic disorders.

Q: What has been the proudest achievement of your career to date? And what specific goals do you hope to achieve in future work?

A: My proudest achievements are when I receive handwritten thank you cards from patients or parents. I would like to collect as many of those as possible in the future.

Q: What advice do you have for medical students who are thinking of beginning a career in allergy and immunology?

A: I cannot think of a better field to pursue for inquisitive young minds. The study of allergy and immunology is the study of how humans cope with their environment. I think this field of study will only become more important in the future as we continue to rapidly change our environment and expose ourselves to increasingly unnatural environments and threats. They will not be disappointed by their decision.

> “I see education efforts as a crucial mission of our centre.”

Amir Hamzah Abdul Latiff

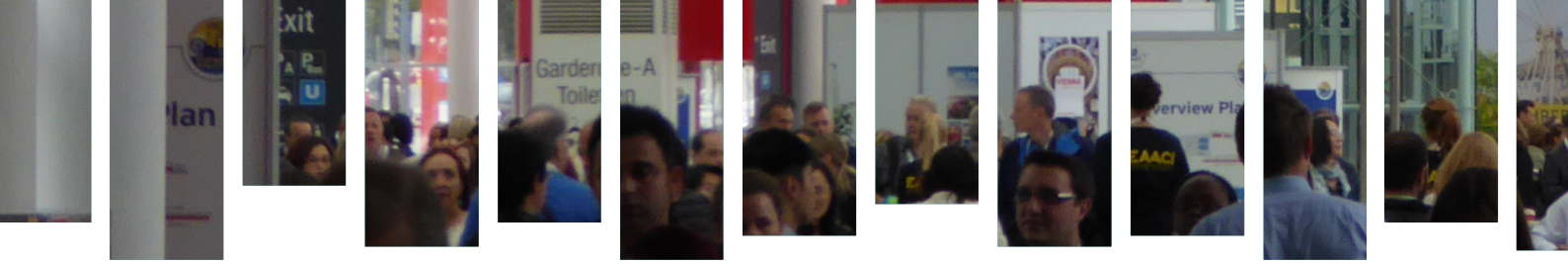
Consultant in Paediatrics, Clinical Immunology and Allergy, Department of Paediatrics, Pantai Hospital, Kuala Lumpur, Malaysia; President, Malaysian Society of Allergy and Immunology (MSAI); Board Member, Asia Pacific Association of Allergy, Asthma and Clinical Immunology (APAAACI).

Q: What first attracted you to working in the allergy and immunology field and then to direct your focus on paediatric medicine?

A: I actually started to specialise in paediatric medicine with the intention to eventually subspecialise in nephrology. Hence for my masters research thesis I settled on the subject of steroid-sensitive nephrotic syndrome (SSNS). I was strongly encouraged to study the immunological aspects of SSNS, whereby it was regarded that the immune system played a role in its mechanism. It was then that I was attracted to the field of clinical immunology and allergy, and pursued this further as a second specialisation.

Q: What areas of research are you currently exploring and what insights do you hope to gain from doing so?

A: Being away from the academic field for nearly a decade meant that exploring research opportunities boiled down to a simpler research area for which data on current prevalence of allergies in Malaysia is sorely needed. This may seem mundane, but given that no new data has emerged in the last 10 years on the prevalence



of allergic diseases in Malaysia, particularly food and drug allergies as well as anaphylaxis, it is imperative to establish these in order to ascertain the burden of allergic diseases, from the health and economic aspects.

Q: How far has our understanding of the factors determining the development of allergies advanced since you first began working in this field?

A: In my nearly 20 years in the field, there have been more questions than answers on the epidemic that is allergies. Whilst many factors have emerged that may throw light on the rising development of allergies and are brought to the forefront, not a single factor enjoys a sense of belief of being more significant from one than the other, be it from microbiomes to climate change. It is more likely that these multiple factors are interplaying, and that combinations of these factors in the right proportions leads to the development of allergies. Translational studies become more relevant towards this understanding and these studies are steadily revealing paths for newer therapeutic measures in the treatment of allergies.

Q: What are the greatest barriers to tackling these allergic diseases and disorders, and how can they be overcome?

A: The greatest barriers to tackling allergic diseases is creating the awareness that handling allergic diseases requires a holistic, integrative, and personalised approach in their diagnoses and treatment. It is not merely a simple organ-based style of treatment when symptoms appear, but seeking pre-morbid situations via clinical history to establish those at risk of developing more complex allergic diseases. In other words, look and seek to understand the patient with allergic diseases rather than the allergic diseases affecting a patient. Thus, it is important that allergic diseases are managed by fully trained allergists.

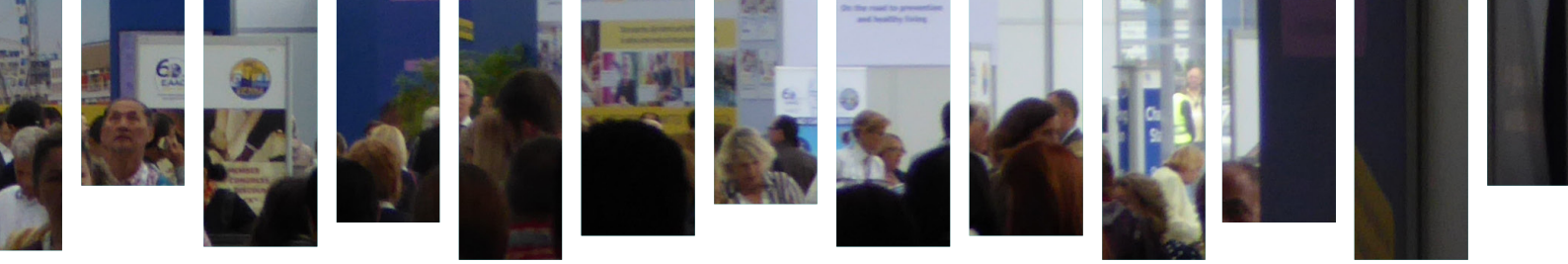
> “In my nearly 20 years in the field, there have been more questions than answers on the epidemic that is allergies.”

Q: Do you anticipate immunotherapy, a potentially curative treatment, to overtake antihistamine medication as the prevalent treatment approach to allergies?

A: This will depend on where we are practising, given the starting cost is never cheap enough to persuade policy makers to employ immunotherapy as the first modality of treatment. Granted it is potentially curative and in the long-term generally cheaper than all conventional anti-allergic medications bundled together. Naturally, not everyone with relevant allergic disorders for which immunotherapy is an option would need to undergo the treatment. Once those patients are identified, then immunotherapy will be advised and commenced sooner rather than later. Nonetheless the high cost factor remains and there needs to be a mechanism of reimbursing these patients, whether it be via insurance or government funding. Fortunately, this is possible in many instances but not exhaustive, and immunotherapy should in principle be available to all patients requiring the treatment.

Q: How would you describe the quality of care given to patients with an allergic disorder or disease in Malaysia, where you currently practise?

A: For as long as allergy and clinical immunology are not recognised as a subspecialty of medicine, there will be an insufficient approach in providing high quality of care to patients with allergic diseases. Once there is a recognition of this subspecialty then will come the realisation that many allergic diseases are not as ‘simple’ as they are made out to be and their understanding requires specialists devoted to this specific field of medicine. This is in tandem with the fact that the pathophysiological mechanism of allergic diseases becomes more complex and treatment will require astute clinical judgement that only allergists and/or clinical immunologists could provide from their extensive training and research. Thus, whilst there is enough quality of care, it is not optimal at its best and there is certainly room for improvement.



Q: In light of your involvement with the Allergy-Free Nation Initiative in Malaysia, do you think that a country free from allergies is achievable?

A: The Allergy-Free Nation (AFN) initiative came about via the Malaysian Society of Allergy and Immunology (MSAI) due to a feeling that the prevalence of allergic diseases is increasing in Malaysia, and that further steps are needed to prevent this rise. As mentioned earlier, good quality data has been scarce or not forthcoming on the current prevalence of allergic diseases in Malaysia. Hence the AFN campaign was launched with an Allergy Awareness Survey amongst a select group of the population in the area around the capital city of Malaysia, i.e. Kuala Lumpur, which exceeded 8,000 people (and still ongoing). The results would hopefully gauge the prevalence and the degree of understanding of allergic diseases amongst the general population. From there on, the second stage of the initiative would be the structuring of a National Allergy Road Map to tackle the problem of allergic diseases from various angles, impressing upon the policy makers to take note of the seriousness of allergic diseases which would have a significant impact on the health of the nation and economy. The AFN initiative is very ambitious and, whilst not achievable for a foreseeable period, there is always the possibility that with time, and discovery of the aetiology of allergic diseases via research co-operation through global networking, a country could be free of allergic diseases.

Q: In the years to come do you expect there to be an increasing demand made on this field of medicine to respond to changing environments which could expose people to new forms of allergens and infections?

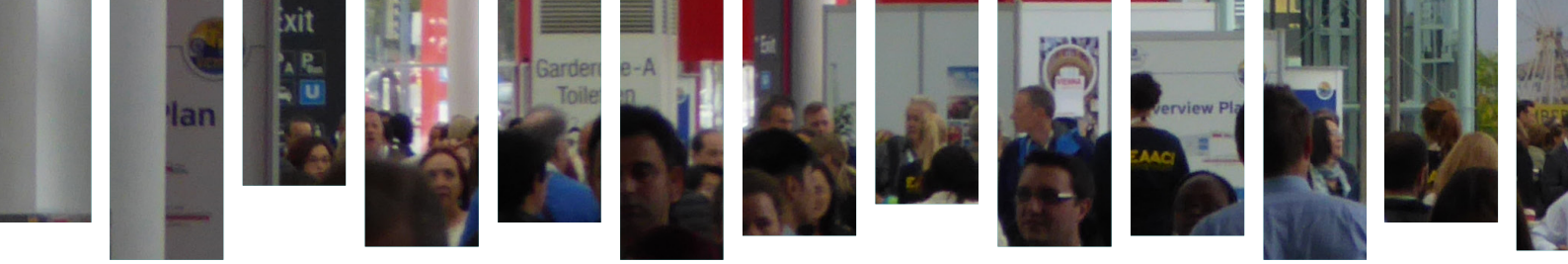
A: I would hope so, and the road is a winding one to say the least, as a more concerted effort is taken to address the basic requirements in delivering high quality management of allergic diseases, both diagnostic and treatment, along with an in-depth consideration of the underdiagnosis and undertreatment of allergic diseases. The demand is certainly there now and it is bound to increase rapidly if no major steps are taken to dampen the rapidity of this epidemic of the 21st century. That rise is certainly related to the changing environments, as has been alluded to, and it may transform into a more challenging and complex situation with an altered natural history of these allergic diseases. We have to act now.

Q: What do you feel has been the greatest achievement in your work to date? What goals have you set for yourself in future work?

A: I still feel that I have made no great achievement until I can convince the policy makers to stand up and take note of the gravity of the situation. Patients suffering from allergic diseases and other immune-mediated diseases (including primary immunodeficiencies, [PID]) need the best possible high quality healthcare system to be in place for them. Putting this in place would be a major goal, and from there on hopefully we would see allergy and clinical immunology recognised as a specialised field of medicine in its own right. Parallel to this would be the follow-on to the AFN initiative, with the Malaysian Allergy Road Map. Another major goal I have is to address the issue of increasing awareness for PID, where allergic diseases can co-exist. PID are still not well recognised and are often underdiagnosed, with our current data suggesting we have only diagnosed 2% of PID patients in Malaysia. This is actually comparable with data from other parts of the world, and emphasises the exact reason why clinical immunology must be recognised; clinical immunologists and allergists are best suited to deal with immune-mediated diseases. These goals would need to be in place very soon, as Malaysia approaches our 2020 vision to be a developed nation.

Q: What advice can you give to medical students considering a career in allergy and immunology?

A: I would not stop medical students considering a career in allergy and immunology given the background painted thus far. We need more medical students specialising in this field, particularly in Malaysia, in order to achieve our 2020 vision.



With their knowledge we can build the nation further, forming a new generation of highly trained clinical immunologists and allergists who are competent in all aspects of clinical management, laboratory skills, and research background, with the ability to teach and generate even more clinical immunologists and allergists to meet the demand of the nation.

Sarah Karabus

Paediatric Allergist and Paediatrician, Christiaan Barnard Memorial Hospital; Senior Lecturer, Allergy Division, Red Cross War Memorial Children's Hospital, Cape Town, South Africa.

Q: What first motivated you to enter the allergy field and then to focus your work in paediatric allergy?

A: My primary training was in paediatrics and as I completed this, the opportunity became available for a fellowship position in paediatric allergy. Allergy was something that I had had precious little exposure to during my paediatric training so it was an opportunity to continue learning. There was only one position available, so if one did not jump at it, the opportunity would only come around again in 2 years.

Q: To what extent do you feel the treatment and care of paediatric allergic disorders have improved since you first began working in this area of medicine?

A: I think that the growing awareness of allergy as an important speciality has led to a greater understanding of the pervasiveness of allergic disease and thus better resources being allocated. Allergy as a speciality has only recently been officially recognised as such in South Africa.

Q: What significant improvements in treatment approaches towards tackling these disorders do you anticipate emerging in the near future?

A: There are numerous emerging treatment approaches. Some of the very exciting fields include the prevention of allergic disease, immunomodulation/immunotherapy, as well as other specific targeted therapies.

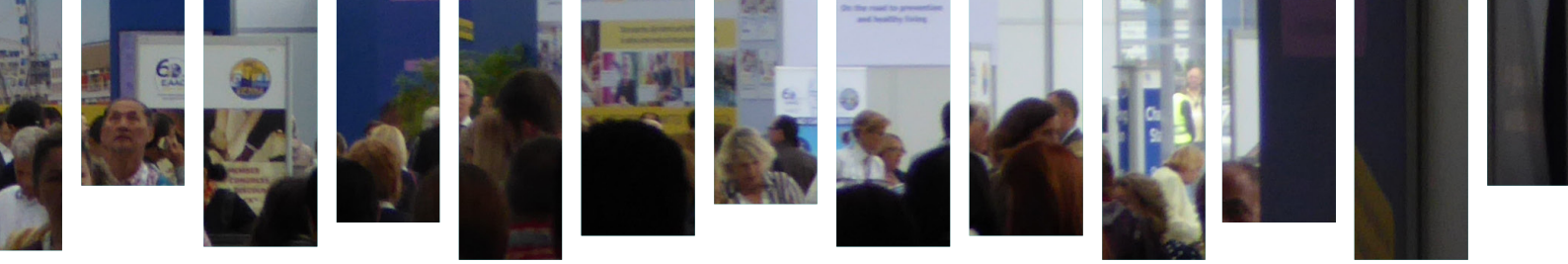
Q: How important are immunotherapy approaches towards allergic disorders and do you feel this treatment option will become increasingly common in clinical practice?

A: Absolutely and it is happening already. Thus far, this is the only possible way that allergic disease can be 'cured' rather than merely treated.

Q: In regards to life-threatening food allergies experienced by children, do you feel that their exposure to the risk of a severe reaction in public spaces, particularly within South Africa where you currently practice, is adequately managed and prepared for?

A: Unfortunately, this is not the case. Public awareness on how to recognise the symptoms of a severe allergic reaction as well as how to manage it is severely lacking. Great work is currently being done in schools during public awareness drives on radio and television but this is driven mainly by the Allergy Society of South Africa (a non-government organisation), private individuals, companies, and other interested groups; therefore scope is limited and is on an *ad hoc* basis. There is very little participation on a national health department level which is sorely needed.

> "Allergy was something that I had had precious little exposure to during my paediatric training so it was an opportunity to continue learning."



Q: What are some of the greatest challenges you currently face in your own practice toward the effective management and treatment of child allergies and how do you intend to tackle them?

A: The main challenges are awareness and education. It takes hours educating a family about the child's condition, about the medications, and the need for good follow-up care. In South Africa another huge obstacle is financial. Most, but not all of the treatments that are available internationally are available here. However, the vast majority of our population are unable to access the care they need due to financial and social constraints.

> "South Africa is a developing country and clearly research is not always the top priority when it comes to funding."

Q: Do you feel that there is an adequate research focus on allergic diseases and disorders in South Africa? How does this compare with other parts of the world?

A: South Africa is a developing country and clearly research is not always the top priority when it comes to funding. However, I do feel that we do very well with our limited resources; our academic units have produced excellent work that has been recognised internationally and continues to do so to date.

Q: Is there any advice you would offer to someone considering entering the field of allergy and immunology?

A: This is an exciting field with new developments every day; you can never be bored in this field!

ADJUVANTS IN ALLERGY: ELEVATING EFFICACY

This symposium took place on 12th June 2016, as a part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress 2016 in Vienna, Austria

Chairperson

Matthias Kramer[1]

Speakers

Ralph Mösges,[2] Randolf Brehler,[3] Thomas Kündig[4]

1. Allergy Therapeutics, Worthing, UK

2. Institute of Medical Statistics, Epidemiology and Computer Science, University Hospital Cologne, Cologne, Germany

3. Department of Dermatology, University Hospital, Münster, Germany

4. Department of Dermatology, Zurich University Hospital, Zurich, Switzerland

Disclosure: Prof Matthias Kramer is an employee of Allergy Therapeutics UK Ltd. Prof Ralph Mösges has received consulting and/or speaker's fees from, and/or conducted contract research for, several companies including ALK, Allergopharma, Bencard, Dr Beckmann, BioTechTools, HAL, Leti, Lofarma, Novartis, Roxall, Merck, and Stallergènes. Prof Randolf Brehler has received consulting and/or speaker's fees from Bencard, ALK, Allergopharma, Almirall, Basilea, Dr Pfleger, Hal, Leti, MSD, MEDA, Novartis, Pierre Fabre, Pohl Boskamp, Siemens, Stallergènes, and Thermo-Fischer. Dr Thomas Kündig has received consulting and speaker's fees from Allergy Therapeutics.

Acknowledgements: Writing assistance was provided by Tabasum Mughal of ApotheCom.

Support: The symposium was sponsored by Allergy Therapeutics. Authors received honoraria for preparation and delivery of their presentations. The views and opinions expressed are those of the authors and not necessarily of Allergy Therapeutics.

Citation: EMJ Allergy Immunol. 2016;1[1]:32-36.

MEETING SUMMARY

This symposium provided an overview of the current and future technologies and treatments used in the field of allergen immunotherapy (AIT). Prof Ralph Mösges explored the concept of pre-seasonal immunotherapy in overcoming the problem of recurrent allergy, focussing on the use of Pollinex® Quattro versus the use of symptomatic treatment according to current guidelines. The use and mechanisms of adjuvants was explored by Prof Randolf Brehler, who discussed the use of adjuvants in AIT including delivery systems, immunopotentiators, and targeted delivery systems to facilitate optimum immune responses with the potential of a lower injection burden and increased efficacy of treatment. Prof Thomas Kündig concluded the symposium with an overview of the future of AIT and the use of virus-like particles (VLP) in harnessing the innate immune system response to protect against allergens.

Ultra-short Course Immunotherapy

Professor Ralph Mösges

The European Medicines Agency (EMA) defines four criteria for the market authorisation application for AIT: the treatment of allergic symptoms; the sustained clinical effect of the treatment; the long-term efficacy and disease-modifying effect of the treatment; and curing the allergy with sustained absence of allergic symptoms in post-treatment years. This is illustrated in the landmark study by Durham et al.[1] where the treatment of rhinoconjunctivitis in response to grass pollen led to an initial decrease in symptoms in the first season, with a sustained clinical effect in Seasons 2 and 3. Long-term efficacy and disease modification continued to be observed in follow-up Seasons 1 and 2.[1]

However, the question that remains is whether AIT is able to cure allergies. An epidemiological study

in 790 German patients in Germany undergoing AIT found that 100% of patients expected a total cure of their allergy after treatment.[2] Evidence of long-lasting clinical efficacy of AIT has been demonstrated in a study where birch pollen immunotherapy resulted in a long-term loss of Bet v 1-specific T helper (Th)2 responses, transient induction of allergen-specific interleukin (IL)-10-producing T receptor 1 cells and synthesis of immunoglobulin (Ig)E-blocking antibodies in serum.[3] In children, pre-seasonal grass pollen immunotherapy for 3 years was effective and a 12-year follow-up after the cessation of immunotherapy in the same study cohort revealed an ongoing clinical benefit.[4] Prospective evaluation of sublingual immunotherapy (SLIT) given for 3, 4, or 5 years showed that in the patients receiving SLIT for 3 years, the clinical benefit persisted for 7 years. In those receiving immunotherapy for 4 or 5 years, the clinical benefit persisted for 8 years, however symptoms reappeared.[5] Revaccinating these patients with therapy resulted in clinical benefit as seen before; however, often adherence to therapy, particularly if it is SLIT, can be a problem.

Data from a survey of 296 allergists in Italy on the use of SLIT to treat allergic rhinitis and asthma revealed that after 1 year of SLIT only 45% of patients remained on therapy and only 15% of patients completed the full therapy course of 3 years.[6] When SLIT adherence was compared with subcutaneous immunotherapy in a large German cohort treated with specific grass pollen immunotherapy, results indicated that there was no difference in the levels of adherence with either modes of administration.[7] Hence, there is a need for AIT that is effective and results in increased adherence.

Ultra-short pre-seasonal subcutaneous therapy may therefore provide a better choice for patients. Pollinex® Quattro is a unique allergy specific immunotherapy with three active components: an allergoid, monophosphoryl lipid A (MPL), and micro crystalline tyrosine. Polymerisation of the allergen to create an allergoid reduces the number and specificity of specific IgE epitopes whilst retaining much of the IgG reactivity, so that the dose can be increased without compromising tolerability. MCT is employed as a natural depot that allows for controlled allergoid release from the injection site. The rapid metabolism of MCT, the half-life of which is approximately 48 hours, permits the short-course nature of Pollinex Quattro. The MPL component acts as an adjuvant whose activity is restricted to the toll-like receptor 4 and stimulates immune response from allergen-specific Th1 cells. Together, these components allow for a reduction in injections per year from more than 20 on average to just 4 pre-seasonal injections, so that within 3 weeks the full dose can be administered. A randomised, double-blind, placebo-controlled Phase IIb study assessing the clinical efficacy of modified short ragweed pollen adsorbed to MCT + MPL illustrated the synergistic effect of adding MPL to MCT by increasing efficacy by a further 25%[8] compared with non-adjuvanted AIT. Similar short-term immunotherapy with allergoids and the addition of MPL improved symptoms in >93% of pollen-allergic patients after 3 years.[9]

The benefits of short-course AIT were further demonstrated in a non-interventional study in adults, adolescents, and children suffering from recurrent allergic rhinitis who had successfully undergone AIT (regardless of route of administration or product) 5–10 years earlier and who could choose either treatment with Pollinex Quattro (four injections) or state-of-the-art symptomatic therapy. The primary endpoint was assessment of the efficacy of AIT with Pollinex Quattro during 30 days of the pollen peak based upon a change in rhinitis or conjunctivitis symptoms or change of medication, as documented in a diary. All patients had access to symptomatic treatment. Combined symptom and medication scores were significantly and consistently lower than those of the control group during 30 days of the peak grass pollen season, demonstrating the efficacy of the tested AIT. The percentage of patients not using additional symptomatic medication during the 2014 and 2015 grass pollen seasons was significantly lower ($p<0.001$) in the group treated with Pollinex Quattro than in the control group, and tolerability assessment by the patients demonstrated that over 80% of patients tolerated injections well or very well, and the remaining indicated moderately tolerating the injections. There were no major safety issues identified, with 30% of patients experiencing injection site reactions.

In summary, results from large clinical trials have shown that revaccination with Pollinex Quattro is successful in treating patients who redevelop an allergy many years after their initial AIT, leading to an improvement in symptoms with a reduction in the frequency of therapy administration. New

advances in AIT exceed the effects of symptomatic treatment according to current guidelines.[10]

Adjuvants in Immunotherapy

Professor Randolf Brehler

An ideal vaccine must initiate an innate immune response capable of directing the adaptive immune response toward efficient inactivation and removal of the pathogen, followed by the development of immune memory. Adjuvants are added to vaccines to enhance the immunogenicity of highly purified antigens that have insufficient immune-stimulatory capabilities.[11] Adjuvants work by extending antigen exposure, increasing antibody titres, enhancing cell-mediated and mucosal immunity, and supporting the production of cytokines. Their use in vaccines reduces the number of injections required for effective immunisation and stabilises vaccine formulations.

Several adjuvants have been used for vaccine development, with aluminium being one. Intraperitoneal delivery of aluminium resulted in activation of IL-1β via the NLRP3 inflammasome, the activation of dendritic cells, promotion of inflammatory monocytes, and enhancement of antigen uptake.[12] Although the ability to drive an antibody titre response is greatly improved by the use of adjuvants, there are some disadvantages; for example it has been suggested that aluminium has a propensity to accumulate in tissues.[13] There are no preclinical models on the kinetics of aluminium localisation after subcutaneous injection based on the allergy formulations that currently exist, therefore this required investigation. The analysis of absorption of aluminium in rats after four injections with a 3–4 day interval showed that the depot of aluminium from the injections persisted at the injection site for 180 days.[13] Extrapolation of this data from rats to humans would predict that an aluminium-containing adjuvant would be retained at the subcutaneous dose site for up to 37 years.[13]

There is discussion about whether a long-lasting allergen depot is necessary for the immunological efficacy of a vaccine or for subcutaneous AIT. In a mouse model, removal of the injection site (ear pinna) as early as 2 hours after administration had no appreciable effect on antigen-specific T and B cell responses, indicating that antigen depot does not play an important role in alum adjuvant activity.[14] Although aluminium is used as an adjuvant for many diseases, oil-in-water emulsions and MPL plus aluminium are also used, with several others currently being investigated.[15]

Delivery retardation of the adjuvant allows the slow and sustained release of an allergen for continuous stimulation of the immune system in order to reduce side effects and to enhance efficacy. In animal experiments, induction of IgG depends preferentially on the aluminium concentration rather than on the allergen concentration.[16] Further investigation in a clinical trial however, was not able to prove that a specific immunotherapy preparation with increased aluminium adjuvant and decreased allergen concentrations was clinically efficacious.[17] Furthermore there is debate about the chronic toxicity of aluminium in the context of neurologic disorders and autoimmune and inflammatory syndrome. It has previously been shown to increase Th2 sensitisation.[18] Its use in patients with renal insufficiency may contribute to its accumulation in the body, particularly in the bone and brain.

An alternative depot adjuvant to aluminium is MCT which forms a highly stable vaccine with allergens and has been shown to facilitate interferon gamma and IL-10 secretion and induce less IgE but similar IgG responses in comparison with aluminium.[19] MCT elicits the Th1 response, is compatible with other adjuvants, and has a proven safety profile. Its half-life (48 hours) allows for a prolonged immune exposure yet with regiment that allows a rapid up-dosing regimen.

Adjuvants, whether they act as delivery systems, immunopotentiators, or vector systems, are a key part of AIT with the ability to stimulate the immune system whilst demonstrating a proven safety profile.

Future Immunotherapy: Virus-Like Particles

Doctor Thomas Kündig

Immunological memory is based upon exposure to an antigen which then results in the proliferation of specific B and T cells and the subsequent formation of memory B and T cells. AIT needs to be able to elicit enough of an immune response to build immunological memory to be successful.

Other than increasing allergen dose, which may have negative safety implications, alternative methods to increase the efficacy of AIT have long been sought after. Direct intralymphatic immunotherapy into a subcutaneous lymph node markedly enhanced protective immune responses, so that both the dose and the number of allergen injections could be reduced, making intralymphatic immunotherapy safer, faster than other forms of immunotherapy, and most importantly, enhancing patient convenience and compliance.[20] Lymph nodes are key structures that co-ordinate the type and specificity of immune responses to an allergen, most importantly, it is in the lymph nodes where antigen-presenting cells such as dendritic cells present the allergen to T and B lymphocytes. Thus, lymph nodes are key structures that vaccines and immunotherapeutic agents must reach to generate antigen-specific responses that can change the course of an allergy. In one clinical trial, patients were randomised to receive either 54 subcutaneous injections with pollen extract over 3 years or 3 intralymphatic injections over 2 months. Results showed that three low-dose intralymphatic allergen administrations increased tolerance to nasal provocation with pollen within 4 months and reduced treatment time from 3 years to 8 weeks.[21] However while a patient only requires three injections, the equipment needed to locate lymph nodes as well as the extra time required for the procedure certainly represent the disadvantage.

With epicutaneous immunotherapy (EPIT), the allergen is delivered to the epidermis where it is picked up by Langerhans cells or dermal dendritic cells and transported to the lymph node. In two clinical trials, the application of 12 and 6 patches of EPIT led to approximately 70% amelioration in allergic symptoms versus only 20% amelioration in the placebo group.[22] In order to achieve this effect, the skin must be prepared by removing the stratum corneum, allowing the allergen to enter the epidermis. This is because the epidermis is highly resistant to molecules passing through it and thus techniques investigated so far have utilised very high concentrations of allergen.

Dangerous pathogens are associated with molecular 'patterns' (PAMPs). These can be in the form of bacterial DNA, viral RNA, and lipopolysaccharides which trigger the immune system to produce a response. PAMPs induce the activation of antigen-presenting cells (dendritic cells and B cells) by activating toll-like receptors. An approach to improving AIT includes the addition of adjuvants. MPL is such an adjuvant. Formulated from the cell wall of the bacterium *Salmonella minnesota*, MPL elicits a strong immune response.

A potential solution to the problems of existing AIT, i.e. poor uptake of subcutaneously injected or orally administered allergen by dendritic cells, skin barriers reducing efficacy of EPIT, patient adherence, and time spent with a patient, is VLP. The function of a virus capsid is to protect the DNA or RNA of the virus and as such, there have been attempts to load bacterial or viral DNA into the capsid to act as an adjuvant in therapy. After subcutaneous injection, a VLP is the perfect size for being transported via lymph vessels into the lymph nodes. Also a quasi-crystalline array of antigenic epitopes strongly enhances B cell responses, as cross-linking numerous B cell receptors can activate B cells to produce antibodies without the need for T cell help. This is known as a 'T cell independent antibody response'. Attaching Fel d1 on the surface of a VLP to desensitise mice showed that the mice were protected against anaphylaxis.[23] This protection is still seen, even in the absence of T cells and can even be transferred from one mouse to another by simply making an adoptive serum transfer. Injecting a VLP loaded with hundreds of allergens on the surface of the particle is surprisingly safe in highly allergic individuals, mainly due to the inability of the particle to diffuse into tissue to cause mast cell degranulation.

The subcutaneous vaccination of humans with VLP loaded with allergen resulted in an increase in IgG, particularly IgG1 and IgG3, subclasses that are typically observed after a viral infection; however, the antibodies to this particle dropped to baseline levels within a year.[21] In order to overcome this, a novel VLP has been developed and testing has revealed that it is able to generate a long-lived IgG response. The use of this particle in a future vaccine, such as the peanut vaccine, may help to further potentiate the immune response and protect allergic individuals.

REFERENCES

1. Durham SR et al. SQ-standardized sublingual grass immunotherapy: Confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. J Allergy Clin Immunol. 2012;129(3):717-725.e5.

2. Sondermann N et al. Erfolgsfaktoren der Adherence bei Hyposensibilisierung. Allergologie. 2011;34(9):441-6.

3. Möbs C et al. Birch pollen immunotherapy results in long-term loss of Bet v 1-specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies. J Allergy Clin Immunol. 2012;130(5): 1108-1116.e6.

4. Eng PA et al. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy. 2006;61(2):198-201.

5. Marogna M et al. Long-lasting effects of sublingual immunotherapy according to its duration: a 15-year prospective study. J Allergy Clin Immunol. 2010;126(5):969-75.

6. Scurati S et al. Adherence issues related to sublingual immunotherapy as perceived by allergists. Patient Prefer Adherence. 2010;4:141-5.

7. Sieber J et al. Medication persistence with long-term, specific grass pollen immunotherapy measured by prescription renewal rates. Curr Med Res Opin. 2011; 27(4):855-61.

8. Patel P et al. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. J Allergy Clin Immunol. 2014; 133(1):121-9.e1-2.

9. Zielen S et al. Short-term immunotherapy with allergoids and the adjuvant monophosphoryl lipid A. Results from a 3-year post-marketing surveillance study. Allergologie. 2007;30(2):S1-8.

10. Prof. Dr. Ralph Mösges. Revaccination With Pollinex® Quattro. NCT02579720. https://clinicaltrials.gov/ct2/show/study/NCT02579720.

11. Pasquale AD et al. Vaccine Adjuvants: from 1920 to 2015 and Beyond. Vaccines (Basel). 2015;3(2):320-43.

12. Liang F, Loré K. Local innate immune responses in the vaccine adjuvant-injected muscle. Clin Transl Immunol. 2016; 5(4):e74.

13. McDougall SA et al. Analysis of aluminium in rat following administration of allergen immunotherapy using either aluminium or microcrystalline-tyrosine-based adjuvants. Bioanalysis. 2016;8(6):547-56.

14. Hutchison S et al. Antigen depot is not required for alum adjuvanticity. FASEB J. 2012;26(3):1272-9.

15. Banday AH et al. Cancer vaccine adjuvants--recent clinical progress and future perspectives. Immunopharmacol Immunotoxicol. 2015;37(1):1-11.

16. Rask C et al. An alternative allergen: Adjuvant formulation potentiates the immunogenicity and reduces allergenicity of a novel subcutaneous immunotherapy product for treatment of grass-pollen allergy. Clin Exp Allergy. 2012;42(9): 1356-68.

17. Kleine-Tebbe J et al. Negative clinical results from a randomised, double-blind, placebo-controlled trial evaluating the efficacy of two doses of immunologically enhanced, grass subcutaneous immunotherapy despite dose-dependent immunological response. Clin Drug Investig. 2014;34(8):577-86.

18. Jensen-Jarolim E. Aluminium in Allergies and Allergen immunotherapy. World Allergy Organ J. 2015;8(1):7.

19. Leuthard D et al. Microcrystalline tyrosine as an adjuvant in allergy immunotherapy: a mouse study. Poster 680. EAACI Annual Meeting, Vienna, Austria, 11-15 June 2016.

20. Senti G et al. Intralymphatic immunotherapy. Curr Opin Allergy Clin Immunol. 2009;9(6):537-43.

21. Senti G et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proc Natl Acad Sci U S A. 2008;105(46):17908-12.

22. Senti G et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol. 2009.124(5) 997-1002.

23. Schmitz N et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. J Exp Med. 2009;206(9):1941-55.

IMPROVING DIAGNOSIS: THE NEXT FRONTIER IN HEREDITARY ANGIOEDEMA MANAGEMENT

This satellite symposium took place on 12th June 2016, as a part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Vienna, Austria

Chairperson

Stephen Jolles[1]

Speakers

Stephen Jolles,[1] Marco Cicardi,[2] Coen Maas[3]

1. University Hospital of Wales, Cardiff, UK

2. University of Milan, Luigi Sacco Hospital, Milan, Italy

3. University Medical Center Utrecht, Utrecht, Netherlands

Disclosure: Dr Stephen Jolles has received support including travel and research grants, consultancy and speaker honoraria from Baxter, Binding Site, Biotest, BPL, CSL Behring, Octapharma, Grifols Phadia/Thermofisher, Shire, SOBI, UCB Pharma, and LFB. Prof Marco Cicardi has received speaker, member of an advisory board, or consultant for Biocryst, CSL Behring, Dyax, Pharming/SOBI, and Shire/ViroPharma. Dr Coen Maas has received speaker honoraria from Bayer and Shire.

Acknowledgements: Writing assistance was provided by Dr Joan Thomas of ApotheCom.

Support: The symposium and publication of this article was sponsored by Shire. Authors received honoraria for preparation and delivery of their presentations. The views and opinions expressed are those of the authors and not necessarily Shire.

Citation: EMJ Allergy Immunol. 2016;1[1]:37-43.

MEETING SUMMARY

Hereditary angioedema (HAE) is a rare autosomal dominant condition caused by a deficiency or dysfunction of C1 esterase inhibitor (C1-INH) that normally blocks activation of C1, the first component of the complement cascade. The condition manifests as recurrent self-limiting episodes of angioedema, without urticaria or pruritus, most commonly affecting the skin or mucosal tissues of the upper respiratory and gastrointestinal tracts.[1] Symptoms are disabling and can be life-threatening when affecting the upper airways.[1] Low awareness of the condition and its resemblance to other disorders typically leads to delays in diagnosis.[2] Multiple mutations of the human *C1-INH* gene (*SERPING1*) have been identified, some of which cause HAE and some of which do not.[1] Genetic testing alone is therefore not diagnostic of HAE and needs to be supplemented with biochemical testing and hereditary information. There are mixed opinions among clinicians and scientists on the utility of genetic testing for diagnosis of HAE. The objective of this symposium was to raise awareness of HAE and its diagnosis, along with the role of genetic testing, familial testing, and future diagnostic methods for this disorder.

Dr Stephen Jolles chaired the symposium and opened with a presentation on current diagnosis of HAE. Prof Marco Cicardi presented on biomarkers that enable earlier diagnosis of HAE; and in the final presentation, Dr Coen Maas discussed the future of HAE diagnosis. Interactive voting and question and answer sessions were used to elicit the opinions of the audience at intervals throughout the symposium, which was concluded with a general discussion session.

Diagnosing Hereditary Angioedema Patients Today

Doctor Stephen Jolles

Initial diagnosis of HAE centres around clinical presentation and physical findings. Patients typically present with self-limiting episodes of angioedema (without urticaria or pruritus), which most commonly affects the skin or mucosal tissues of the upper respiratory and gastrointestinal tracts. Diagnosis is supported by biochemical testing that should ideally be conducted twice, with at least 1 month between tests. A family history of HAE is supportive of diagnosis, but not required. HAE affects approximately 1 in 50,000 people, with equal prevalence in men and women.[3-5] Unfortunately, diagnosis is often delayed, typically by 10–18 years.[3-5] Type I HAE is the most prevalent, accounting for about 85% of cases, and is characterised by low C1-INH and C4 levels.[3-5] Type II HAE (15% of cases) presents with normal or elevated but dysfunctional C1-INH and low C4 level.[3-5]

HAE has an autosomal dominant inheritance pattern, with one copy of the affected gene being sufficient to cause the disorder. HAE can also arise from spontaneous mutations in people with no family history of the disorder, accounting for 20–25% of HAE cases.[6] Inherited forms of HAE are caused by a mutation in the *SERPING1* (*serine proteinase inhibitor clade G*) gene, which is mapped to chromosome 11 (11q12–q13.1). *SERPING1* contains eight exons and seven introns and is prone to large deletions and duplications.[7] Type I HAE is caused by mutations throughout *SERPING1* that results in impaired protein folding or secretion.[7] Mutations in Type II HAE involve the active site of exon 8, which results in a dysfunctional protein that impairs binding to proteases.[7] Interestingly, about 10% of people with *SERPING1* mutations are asymptomatic.[7]

Over the years, a variety of different methods have been used for the diagnosis and classification of HAE, including whether or not a particular drug is successful in treating idiopathic histaminergic angioedema; biochemistry and immunology testing of acquired C1-INH deficiency; biochemistry exclusion (+/-) of angiotensin-converting enzyme (ACE)-induced acquired angioedema (AAE); history and biochemistry of Type I and Type II HAE; genetics of hereditary Factor XII (FXII) angioedema; and biochemistry and genetics of normal C1-INH-HAE. Different tests are therefore required to reach a diagnosis depending on the setting. In routine immunology and biochemistry laboratories, protein (C3, C4, C1-INH) and functional (fC1-INH) tests are generally available. Protein tests require a serum sample, measured by nephelometry or radial immunodiffusion, which, although may be subject to interference with lipidaemia, haemolysis, or icterus, are generally very reliable.[8] Functional tests (required for Type II HAE diagnosis) are performed on serum samples by sandwich enzyme-linked immunosorbent assay (ELISA) (of activated C1s); the main issue with this test is sample delay, so fresh samples need prompt freezing if transport is needed and a delay before analysis is anticipated.[8] New functional tests in development aim to mirror more closely pathways of interest in HAE and use biotinylated activated *FXII* (*FXII-a*) or biotinylated kallikrein bound to avidin-coated plates for the detection of bound C1-INH.[8]

There are several additional causes of low C4 other than HAE. These include AAE, systemic lupus erythematosus, vasculitis, antiphospholipid syndrome, cryoglobulinaemia (essential or secondary, e.g. to hepatitis B virus and hepatitis C virus), cold agglutinin disease (Immunoglobulin M red blood cell), C4 nephritic factor (C4b2a), viral infections with parvovirus, genetic null alleles (C4a and C4b), and rarely, advanced liver disease.[9] In AAE, the expected patient profile would be low C4, low C1q, both antigenic and functionally low C1-INH, and anti-C1 INH antibodies (in 70% of patients). AAE is a disease with late onset (>40 years of age) and there is usually no family history of the disorder.[10,11]

There are two categories of HAE with normal C1-INH-HAE with *FXII* mutations and without *FXII* mutations.[4] HAE with normal C1-INH presents predominantly in adulthood and may have more frequent involvement of the tongue, uvula, and face, but there is no erythema multiforme.[4] Idiopathic angioedema needs to be excluded as this disorder also presents with normal C1-INH.[4]

In conclusion, clinical history, physical examination, and supportive family history are central in the diagnosis of HAE. Frontline biochemical testing involves C4, C1-INH, and fC1-INH, optimally repeated a month or more apart. In patients with normal results, there may be an additional role for testing during an attack, because a normal C4 during an attack in HAE is an extremely rare

event. Some current diagnostic tests may have utility in therapeutic monitoring, but are unable to predict disease severity or explain variability between patients.

During his presentation, Dr Jolles asked the audience whether or not genetic testing was available to them in their practice. A total of 56% of the audience responded 'yes', while 44% do not have genetic testing available. Dr Jolles then asked the audience the most common reason for them conducting genetic testing: the majority (50%) voted for diagnostic uncertainty; 14% for routine part of work-up; 14% for family testing; 6% to rule out HAE; and 16% never use genetic testing.

Biomarkers: Improving Diagnosis

Professor Marco Cicardi

There are various approaches available to diagnose angioedema. These include the identification of aetiology, pathogenic mechanisms, and phenotypes, and the stratification of phenotypes.

Identification of Aetiology

Angioedema due to C1-INH deficiency was identified by Virginia Donaldson as the biomarker that defines a group of patients with the phenotype of recurrent angioedema. Two types of angioedema due to C1-INH deficiency exist: the inherited autosomal dominant form (HAE), and the non-inherited form caused by anti-C1 INH antibodies (AAE) and/or lymphoproliferative disease.[12] HAE due to a mutation in the *FXII* gene (known as the *Thr328Lys* mutation) has been traced back to a gene rearrangement in a single person. People with this type of HAE have a different phenotype to those with HAE due to C1-INH deficiency.[13,14] Patients with angioedema due to deficiencies in ACE can be identified by the pattern of relapses after discontinuation of ACE-inhibitor therapy.[15]

Identification of Pathogenetic Mechanisms

Bradykinin is the main mediator of angioedema.[16] In HAE with FXII mutations, *FXII* mutant proteins are cleaved and rapidly activated by plasmin and escape inhibition by C1-INH, thereby causing excessive bradykinin formation.[17] In ACE-inhibitor-related angioedema, catabolism of bradykinin is reduced due to inhibition of ACE (which degrades bradykinin). Overall levels of bradykinin are increased, but cleavage of the precursor to bradykinin (high molecular weight kininogen) is not increased.[18,19] Idiopathic histaminergic angioedema is diagnosed when angioedema without wheals stops recurring upon continuous treatment with an H1 histamine receptor blocker (up to 4-times the approved dose).[3]

Identification of Phenotypes

Phenotypes can be used to diagnose or sub-categorise groups of patients. In a nationwide survey of 983 Italian patients with HAE (87% with Type I and 13% with Type II), a diagnosis of C1-INH deficiency could be made based on reduction in C1-INH function (median value: 20% for Type I and 19% for Type II).[20] Antigenic C1-INH, however, was within the normal range for Type II patients (median value: 96%) but not for Type I patients (median value: 21%), demonstrating the value of this technique in categorising Type I and Type II patients.[20] In patients with *FXII* mutations, there is a large difference in phenotype, as male patients rarely experience oedema whereas female patients almost always do.[21]

Several different forms of angioedema can therefore be identified. Diagnosis of acquired idiopathic non-histaminergic angioedema is based on the exclusion of C1-INH deficiency, ACE inhibitor therapy, evidence for causative agents, absence of family history of angioedema, and absence of mutations in the FXII gene.[22] In addition, there are two groups of patients, those that respond to anti-histamine treatment and those who do not.[23] Bradykinin has been shown to be an essential mediator in most subtypes of angioedema without wheals (Figure 1).[22]

Stratification of Phenotypes

Recurrent angioedema without urticaria is associated with degradation of bradykinin, which is broken down (cleaved) by kallikrein.[12] Cleavage results in two-chain high molecular weight kininogen molecules that can be visualised by electrophoresis and used as a biomarker for different forms of angioedema.[23] In patients with C1-INH HAE, kininogen is a potential biomarker for disease severity, as patients with fewer symptoms have lower levels of cleaved kininogen than patients who are more symptomatic.[24] Several other proteins have been shown to be involved in the bradykinin-forming cascade, including the plasminogen activator urokinase and

vascular endothelial-like growth factor (VEGF); VEGF could potentially be used as a biomarker to distinguish different forms of angioedema or disease severity.[25-27]

In conclusion, C1-INH deficiency, mutations in *FXII*, and treatment with ACE-inhibitors are diagnostic of specific forms of angioedema. Evidence of histamine as the mediator distinguishes one form of AAE. Other forms of angioedema are defined only by the clinical profile and the exclusion of specific diagnoses. Markers to stratify patients with angioedema are still only partially identified and need to be further refined.

Question and Answer Session

Prof Cicardi was asked if he could provide further insight into the classification of non-histaminergic angioedema, and responded that the important issue is that in these patients, a mechanism exists that is different from the mechanism in patients that respond to antihistamine. Histamine may play a role in these patients, but is not the main mediator.

When asked if the kinetics of *C1-INH* and *fC1-INH* differ upon administration of C1-INH in people with hereditary versus those with AAE, Prof Cicardi responded that the kinetics are very different within patients. Patients with C1-INH deficiency demonstrate much more rapid catabolism of C1-INH than healthy people.

In response to the question of whether or not high molecular weight kininogen can serve as a prognostic biomarker, Prof Cicardi responded that kininogen may serve as an acceptable biomarker in the future, but the technical application and sensitivity of the assay needs improvement.

Looking Towards a Future of Improved Risk Stratification and Management

Doctor Coen Maas

As presented by Prof Cicardi, bradykinin is an important disease mediator in HAE. Accurate detection of bradykinin production during an angioedema attack, however, is challenging as its production is localised and difficult to detect systemically.[28] Bradykinin is also rapidly broken down by kinases, which makes detection even more difficult.

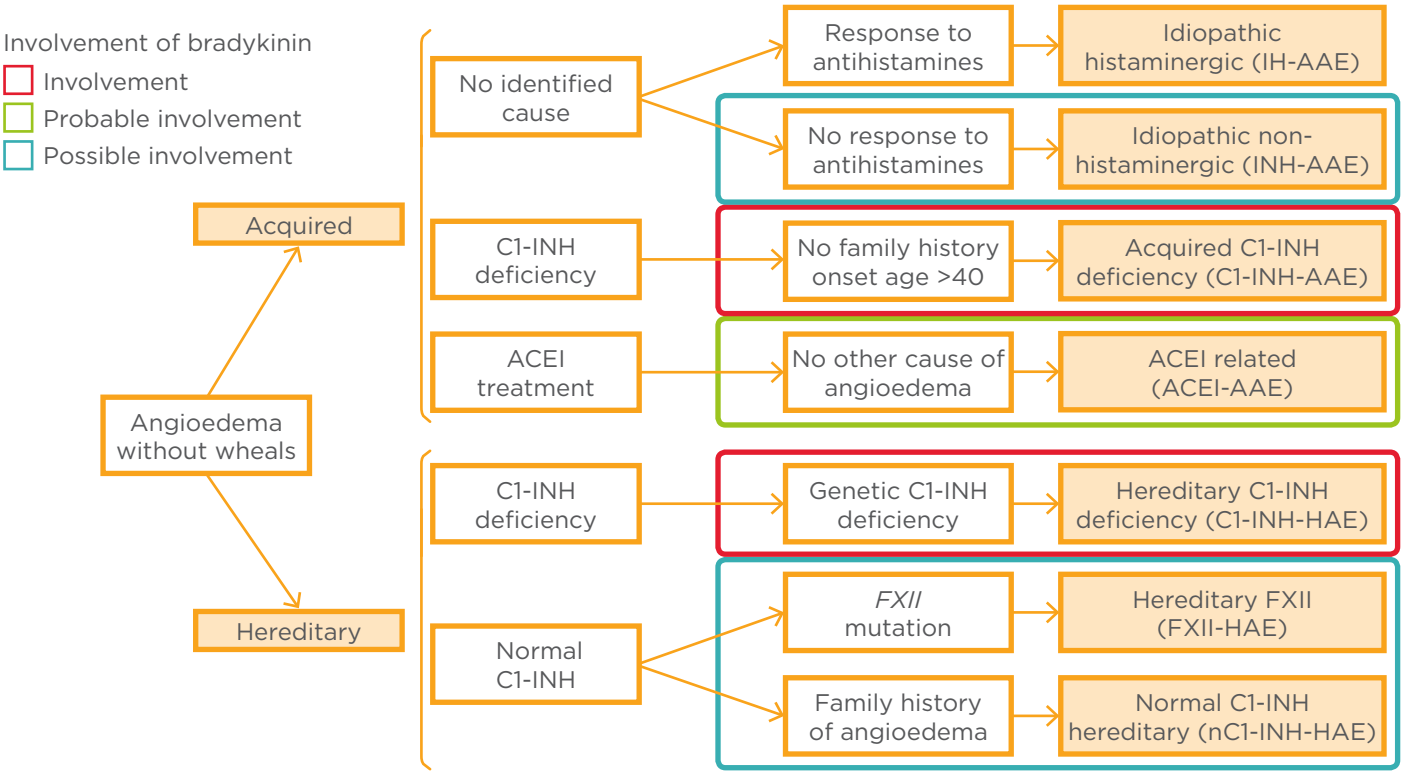


Figure 1: Classification of angioedema without wheals and involvement of bradykinin.[22]
ACEI: angiotensin-converting enzyme inhibitor; FXII: Factor XII; INH: esterase inhibitor; AAE: acquired angioedema; HAE: hereditary angioedema.

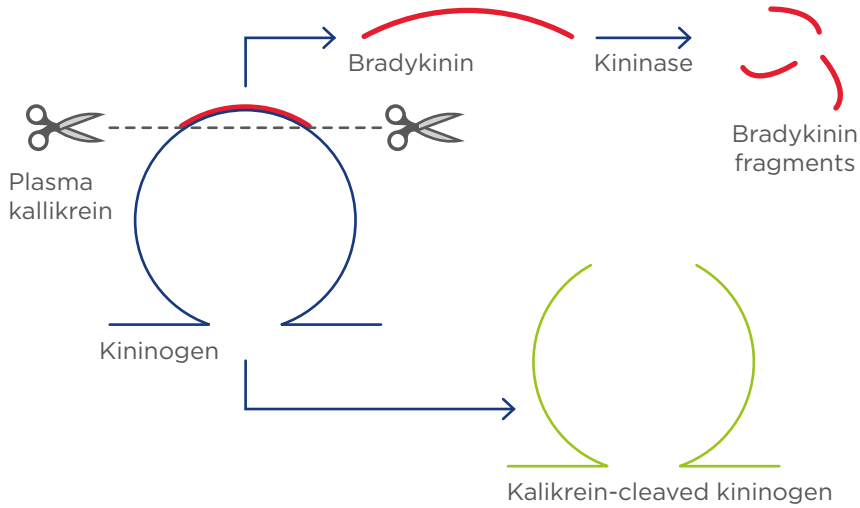


Figure 2: Cleavage of kininogen, producing high molecular weight cleaved kininogen.

Bradykinin is the end-product of the contact activation system that results from cleavage from its precursor protein, kininogen, by kallikrein. This process results in the production of high molecular weight cleaved kininogen (Figure 2). High molecular weight cleaved kininogen may be a suitable biomarker for contact activation as it is insensitive to kinases and readily detectable in the systemic circulation of healthy subjects.

In addition to cleavage by kallikrein, during an angioedema attack, kininogen is cleaved by several other enzymes and this may occur without the accompanied release of bradykinin. One of these enzymes is plasmin, and cleavage of kininogen by either kallikrein or plasmin results in similarly sized products when assayed by Western blotting.[29] Evaluation of kininogen cleavage using this method is therefore not adequate to accurately measure bradykinin production, and an assay with small (17 kDa) highly-specific monoclonal antibodies known as nanobodies is being developed to accurately differentiate kallikrein-cleaved kininogen.[30]

FXII is the initiator of the contact activation system, and collective evidence points towards a role for *FXII* in angioedema attacks. This enzyme normally has several cleavage sites. People with mutations in *FXII* have an additional cleavage site in FXII, which causes the enzyme to fragment and activate more rapidly.[17] In three types of FXII-HAE, mutants of *FXII* that are associated with angioedema were found to be particularly sensitive to activation by plasmin, resulting in higher levels of FXII. When no pathological mutations are present, several other enzymes may also be involved in FXII cleavage, including neutrophil elastase.[30] Indeed, studies indicate that neutrophil elastase and kallikrein act synergistically to activate *FXII*.[30]

To summarise, kallikrein-cleaved kininogen is a circuit marker for bradykinin production and a potential surrogate marker for HAE. It is important, however, to understand that cleavage may not always represent bradykinin formation and the bradykinin-forming mechanism is best analysed under controlled conditions to prevent peri-analytical artefacts, tailored sampling procedures may be required. The study of *FXII* is key to understanding disease mechanisms in HAE, and may lead to identification of the underlying cause of attacks and, as yet, unidentified mechanisms in this disease.

Question and Answer Session

Dr Maas was asked if there was a marker for risk of angioedema attack and responded that studies investigating the natural triggers of FXII activation have demonstrated that plasminogen levels correspond well with disease activity.[17] In patients with HAE and *FXII* mutations, the risk corresponds with the enzyme that triggers *FXII*. Different triggers may activate FXII in different patients. In general, however, bradykinin production is a good surrogate marker for disease activity.

A member of the audience asked Dr Maas if patients have co-existent histaminergic and bradykinin-activated HAE. Dr Maas responded that there is a mechanistically strong overlap

between histaminergic reactions and the formation of bradykinin, and that this has been observed in patients with anaphylactic shock (without angioedema).[31] In animal models, at least 50% of the hypotension in anaphylactic shock is directly attributable to bradykinin.[32] Bradykinin may therefore be secondary to a histaminergic reaction in patients.

When asked if taking samples during an attack would improve the quality of analysis, Dr Maas responded that it would. However, where plasma samples are useful, serum samples have the caveat that they will be fully clotted and all kininogen will be cleaved. However, samples taken during an attack can be informative in detailing the difference between the active and inactive phases of angioedema.

Finally, to a question of whether biomarkers will be used to aid treatment of angioedema, Dr Maas responded that it was possible, but the amount of bradykinin required in patients with idiopathic angioedema needs to be elucidated first.

Discussion Q&A and Interactive Voting

Interactive Voting

Dr Jolles opened the session and asked the audience what proportion of family members have been tested in their clinical cohorts. There was wide variation in the proportion of clinicians that conduct genetic testing and those that do not. Only 17% of the audience have conducted genetic testing on 75–100% of family members, while 11% have conducted no genetic testing on family members.

Dr Jolles then asked the audience what proportion of their patients should have a genetic diagnosis of HAE. Just over 44% of the audience responded that >75% of their patients should have a genetic diagnosis, compared with 21% that thought that <10% should be tested.

Finally, Dr Jolles asked the audience what the main barriers to genetic testing were. The main barriers were cost (39% of the audience) and availability (34%). Difficulties in testing family members was voted by 18% of the audience, and only 9% thought it was unnecessary.

Discussion Q & A

Several questions were posed by members of the audience:

Q: How useful is genetic testing in patients with an HAE clinical phenotype and clinical manifestations, but who have normal C4, C1-INH, and fC1-INH?

Prof Cicardi responded that the diagnosis of C1-INH deficiency is based on a biochemical diagnosis, so genetic testing will not necessarily help these patients. It can, however, determine if the cause of C1-INH deficiency is genetic, which may be useful if there is no family history. A test for FXII could, however, be useful and may tell you why a patient has angioedema.

Q: Is biochemical testing in C1-INH deficiency sufficient for a diagnosis of HAE?

Prof Cicardi responded that for many patients, genetic testing is not useful. A particular level of C1-INH deficiency has to be reached before angioedema occurs and this cannot be predicted by genetic testing. Even if a mutation in *C1-INH* is demonstrated, it does not mean that the activity of the gene is modified.

Dr Jolles said that genetic testing could be useful for exclusion purposes in cases that are difficult to diagnose. In addition, novel mutations that are found and associated with an appropriate clinical presentation and biochemical results and that are then included in genetic databases would increase the probability of being able to more accurately diagnose HAE in the future.

Q: What should be the approach in patients in which a new mutation variation is found? And, secondly, in patients in which no mutations are found for C4 and C1-INH?

Prof Cicardi responded that segregation studies need to be conducted first to evaluate the relationship between any new mutation and the function of *C1-INH*, and, in the second case, Dr Jolles highlighted that genetic testing does have limitations in that only exons are generally evaluated; mutations in introns or upstream of the gene will not be detected.

Q: Should genotyping be the preferred diagnostic test for newborns and children?

Prof Cicardi responded that infants (<1 year of age) may have C1-INH levels that are inconclusive. In these patients, if there is already a family history

of the disease, genetic testing may be the most precise method of diagnosis. After 1 year of age, C1-INH function can be used.

Symposium Summary

Dr Jolles summarised the presentations and discussion from the symposium:

- HAE is caused by C1-INH deficiency or dysfunction
- Current diagnosis is based on clinical presentation, C4 and C1-INH testing, and/or family testing: it is important that family members are encouraged to be tested for HAE
- Genetic testing is not routinely conducted, possibly due to lack of availability, cost, and that a firm diagnosis can be established in most cases of Type 1 and 2 HAE with biochemical testing
- Biomarkers could potentially help to identify triggers for HAE attacks and guide clinical treatment
- Kallikrein-cleaved kininogen is a potentially attractive surrogate marker for bradykinin formation, and therefore HAE
- Research developments are improving our understanding of the processes involved in HAE

REFERENCES

1. Nzeako UC et al. Hereditary angioedema: a broad review for clinicians. Arch Intern Med. 2001;161(20):2417-29.
2. Zanichelli A et al. Hereditary angioedema with C1 inhibitor deficiency: delay in diagnosis in Europe. Allergy Asthma Clin Immunol. 2013;9(1):29.
3. Agostoni A et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. J Allergy Clin Immunol. 2004; 114(3 Suppl):S51-131.
4. Zuraw BL, Christiansen SC. Pathogenesis and laboratory diagnosis of hereditary angioedema. Allergy Asthma Proc. 2009;30(5):487-92.
5. Jolles S et al. A UK national audit of hereditary and acquired angioedema. Clin Exp Immunol. 2014;175(1):59-67.
6. Banerji A. Hereditary angioedema: classification, pathogenesis, and diagnosis. Allergy Asthma Proc. 2011; 32(6):403-7.
7. López-Lera A et al. SERPING1 mutations in 59 families with hereditary angioedema. Mol Immunol. 2011;49(1-2):18-27.
8. Joseph K et al. A novel assay to diagnose hereditary angioedema utilizing inhibition of bradykinin-forming enzymes. Allergy. 2015;70(1):115-9.
9. Nusinow SR et al. The hereditary and acquired deficiencies of complement. Med Clin North Am. 1985;69(3):487-504.
10. Malbran A et al. Acquired angioedema: observations on the mechanism of action of autoantibodies directed against C1 esterase inhibitor. J Allergy Clin Immunol. 1988;81(6):1199-204.
11. Melamed J et al. The metabolism of C1 inhibitor and C1q in patients with acquired C1-inhibitor deficiency. J Allergy Clin Immunol. 1986;77(2):322-6.
12. Cugno M et al. C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. Trends Mol Med. 2009;15(2):69-78.
13. Cichon S et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. Am J Hum Genet. 2006;79(6):1098-104.
14. Kiss N et al. Novel duplication in the F12 gene in a patient with recurrent angioedema. Clin Immunol. 2013;149(1): 142-5.
15. Beltrami L et al. Long-term follow-up of 111 patients with angiotensin-converting enzyme inhibitor-related angioedema. J Hypertens. 2011;29(11):2273-7.
16. Cugno M et al. Activation of the coagulation cascade in C1-inhibitor deficiencies. Blood. 1997;89(9):3213-8.
17. de Maat S et al. Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations. J Allergy Clin Immunol. 2016;[Epub ahead of print].
18. Nussberger J et al. Plasma bradykinin in angio-oedema. Lancet. 1998;351(9117): 1693-7.
19. Nussberger J et al. Bradykinin-mediated angioedema. N Engl J Med. 2002;347(8): 621-2.
20. Zanichelli A et al. A nationwide survey of hereditary angioedema due to C1 inhibitor deficiency in Italy. Orphanet J Rare Dis. 2015;10:11.
21. Bork K et al. Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations. Allergy. 2015;70(8):1004-12.
22. Cicardi M et al. Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. Allergy. 2014;69(5): 602-16.
23. Zhang JC et al. Two-chain high molecular weight kininogen induces endothelial cell apoptosis and inhibits angiogenesis: partial activity within domain 5. FASEB J. 2000;14(15): 2589-600.
24. Suffritti A. Manuscript in preparation.
25. Kaplan AP, Ghebrehiwet B. The plasma bradykinin-forming pathways and its interrelationships with complement. Mol Immunol. 2010;47(13):2161-9.
26. Joseph K. J Allergy Clin Immunol. In press.
27. Loffredo S et al. Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema. Allergy. 2016;71(7):989-96.
28. Nussberger J et al. Local bradykinin generation in hereditary angioedema. J Allergy Clin Immunol. 1999;104(6):1321-2.
29. Personal communication. University Medical Centre, Utrecht.
30. Maas C. Manuscript in preparation.
31. Sala-Cunill A et al. Plasma contact system activation drives anaphylaxis in severe mast cell-mediated allergic reactions. J Allergy Clin Immunol. 2015; 135(4):1031-43.e6.
32. Oschatz C et al. Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. Immunity. 2011;34(2):258-68.

Award Winners at the European Academy of Allergy & Clinical Immunology Congress 2016

Over the 4 days of the EAACI 2016 congress, prizes were awarded to the best abstract presentations in the Oral Abstract Session, Poster Discussion Session, and Thematic Poster session. This amounted to a total of 124 abstract award winners who received a certificate and €200 prize money. In each session type, prizes were awarded to the best single abstract submitted under the various session titles. Winners were selected by two session chairs according to a criteria evaluating the scientific content and methodology of the research, its novelty and originality, and the ability of the presenter to present the abstract concisely and clearly as well as answering any questions. Due to the high volume of award winners, not every individual can be recognised but below is a selection of some of the significant research presented this year.

Thematic Poster Session

From the 'Learning from the case reports' session, Dr Claus R. Johnsen and his team (Denmark) were the abstract prize winners for their presentation: 'Severe food allergy to water chestnut - water caltrop (Singoda flour): a case report'. The abstract discussed a rare case of a 37-year-old man who presented oral allergy symptoms alongside other symptoms, such as vomiting and abdominal cramps, within the 2 hours following the intake of legumes battered in water chestnut (singoda) flour. It is a widely used plant across Asia, Australia, tropical Africa, and islands of the Pacific and Indian Oceans and is considered a 'safe' food for allergic subjects. The patient had previously experienced two severe reactions after eating water chestnut products and a series of clinical tests, including a food challenge and skin-prick, confirmed the diagnosis of the allergy. To the knowledge of Dr Johnsen and the research team this is the first case presented with a food allergy to water chestnut, also known as water caltrop, and this may be an underdiagnosed allergy.

To the knowledge of Dr Johnsen and the research team this is the first case presented with a food allergy to water chestnut, also known as water caltrop, and this may be an underdiagnosed allergy.

Ms Samantha Tyler and colleagues (UK) received the award in the 'Allergy epidemiology' category for their presentation 'Innovative nasal filters allow for allergen exposure monitoring and are acceptable to wear'. The research highlighted in the presentation explored the feasibility of individuals wearing a nasal filter over the course of a day to assess the full spectrum of allergens which are breathed in during a

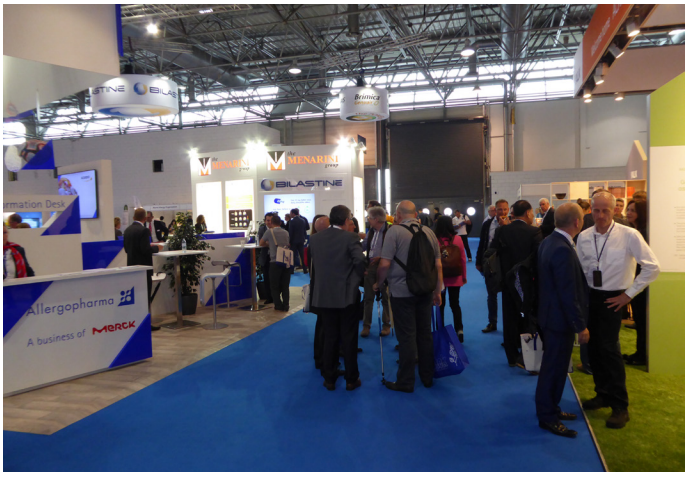
normal daily routine. The researchers sought to find a new and innovative approach to the assessment of allergen exposure that would take into account a much wider range of allergens than the amount yielded from analysing a common sample type, settled dust. The nasal filter consisted of a membrane that removed particles by means of interception and impaction, and the researchers found that significant levels of allergens were readily detectable in the nasal filter once extracted. The majority of volunteers who wore the device forgot they were wearing it within 60 minutes of putting it on and they did not experience a difference in breathing resistance. These filters could be a simple and easily wearable method that could potentially allow for a wider spectrum of allergen sources to be investigated and assist in a better understanding of their role in the development of allergic disease.

The 'Pediatric anaphylaxis: the cold as a trigger' presentation won Dr Joana Cosme et al. (Portugal) the award in the category of 'Aspects of paediatric atopic manifestation, management and quality of life'. It reports on a case of anaphylaxis induced in a 14-year-old girl by cold stimuli, a severe manifestation of cold urticaria which is rarely presented in a patient of this age. The girl was referred to an allergy clinic after twice losing consciousness and generalised urticarial lesions after swimming in the ocean. A series of successive episodes experienced by the patient is then described in the research, including an episode of angioedema localised to the lips after eating an ice cream, a second angioedema episode of the thumb after carrying a glass of cold water, and an episode after a walk on a windy day. After the diagnosis of cold-induced urticarial and anaphylaxis was established by an ice cube provocation test, the patient was treated with second generation antihistamines, with a higher dosage given during the colder seasons. After beginning treatment, no more episodes of a loss of consciousness occurred with only two episodes of urticaria after exposure to windy weather. No more symptoms following consumption of cold drinks or foods were presented. The patient was also prescribed an adrenaline auto-injector and was advised to limit cold exposure during cold weather.

Oral Abstract Sessions

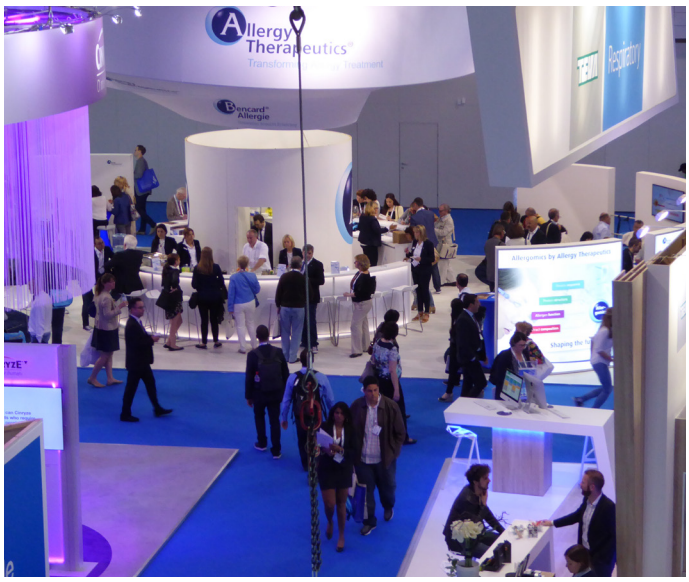
Dr Valérie Verhasselt and her team's presentation (France) 'Early oral exposure to house dust mite allergen through breast milk: a potential risk factor for allergic sensitization and respiratory allergies in children' abstract presentation was deemed the best in the 'Prediction and prevention of childhood atopic disease' category. The abstract reports on a study investigating whether early oral exposure to a major house dust mite (HDM) allergen through breast milk modifies the risk of allergic sensitisation and respiratory allergies in children. Children breastfed by mothers with a history or asthma or allergies with high levels of the *Dermatophagoides Pteronyssinus* 1 HDM allergen in their milk were compared to those with mothers who had low levels of the allergen, as confirmed by enzyme-linked immunosorbent assay. The results showed that high levels of *D. pteronyssinus* 1 in milk had an increased risk of asthma or allergic rhinitis. It also showed an increased risk of allergic sensitisation in children at 5-years-old who had been breastfed by mothers with high levels of the allergen. It was concluded that oral exposure to the HDM allergen through breast milk may contribute to the subsequent development of respiratory allergies in children. These findings are considered by the researchers to provide the basis for future research into inhibiting the priming activity of mothers' milk with the HDM allergen present.

In the 'Food allergens: Biochemical characterisation and allergenicity' session, Prof Inomata Naoko and coworkers (Japan) won the award. The presentation was titled: 'High prevalence of peamaclein sensitization in fruit allergy with negative IgE reactivity to Bet v 1 homologues and profilin'. Peamaclein was recently reported to be a new allergen in peach allergy but proteins homologous to peach peamaclein have been found in many other plants. The research team investigated the sensitisation to paemaclein in fruit allergy patients with negative Immunoglobulin E reactivity to Bet v I homologues and the profilin protein, which are marker allergens for pollen-food allergy syndrome. They found a high prevalence of sensitisation among patients who presented these markers indicating that those sensitised might also have allergies to multiple fruits and that peamaclein could be a cross-reactive allergen.



Poster Discussion Sessions

The prize winner in the 'Allergy and asthma in children' category was Dr Iana Markevych and colleagues (Germany) for their presentation 'Early life travelling does not increase risk of asthma, allergies and atopy until 15 years: results from GINIplus and LISAplus'. The research offered a response to speculation that travelling to places substantially different from a person's home environment in terms of climate, vegetation, and food early in life could increase the exposure to future unknown allergens. This could then promote the development of allergies. The researchers collected detailed data on individuals travelling around and out of Europe during the first 2 years of their life alongside a range of atopic outcomes and potential confounders for up to 15 years. The conclusions drawn from their longitudinal analysis of 5,996 people indicated that travelling abroad appears to be neither adverse nor beneficial in relation to the development of allergies. Although replication is needed in future epidemiological studies, the research could be important for parents considering travelling with their small child who worry about the risk of development of various atopic conditions.



The conclusions drawn from their longitudinal analysis of 5,996 people indicated that travelling abroad appears to be neither adverse nor beneficial in relation to the development of allergies.

Dr Shintaro Suzuki and others (Sweden) were awarded the prize for the abstract presentation 'Severity of allergic asthma is associated to multiple dog and/or cat allergen component sensitization' in the 'Factors associated with asthma severity' category. The goal of the study that was presented was to determine the distribution of dog and cat allergen component sensitisation in a random population of adults and relate the degree of sensitisation to allergic asthma severity. The patient cohort consisted of 2,006 people, 744 of them had asthma and 1,262 did not. Those with allergic asthma were screened for allergen-specific IgE to cat and dog and those with a measurement of serum IgE >0.35 kU_A/L were screened for the specific cat and dog allergy components: Fel d 1, 2, 4 and Can f 1, 2, 3, 5, respectively. Sensitisation to two or more cat or dog allergen components was associated with an increased prevalence of asthma combined with allergic rhinitis. A combined sensitisation to cat and dog allergens increased the risk of expressing more signs of asthma severity.

Sensitisation to two or more cat or dog allergen components was associated with an increased prevalence of asthma combined with allergic rhinitis.

CLIMATE CHANGE IMPACT ON POLLEN IN THE AIR: A MODELLING STUDY

***Mikhail Sofiev**

Finnish Meteorological Institute, Helsinki, Finland
**Correspondence to mikhail.sofiev@fmi.fi*

This study considered possible changes in atmospheric transport of aeroallergens that can be expected due to climate change. The ongoing and forthcoming meteorological changes will affect all stages of pollen formation and distribution (Figure 1), but the analysis leaves out the processes of plant development and pollen formation, thus concentrating only on the atmospheric transport.[1]

Analysis of meteorological trends in the past and those expected for the future highlighted:

- A reduction of mean wind speed, with simultaneous empowering of storms
- An increase of specific humidity but uncertain trends of relative humidity in which reduction was noticeable in spring in Central and Northern Europe and in summer in the South
- An increase of duration and severity of dry spells, with simultaneous increase in power of individual rains. The total precipitation amount stays almost constant or grows

Estimating the climate impact on pollen dispersion and deposition can only be based on model simulations because this impact is not observable; meteorology affects the plant habitat and pollen production much more than its transport in the air. Distinguishing between these processes using only pollen observations is impossible. Model assessments can reveal the impact of each factor separately but it is hard to demonstrate that the obtained results represent the reality. However, pollen behaviour in the atmosphere is comparatively well established; basic physical laws are valid regardless of the atmospheric conditions and their current representation in dispersion models is sufficient for pollen calculations.[2]

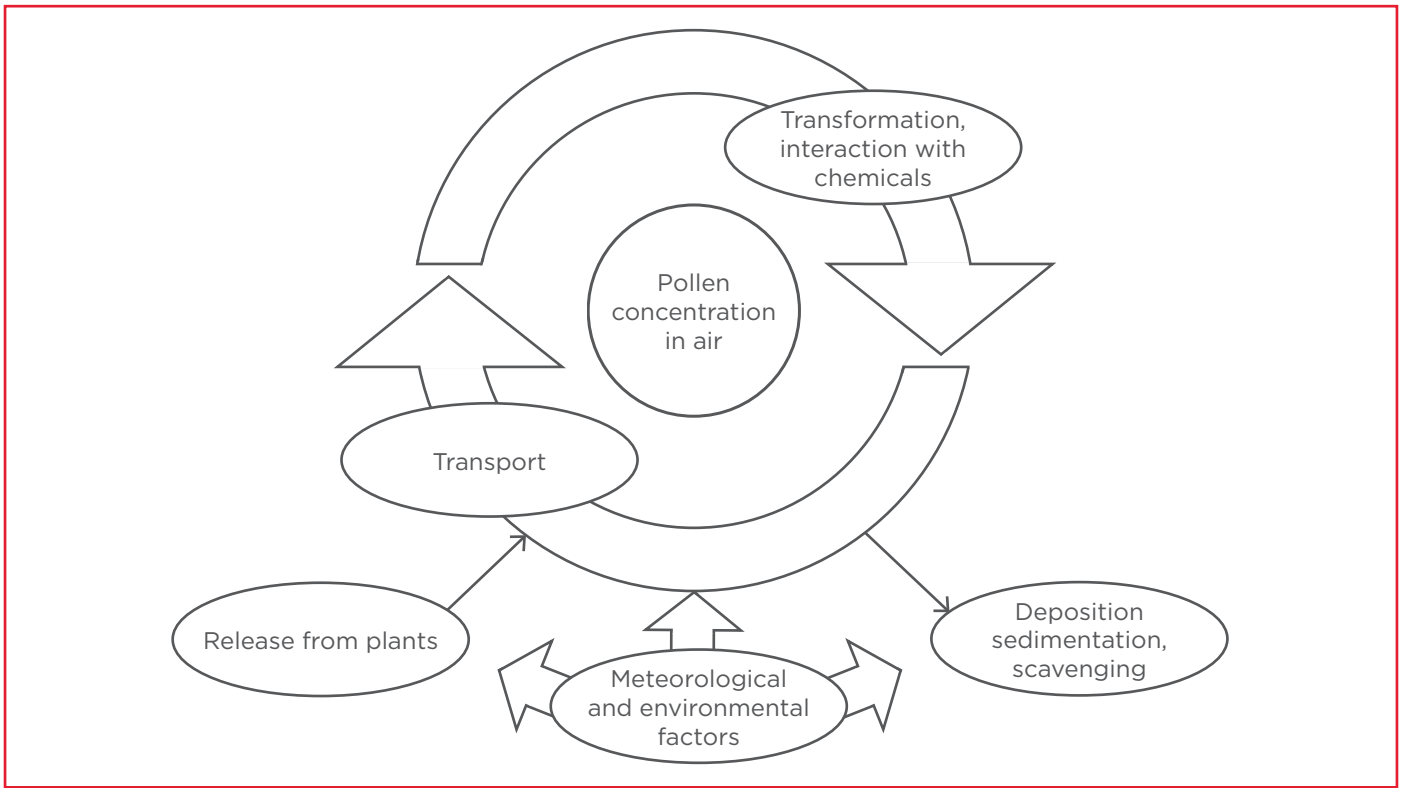


Figure 1: A schematic view of the main parts of pollen life cycle in the atmosphere.
Modified from Sofiev and Bergmann[3]

Therefore, transport with wind, mixing, and deposition can be analysed with confidence. The water budget of the flying grain and its water exchange with the surrounding air are studied much less, thus making it difficult to analyse the trends due to changing hydrological cycle and air humidity.

Keeping in mind the above uncertainties, only the most pronounced and physically grounded conclusions can be taken forward. In particular, the impact of the meteorological trends on pollen dispersion in Europe will probably lead to a reduction of the pollen transport distance and a corresponding restructuring of the load pattern. One can expect lower concentrations far away from the sources but the changes in the near fields are more uncertain due to competition of various factors. Pollen dispersion modelling over the last 33 years still suggests, in most cases, that the near-source concentrations grew by ~0.1% per year, with simultaneous reduction in remote regions. This conclusion is in the general agreement with other studies made for 'conventional' pollutants.

REFERENCES

1. Sofiev M, Prank M. "Impacts of climate change on aeroallergen dispersion, transport and deposition", Beggs P. (Ed), Impacts of Climate Change on Allergens and Allergic Diseases (2016), Cambridge: Cambridge University Press.

2. Sofiev M et al. Towards numerical forecasting of long-range air transport of birch pollen: theoretical considerations and a feasibility study. Int J Biometeorol. 2006;50(6):392-402.

3. Sofiev M, Bergmann KC. (Eds), Allergenic pollen: A review of production, release, distribution and health impact (2013), Berlin:Springer-Verlag.

THE ROLE OF THE ALLERGIST IN EOSINOPHILIC OESOPHAGITIS

***Ingrid Terreehorst**

ENT Department, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
**Correspondence to i.terreehorst@amc.uva.nl*

Eosinophilic oesophagitis (EoE) is an emerging disorder, affecting both adults and children. Therapy consists of local steroids, various diets, and, in case of stenosis, dilation of the oesophagus. Elemental diets induce a histological remission of 90.8%, while the so-called six food elimination diet (the avoidance of wheat, milk, eggs, soy, peanuts/tree nuts, fish, and sea food) has a remission rate of 74%. This would support food allergy as a cause of EoE. Furthermore, almost 75% of children and up to 91% of adults express specific immunoglobulin (Ig)E against environmental allergens and food allergens.

Nevertheless, diets directed by specific IgE test results against food allergens have failed so far. In a study by van Rhijn et al. all patients received a tailor-made diet based on the result of a microarray assay (ISAC). Only one of the participating patients showed a histological response in the biopsy of the oesophagus, all other patients showed no improvement. This result is supported by other studies investigating the usefulness of skin-prick testing, patch testing, and specific IgE in serum (CAP, RAST). Furthermore, anti-IgE was just as effective as placebo when used as treatment in EoE while this therapy has proven its usefulness in severe asthma and chronic urticaria.

An explanation for the failing of specific IgE testing as guidance for a diet in EoE could be the failure of the allergens themselves. It is well known that both tests for inhalant and food allergens can be false positive and false negative. A false positive result can be explained by the presence of minor allergens in an extract that can bind specific IgE but have no biological activity, for instance carbohydrate cross-reactive determinants. Using fresh food for skin testing could induce the problem of contamination, e.g. using the same knife for slicing both fruit and nuts.

The different varieties of apple for instance can cause a false negative test, since some

apple species contain fewer allergens than others. Other causes for false negative tests are the disappearance of allergens after sterilisation (heat-labile allergens) and defatting (oleosins), in addition to problems regarding the reproducibility of allergen extracts in general.

One of the roles of the allergist is to consider these factors and optimise testing in EoE, not only by improving extracts but also through reading allergy tests within 20 minutes, which is fitting for IgE mediated diseases, and to do late readings in order to evaluate the non-IgE mediated pathophysiological mechanisms. However, results of this delayed reading have so far been disappointing.

The other roles of the allergist therefore remain of importance, treating concomitant allergic diseases such as allergic rhinitis, asthma, and eczema according to international guidelines. Furthermore, together with the dietician the allergist is required to advise patients in maintaining a balanced diet by means of introducing other foods to replace the components that have to be avoided and if necessary, to support the patients with supplements. Finally, allergists are important in the process of reintroducing food into a patient's diet.

In conclusion, it can be stated that the role of the allergist is to support and assist the gastroenterologist, together with the dietician, to optimise diagnostics and treatment for the EoE patient.

DUPILUMAB IMPROVES HEALTH-RELATED QUALITY OF LIFE AND ABSENTEEISM IN NASAL POLYPOSIS PATIENTS: RESULTS FROM A PHASE IIA TRIAL

***Claus Bachert**

Head of Upper Airways Research Laboratory and Chief of Clinics ENT-Department, University Hospital Ghent, Ghent, Belgium
**Correspondence to Claus.Bachert@ugent.be*

RESEARCH OBJECTIVES

Chronic rhinosinusitis with nasal polyposis (CRSwNP) affects between 1% and 4% of the general population.[1-3] The CRSwNP symptoms are chronic, and substantially impair health-related quality of life (HRQoL),[3] which has been reported for patients both in the USA[4] and EU.[5] The impact of CRSwNP on HRQoL has been shown to be comparable to congestive heart failure and chronic obstructive pulmonary disease patients.[4] Medical interventions for CRSwNP are often inadequate; the impact of intranasal corticosteroids in reducing polyp size and impairment of sense of smell is limited.[6] Short courses of oral corticosteroids (OCS) are recommended as adjunctive therapy in severe disease;[7] however, there is little rigorous clinical support for a positive benefit/risk profile for OCS use. It has been indicated that the duration of clinical benefit is variable and may decrease with repeated courses of treatment.[3] When medical treatments prove inadequate and appropriately directed medical management fails or is contraindicated, as is frequently the case, surgery is the only remaining alternative for symptomatic nasal polyposis (NP) patients.[2] Although the benefits of surgery for NP have been established, polyp regrowth with recurrence of symptoms is reported to occur in 40–90% of patients. A history of asthma, aspirin sensitivity, and previous sinus surgery have all been found to be predictive factors for polyp recurrence;[8] similarly to high immunoglobulin (Ig)E, serum-IgE and interleukin-5 concentrations in the polyp tissue.[9] A proof-of-concept Phase IIa study demonstrated the efficacy of dupilumab in treating patients with CSwNP. When added to a background of mometasone furoate nasal spray (MFNS), dupilumab reduced nasal polyp burden.[10] The objective of the current analysis was to assess

the effect of dupilumab on HRQoL outcomes and productivity in patients with CSwNP.

RESEARCH PROCESS

In a multicentre, international, randomised, double-blind, placebo-controlled, parallel-group, Phase IIa study, adults with CSwNP received either dupilumab (n=30) or placebo (n=30) on a background of MFNS for 16 weeks (NCT01920893).[10] Chronic rhinosinusitis-specific QoL was measured using the Sino-Nasal Outcomes Test (SNOT-22); general HRQoL was assessed using the self-rated EuroQoL-5D visual analogue scale (EQ-5D VAS), and the 36-Item Short Form Health Survey (SF-36). Absenteeism was defined as annualised mean number of days missed from work as a consequence of CSwNP (days/patient/year).

CONCLUSIONS

Dupilumab demonstrated efficacy in patients with CSwNP, considering both clinical and radiographic outcomes. Dupilumab also resulted in a significant improvement in SNOT-22 score, self-rated EQ-5D VAS, and mental health component summary score of the SF-36 when compared with placebo. Employed patients receiving dupilumab had significantly fewer sick leave days and improved productivity. Injection site reactions, headache, and nasopharyngitis were the most frequently reported adverse events with dupilumab.

Broader Implications of the Results

There are a limited number of therapies available for patients with CSwNP. Furthermore, the current standard of care, in many patients, has only modest effects on the symptoms, which substantially impact HRQoL. Therefore, newer therapies are required that not only impact the clinical outcomes, but also improve HRQoL and productivity.

Acknowledgments

ClinicalTrials.gov identifier: NCT01920893. Sponsored by Sanofi and Regeneron Pharmaceuticals, Inc. Medical writing assistance provided by Ronald van Olffen, PhD, of Excerpta Medica, funded by Sanofi and Regeneron Pharmaceuticals, Inc.

REFERENCES

1. Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. A review of 6,037 patients. J Allergy Clin Immunol. 1977; 59(1):17-21.

2. Fokkens WJ et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology. 2012;50(1):1-12.

3. Peters AT et al. Diagnosis and management of rhinosinusitis: a practice parameter update. Ann Allergy Asthma Immunol. 2014;113(4):347-85.

4. Soler ZM et al. Health state utility values in patients undergoing endoscopic sinus surgery. Laryngoscope. 2011;121(12):2672-8.

5. Khan A et al. The GALEN sinusitis cohort: impact on quality of life in patients with chronic rhinosinusitis with nasal polyps (CRSwNP). Abstract 2331. European Academy of Allergy and Clinical Immunology Congress 2015, 6-10 June 2015.

6. Lund VJ, "Where are we in the medical treatment of nasal polyps," Önerc TM et al. (eds.), Nasal Polyposis: Pathogenesis, Medical and Surgical Treatment (2010), Berlin: Springer-Verlag, pp.238-48.

7. Alobid I, Mullol J. Role of medical therapy in the management of nasal polyps. Curr Allergy Asthma Rep. 2012;12(2):144-53.

8. Wu AW et al. Factors affecting time to revision sinus surgery for nasal polyps: a 25-year experience. Laryngoscope. 2014;124(1):29-33.

9. Bachert C et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. J Allergy Clin Immunol. 2010;126(5):962-8.

10. Bachert C et al. Effect of subcutaneous dupilumab on nasal polyp burden in patients with chronic sinusitis and nasal polyposis: A randomized clinical trial. JAMA. 2016;315(5):469-79.

EDITOR'S PICK

This article provides a very good and concise clinical review of drug allergy. Many aspects of drug allergies are well described, including the establishment of diagnosis and the management of some of the most common drug hypersensitivity reactions, such as allergic reactions to acetylsalicylic acid, non-steroidal anti-inflammatory drugs, and some antibiotics. The article is well presented and provides a simple table that clearly illustrates the potential processes that underlie the development of some drug allergies. This is a must-read for any clinicians or researchers working in the area of drug allergies.

Prof Jacques Bouchard

A REVIEW OF DRUG ALLERGIES: DIAGNOSIS AND MANAGEMENT

***Arvind Bamanikar**

Department of Medicine, Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India
**Correspondence to bamanikar@gmail.com*

Disclosure: The author has declared no conflicts of interest.
Received: 21.02.16 **Accepted:** 23.06.16
Citation: EMJ Allergy Immunol. 2016;1[1]:52-57

ABSTRACT

Drug allergy, in clinical practice, includes a wide spectrum of immunologically-mediated hypersensitivity reactions, also called drug hypersensitivity reactions (DHRs). It can present with diverse clinical manifestations and can have various underlying pathophysiological mechanisms. Drug allergies often require a number of investigations and this can sometimes lead to a delay in treatment of the original disease. Drug allergy can affect quality of life too. It does contribute to significant morbidity and even mortality which is largely avoidable. Meticulous relevant details in history and clinical examination are often rewarding in arriving at the correct diagnosis. Patients with underlying chronic airways diseases such as asthma may benefit from skin tests and graded allergen challenges. Procedures to induce drug tolerance are sometimes helpful in the drug allergy management. Likelihood of cross-reactivity among drugs should be taken into account while choosing alternative medication. Measures for drug allergy reactions are mostly supportive and usually include topical corticosteroids and oral antihistamines. However, systemic corticosteroids may be required in severe DHRs along with adrenaline in the event of anaphylaxis. The most effective approach towards the problem of 'drug allergy' is discontinuing or avoiding the offending culprit. Procedures to induce drug tolerance may be considered as a temporary measure toward tolerance to the offending drug if there is no alternative available.

This article aims to provide a simple clinical review of drug allergy and plan of action for the diagnosis as well as management of some of the most common DHRs, such as allergies to acetylsalicylic acid, non-steroidal anti-inflammatory drugs, penicillins, sulpha drugs, cephalosporin, contrast media in imaging procedures, anaesthetic agents, and vaccines.

Keywords: Allergy, cephalosporins, drug hypersensitivity reactions (DHRs), penicillins.

INTRODUCTION

Drug allergy or drug hypersensitivity reactions (DHRs) consist of any harmful or unintended reactions to a drug that are known to occur at doses used for prevention, diagnosis, or treatment.[1] DHRs or 'adverse drug reactions' are the terms often used interchangeably and essentially consist of adverse effects of drugs on the body which resemble allergic reactions in clinical practice. Drug allergies are specified adverse reactions, for which a definite immunological mechanism (either drug-specific antibody or T cell) is demonstrated.[2] DHRs are common in clinical practice and reported in up to 15–25% of patients, with serious reactions occurring in 7–13% of these patients.[3,4]

DHRs are either predictable reactions that may occur in anyone (Type A) or unpredictable that will occur in only susceptible individuals (Type B). Unpredictable reactions are estimated to occur in approximately 20–25% of patients who experience DHRs,[1,5,6] while drug allergy accounts for approximately 5–10% of all DHRs.[7] Clinically, DHRs are classified as immediate types which typically occur within 1–6 hours after the last drug administration. They manifest in the form of urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm, gastrointestinal symptoms (nausea, vomiting, diarrhoea, abdominal pain), anaphylaxis, and anaphylactic shock. Non-immediate DHRs occur as delayed onset urticaria, maculopapular type eruptions, fixed drug eruptions, vasculitis, toxic epidermal necrolysis (TEN), Stevens–Johnson syndrome (SJS), drug reaction with eosinophilia and systemic symptoms (DRESS), acute generalised exanthematous pustulosis, symmetrical drug-related intertriginous, and flexural exanthemas. In these reactions, internal organs can be affected either alone or with skin manifestations (DRESS, vasculitis) and include hepatitis, acute kidney injury and acute renal failure, pneumonitis, anaemia, neutropenia, and thrombocytopenia; they may occur as early as 1 hour after the initial drug administration.[1] Predictable drug reactions are the most common type of DHR and are usually dose dependent and related to the known side effects, overdose, and drug interactions. Unpredictable reactions occur in an estimate of 20–25% of patients who experience DHRs; generally, these reactions are not related to the pharmacologic actions of the drug.[1,5,6]

Drug allergy is classified by the Gell and Coombs classification of human hypersensitivity, illustrated in Table 1 below.[2] This classification system consists of immediate-type reactions (immunoglobulin [Ig]-E mediated), cytotoxic reactions (IgG/M-mediated), immune complex reactions, and delayed-type hypersensitivity reactions (cellular immune-mediated).[8-10]

Table 1: Classification of drug allergies.

Reaction type	Type of immune response	Pathophysiology	Clinical symptoms	Typical chronology of the reaction
I	IgE	Mast cell and basophil degranulation	Anaphylactic shock, angioedema, urticaria, bronchospasm	Within 1–6 hours after the last intake of the drug
II	IgG and complement	IgG and complement-dependent	Cytopenia	5–15 days after the start of the offending drug
III	IgM or IgG and complement or FcR	Cytotoxicity and deposition of immune complexes	Serum sickness, urticaria	7–8 days
			Vasculitis	7–21 days after the start of the offending drug
IVa	Th1 (IFN-c)	Monocytic inflammation	Eczema	1–21 days after the start of the offending drug
IVb	Th2 (IL-4 and IL-5)	Eosinophilic inflammation	Maculopapular exanthema	1 to several days after the start of the offending drug
			DRESS	2–6 weeks after the start of the offending drug

Table 2 continued.

Reaction type	Type of immune response	Pathophysiology	Clinical symptoms	Typical chronology of the reaction
IVc	Cytotoxic T cells (perforin, granzyme B, FasL)	Keratinocyte death mediated by Tcells CD4 or CD8	Maculopapular exanthema	1–2 days after the start of the offending drug for fixed drug eruption
			SJS/TEN, pustular exanthema	4–28 days after the start of the offending drug
IVd	T cells (IL-8/CXCL8)	Neutrophilic inflammation	AGEP	Typically, 1–2 days after the start of the offending drug (but could be longer)

Ig: immunoglobulin; FcR: fc receptor; FasL: fas ligand; SJS: Stevens–Johnson syndrome; Th1: T helper Type 1; Th2: T helper Type 2; DRESS: drug reaction with eosinophilia and systemic symptoms; TEN: toxic epidermal necrolysis; IFN-c: interferon-c; IL: interleukin; AGEP: acute generalised exanthematous pustulosis.

The pharmacologic interaction with immune receptors ('p-i concept') has also been proposed in addition to drug hypersensitivity classification. In this p-i concept scheme, a drug binds non-covalently to a T cell receptor.

This leads to an immune response via interaction with a major histocompatibility receptor, however no sensitisation is involved because the stimulation of memory and effector T cells happens directly, which is similar to the concept of superantigens.[4,5]

Unlike immune-mediated drug reactions, pseudoallergic reactions are not associated with the production of antibodies or sensitised T cells, but are often clinically indistinguishable from DHRs. Non-steroidal anti-inflammatory drugs (NSAIDs), and angiotensin-converting enzyme inhibitors are common causes of these non-allergic reactions.[6,11,12]

RISK FACTORS

An increased risk of developing a drug allergy is associated with host factors (e.g. age, gender, genetic polymorphisms, certain viral infections) and drug-related factors (e.g. frequency of exposure, route of administration, molecular weight). Drug allergy commonly occurs in young and middle-aged adults, and is more often found in women than men. Human leukocyte antigen, HIV, and the Epstein–Barr virus have also been linked to increased risk of building immunologic response and reactions to drugs. Genetic polymorphisms in drug metabolism influence susceptibility to drug allergy. Drug administration via topical, intramuscular, and intravenous methods are more likely to cause allergic drug reactions than oral administration while intravenous administration is associated with more severe reactions. Prolonged high doses or frequent use of drug are more likely to develop hypersensitivity reactions than a single large dose. In addition, large macromolecular drugs (e.g. insulin or horse antisera) or drugs that form haptens (bind to tissue or blood proteins and elicit an immune response), such as penicillin, are also associated with a greater likelihood of causing hypersensitivity reactions.[6,8,13-16]

Diagnosis

A thorough history and identification of clinical signs and symptoms that are compatible with drug-induced allergic reactions form the main diagnostic method. Diagnostic tests include skin testing and if warranted, graded challenges, as well as the induction of drug tolerance procedures which are advocated on the basis of history and physical examination results. A history should include details of all prescription and over-the-counter drugs taken by the patient. It is often rewarding to know the dates of drug intake, formulation of the drug(s) taken, dosage, and route of drug intake. Clinical symptoms and signs developed with their timing and duration in relation to drug intake, as well as previous drug exposures and reactions, are also recorded.[1,5,7,16] A thorough and careful physical examination can help to define possible mechanisms underlying the reaction and guide subsequent investigations and diagnostic testing. The most common clinical manifestations of drug allergy are seen on the skin[1,7,12] and sometimes mucous membranes.

Table 2: Differential diagnosis of drug allergy and a list of conditions to consider.

IgE-mediated drug allergy	Non-IgE-mediated reactions
(Urticaria, angioedema, anaphylaxis, bronchospasm): • Carcinoid syndrome • Insect bites/stings • Mastocytosis • Asthma • Food allergy • Scombroid fish poisoning • Latex allergy • Infection (EBV, hepatitis A, B, and C, gastrointestinal parasites)	(Exanthema, DRESS, SJS, TEN): • Acute graft-versus-host disease • Kawasaki disease • Still's disease • Psoriasis • Insect bites/stings • Viral infection • Streptococcal infection

IgE: immunoglobulin E; EBV: Epstein–Barr virus; SJS: Stevens–Johnson syndrome; TEN: toxic epidermal necrolysis; DRESS: drug rash with eosinophilia and systemic symptoms.

The most common cutaneous manifestation is generalised exanthema (as a maculopapular rash), which appears between a few days and 3 weeks after drug exposure, usually starting on the trunk and eventually spreading to the limbs. Urticaria and angioedema that result from both IgE-mediated and non-IgE-mediated mechanisms are also common. SJS and TEN are the most severe forms of drug reactions. SJS usually begins with a maculopapular rash that often progresses to bullae, mucous membrane ulcerations, conjunctivitis, fever, sore throat, and fatigue. TEN is a rare condition with similarity to SJS, however TEN causes a large portion of the epidermis to denude from the layers below, leading to extensive skin sloughing and a scalded skin appearance. The most common culprit for SJS and TEN are sulphonamides and as such should be avoided by the patient in the future.[1] Since the clinical manifestations of drug allergy are mostly variable, it is necessary to exclude other conditions that can mimic drug-induced allergic reactions. Table 2 shows conditions in the differential diagnosis of drug allergy.[6]

DIAGNOSTIC TESTS

IgE-mediated (Type I) reactions can be diagnosed by diagnostic procedures such as skin-prick testing and intradermal injection of the allergen. Skin testing guidelines are standardised for penicillin. The usefulness of skin tests for local anaesthetic muscle relaxants are limited due to negative results being more frequent, but they are very sensitive for insulin or monoclonal antibodies. On the contrary, a negative test result is useful for ruling out penicillin allergy and high molecular weight proteins, although it does not exclusively rule out the presence of a specific allergen. Serum-specific IgE tests are available for some drugs and are often expensive and less sensitive than skin tests. It is necessary to remember that most of these *in vitro* tests are not optimally validated for drug allergy testing.[1,16]

Patch testing involves placing the allergen (at non-irritant concentrations) on the patient's back for 48 hours and then assessing any reactions. This type of testing is useful for the diagnosis of various delayed (Type IV) skin reactions, but is often not helpful for the diagnosis of SJS or TEN.[11,12,16,17] The measurement of histamine and tryptase levels in the blood have been useful in confirming acute IgE-mediated reactions, particularly anaphylaxis; however, negative results do not necessarily rule out acute allergic reactions. Haemolytic anaemia may also be confirmed with a positive direct and/or indirect Coombs test.[1,12,16]

The potential role of the basophil activation test (the quantification of basophil activation by flow cytometry) in the diagnosis of drug allergy has been recently studied because basophils are involved in both immune-mediated and non-immune-mediated reactions. Although some evidence suggests that the test is useful for evaluating possible allergies to β-lactam antibiotics, NSAIDs, and muscle relaxants, further confirmatory studies are needed before it is widely accepted as a diagnostic tool.[1,18,19]

MANAGEMENT OF COMMON DRUG ALLERGIES

The most effective strategy is to avoid or discontinue the offending drug. If an alternative medication is available (with unrelated chemical structures), cross-reactivity among drugs should be taken into consideration when substituting the agent.[1,12] Additional therapies, for example topical corticosteroids and oral antihistamines, may improve cutaneous symptoms. In the event of anaphylaxis, adrenaline is administered by intramuscular injection into the lateral thigh. Systemic corticosteroids are normally used to treat severe systemic reactions but should not be given prior to or as a substitute to adrenaline in the treatment of anaphylaxis.

Prevention of Future Reactions

Prevention of future reactions is an important part of patient management. Allergy bracelets/necklaces should be considered, particularly if the patient has a history of severe forms of drug-induced allergic reactions.[16] Procedures to induce drug tolerance are expected to modify a patient's immunologic or non-immunologic response to a drug temporarily, through the administration of incremental doses of the drug. Most regimens begin with a very dilute concentration of the drug, then the dose is increased every 15–20 minutes or so, usually with double the dosage until a full therapeutic dose has been administered after 3–8 hours. Drug tolerance-induction procedures and graded challenges are potentially harmful and should only be performed by experienced personnel in facilities with resuscitative equipment readily available.[1,20]

SPECIFIC DRUG REACTIONS

Acetylsalicylic Acid and Non-Steroidal Anti-Inflammatory Drugs

Acetylsalicylic acid (ASA) and NSAIDs can cause urticaria, angioedema, and anaphylaxis. Patients suffering from chronic airway diseases such as asthma, rhinitis, and sinusitis, may react to ASA and NSAIDs that inhibit cyclo-oxygenase (COX)-1. The management of these patients involves avoidance of aspirin and NSAIDs, as well as aggressive treatment of the underlying respiratory disorder. Selective COX-2 inhibitors almost never cause reactions and can typically be taken safely by patients with ASA/NSAID allergy.

Sulphonamides

Sulphonamide antibiotics are often associated with delayed cutaneous maculopapular eruptions, SJS, and TEN. Patients infected with HIV are at increased risk of developing cutaneous reactions to sulphonamide antibiotics. Trimethoprim-sulfamethoxazole is the drug of choice for the treatment of a number of HIV-associated infections, therefore many HIV-positive patients with a history of adverse reactions to sulphonamides still require treatment with this antibiotic. Induction of drug tolerance procedures can be used to safely administer trimethoprim-sulfamethoxazole to HIV-positive patients with a history of adverse reactions to the antibiotic.

Penicillin

Penicillin is the most frequent drug allergy, affecting approximately 10% of patients. Ideally, management of the patient with penicillin allergy should include penicillin skin testing. Approximately 90% of patients have negative penicillin skin test responses and can safely receive cephalosporins as well as other β-lactam agents. Carbapenems may be administered as a graded challenge after prophylactic skin tests.[21,22] Monobactams are generally well-tolerated by patients with penicillin allergy, except if they have had an allergic reaction to ceftazidime.[23-25] Second or third-generation cephalosporins may also be considered, since the degree of cross-reactivity with these agents and penicillin has been shown to be lower than with first-generation agents.[1,26]

Cephalosporins

Cephalosporins can commonly cause maculopapular rashes and drug fever; urticaria is less common and anaphylaxis is rarely seen.[26] In subjects with cephalosporin allergy, there is limited cross-reactivity on immunological testing between second and third-generation cephalosporins and penicillins, but this does not necessarily indicate clinical reactivity.[26] If skin testing is positive and no alternative drug exists, induction of drug tolerance procedures may be attempted.[1,7]

Radiocontrast Media

Pseudoallergic and allergic reactions to radiocontrast media can usually be prevented through the use of pre-treatment with medication, which includes oral corticosteroids and H_1-antihistamines. Agents with low osmolarity are better for use in such circumstances.[1,7]

Anaesthetics

True allergic reactions to local or general anaesthetics are very rare; reactions are usually due to preservatives in the medication or due to adrenaline. Allergy to inhaled anaesthetics is not reported in the current literature.[27]

Vaccines

The seasonal flu vaccine or yellow fever vaccine may contain small quantities of egg protein, for which allergic response may be evoked.

CONCLUSIONS

Diagnosis relies on a meticulous history and physical examination. Skin testing, and if warranted graded challenges of allergen, and induction of drug tolerance procedures may be required in some instances. The mainstay of treatment for drug allergy is avoidance of the offending drug. When available, alternative medications with unrelated chemical structures should be substituted. Cross-reactivity among drugs should be taken into consideration when choosing alternative medications. If a particular drug to which the patient is allergic is indicated and there is no suitable alternative, procedures of induction of drug tolerance may be considered for producing temporary tolerance to the drug.

REFERENCES

1. Khan DA, Solensky R. Drug allergy. J Allergy Clin Immunol. 2010;125(2 Suppl 2): S126-37.

2. Demoly P et al. International Consensus on drug allergy. Allergy. 2014;69(4): 420-37.

3. Schmid DA et al. Transfection of drug-specific T-cell receptors into hybridoma cells: tools to monitor drug interaction with T-cell receptors and evaluate cross-reactivity to related compounds. Mol Pharmacol. 2006;70(1):356-65.

4. Sieben S et al. Delayed-type hypersensitivity reaction to paraphenylenediamine is mediated by 2 different pathways of antigen recognition by specific alphabeta human T-cell clones. J Allergy Clin Immunol. 2002;109(6): 1005-11.

5. Vervloet D, Durham S. Adverse reactions to drugs. BMJ. 1998;316(7143):1511-4.

6. Sylvia LM, "Drug allergy, Pseudo allergy, and Cutaneous diseases," Tisdale JE, Miller DA (eds.), Drug-Induced Diseases: Prevention, Detection, and Management (2010), Bethesda: American Society of Health-System Pharmacists, pp.21-97.

7. Riedl MA, Castillas AM. Adverse drug reactions: types and treatment options. Am Fam Physician. 2003;68(9):1781-90.

8. Pichler WJ. Delayed drug hypersensitivity reactions. Ann Intern Med. 2003;139(8):683-93.

9. Posadas SJ, Pichler WJ. Delayed drug hypersensitivity reactions - new concepts. Clin Exp Allergy. 2007;37(7):989-99.

10. Friedmann PS, Ardern-Jones M. Patch testing in drug allergy. Curr Opin Allergy Clin Immunol. 2010;10(4):291-6.

11. Schnyder B. Approach to the patient with drug allergy. Immunol Allergy Clin North Am. 2009;29(3):405-18.

12. Barranco P, Lopez-Serrano MC. General and epidemiological aspects of allergic drug reactions. Clin Exp Allergy. 1998;28(Suppl 4):61-2.

13. Adkinson NF Jr. Risk factors for drug allergy. J Allergy Clin Immunol. 1984; 74(4 Pt 2):567-72.

14. Pirmohamed M, Park BK. Adverse drug reactions: back to the future. Br J Clin Pharmacol. 2003;55(5):486-92.

15. Mirakian R et al.; BSACI. BSACI guidelines for the management of drug allergy. Clin Exp Allergy. 2009;39(1):43-61.

16. Barbaud A. Drug patch testing in systemic cutaneous drug allergy. Toxicology. 2005;209(2):209-16.

17. Sanz ML et al. Cellular tests in the diagnosis of drug hypersensitivity. Curr Pharm Des. 2008;14(27):2803-8.

18. Hausmann OV et al. The basophil activation test in immediate-type drug allergy. Immunol Allergy Clin North Am. 2009;29(3):555-66.

19. Aberer W, Kranke B. Provocation tests in drug hypersensitivity. Immunol Allergy Clin North Am. 2009;29(3):567-84.

20. Atanaskovic-Markovic M et al. Tolerability of imipenem in children with IgE-mediated hypersensitivity to penicillins. J Allergy Clin Immunol. 2009;124(1):167-9.

21. Frumin J, Gallagher JC. Allergic cross-sensitivity between penicillin, carbapenem, and monobactam antibiotics: what are the chances? Ann Pharmacother. 2009;43(2):304-15.

22. Saxon A et al. Imipenem cross-reactivity with penicillin in humans. J Allergy Clin Immunol. 1988;82(2):213-7.

23. Saxon A et al. Lack of cross-reactivity between aztreonam, a monobactam antibiotic, and penicillin in penicillin-allergic subjects. J Infect Dis. 1984;149(1): 16-22.

24. Adkinson NF Jr. Immunogenicity and cross-allergenicity of aztreonam. Am J Med. 1990;88(3C):12S-15S.

25. Kelkar PS, Li JT. Cephalosporin allergy. N Engl J Med. 2001;345(11):804-9.

26. Romano A et al. IgE-mediated hypersensitivity to cephalosporins: cross-reactivity and tolerability of penicillins, monobactams, and carbapenems. J Allergy Clin Immunol. 2010;126(5):994-9.

27. Warrington R, Silviu-Dan F. Drug allergy. Allergy Asthma Clin Immunol. 2011;7 Suppl 1:S10.

ATOPIC DERMATITIS: THE NEED FOR A SUB-SAHARAN PERSPECTIVE

***Richard E. Paul,[1,2] Anavaj Sakuntabhai[1,2]**

1. Institut Pasteur, Functional Genetics of Infectious Diseases Unit, Paris, France
2. Centre National de la Recherche Scientifique, Unité de Recherche Associée, Paris, France
**Correspondence to richard.paul@pasteur.fr*

Disclosure: The authors have declared no conflicts of interest.
Received: 18.03.16 **Accepted:** 24.06.16
Citation: EMJ Allergy Immunol. 2016;1[1]:58-64.

ABSTRACT

Atopic dermatitis (AD) is one of the most common non-infectious diseases in the world. For over two decades there has been considerable mobilisation to create a robust framework to address this global problem (the International Study of Asthma and Allergies in Childhood [ISAAC] consortium). However, information about Sub-Saharan Africa remains sparse, likely reflecting the increased focus placed on infectious diseases. However, this region harbours the greatest environmental and genetic diversity and thus offers enormous potential for understanding the differential environmental impact on human populations predisposed to allergic diseases. Moreover, it is increasingly clear that many pathologies share the same genetic determinants and this spans both non-infectious and infectious diseases. In this review, we discuss the comparative genetics of the allergic diseases and then expand into infectious diseases, notably malaria. We discuss the considerable overlap in the identified genetic determinants of AD and malaria and develop a hypothesis based on the importance of saliva from mosquito bites, arguably the most prevalent allergen in the region. Following the completion of the first phase of the African Genome project, we stress the significance of more focus on allergic diseases in the region, which will certainly generate an abundance of novel insight into the environmental and genetic determinants of allergy and may also contribute to our understanding of arthropod-borne infectious diseases.

Keywords: Atopic dermatitis (AD), malaria, Africa, human genetics, mosquito bites.

INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder that is often associated, either sequentially or concomitantly, with asthma and allergic rhinoconjunctivitis. The global significance of these non-communicable allergic diseases led to the establishment of the International Study of Asthma and Allergies in Childhood (ISAAC) consortium in the 1990s.[1] The aim of ISAAC was to establish baseline rates of asthma, rhinitis, and eczema to enable global comparisons, assessment of future trends, and to provide a framework for aetiological research into lifestyle, environmental, genetic, and medical care factors affecting these diseases.[2] However, at its inception (Phase 1), only three Sub-Saharan countries (Nigeria, Kenya, and Ethiopia) were included, likely because of the general focus on infectious diseases that impose the major health burden in this region. In Phases 2 and 3, several other countries joined and yet information on the allergy status remains sparse. Point prevalent studies were carried out in 12 Sub-Saharan countries, almost all in children aged 13–14 years old and in urban settings. Rates ranged from 4.7% in the Sudan to 19% in Ethiopia, with a mean of 14.5%.[3,4] Only two studies have however, examined AD trends over time (Phase 1 in 1995 and Phase 3 in 2001–2). In Nigeria there was an increase in lifetime prevalence of itchy rash (7.7% to 10.2%) in children 6–7 years old but a strong decrease (26.1% to 18%) in the 13–14 year olds. Physician-diagnosed AD decreased significantly in both age groups.[5] By contrast, the Kenyan study on 13–14 year olds in rural cohorts revealed a similar increase in AD to that found in the most studied African countries,[6] Morocco and South Africa;[7] lifetime itchy rash increased from 23.8% to 28.5% and physician-diagnosed AD from 13.9% to 28.5%.

The data on African populations is thus sparse and needs to be improved, not only for the specific public health burden that allergy may impose on the region, but also because studies in Africa may be pertinent for allergy worldwide. AD is very prevalent in African-American children,[8] highlighting ethnic differential susceptibility. The human genome was shaped by the selective forces present prior to the early human migrations out of Africa, when infectious diseases were likely of significant importance. Such selection of strong pro-inflammatory responses by infectious diseases may have negative consequences in the form of allergic diseases worldwide today.[9] The development of AD has been associated with genetic polymorphisms, skin barrier dysfunction, environmental exposures, and host immune dysregulation. The pathophysiology of AD may be associated with a child's sensitisation to specific environmental or food allergens in association with skin barrier dysfunction. Africa is an important region to study complex diseases such as AD, because of its high genetic diversity, large variation in climate, and environmental conditions.[10] The importance of both human genetic and environmental determinants of AD makes the dearth of information from Sub-Saharan Africa all the more frustrating. Following the completion of the first detailed characterisation of the African genome, it is now possible to benefit from particularities of the African genome and environment to address genetic and environmental factors that contribute to complex multifactorial diseases.[11] Sub-Saharan Africa has a very particular environment and one that needs special attention, largely because of the high incidence of infectious diseases. As we will discuss here, the likelihood that there are shared human genetic determinants underlying infectious and non-infectious diseases should generate an increased capacity to identify genes of interest. Furthermore, infectious agents represent a facet of the environment that is rarely considered, likely because of the relatively low prevalence in societies where the majority of allergic disease research is carried out.

COMPARATIVE GENETICS OF ATOPIC DERMATITIS AND ALLERGIC DISEASES

Genome-Wide Association Studies

It is widely recognised that common multifactorial diseases are caused by multiple genetic and environmental factors and interactions among all these factors. With the development of genotyping technologies, genome-wide association studies (GWAS) have become the method of choice to identify complex disease associated genes using single nucleotide polymorphisms (SNPs) as biomarkers.[12] One of the interesting features of GWAS is that the same loci have been found to be associated with several diseases (e.g. cancers, cardiovascular diseases, autoimmune diseases), suggesting that genes with a pleiotropic effect may be more frequent than anticipated and may play a key role in basic pathophysiological mechanisms underlying a number of diseases. AD is often associated with asthma and allergic rhinitis, and it is likely that these three diseases share common genetic and environmental determinants.[13] The identification of pleiotropic genes that are likely to influence master regulators of biological processes is therefore of major importance. Studying together diseases that are suspected to share common genetic determinants can facilitate the characterisation of such genes.

There have been several GWAS studies of AD in European, Japanese, and Chinese populations.[14-16] A total of 19 susceptibility genes have been identified, suggesting that AD is influenced by genes involved in epidermal barrier functions (e.g. filaggrin [FLG]) and innate and adaptive immunity amongst others (notably, interleukin [IL]-1 signalling and nerve growth factor signalling).[17] The adaptive immune response in AD is associated with increased expression of the T helper 2 (Th2) cell cytokines, IL-4 and IL-13.[18] Recent studies have shown a significantly greater number of *IL-4*, *IL-5*, and *IL-13* messenger RNA (mRNA)-expressing cells in acute skin lesions, and an increased number of Th2 cells expressing *IL-4* and *IL-13* mRNA in unaffected skin of AD patients.[19] Allergen-specific immunoglobulin (Ig)E plays a crucial role in the pathogenesis of allergic diseases by binding allergens and initiating immunological responses. Approximately 80% of patients with AD have elevated serum IgE and/or immediate skin test reactivity to allergens.[18] There is a strong genetic contribution to the variability of the total IgE level. Several GWAS for total IgE levels have identified two associated susceptibility loci, 1q23 and 5q31, in European and mixed populations including African-American and Hispanic individuals.[20-22] In particular, a functional SNP in 1q23 significantly influenced the cell surface expression of Fc fragment of IgE, high-affinity receptor (FCεR1A) on basophils, and the regulatory mechanism of FCεR1A expression via GATA2.[20]

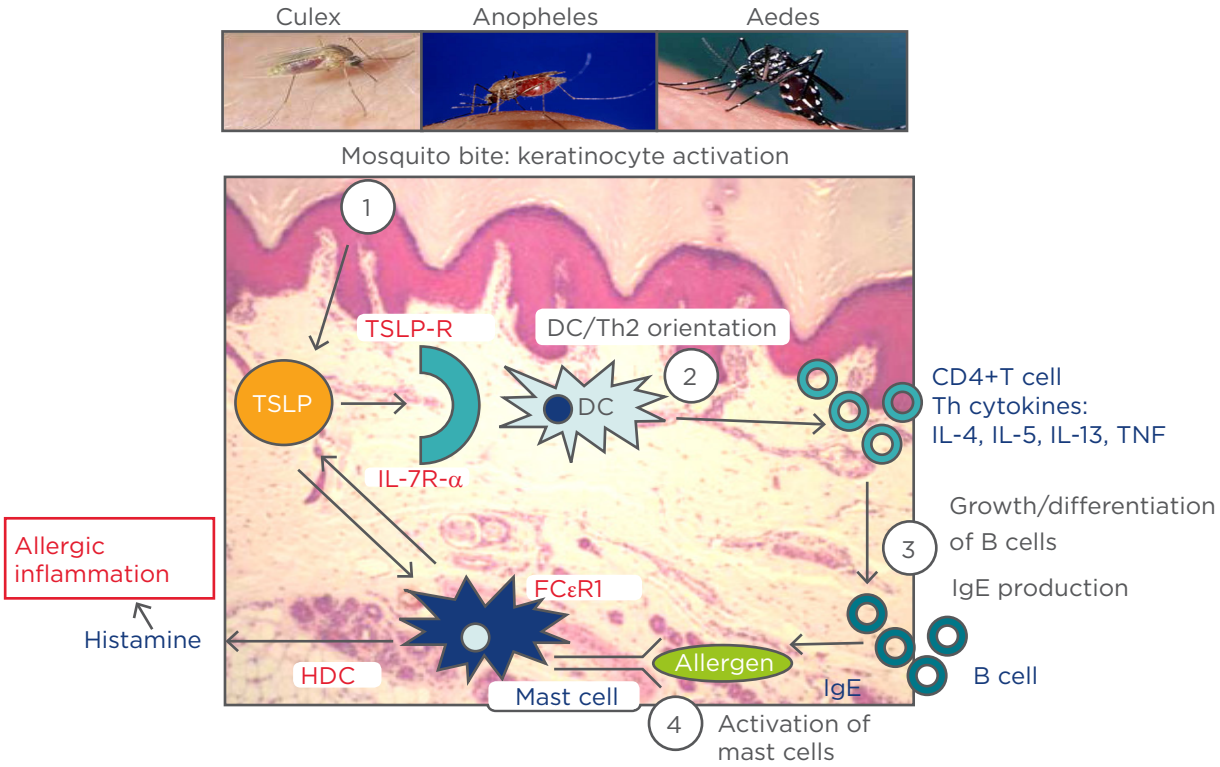


Figure 1: A schematic representation of the proposed key role played by mosquito bites in allergic inflammation.

TSLP: thymic stromal lymphopoietin; TSLP-R: thymic stromal lymphopoietin receptor; IL: interleukin; IL-7R-α: interleukin-7 receptor alpha; DC: dendritic cell; HDC: histidine decarboxylase; TNF: tumour necrosis factor; IgE: immunoglobulin E; Th2: T helper Type 2; FcεRI: high-affinity IgE receptor.

Recent GWAS have revealed a number of susceptibility loci shared by several allergic diseases and phenotypes for bronchial asthma, allergic rhinitis, the number of eosinophils, allergic sensitisation, and total IgE levels. Moreover, there are significant genome-wide susceptibility regions overlapping AD and other allergy-related phenotypes. Of particular interest is the susceptibility loci 5q31.1 that contains *RAD50* and *IL-13*, amongst others.[23-25] The IL-4, IL-13, STAT6 pathway has been reported to be associated with IgE and asthma and the *IL-13* gene is the only one found significantly associated with both asthma and IgE by the GABRIEL consortium GWAS.[26] The association of *STAT6* with IgE reported by a previous GWAS was replicated by the GABRIEL consortium.[20,26]

Filaggrin and Thymic Stromal Lymphopoietin

FLG plays an important role in the skin's barrier function and *FLG* loss-of-function mutations have been associated with an increased risk of developing persistent AD.[27] FLG binds to and condenses the keratin cytoskeleton to form tight bundles, flattening and strengthening the cells to create a strong barrier. Subsequent proteolysis leads to further degradation into hygroscopic amino acids, which constitute one element of natural moisturising factor. A defect in the production of FLG creates a more porous skin surface, resulting in epicutaneous sensitisation to environmental allergens and activating the host immune system, thereby resulting in local inflammation, pruritus, and visible skin lesions. *FLG* loss-of-function mutations are more commonly found in individuals of European and Asian ancestry compared with those of African ancestry.[28] Indeed, of the four most common European mutations (*R501X*, *2282del4*, *R2447X*, and *S3247X*), at least one was found in 27.5% of whites compared with 5.8% of African-Americans.[29]

In addition to FLG, the *thymic stromal lymphopoietin* (*TSLP*) gene was found to be located adjacent to a region (5q11.1) associated with AD. TSLP is a cytokine produced by epithelial cells (such as keratinocytes and bronchial epithelial cells) and has an important role in

conditioning dendritic cell (DC) maturation. TSLP-activated DCs induce inflammatory Th2 cells that produce the classical Th2 cytokines IL-4, IL-5, and IL-13, in addition to large amounts of tumour necrosis factor (TNF). Interestingly, mast cells activated by IgE binding to the high-affinity IgE receptor (FcεRI) expressed high levels of TSLP.

TSLP promotes Th2 cell responses associated with immunity to some helminth parasites and the pathogenesis of a number of inflammatory diseases such as AD. Indeed, increased expression of TSLP has been strongly associated with AD as well as other allergic diseases, including asthma and allergic rhinitis.[30-32] Although FLG protein contributes to the skin barrier, TSLP expression occurs after antigen sensitisation through a disrupted skin barrier and subsequently promotes the immune responses resulting in inflammation that leads to AD. In AD, epithelial cells markedly increase TSLP expression in response to inflammation, leading to macrophage activation, DC maturation, induction of inflammatory Th2 cells, and eventual chemoattraction of a suite of innate immune cells, such as eosinophils, neutrophils, and mast cells, with resulting pathological effects. Genetic variants resulting in diminished TSLP activity were protective against persistent AD even in individuals with the *FLG* loss-of-function mutation.[32,33] Therefore, TSLP is a master regulator of allergic inflammation in the skin and can significantly influence the AD phenotype.

INFECTIOUS DISEASES AND ALLERGIC RISK

There are many examples of infectious agents that impact upon allergic risk. Severe respiratory syncytial virus infection in infants increases the risk of allergic rhinoconjunctivitis and allergic asthma.[34] Measles,[35] hepatitis A, and tuberculosis seemingly reduce atopy,[36] whereas HIV infection is associated with increased risk of asthma and AD.[37] Helminth infections are considered to reduce clinical expression of an atopic tendency.[38] The relationship of *Staphylococcus aureus* and patients with AD is unusual in that *S. aureus* colonisation is both a cause and a consequence of allergic skin inflammation.[39] The allergic skin inflammation associated with AD leads to increased colonisation by *S. aureus* notably because of skin barrier dysfunction and ineffective innate immune responses. *S. aureus* in turn produces exotoxins (superantigens) that can penetrate the skin barrier and contribute to the persistence and exacerbation of allergic skin inflammation.[40] Of particular interest are pathogens that are transmitted by haematophagous arthropods (mosquitoes, sandflies, ticks) because of the allergenic nature of their saliva.

Lessons from Malaria

Although the human genetic susceptibility to malaria has classically focussed on the haemoglobinopathies (sickle cell, beta and alpha thalassaemia), there is an unusual relationship between allergy and the mosquito-borne protozoan parasite, *Plasmodium falciparum*, the aetiological agent of lethal tertian human malaria. Several lines of evidence support the concept that susceptibility to malaria and atopy may be related to the same immunological defect and/or influence one another.

Impact of allergy on malaria infection outcome

At the phenotypic level, there is evidence that allergy impacts upon risk of malaria. In Ethiopia, atopic children had a higher prevalence of malaria attacks,[41] while in Tanzania maternal malaria had a protective effect on wheezing in 4-year-old children.[42] AD, and to a lesser extent asthma, were found to increase the risk of persistent clinical malaria episodes, suggesting interference with the developmental of clinical immunity to the parasite.[43] Finally, there is some evidence that IgE levels are elevated during malaria infections and that the mosquitoes as well as the parasites contribute to this state and thus may exacerbate the immunological environment.[44,45] Notably, individuals classified as having moderate or severe symptoms of AD (assessed using the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for sub-tropical countries) had significantly higher specific IgE levels against *Culex, Aedes*, and *Anopheles* salivary gland extract.[43,45]

Role of immunoglobulin E

Several findings suggest that IgE could play a detrimental role during malaria disease development. This was supported by data showing that IgE levels were much reduced amongst patients with uncomplicated malaria in comparison with those suffering from severe malaria.[46] Furthermore, immunohistological studies on brain sections revealed the presence of IgE deposits in

brain microvessels and on infected erythrocytes from cerebral malaria patients as well as in placentas infected with *P. falciparum*.[47] Various immune complexes, which consist of either IgE and antigen aggregates or IgE with IgG anti-IgE, could bind to Fc receptors expressed on monocytes that become activated, giving rise to TNF-α secretion.[46] In addition, IgE levels were found to be higher in cerebral *P. falciparum* malaria when compared with uncomplicated malaria.[48]

Shared genetics

A mouse model (NC/Jic) for human atopic disease was found to be susceptible to murine malaria and a major quantitative trait locus (derm1) for atopic disease mapped close to the region controlling parasitaemia (char1 or pymr) on mouse chromosome 9.[49,50] Genome scan linkage studies revealed four regions linked to malaria phenotypes. 5p15–p13 and 13q13–q22 were found to be associated with clinical malaria and 5q31–q33 and 12q21–q23 with parasite density.[51] While these regions are extensive and contain many putative candidate genes, it is remarkable that all four regions overlap with those that have been previously identified to be involved in asthma/atopy and especially IgE levels,[52,53] suggesting that common mechanisms may be involved between both pathogenic mechanisms. Moreover, a logarithm of the odds (LOD) score >1 was found using both analytical methods for the region containing the high-affinity receptor for IgE (FcεR1B on 11q12.1). The regions 13q13–q22 and 12q21–q23 contain genes known to increase total serum IgE levels, namely *PHF1153* and *STAT6* genes.[54] The Stat6 protein plays a central role in exerting IL-4 mediated biological responses. The *IL-4* gene is located on 5q31 together with important cytokines (IL-13, IL-5, IL-9), a region found to be linked to parasite density in this and previous studies and consistently reported to be linked to asthma-related phenotypes.[55] The cluster of cytokines on 5q31, namely IL-4 and IL-13, are major cytokines promoting the differentiation of Th2 cells that are involved in the allergic response. Genetic variants in *IL-4* and *IL-13* genes on 5q31 and *STAT6* on 12q21 have been consistently found associated with total IgE levels and this is confirmed by GWAS of this phenotype.[20,56]

Of particular interest is the 5p13 linkage region; this includes several genes involved in innate immunity and notably the interleukin-7 receptor (*IL-7R*). IL-7R plays a crucial role in signal transduction of TSLP. TSLP receptor chain alone binds to TSLP at low affinity. A combination of TSLP receptor and IL-7R-α chain results in high-affinity binding. Fine mapping of this 5p region using Illumina® GoldenGate revealed an association with *IL-7R* at a LOD score of ~5 (unpublished data).

MOSQUITO BITES, ATOPIC DERMATITIS, AND MALARIA: AN UNDERLYING MECHANISM

It is recognised that the type of immune balance driven by the parasite operates at a very early stage post parasite delivery. The response of sentinel cells, such as DCs, thus determines the evolution of the immune response and can lead to protection, tolerance, or immunopathology. Mosquito saliva contains pharmacologically active proteins and peptides[57] which provoke a localised allergic reaction in the skin, and injection of saliva into the skin during a mosquito bite induces the production of IgE and IgG antibodies as well as dermal hypersensitivity reactions.[58] This suggests that the saliva can orientate the immune response towards a Th2 profile.

The combination of this information leads us to propose a mechanism underlying the observed shared associations between AD and malaria (Figure 1). Mosquito bites will lead to activation of keratinocytes resulting in localised production of TSLP that subsequently orientates immature DCs to a Th2 profile. These in turn induce inflammatory Th2 cells that produce associated Th2 cytokines, which result in differentiation of B cells that generate IgE. Cross-linking of IgE on the high affinity receptors on mast cells causes their activation and release of both TSLP and histamine. Increased levels of histamine in plasma and tissue, derived from basophils and mast cells, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[59] The release of TSLP by mast cells will in turn stimulate Th2 DC orientation and so forth. Perturbation of the Th1/Th2 balance will certainly impact upon the long-term development of immunity to malaria parasites and the short-term response to infection. Furthermore, the itch and scratch cycle characteristic of AD will be exacerbated by mosquito bites and saliva, which

is a highly prevalent 'environmental' allergen in Sub-Saharan Africa and elsewhere. Mosquito bites will thus likely increase AD in individuals genetically predisposed and with resultant increased severity of malaria disease. Whilst the same relationship would be predicted for *P. falciparum* endemic settings out of Africa, it may not be the case for the other major malaria parasite, spp. *Plasmodium vivax*, which induces a very different immunological response. It is, however, still unclear whether the parasites themselves play an active role in such immune deviation, exacerbate AD in turn and the extent to which the parasites themselves benefit from the increased severity of disease associated with such an inflammatory terrain: the production of specialised parasites stages, necessary for transmission from human to mosquito, was reduced in individuals with elevated IgE.[45] Further research on the physiological link between malaria parasite infection outcome and AD and other IgE-associated pathologies would be invaluable. Underlying details notwithstanding, it is promising to note that certain antihistamines have been effective against malaria and the current anti-malarial of choice, artesunate, against asthma.[60,61] It remains to be seen whether next-generation allergic disease treatments (IL-4/IL-13 agonists) also have beneficial action against malaria parasites.

CONCLUDING REMARKS

The underlying causes of AD are multifactorial and yet it has been recognised for some time that environmental factors play an important role and increase or decrease risk in individuals with a genetic predisposition. Sub-Saharan Africa has received relatively little attention and yet represents a region that is rich in environmental and genetic diversity. A major challenge in diagnosis of AD is the plethora of different aetiologies that share similar symptoms. HIV/AIDS associated cutaneous symptoms in particular, may pose a significant challenge for AD differential diagnosis in Africa.[8] Further focus on allergic diseases in this region would be of immense value to our understanding of the genetic and environmental basis to allergic diseases and will likely contribute to our understanding of infectious diseases and most notably those transmitted by biting arthropods.

REFERENCES

1. Asher MI et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. Lancet. 2006;368(9537):733-43.

2. International Study of Asthma and Allergies in Childhood. Available at: http://isaac.auckland.ac.nz/ Last accessed: 10 March 2016.

3. Ait-Khaled N et al. Prevalence of symptoms of asthma, rhinitis and eczema in 13- to 14-year-old children in Africa: The International Study of Asthma and Allergies in Childhood Phase III. Allergy. 2007;62(3):247-58.

4. Mavale-Manuel S et al. Asthma and allergies in schoolchildren of Maputo. Allergy. 2007;62(3):265-71.

5. Falade AG et al. Trends in the prevalence and severity of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. J Natl Med Assoc. 2009;101(5):414-8.

6. Esamai F et al. Prevalence of asthma, allergic rhinitis and dermatitis in primary school children in Uasin Gishu district, Kenya. East Afr Med J. 2002;79(10):514-8.

7. Deckers IA et al. Investigating international time trends in the incidence and prevalence of atopic eczema 1990-2010: A systematic review of epidemiological studies. PLoS One. 2012;7(7):e39803.

8. Siegfried EC, Hebert AA. Diagnosis of atopic dermatitis: Mimics, overlaps, and complications. J Clin Med. 2015;4(5):884-917.

9. Le Souëf PN et al. Evolutionary adaptation of inflammatory immune responses in human beings. Lancet. 2000;356(9225):242-4.

10. Campbell MC, Tishkoff SA. African genetic diversity: Implications for human demographic history, modern human origins, and complex disease mapping. Annu Rev Genomics Hum Genet. 2008;9:403-33.

11. Gurdasani D et al. The African Genome Variation Project shapes medical genetics in Africa. Nature. 2015;517(7534):327-32.

12. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med. 2009;360(17):1759-68.

13. Guilloud-Bataille ME et al. Evidence for linkage of a new region (11p14) to eczema and allergic diseases. Hum Genet. 2008;122(6):605-14.

14. Sun LD et al. Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. Nat Genet. 2011;43(7):690-4.

15. Paternoster L et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. Nat Genet. 2011;44(2):187-92.

16. Hirota T et al. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. Nat Genet. 2012;44(11):1222-6.

17. Tamari M, Hirota T. Genome-wide association studies of atopic dermatitis. J Dermatol. 2014;41(3):213-20.

18. Leung DY. New insights into atopic dermatitis: Role of skin barrier and immune dysregulation. Allergol Int. 2013;62(2):151-61.

19. Hamid Q et al. In vivo expression of IL-12 and IL-13 in atopic dermatitis. J Allergy Clin Immunol. 1996;98(1):225-31.

20. Weidinger S et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet. 2008;4(8):e1000166.

21. Granada M et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart

Study. J Allergy Clin Immunol. 2012;129(3): 840-5.

22. Levin AM et al. A meta-analysis of genome-wide association studies for serum total IgE in diverse study populations. J Allergy Clin Immunol. 2013¬; 131(4):1176-84.

23. Torgerson DG et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet. 2011; 43(9):887-92.

24. Hirota T et al. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. Nat Genet. 2011; 43(9):893-6.

25. Ramasamy A et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. J Allergy Clin Immunol. 2011; 128(5):996-1005.

26. Moffatt MF et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363(13):1211-21.

27. Irvine AD et al. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med. 2011;365(14):1315-27.

28. Brown SJ, McLean WH. One remarkable molecule: Filaggrin. J Invest Dermatol. 2012;132(3 Pt 2):751-62.

29. Margolis DJ et al. The persistence of atopic dermatitis and filaggrin (FLG) mutations in a US longitudinal cohort. J Allergy Clin Immunol. 2012;130(4):912-7.

30. Esnault S et al. Thymic stromal lymphopoietin (TSLP) as a bridge between infection and atopy. Int J Clin Exp Pathol. 2008;1(4):325-30.

31. Barnes KC. An update on the genetics of atopic dermatitis: Scratching the surface in 2009. J Allergy Clin Immunol. 2010; 125(1):16-29.

32. Gao PS et al. Genetic variants in thymic stromal lymphopoietin are associated with atopic dermatitis and eczema herpeticum. J Allergy Clin Immunol. 2010; 125(6):1403-7.

33. Margolis DJ et al. Thymic stromal lymphopoietin variation, filaggrin loss of function, and the persistence of atopic dermatitis. JAMA Dermatol. 2014;150(3): 254-9.

34. Stein RT et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet. 1999;354(9178): 541-5.

35. Shaheen SO et al. Measles and atopy in Guinea-Bissau. Lancet. 1996; 347(9018):1792-6.

36. McIntire JJ et al. Immunology: Hepatitis A virus link to atopic disease. Nature. 2003; 425(6958):576.

37. Siberry GK et al. Increased risk of asthma and atopic dermatitis in perinatally HIV-infected children and adolescents. Clin Immunol. 2012;142(2):201-8.

38. Smits HH et al. Chronic helminth infections protect against allergic diseases by active regulatory processes. Curr Allergy Asthma Rep. 2010;10(1):3-12.

39. Gould HJ et al. The allergic march from Staphylococcus aureus superantigens to immunoglobulin E. Chem Immunol Allergy. 2007;93:106-36.

40. Lin YT et al. Role of bacterial pathogens in atopic dermatitis. Clin Rev Allergy Immunol. 2007;33(3):167-77.

41. Haileamlak A et al. Early life risk factors for atopic dermatitis in Ethiopian children. J Allergy Clin Immunol. 2005;115:370-6.

42. Sunyer J et al. Prenatal risk factors of wheezing at the age of four years in Tanzania. Thorax. 2001;56:290-5.

43. Herrant M et al. Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria. BMJ Open. 2013;3(7):e002835.

44. Lawaly R et al. Impact of mosquito bites on asexual parasite density and gametocyte prevalence in asymptomatic chronic Plasmodium falciparum infections and correlation with IgE and IgG titers. Infect Immun. 2012;80(6):2240-6.

45. Herrant M et al. Risk factors associated with asthma, atopic dermatitis and rhinoconjunctivitis in a rural Senegalese cohort. Allergy, Asthma Clin Immunol. 2015; 11(1):24.

46. Perlmann P et al. IgE and tumor necrosis factor in malaria infection. Immunol Lett. 1999;65:29-33.

47. Maeno Y et al. IgE deposition in brain microvessels and on parasitized erythrocytes from cerebral malaria patients. Am J Trop Med Hyg. 2000;63(3-4):128-32.

48. Desowitz RS. Plasmodium-specific immunoglobulin E in sera from an area of holoendemic malaria. Trans R Soc Trop Med Hyg. 1989;83(4):478-9.

49. Ohno T et al. Chromosomal mapping of the host resistance locus to rodent malaria (Plasmodium yoelii) infection in mice. Immunogenetics. 2001;53(9): 736-40.

50. Kohara Y et al. A major determinant quantitative-trait locus responsible for atopic dermatitis-like skin lesions in NC/Nga mice is located on Chromosome 9. Immunogenetics. 2001;53(1):15-21.

51. Sakuntabhai A et al. Genetic determination and linkage mapping of Plasmodium falciparum malaria related traits in Senegal. PLoS One. 2008;3(4): e2000.

52. Jang N et al. Polymorphisms within the PHF11 gene at chromosome 13q14 are associated with childhood atopic dermatitis. Genes Immun. 2005;6(3): 262-4.

53. Friberg C et al. Analysis of chromosome 5q31-32 and psoriasis: Confirmation of a susceptibility locus but no association with SNPs within SLC22A4 and SLC22A5. J Invest Dermatol. 2006;126(5):998-1002.

54. Chen W, Hershey GKK. Signal transducer and activator of transcription signals in allergic disease. J Allergy Clin Immunol. 2007;119(3):529-41.

55. Bouzigon E et al. Meta-analysis of 20 genome-wide linkage studies evidenced new regions linked to asthma and atopy. Eur J Hum Genet. 2010;18(6):700-6.

56. Holloway JW et al. Genetics of allergic disease. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S81-94.

57. Ribeiro JM. Role of saliva in blood-feeding by arthropods. Annu Rev Entomol. 1987;32:463-78.

58. Reunala L et al. Passive transfer of cutaneous mosquito bite hypersensitivity by IgE anti-saliva antibodies. J Allergy Clin Immunology. 1994;94:902-6.

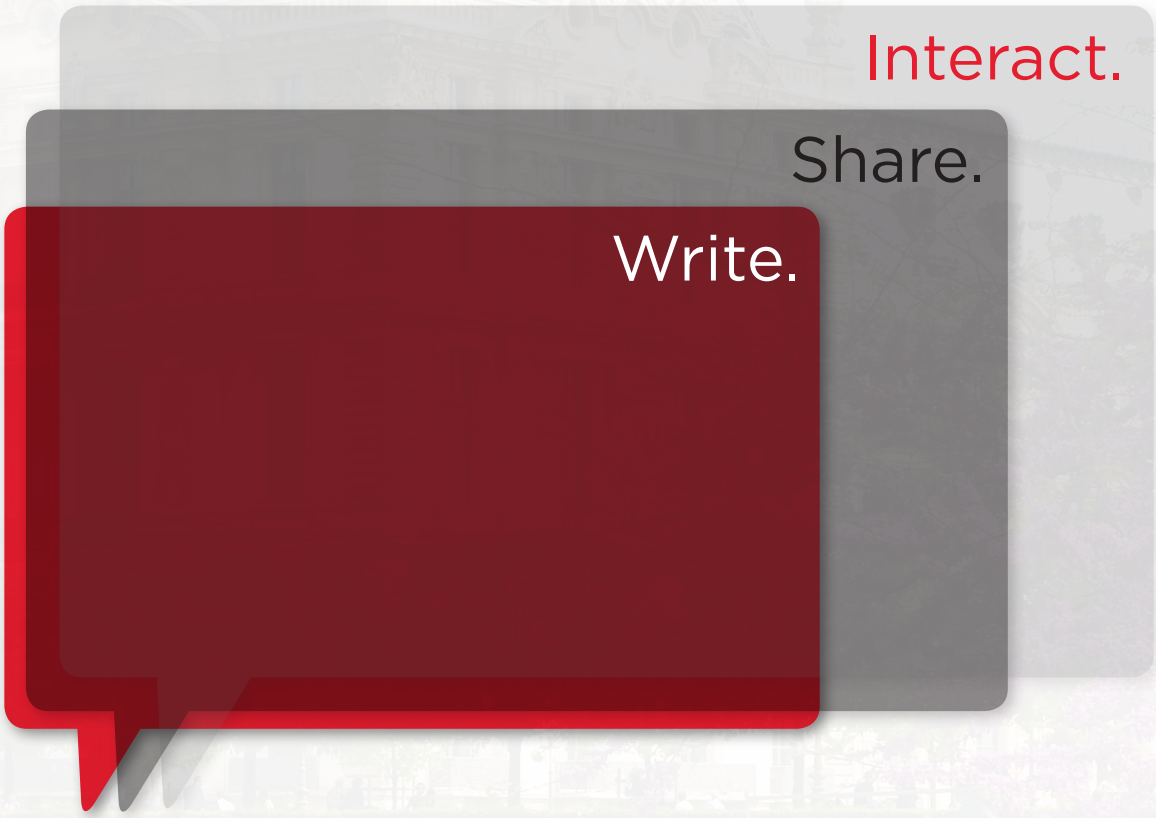
59. Mecheri S. Contribution of allergic inflammatory response to the pathogenesis of malaria disease. Biochim Biophys Acta. 2012;1822(1):49-56.

60. Ho WE et al. Anti-malarial drug artesunate ameliorates oxidative lung damage in experimental allergic asthma. Free Radic Biol Med. 2012;53(3):498-507.

61. Chong CR et al. A clinical drug library screen identifies astemizole as an antimalarial agent. Nat Chem Biol. 2006; 2(8):415-6.

EMJ EUROPEAN MEDICAL JOURNAL

Would you like to write for the EMJ blog?



Get involved today:
emjreviews.com/blog

Follow us:

www.emjreviews.com

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

IN SEARCH OF BIOMARKERS IN EOSINOPHILIC OESOPHAGITIS: WE ARE NOT THERE YET!

Neeti Bhardwaj,[1] *Gisoo Ghaffari[2]

1. Department of Pediatrics, Division of Allergy and Immunology, Pennsylvania College of Medicine, Hershey, Pennsylvania, USA
2. Department of Medicine, Division of Pulmonary, Allergy, Critical Care Medicine, Pennsylvania College of Medicine, Hershey, Pennsylvania, USA
**Correspondence to gghaffari@hmc.psu.edu.*

Disclosure: The authors have declared no conflicts of interest.
Received: 23.03.16 **Accepted:** 22.06.16
Citation: EMJ Allergy Immunol. 2016;1[1]:66-73.

ABSTRACT

Background: Eosiniphilic oesophagitis (EoE) is an immune-mediated disease with a complex pathophysiology. The accepted standard for objectively monitoring inflammation associated with this disorder is the number of eosinophils in oesophageal tissue biopsies obtained endoscopically. There is a need for alternative biomarkers that effectively correlate with disease activity and can hopefully be obtained non-invasively. The aim of this study is to review the literature on various biomarkers of EoE, with respect to their correlation to disease activity and response to treatment.

Methods: A literature search was performed using PubMed and OVID with keyword combinations of EoE and various potential biomarkers. Between 2006 and 2015, 39 studies that investigated the correlation of various tissue and serum biomarkers with EoE disease were identified.

Results: A number of candidates have emerged as potential biomarkers of inflammation in EoE. Eotaxin-3, interleukin (IL)-5, IL-13, microRNAs, and mast cell mediators have shown the most promise. Studies on these markers are quite heterogeneous in terms of methodology, with use of invasively as well as non-invasively obtained specimens.

Conclusion: The quest for an ideal biomarker for EoE continues. Establishment of normal values, effects of concomitant atopic diseases, age and gender, and validation of methodology of the tests are some of the challenges that future research should address.

Keywords: Eosinophilic oesophagitis (EoE), biomarkers, eotaxin-3, microRNA (miRNA), mast cell (MC), cytokines.

INTRODUCTION

Eosinophilic oesophagitis (EoE) is a chronic immune-mediated disease characterised by oesophageal dysfunction, marked oesophageal eosinophilic infiltration (≥15 eosinophils/high-power field [hpf]), and variable response to acid suppression therapy.[1,2] It affects both children and adults. The presenting symptoms, which vary by age, include difficulty eating, failure to thrive, chest and/or abdominal pain, dysphagia, and food impaction.[3] Diagnostic guidelines published in 2007,[1] and revised in 2011,[4] require demonstration of oesophageal eosinophilia in the absence of gastro-oesophageal reflux disease (GORD) as determined through a clinical trial with proton pump inhibitors (PPIs) or the use of pH-monitoring studies. EoE is associated with other atopic diseases, and many patients have evidence of food and aeroallergen sensitivities. T helper 2 (Th2) cells and their associated cytokines are involved in the pathogenesis of EoE.[3]

Currently, oesophagogastroduodenoscopy and histological examination of oesophageal mucosal biopsies are required to establish the diagnosis, objectively assess response to therapy, document disease remission, and evaluate symptom

recurrence.[1,5] Reliable, non-invasive biomarkers have not yet been identified. An ideal biomarker for EoE should correlate with disease activity, reflect changes with therapy, have high sensitivity and specificity, be reproducible and cost-effective, and be performed on non-invasively and easily obtained specimen.[5] A non-invasive biomarker continues to be elusive, although some promising candidates have emerged since our previous publication in 2012.[6] In this article, we attempt to provide a summary of what is currently known about potential biomarkers of oesophageal inflammation among EoE patients. The complex pathogenesis of EoE provides numerous candidates. We have reviewed studies that used tissue and/or serum samples from patients who were diagnosed with EoE based on the widely accepted and practiced criteria per consensus recommendations published in 2007[1] and revised in 2011.[4] A brief synopsis of the literature search is provided in Table 1. These studies not only have future diagnostic ramifications; they also provide valuable insight into the pathogenesis of EoE.

EOTAXINS

Eotaxins are chemoattractants shown to co-operate with interleukin (IL)-5 in tissue recruitment of eosinophils.[7] Eotaxins 1, 2, and 3 have been investigated as potential biomarkers. The blood levels of eotaxins 1 and 2 did not correlate with oesophageal eosinophilia in a cross-sectional analysis of 47 paediatric patients with EoE.[8] Studies on tissue expression of these two eotaxins have yielded mixed results in terms of correlation with disease activity.[9-11] Eotaxin-3 however, has emerged as one of the most promising candidates as a biomarker for EoE. Oesophageal inflammation in EoE is driven by Th2 pathway in response to allergen exposure, leading to release of eotaxin-3 from oesophageal eosinophils.[12]

Table 1: Summary of studies on potential biomarkers for eosinophilic oesophagitis.

Name of biomarker	Number of studies	Tissue/serum based	Number of studies showing correlation with disease activity	Number of studies showing response to steroid treatment (if studied)
Eotaxin-3	13	11/2	10: positive correlation	4: downregulation
Eotaxins 1 and 2	4	3/1	3: positive correlation	1: downregulation of *CCL11* (eotaxin-1) gene expression
IL-5	10	5/5	6: positive correlation 2: no correlation	3: downregulation
IL-13	5	4/1	2: positive correlation 1: no correlation	2: decrease in mRNA levels
EDN	5	2/3	4: positive correlation 1: no correlation	1: decrease in serum levels
MicroRNA	2	1 mixed/1 tissue	*miR-21* and *miR-223* upregulated, *miR-375* downregulated in tissue. *miR-146a*, *miR-146b,* and *miR-223* increased in plasma	Tissue *miR-675* induced by and *miR-375* normalised with disease remission. Plasma *miR-146a* and *miR-223* normalised with remission, *miR-146b* remained elevated
Mast cells	9	8/1	7: positive correlation 1: no correlation	2: decreased tissue mast cell numbers with treatment

EDN: eosinophil-derived neurotoxin; IL: interleukin.

Genome-wide microarray expression analysis in 2006[13] showed markedly increased expression of eotaxin-3 encoding gene in EoE patients. Moreover, eotaxin-3 tissue expression was shown to positively correlate with oesophageal eosinophil numbers.[14] IL-13 stimulation of oesophageal epithelial cells led to increased production of eotaxin-3.[15] A study in 2008 demonstrated that eotaxin-3 oesophageal gene expression levels in EoE patients were down regulated upon steroid treatment.[11] Eotaxin-3 messenger RNA (mRNA) levels had 89% sensitivity for distinguishing patients with and without EoE.[16] Non-invasive analysis of blood eotaxin-3 levels showed significant correlation with oesophageal eosinophil density in a prospective cross-sectional analysis on 47 paediatric patients.[8] Eotaxin-3 has been investigated as a marker to differentiate EoE from GORD. Bhattacharya et al.[10] showed that the mean mRNA expression levels of eotaxin-3 were markedly elevated in patients with EoE as compared with patients with GORD and healthy control groups.

In a case control study, Dellon et al.[17] used immunohistochemistry to compare the density of eotaxin-3 among EoE and GORD patients. Eotaxin-3 density was higher in EoE than in GORD, although the correlation with eosinophil count was weak. Another study published in 2014[18] investigated use of eotaxin-3 to differentiate EoE from proton pump inhibitor-responsive oesophageal eosinophilia (PPI-ROE). While oesophageal tissue from EoE patients showed significantly higher expression of eotaxin-3 compared with controls, it could not distinguish EoE from PPI-ROE. More recently, Moawad et al.[19] also published data on a retrospective study on patients with EoE, GORD, and PPI-ROE. Eotaxin-3 staining scores were significantly higher for EoE patients compared to GORD (p=0.002), whereas there was a trend towards significance between EoE and PPI-ROE (p=0.054). Molina-Infante et al.[20] studied gene expression of eotaxin-3 at baseline and after omeprazole 40 mg twice a day (b.i.d) for 8 weeks. Eotaxin-3 expression was indistinguishable between EoE and PPI-ROE at baseline. PPI therapy significantly decreased eotaxin-3 expression in PPI-ROE and in steroid-responsive EoE.[20] Eotaxin-3 has been investigated for its potential as a non-invasive marker. There were no significant differences between serum levels of eotaxin-3 among EoE cases and controls, and among cases before and after treatment.[21] There is a preponderance of evidence for the positive correlation of eotaxin-3 tissue expression and disease activity in terms of oesophageal eosinophilia and response to corticosteroid therapy, however, its potential as a non-invasive marker is less promising.

CYTOKINES

A number of studies have examined potential correlation of EoE with pathogenically related cytokines including IL-5, IL-13, IL-15, CCL5, and GM-CSF in the peripheral circulation.[22] IL-5 plays a critical role in eosinophil trafficking into inflammatory sites along with other Th2 cytokines, IL-4, and IL-13.[23] Tissue IL-5 mRNA expression levels were found to be significantly elevated in patients with EoE compared with GORD and controls.[10] Lucendo et al.[11] in 2008 showed that oesophageal *IL-5* gene expression levels were variably downregulated after topical steroid treatment. Another study in 2011[16] showed a 4-fold induction of oesophageal transcripts of IL-5 receptor alpha in individuals with EoE. Serum levels of IL-5 in EoE patients have also been studied. In 2006, Konikoff et al.[8] showed that blood levels of IL-5 did not correlate with oesophageal eosinophil density and were not increased in active EoE versus controls or those with inactive EoE. Bullock et al.[14] showed that EoE patients with active disease had an increased percentage of CD4+ T cells expressing IL-5 compared with those in disease remission. Huang et al.[24] demonstrated significantly increased levels of plasma IL-5 in EoE versus GORD. However, a longitudinal study in children with EoE showed that serum IL-5 levels in patients with EoE were not statistically different from those of the controls.[25] IL-13 is a Th2 cell derived cytokine also involved in eosinophil trafficking to inflammatory sites.[23] Gupta et al.[9] in 2006 showed that IL-13 mRNA was similar between controls and patients with EoE. However, Blanchard et al.[15] in 2007 found IL-13 mRNA levels to be markedly increased in oesophageal biopsy specimens from EoE patients compared with those from healthy individuals, with reversal following glucocorticoid treatment. In fact, the efficacy of anti-IL-13 therapy could be assessed with an IL-13-induced transcriptome. Moreover, IL-13 stimulation of oesophageal epithelial cells was shown to induce eotaxin-3 production.[15] IL-5 and IL-13 gene expression in the oesophagus was studied at baseline and at 8 weeks following treatment with omeprazole 40 mg b.i.d in adult patients with EoE phenotype.[20] PPI therapy

significantly downregulated oesophageal *IL-5* and *IL-13* gene expression in PPI-ROE, similar to that seen in steroid-responsive EoE. Another study in 2011 identified IL-13 as one of the eight cytokines whose blood levels retrospectively distinguished EoE patients from healthy controls with 100% specificity and sensitivity. It proposed the development of a cytokine panel scoring system for predicting the diagnosis of EoE.[16] Dellon et al.[21] in 2015 evaluated serum IL-5, 6, 9, and 13 levels (among a panel of several serum biomarkers). No significant differences in assay values were seen between EoE cases and controls, or before and after treatment values among cases.[21] Studies done so far on peripheral cytokine measurements do not establish a consistent correlation between peripheral levels and oesophageal disease activity. Moreover, peripheral cytokine measurement is affected by the confounding factors of concomitant allergic conditions; hence, it is very difficult to establish threshold levels. However, tissue expression of *IL-5* and *IL-13* mRNA could potentially be used for diagnosis as well as monitoring response to therapy in patients with EoE.

The cytokines could be useful targets for treatment options. The humanised monoclonal immunoglobulin (Ig)G antibody against human IL-5 (mepolizumab) has been investigated as a potential therapeutic intervention in EoE. In a randomised, placebo-controlled, double-blind trial in 2010, Straumann et al.[26] demonstrated a marked decrease in mean oesophageal eosinophilia (p=0.03) in the mepolizumab group compared with placebo group 4 weeks after initiation of treatment which consisted of two intravenous infusions of 750 mg mepolizumab 1 week apart. Limited improvement of clinical symptoms was seen. Assa'ad et al.[27] performed an international, multicentre, double-blind, randomised, prospective study of 59 children. Patients received an infusion every 4 weeks (a total of 3 infusions) of 0.55, 2.5, or 10 mg/kg mepolizumab. Peak and mean oesophageal intraepithelial eosinophil counts decreased significantly to 40.2±5.17 and 9.3±1.25 per hpf, respectively (p<0.0001). There was no placebo group in this trial.[27] Rothenberg et al.[28] investigated intravenous anti-IL-13 monoclonal antibody QAX576 in treatment of EoE. The mean oesophageal eosinophil count decreased by 60% with QAX576 versus an increase of 23% with placebo (p=0.004), and the decrease was sustained up to 6 months. There was a trend towards clinical improvement of dysphagia. While these results are encouraging, we need larger placebo-controlled trials to further define the potential therapeutic role of anti-IL-5 and anti-IL-13 antibodies in EoE.

PRODUCTS OF EOSINOPHIL DEGRANULATION

Eosinophil-derived neurotoxin (EDN) is an eosinophil granule-derived secretory protein with ribonuclease and antiviral activity.[29] Yang et al.[30] in 2008 demonstrated that EDN enhances antigen-specific Th2-biased immune responses. Extracellular EDN has been used as an indicator of eosinophil activation and degranulation *in vitro*. Increased levels of EDN in body fluids have been observed in patients with various eosinophil-associated diseases.[31]

A prospective, cross-sectional analysis on 47 paediatric patients undergoing endoscopic evaluation of possible EoE showed that plasma EDN levels significantly correlated with oesophageal eosinophil density and were increased in patients with active EoE versus controls.[8] Similarly, Subbarao et al.[25] found that serum EDN levels were significantly elevated in children with EoE compared with controls. However, in a recent study in 2015, serum levels of EDN were not found to be significantly different between EoE patients and control, and between pre and post-treatment EoE cases.[21] Tissue EDN expression in EoE patients has also been examined. Alexander et al.[32] in 2008 published a study on immunofluorescence (IF) staining of oesophageal biopsy specimens for EDN in four groups of patients: normal, low-level eosinophilia GORD patients, EoE patients with dysphagia with response to topical steroid therapy, and classic EoE patients with 25–100 eosinophils/hpf. EDN scores were found to be higher in the latter two categories, suggesting that extracellular EDN IF staining is a better marker for EoE than maximum eosinophil count.[32] Another IF study suggested that tissue eosinophils may underestimate how extensively eosinophils are involved, particularly in individuals with marked eosinophil degranulation.[31] The above studies do establish a positive correlation of tissue EDN with EoE disease activity, however further studies are needed to define its place in the diagnostic algorithm of EoE. Schlag et al.[33] evaluated serum levels of eosinophil cationic protein (ECP) and mast cell (MC) tryptase as markers for response to topical steroid treatment in EoE patients. Serum ECP levels were shown to correlate significantly

with oesophageal eosinophil counts compared with serum MC tryptase levels.

MICRO RNAS

MicroRNAs (miRNAs) are single-stranded RNA molecules 19–25 nucleotides long, that regulate post-transcriptional gene silencing of target genes.[34] Multiple studies have demonstrated that EoE is associated with marked changes in tissue-specific gene expression, referred to as the EoE transcriptome.[13,15] In 2012, Lu et al.[35] showed that miRNA-21 and miRNA-223 were the most upregulated miRNAs in EoE patients, while miR-375 was the most downregulated. This miRNA signature correlated with the degree of tissue eosinophilia, was distinct from patients with chronic non-EoE, and was largely reversible with glucocorticoid therapy.[35] Levels of miRNA-146a, miRNA-146b, and miRNA-223 were upregulated in plasma of EoE patients compared with healthy controls.[35,36] The levels normalised with disease remission and inversely correlated with the degree of allergic inflammation.[36] Interestingly, miRNA-375 expression inversely correlates with the degree of allergic inflammation in EoE, as measured by oesophageal eosinophil levels, the gene expression levels *IL-5* and *IL-13*, and MC-specific enzymes.[37] They further analysed oesophageal epithelial miRNA and mRNA from five paired biopsies pre and post-treatment with glucocorticoids, and found that 32 miRNAs were significantly upregulated and 4 were downregulated in pre-treated biopsies, with miRNA-214 being the most upregulated (150-fold).[38] Taken together, these studies propose circulating miRNAs as promising candidates for non-invasive biomarkers of EoE due to their disease-specific dysregulation and their relative stability compared with mRNAs. Further studies will help refine their clinical utility.

MAST CELLS

MCs are important in the pathogenesis of EoE as they produce an abundance of cytokines that activate eosinophils and molecules that directly promote tissue remodelling.[39,40] Studies quantifying intraepithelial MCs using anti-tryptase antibodies have shown higher counts among EoE patients than GORD and control groups.[9,41] Abonia et al.[42] generated transcriptome expression profiles of the MC proteases carboxypeptidase A3 and tryptase that were shown to correlate with MC levels and distinguished EoE patients from controls. Treatment of patients with fluticasone propionate normalised levels of MCs and MC transcriptome in responder patients.[42] Aceves et al.[43] showed significantly increased numbers of tryptase-positive MCs in oesophageal smooth muscle of EoE patients, with a significant reduction in numbers following the use of topical corticosteroid. Moreover, tryptase positive MCs were shown to express tumour necrosis factor (TNF)-β1 which increased contractility of cultured human oesophageal smooth muscle cells *in vitro*.[43] In another study, expression of several MC-associated genes in biopsy specimens from patients with EoE without treatment were significantly increased compared with control subjects, followed by significant reduction by treatment with swallowed fluticasone.[44] Dellon et al.[17] in 2012 showed that patients with EoE had substantially higher levels of myelin basic protein (MBP) staining than GORD patients. Moreover, MBP density and eosinophil count correlated (r=0.81, p=0.001). In a subsequent study in 2014, oesophageal tissue from EoE patients was shown to have substantially higher levels of MBP and tryptase than controls on immunohistochemical analysis, compared with controls. However, these markers could not distinguish EoE from PPI-ROE.[18] Dellon et al.[21] in 2015 did not find any difference in plasma MBP levels between cases and controls, and between pre and post-treatment EoE cases. The above studies show that MC products and dysregulated transcriptomes in EoE patients could potentially be useful for disease diagnosis and monitoring.

ABSOLUTE EOSINOPHIL COUNT

Numerous studies have documented absolute and relative peripheral blood eosinophil counts in patients with EoE. The reported incidence of peripheral blood eosinophilia (PBE) shows a wide range of 10–100% over different age groups.[5] Baxi et al.[45] reported a 67% incidence of PBE in children and adolescents in 2006. A prospective cross-sectional analysis of paediatric patients undergoing endoscopic evaluation of possible EoE showed that absolute eosinophil count (AEC) levels significantly correlated with oesophageal eosinophil density.[8] In general, the reported degree of PBE when present in patients with EoE is modest.[5] While there appears to be a correlation between active oesophageal disease and peripheral eosinophil count, this parameter alone has limited potential as a disease marker. Especially since it may be affected by atopic disease in

general, and may not be specific to oesophageal mucosal inflammation.[22]

There is very limited data correlating PBE with response to therapy. More data is needed regarding the effect of therapy for EoE on AEC in the context of eosinophil counts on oesophageal mucosal biopsies.

FRACTIONAL EXHALED NITRIC OXIDE

Fractional exhaled nitric oxide (FeNO) has been used clinically to monitor asthma inflammation. Results of FeNO were compared with oesophageal biopsy results among 51 patients. Exhaled nitric oxide was shown to have high specificity (87%) and negative predictive value (78%).[46] In a prospective multicentre study, FeNO levels and symptom scores were measured among non-asthmatic EoE patients undergoing topical corticosteroid therapy.[47] A statistically significant difference was found between pre and post-treatment FeNO levels (20.3 ppb [parts per billion] versus 17.6 ppb, p=0.009). However, the FeNO levels were not found to confidently predict a clinical or histological response.

OTHER MARKERS

Various other cytokines, chemokines, and cell products are under investigation as potential markers of disease activity and response to therapy. Protheroe et al.[48] developed a monoclonal antibody specific to an eosinophil secondary granule protein eosinophil peroxidase and used it to identify intact eosinophils and detect eosinophil degranulation in formalin-fixed specimens. They developed a histopathologic scoring system to identify patients whose clinical course was suggestive of a diagnosis of EoE, but failed to reach the critical threshold of ≥15 eosinophils per hpf.[48] Huang et al.[24] studied plasma fibroblast growth factor basic levels by cytometric bead array to differentiate EoE from GORD. Another protein of interest is FK506-binding protein 51 (FKBP51). A study in 2010 demonstrated increased oesophageal FKBP51 mRNA levels in topical glucocorticoid responders compared with control subjects and patients with untreated active EoE, suggesting that increased FKBP51 transcript levels could be used to distinguish glucocorticoid responders from untreated patients with active EoE.[49] Merves et al.[50] reported increased expression of autophagy-related gene product 7 (ATG7) in active EoE compared with controls and EoE in remission. Zukerberg et al.[51] demonstrated that intrasquamous IgG4 deposits may be useful as an adjunctive marker to distinguish GORD from EoE. Intrasquamous extracellular IgG4 deposits were seen in 76% of EoE cases compared with none of the GORD cases. Moreover, EoE patients on treatment were less likely to be positive for this marker.[51] Group 2 innate lymphoid cells are a recently discovered group of lineage-negative cells that express the chemoattractant receptor homologous molecule expressed on Th2 lymphocytes. Recently it has been shown that these cells can be successfully quantified in oesophageal biopsy specimens, and their expression is significantly higher among patients with active EoE versus inactive EoE, and also higher than expression among controls and PPI-ROE patients.[52] The oesophageal string test was investigated as a minimally invasive clinical device to measure oesophageal inflammation among children by Furuta et al.[53] MBP-1, EDN, ECP, and eosinophil peroxidase were measured in luminal effluents eluted from oesophageal string tests and extracts of mucosal biopsies. The levels of eosinophil-derived proteins in luminal secretions were found to be reflective of mucosal inflammation. The immune mechanism involved in the pathogenesis of EoE is associated with differential expression of various inflammatory and epithelial-derived genes. Matoso et al.[54] used gene expression microarray and reverse transcription polymerase chain reaction to screen oesophageal biopsies from paediatric EoE patients before and after treatment with topical steroids. Overexpression of *ALOX15* and TNF-α-induced factor 6, and underexpression of filaggrin, *SLURP1*, and *CRISP3* was noted in EoE. They subsequently demonstrated that positive *ALOX15* expression is more prevalent in EoE than in GORD, and could be a valuable marker to differentiate between the two conditions.

CONCLUSIONS

The current standard of care for diagnosis of EoE and monitoring of response to treatment dictates the enumeration of eosinophils in a mucosal biopsy specimen obtained endoscopically. The pathogenesis of EoE is complex and hence a plethora of biomarkers are being studied to objectively assess the inflammation associated with this disease. The above review attempts to summarise the vast body of literature available

on potential biomarkers for EoE. Eotaxin-3, IL-5, IL-13, and lately, miRNAs have all shown promise in this direction. The search continues for a biomarker which could be obtained by a non-invasive or minimally invasive means, and accurately reflect the clinicopathologic diagnosis of EoE. Establishment of normal values, effects of concomitant atopic diseases, age and gender, and validation of methodology of the tests, are some of the challenges that future research should address.

REFERENCES

1. Furuta GT et al. Eosinophilic esophagitis in children and adults: A systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133:1342-63.

2. Assa'ad AH et al. Pediatric patients with eosinophilic esophagitis: An 8-year follow-up. J Allergy Clin Immunol. 2007; 119(3):731-8.

3. Abonia JP, Rothenberg ME. Eosinophilic esophagitis: Rapidly advancing insights. Annu Rev Med. 2012;63:421-34.

4. Liacouras CA et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011;128:3-22.

5. Gupta SK. Noninvasive markers of eosinophilic esophagitis. Gastrointest Endosc Clin N Am. 2008;18(1):157-67.

6. Bhardwaj N, Ghaffari G. Biomarkers for eosinophilic esophagitis: A review. Ann Allergy Asthma Immunol. 2012;109:155-9.

7. Rankin SM et al. Eotaxin and eosinophil recruitment: Implications for human disease. Mol Med Today. 2000;6(1):20-7.

8. Konikoff MR et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2006;4(11): 1328-36.

9. Gupta SK et al. Cytokine expression in normal and inflamed esophageal mucosa: A study into the pathogenesis of allergic eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2006;42(1):22-6.

10. Bhattacharya B et al. Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease. Hum Pathol. 2007;38(12):1744-53.

11. Lucendo AJ et al. Treatment with topical steroids downregulates IL-5, eotaxin-1 /CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. Am J Gastroenterol. 2008;103(9):2184-93.

12. Cianferoni A, Spergel J. Eosinophilic esophagitis: A comprehensive review. Clin Rev Allergy Immunol. 2016;50(2):159-74.

13. Blanchard C et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest. 2006;116(2):536-47.

14. Bullock JZ et al. Interplay of adaptive th2 immunity with eotaxin-3/c-C chemokine receptor 3 in eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2007;45:22-31.

15. Blanchard C et al. IL-13 involvement in eosinophilic esophagitis:Transcriptome analysis and reversibility with glucocorticoids. J Allergy Clin Immunol. 2007;120:1292-300.

16. Blanchard C et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. J Allergy Clin Immunol. 2011;127:208-17.

17. Dellon ES et al. Diagnostic utility of major basic protein, eotaxin-3, and leukotriene enzyme staining in eosinophilic esophagitis. Am J Gastroenterol. 2012;107(10):1503-11.

18. Dellon ES et al. Markers of eosinophilic inflammation for diagnosis of eosinophilic esophagitis and proton pump inhibitor-responsive esophageal eosinophilia: A prospective study. Clin Gastroenterol Hepatol. 2014;12(12):2015-22.

19. Moawad FJ et al. Comparison of eotaxin-3 biomarker in patients with eosinophilic oesophagitis, proton pump inhibitor-responsive oesophageal eosinophilia and gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2015;42(2):231-8.

20. Molina-Infante J et al. Proton pump inhibitor-responsive oesophageal eosinophilia correlates with downregulation of eotaxin-3 and Th2 cytokines overexpression. Aliment Pharmacol Ther. 2014;40(8):955-65.

21. Dellon ES et al. Utility of a noninvasive serum biomarker panel for diagnosis and monitoring of eosinophilic esophagitis: A prospective study. Am J Gastroenterol. 2015;110:821-7.

22. Menard-Katcher C, Furuta GT. Non- and semi-invasive methods of monitoring eosinophilic esophagitis. Dig Dis. 2014; 32:102-6.

23. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006; 24:147-74.

24. Huang JJ et al. Fibroblast growth factor as an improved disease indicator for eosinophilic esophagitis compared with Eotaxin-3 and IL-5. J Allergy Clin Immun. 2010;125:Ab162.

25. Subbarao G et al. Exploring potential noninvasive biomarkers in eosinophilic esophagitis in children. J Pediatr Gastroenterol Nutr. 2011;53:651-8.

26. Straumann A et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. Gut. 2010;59(1):21-30.

27. Assa'ad AH et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. Gastroenterology. 2011;141(5):1593-604.

28. Rothenberg ME et al. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. J Allergy Clin Immunol. 2015;135(2):500-7.

29. Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol. 2009; 101:81-121.

30. Yang D et al. Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. J Exp Med. 2008;205(1):79-90.

31. Kephart GM et al. Marked deposition of eosinophil-derived neurotoxin in adult patients with eosinophilic esophagitis. Am J Gastroenterol. 2010;105:298-307.

32. Alexander J et al. Is immunofluoresence staining for eosinophil derived neurotoxin useful in the diagnosis of eosinophilic esophagitis? Am J Gastroenterol. 2008; 103:S68.

33. Schlag C et al. Serum eosinophil cationic protein is superior to mast cell tryptase as marker for response to topical corticosteroid therapy in eosinophilic esophagitis. J Clin Gastroenterol. 2014; 48:600-6.

34. Sayed D, Abdellatif M. MicroRNAs in development and disease. Physiol Rev. 2011;91:827-87.

35. Lu TX et al. MicroRNA signature in patients with eosinophilic esophagitis, reversibility with glucocorticoids, and assessment as disease biomarkers. J Allergy Clin Immunol. 2012;129:1064-75.

36. Lu TX et al. MiR-375 is downregulated in epithelial cells after IL-13 stimulation and regulates an IL-13-induced epithelial transcriptome. Mucosal Immunol. 2012; 5(4):388-96.

37. Lu TX, Rothenberg ME. Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases. J Allergy

Clin Immunol. 2013;132:3-14.

38. Lu S et al. MicroRNA profiling in mucosal biopsies of eosinophilic esophagitis patients pre and post treatment with steroids and relationship with mRNA targets. PLoS One. 2012;7:e40676.

39. Aceves SS et al. Esophageal remodeling in pediatric eosinophilic esophagitis. J Allergy Clin Immunol. 2007; 119:206-12.

40. Chehade M et al. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2007;45(3):319-28.

41. Kirsch R et al. Activated mucosal mast cells differentiate eosinophilic (allergic) esophagitis from gastroesophageal reflux disease. J Pediatr Gastroenterol Nutr. 2007;44:20-6.

42. Abonia JP et al. Involvement of mast cells in eosinophilic esophagitis. J Allergy Clin Immunol. 2010;126:140-9.

43. Aceves SS et al. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-beta1, and increase esophageal smooth muscle contraction. J Allergy Clin Immunol. 2010;126:1198-204.

44. Hsu Blatman KS et al. Expression of mast cell-associated genes is upregulated in adult eosinophilic esophagitis and responds to steroid or dietary therapy. J Allergy Clin Immunol. 2011;127(5):1307-8.

45. Baxi S et al. Clinical presentation of patients with eosinophilic inflammation of the esophagus. Gastrointest Endosc. 2006;64(4):473-8.

46. Lanz MJ et al. Measurement of exhaled nitric oxide in the evaluation for eosinophilic esophagitis in children. Ann Allergy Asthma Immunol. 2012;109(1): 81-2.

47. Leung J et al. Assessment of fractionated exhaled nitric oxide as a biomarker for the treatment of eosinophilic esophagitis. Allergy Asthma Proc. 2012;33(6):519-24.

48. Protheroe C et al. A novel histologic scoring system to evaluate mucosal biopsies from patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2009;7(7):749-55.

49. Caldwell JM et al. Glucocorticoid-regulated genes in eosinophilic esophagitis: A role for FKBP51. J Allergy Clin Immunol. 2010;125(4):879-88.

50. Merves JF et al. ATG7 Gene expression as a novel tissue biomarker in eosinophilic esophagitis. Am J Gastroenterol. 2016; 111(1):151-3.

51. Zukerberg L et al. Esophageal intrasquamous IgG4 deposits: An adjunctive marker to distinguish eosinophilic esophagitis from reflux esophagitis. Histopathology. 2016;67(7): 968-76.

52. Doherty TA et al. Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. J Allergy Clin Immunol. 2015;136(3):792-4.

53. Furuta GT et al. The oesophageal string test: A novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut. 2013; 62:1395-405.

54. Matoso A et al. Expression microarray analysis identfies novel epithelial-derived protein markers in eosinophilic esophagitis. Mod Pathol. 2013;26(5): 665-76.

TREATMENT OPTIONS IN EOSINOPHILIC OESOPHAGITIS

Anna M. Lipowska,[1] Robert T. Kavitt,[1] *Michael F. Vaezi[2]

1. Section of Gastroenterology, Hepatology, and Nutrition, University of Chicago, Chicago, Illinois, USA
2. Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University Medical Center, Nashville, Tennessee, USA
**Correspondence to Michael.Vaezi@Vanderbilt.edu*

Disclosure: The authors have declared no conflicts of interest.
Received: 04.03.16 **Accepted:** 22.06.16
Citation: EMJ Allergy Immunol. 2016;1[1]:74-81.

ABSTRACT

Eosinophilic oesophagitis (EoE) is an increasingly prevalent chronic inflammatory disorder diagnosed by the presence of oesophageal symptoms and eosinophilic inflammation on endoscopic histology. Treatment of EoE centres around the '3 D's': drugs, diet, and dilation, which aim to both improve symptoms and prevent potential complications. Potential pharmacologic therapies include acid suppressing agents and corticosteroids, among others. Dietary strategies comprise the elemental diet, the empiric elimination diet, and the allergy testing-directed elimination diet. The therapeutic landscape of EoE is rapidly changing as our understanding of the disease evolves. This review aims to provide a comprehensive discussion of existing EoE therapies and to outline an approach to EoE management.

Keywords: Eosinophilic oesophagitis (EoE), proton pump inhibitors (PPIs), endoscopy.

INTRODUCTION

Eosinophilic oesophagitis (EoE) is a chronic condition characterised by eosinophilic inflammatory changes of the oesophagus with associated oesophageal symptoms. It is a relatively new disease, first described in a 1978 case report of a patient with eosinophilic infiltration of the oesophagus.[1] Since its discovery the number of cases has continued to increase, and currently the prevalence of EoE worldwide is estimated to be 0.5–1 cases in every 1,000 people.[2] The disease is most frequently found among males of Caucasian descent.

Treatment of EoE focusses on symptom control and decreasing oesophageal inflammation. The mainstay treatment of EoE can be divided into three main categories: pharmacologic therapies which include both topical and systemic agents, dietary modification, and endoscopic interventions (Table 1). Choosing between these therapeutic options depends on several factors, including patient preference and clinical experience, among others. Certain treatment strategies such as the use of proton pump inhibitors (PPIs) are used early on as part of the initial diagnostic evaluation, while most endoscopic interventions, including endoscopic dilation, are reserved as second-line therapy.[3] Dietary therapies range from the strictest elemental diet using an amino acid based formula, to the six-food elimination diet (SFED), and the tailored allergy testing-directed elimination diet. This review aims to provide a comprehensive discussion of existing EoE therapies and outline an approach to EoE management.

PHARMACOLOGIC THERAPY

Pharmacologic therapies in EoE aim to decrease oesophageal inflammation and alleviate symptoms. PPIs are initially recommended to help establish the diagnosis, but are not used as a sole treatment of EoE with the exception of the disease subtype PPI-responsive oesophageal eosinophilia (PPI-ROE). The mainstay of pharmacologic treatment for EoE involves corticosteroids, which several studies have confirmed to improve both oesophageal symptoms and histologic features of EoE. Histology has been a common metric of following patients during therapy, with the goal of achieving eosinophil reduction. Several newer pharmacologic options, such as the chemoattractant receptor homologous molecule expressed on T Helper type 2 cells (CRTh2)

antagonist, have emerged in recent years with promising results, but remain experimental to date.[4]

Proton Pump Inhibitors

Patients with gastro-oesophageal reflux disease (GORD) are often found to have an elevated level of eosinophil infiltration in oesophageal tissue, a consequence of mucosal exposure to acidic contents and a presentation that can mimic EoE.[5,6] During the diagnostic process, attempts to distinguish GORD from EoE are made by assessing responsiveness to PPIs. However, the benefits of PPI therapy appear to reach beyond their acid-suppressing effect. PPIs are thought to play an anti-inflammatory role through several mechanisms, including inhibiting cytokine production from the oesophageal epithelium, impairing neutrophil phagocytosis, and exhibiting anti-oxidant effects.[7,8]

Recent studies have revealed a subtype of EoE known as PPI-responsive oesophageal eosinophilia, defined by the attainment of histologic remission with the use of PPIs.[9,10] Recognising this phenomenon, the latest guidelines recommend a 2-month PPI course followed by repeat endoscopy with biopsies to exclude PPI-ROE.[11] In up to one-third of EoE patients who are found to have PPI-responsive disease, new data are emerging which supports long-term PPI therapy. A recent retrospective cohort study demonstrated that 73% of PPI-ROE patients remained in remission over a 12-month follow-up period and the majority of patients who relapsed re-achieved remission with PPI dose escalation, suggesting that PPI-ROE patients may require maintenance PPI therapy.[12]

Topical Corticosteroids

Topical corticosteroids are used as first-line agents and remain the mainstay of therapy in PPI-unresponsive EoE.[13-15] Studies have found reduced oesophageal lamina propria remodelling and tissue fibrosis with topical steroid use.[16,17] Fluticasone and budesonide are the two most commonly used topical agents in EoE with the largest amount of supporting evidence.[18-28]

In children, oral budesonide significantly improved symptom, endoscopy, and histology scores compared with placebo.[19] In the adult population with active EoE, budesonide was found to induce clinical and histological remission.[20] Long-term budesonide use has also been found to maintain clinical and histologic remission while being well-tolerated by patients in a 50-week trial.[29] Recommended doses of budesonide administered as a viscous suspension are 1 mg daily for children <10 years old and 2 mg daily for older children and adults.[14]

Fluticasone is dispensed through a metered-dose inhaler into the mouth and then swallowed, at 88–440 µg 2 to 4-times daily to children and 440–880 µg twice daily to adults.[14] In one of the earliest trials comparing fluticasone with placebo in paediatric patients, fluticasone induced remission in 50% of study participants compared with just 9% in the placebo group.[24] A more recent multicentre trial indicated that 65% of patients receiving fluticasone achieved complete remission after 3 months.[25] Interestingly, a systematic review and meta-analysis comparing five randomised controlled trials (RCTs) indicated that while topical steroids provoke histological remission, they may not significantly improve clinical symptoms.[30] Overall, >40% of patients have been found to have steroid refractory EoE and 91% of patients experienced a recurrence of symptoms 9 months after completing treatment, prompting the question of the need for alternative therapeutic agents.[30,31]

The most commonly cited side effect of topical corticosteroids is oral and oesophageal candidiasis, although patients may also be at a rare risk of adrenal axis suppression and reduction in bone density.[32] Based on these findings, the length of steroid treatment should be decided on a case-by-case basis with close monitoring for side effects.

Systemic Corticosteroids

The use of systemic corticosteroids to treat EoE is limited given their expanded side effect profile and analogous benefits compared with topical agents. In the one existing RCT comparing oral prednisone to topical fluticasone, histological and clinical improvement was seen in both groups after 4 weeks of treatment and there was no significant difference between the groups in time-to-relapse.[22] However, 40% of patients on prednisone experienced adverse effects of the medication, including weight gain and Cushingoid features, compared with only 15% of fluticasone patients who developed oesophageal candidiasis and no systemic effects. With this in mind, in practice systemic steroids have been reserved for severe refractory cases of EoE, and when used are administered at high doses, for example prednisone at 1-2 mg/kg.[11]

Table 1: Eosinophilic oesophagitis treatment summary.

Therapy	Recommendation
Pharmacologic therapy	
PPIs	• Initial 2-month course followed by repeat endoscopy with biopsies to distinguish diagnosis from GORD • Recommended therapy for subset of patients with PPI-ROE
Topical corticosteroids*	• Used for initial and maintenance therapy in PPI-unresponsive EoE • Budesonide viscous suspension 1 mg/day for children or 2 mg/day for adults, typically in a divided dose or • Fluticasone via metered-dose inhaler 88–440 µg/day in a divided dose for children or 880–1760 µg/day in a divided dose for adults
Systemic corticosteroids	• Reserved for severe refractory cases given side effect profile
Leukotriene receptor antagonists	• Not recommended
Immunomodulators	• Not recommended
Biologics	• Under clinical investigation
Anti-IgE antibody	• Under clinical investigation
Dietary therapy	
Elemental diet	• Amino acid based allergen-free formula followed by slow reintroduction of foods • Most effective but also most strict, causing difficulty with adherence
Testing-directed elimination diet	• Elimination of food groups based on allergy testing • Overall poor efficacy and the least favoured of the three dietary regimens
Empiric elimination diet	• Six most commonly allergenic food groups (milk, soy, egg, wheat, peanuts/tree nuts, shellfish/fish) are removed from the diet and slowly, individually reintroduced after a symptomatic and histologic response • Validated in adults, better concordance than with the elemental diet
Endoscopic treatment	
Endoscopic dilation	• Usually reserved for patients who relapse on pharmacologic or dietary therapy • First-line therapy if high-grade strictures present

GORD: gastro-oesophageal reflux disease; PPIs: proton pump inhibitors; IgE: immunoglobulin E; EoE: eosinophilic oesophagitis. PPI-ROE: PPI-responsive oesophageal eosinophilia.
*Note that no US Food and Drug Administration (FDA)-approved therapies for EoE have been approved to date. The dosing listed above is based on 2013 American College of Gastroenterology (ACG) published guidelines.

Leukotriene Antagonists, Biologics, and Other Therapies

Montelukast, a leukotriene D4 receptor antagonist, has been studied as a potential therapeutic target in EoE, since leukotriene D4 is a chemotactic factor for eosinophils and could play a role in the disease pathogenesis.[15] One small prospective study of 12 patients demonstrated a symptomatic but not histologic benefit, and noted the occurrence of multiple side effects.[33] Furthermore, when administered to patients in remission who completed a 6-month course of fluticasone treatment, montelukast did not succeed

in maintaining clinical or histological remission.[34] Based on this evidence, the use of leukotriene receptor antagonists is not a recommended treatment regimen.

Multiple biologics have been studied in EoE, with the most researched being interleukin (IL)-5 antibodies. Cytokine IL-5 is a key eosinophil growth and activation factor.[35] Mepolizumab, a humanised monoclonal antibody against IL-5, was studied in two small RCTs of adult and paediatric EoE patients.[36,37] Both trials demonstrated a significant reduction in oesophageal eosinophilia in the treatment group, and while the drug was well-tolerated in the adult population there was no evidence of symptomatic improvement. In a RCT in the paediatric population of reslizumab, an IL-5 neutralising antibody, there was a statistically significant decline in eosinophil numbers after treatment, but no significant improvement in symptoms compared with placebo.[38] The use of the tumour necrosis factor alpha inhibitor, infliximab, was described in three patients of refractory EoE, determining no reduction in either eosinophilic infiltration or symptom scores.[39] To date, biologic therapies are currently under study and are not recommended for routine use. There are currently several ongoing trials in EoE, including an advanced examination of the role of infliximab in EoE, as well as studies investigating novel agents such as the study of dupilumab, a fully human monoclonal antibody directed against IL-4 receptors.

Very limited data exists regarding the use of immunomodulators to treat EoE. A case series of three patients treated with azathioprine or 6-mercaptopurine resulted in all three patients experiencing clinical and histological remission.[40] However, given the significant side effect profile of these agents with little accompanying data on their benefit, these agents need to be studied more extensively before they become recommended therapy.

Omalizumab, an anti-immunoglobulin E antibody, is one potential therapeutic target in EoE. A case report of two patients noted improvement in symptoms after omalizumab treatment; however, no histologic change was found.[41] Additionally, no RCTs exist involving omalizumab in the current literature. Furthermore, given that EoE is a transmural disease, new fibroblast-targeted therapies, such as JAK-STAT6 pathway inhibitors, are currently under investigation with the potential of treating both the epithelial fibrosis and the subepithelial fibrosis.[42] Finally, OC000459 is a receptor antagonist to the CRTh2 which mediates chemotaxis of inflammatory cells, including eosinophils. This agent has been more extensively studied in asthmatic patients; however, a RCT of 26 patients demonstrated a moderate but significant improvement in oesophageal eosinophilia and its symptoms after 8 weeks.[43]

DIETARY THERAPY

EoE is strongly associated with multiple allergic conditions, including atopic dermatitis, asthma, and food allergies. The avoidance of potential food allergens through dietary modification in patients with EoE has been used as a non-pharmacologic treatment strategy. Patients are most effectively managed by a multi-disciplinary team including allergy specialists and registered dieticians to help implement and oversee dietary therapy. Three distinct dietary approaches have been studied in the treatment of EoE: elemental diet, testing-directed elimination diet, and the empiric elimination diet.

Elemental Diet

Patients on the elemental diet are placed on an amino acid based allergen-free formula. The elemental diet has been found to be the most effective of all diets in EoE, with 90.8% of patients achieving histologic remission.[44] The diet's efficacy has been most extensively studied in children, demonstrating a significant improvement in both symptoms and histologic findings.[45,46] Only one study in the existing literature verified the benefit of the elemental diet in adults, with complete or near complete endoscopic response in 72% of patients.[47] Beyond decreasing eosinophil infiltration, there may be evidence that the diet also decreases the degree of oesophageal fibrosis.[48]

Once patients achieve clinical and histological improvement, foods are slowly introduced starting with the least allergenic. The main disadvantage of this regimen lies in its strict guidelines, avoidance of solid food, and overall poor tolerability particularly in the adult population. Consequently, practitioners often reserve it for patients who fail other therapies.

Testing-Directed Elimination Diet

Allergy testing-directed elimination diets are based on the results of allergy testing from

radioallergosorbent or skin-prick tests. In a large study of the paediatric population, 49% of participants had normalisation of their oesophageal biopsy findings, after the exclusion of participants treated with an elemental diet.[49] The largest prospective study of adults utilising an individualised diet based on three different skin tests demonstrated a poor response, with 66% of patients having neither a clinical nor histological response to the dietary therapy.[50] A meta-analysis of 14 studies, including both paediatric and adult patients, found the diet's overall efficacy to be 45.5%.[43]

One limitation of this approach lies in the origin of the diet design, as skin prick tests are only successful in identifying foods causing allergic symptoms in approximately 13% of cases.[51] Combined with its suboptimal results compared to the other available dietary therapies, the testing-directed elimination diet has fallen out of favour for non-paediatric patients in general practice.

Empiric Elimination Diet

Compared with allergy testing, the best way to identify food allergens has been confirmed to be through food reintroduction.[51] The most extensively studied empiric elimination diet is the SFED where the six most commonly allergenic food groups (milk, soy, egg, wheat, peanuts/tree nuts, and shellfish/fish) are removed from the patient's diet. To date, extending this diet to avoid rye and barley, or implementing a gluten-free diet, has not been proven to be effective in improving clinical or histologic outcomes in EoE.[52] After confirmed symptomatic and histologic response, on average 6 weeks later the food groups are slowly and individually reintroduced while monitoring for disease reoccurrence. An individualised approach to food reintroduction can be applied given no existing guidelines outlining the order in which food can be reintroduced. However, it should be noted that milk and wheat products are usually added last, as they are the most common source for allergies.[53]

The SFED was first studied in children, where 74% of the study participants experienced improvement of oesophageal inflammation.[54] Subsequent studies validated comparable findings in adults, with the most common food triggers identified as milk and wheat.[51,55] A group investigating the four-food elimination diet (FFED) found its effectiveness to be 54%, with a total of 72% of patients improving if the SFED was applied to the FFED non-responders.[56] A recognised advantage of the empiric elimination diet is significantly improved concordance compared to the elemental diet. Additionally, once specific food allergens are identified, long-term avoidance of the offending agents has shown to produce both clinical and histopathological remission.[55]

ENDOSCOPIC TREATMENT

Endoscopic dilation is usually reserved for patients who relapse on pharmacologic or dietary regimens, and occasionally as first-line therapy if high-grade strictures are initially present. Dilation is performed using one of three types of dilators: mercury or tungsten-filled bougies, wire-guided polyvinyl dilators, or endoscopically-guided balloon dilators.[57] The safety of these procedures has been verified with rare incidence of major complications.[58] A meta-analysis of RCTs of dilations in EoE confirmed that perforation rates remain <1%.[59,60] Less threatening complications such as post-procedural chest pain can occur in up to 75% of patients, but have been found to be well-tolerated by patients.[61]

Retrospective studies have shown that dilation success, defined by symptom resolution, occurs in 83–93% of patients.[61,62] In the absence of severe oesophageal stricturing disease, it is reasonable to consider initial treatment with medical or dietary therapy before proceeding to dilation.[14] A RCT of newly diagnosed EoE patients with mild-to-moderate strictures on PPIs and fluticasone, randomised to dilation or no dilation at time of endoscopy, demonstrated no significant reduction in dysphagia scores between the treatment groups, highlighting that patients without severe strictures may not benefit from endoscopic therapy.[63]

Given frequent recurrence of symptoms post-dilation in 20–25% of patients, serial dilations are often performed to achieve a symptom response.[59,64] In a retrospective study of steroid-naïve EoE patients, the authors used endoscopic dilation as a long-term therapy and maintained patients' symptoms over 13 years with biennial dilations.[65] Sequential dilations are necessary as the intervention does not itself inhibit the pathophysiology of the disease and does not prevent future stricture formation. Studies showed that dilation did not influence the histologic presentation of EoE, with no reduction in oesophageal eosinophilia after the procedure.[61,66]

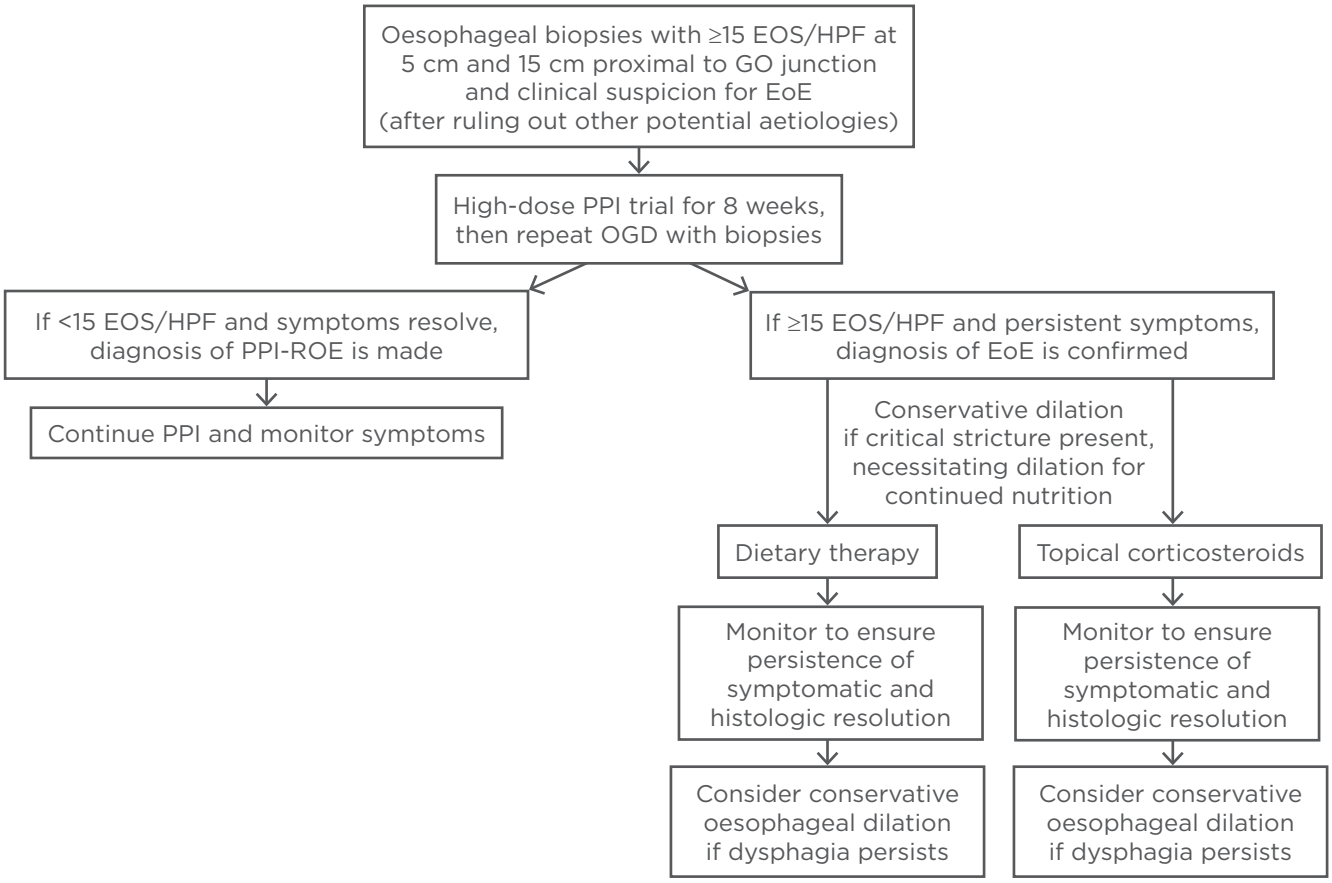


Figure 1: Algorithm for diagnosis and management of eosinophilic oesophagitis.
GO: gastro-oesophageal; EOS/HPF: eosinophils per high-power field; PPI: proton pump inhibitor; EoE: eosinophilic oesophagitis; OGD: oesophagogastroduodenoscopy.
Modified from Kavitt RT et al. with permission.[67]

Thus, when choosing a therapy, it is important to note that oesophageal dilation is thought to treat the clinical symptoms of EoE without changing the course of the underlying disease process.

CONCLUSION

EoE has been defined as a clinicopathological diagnosis, incorporating assessment of eosinophilic burden on histology and patient symptoms and presentation.[14] EoE treatment seeks to target the underlying inflammatory process and suspected triggers, such as food allergens. In the treatment of EoE, a multidisciplinary and collaborative care team comprising of gastroenterologists, allergy/immunology specialists, and dieticians is vital to a successful treatment of comorbid allergic diatheses. Ultimately the goal of treatment is to improve patients' quality of life by reducing symptoms and preventing complications. Current therapeutic options suggest initiating a course of PPIs to confirm the diagnosis and using topical corticosteroids as the mainstay of treatment (Figure 1). The majority of research in dietary therapies has been conducted in the paediatric population, and more data is needed to confirm their efficacy in adults. Future therapies will be based on an improved understanding of the disease pathogenesis, and treatment recommendations will continue to evolve with ongoing research and the development of novel promising therapies.

REFERENCES

1. Landres RT et al. Eosinophilic esophagitis in a patient with vigorous achalasia. Gastroenterology. 1978;74(6):1298-301.
2. Dellon ES. Epidemiology of eosinophilic esophagitis. Gastroenterol Clin North Am. 2014;43(2):201-18.
3. Dellon ES. Diagnosis and management of eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2012;10(10):

1066-78.

4. Dellon ES, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. Gastroenterology. 2014; 147(6):1238-54.

5. Kandulski A, Malfertheiner P. Gastroesophageal reflux disease--from reflux episodes to mucosal inflammation. Nat Rev Gastroenterol Hepatol. 2012;9(1): 15-22.

6. Molina-Infante J et al. Proton pump inhibitor-responsive oesophageal eosinophilia: An entity challenging current diagnostic criteria for eosinophilic oesophagitis. Gut. 2016;65(3):524-31.

7. Cheng E et al. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. Gut. 2013;62(6):824-32.

8. Kedika RR et al. Potential anti-inflammatory effects of proton pump inhibitors: A review and discussion of the clinical implications. Dig Dis Sci. 2009; 54(11):2312-7.

9. Molina-Infante J et al. Review article: proton pump inhibitor therapy for suspected eosinophilic oesophagitis. Aliment Pharmacol Ther. 2013;37(12): 1157-64.

10. Kia L, Hirano I. Distinguishing GERD from eosinophilic oesophagitis: concepts and controversies. Nat Rev Gastroenterol Hepatol. 2015;12(7):379-86.

11. Dellon ES et al. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). Am J Gastroenterol. 2013;108(5): 679-92.

12. Molina-Infante J et al. Long-term loss of response in proton pump inhibitor-responsive esophageal eosinophilia is uncommon and influenced by CYP2C19 genotype and rhinoconjunctivitis. Am J Gastroenterol. 2015;110(11):1567-75.

13. Furuta GT et al. Eosinophilic esophagitis in children and adults: A systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133(4):1342-63.

14. Liacouras CA et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011;128(1): 3-20 e6; quiz 21-2.

15. Redd M, Schey R. Eosinophilic esophagitis: current treatment. Dig Dis Sci. 2013;58(3):613-20.

16. Aceves SS et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. Allergy. 2010;65(1): 109-16.

17. Kagalwalla AF et al. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. J Allergy Clin Immunol. 2012;129(5): 1387-96.e7.

18. Elliott EJ et al. Non-surgical interventions for eosinophilic esophagitis. Cochrane Database Syst Rev. 2010(3): CD004065.

19. Dohil R et al. Oral viscous budesonide is effective in children with eosinophilic esophagitis in a randomized, placebo-controlled trial. Gastroenterology. 2010; 139(2):418-29.

20. Straumann A et al. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. Gastroenterology. 2010;139(5):1526-37.

21. Dellon ES et al. Viscous topical is more effective than nebulized steroid therapy for patients with eosinophilic esophagitis. Gastroenterology. 2012;143(2):321-4.

22. Schaefer ET et al. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. Clin Gastroenterol Hepatol. 2008;6(2):165-73.

23. Peterson KA et al. Comparison of esomeprazole to aerosolized, swallowed fluticasone for eosinophilic esophagitis. Dig Dis Sci. 2010;55(5):1313-9.

24. Konikoff MR et al. A randomized, double-blind, placebo-controlled trial of fluticasone propionate for pediatric eosinophilic esophagitis. Gastroenterology. 2006;131(5):1381-91.

25. Butz BK et al. Efficacy, dose reduction, and resistance to high-dose fluticasone in patients with eosinophilic esophagitis. Gastroenterology. 2014;147(2):324-33.

26. Alexander JA et al. Swallowed fluticasone improves histologic but not symptomatic response of adults with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2012;10(7):742-9.

27. Moawad FJ et al. Randomized controlled trial comparing aerosolized swallowed fluticasone to esomeprazole for esophageal eosinophilia. Am J Gastroenterol. 2013;108(3):366-72.

28. Gupta SK et al. Efficacy and safety of oral budesonide suspension in pediatric patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2015;13(1): 66-76.

29. Straumann A et al. Long-term budesonide maintenance treatment is partially effective for patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2011;9(5):400-9.

30. Murali AR et al. Topical steroids in Eosinophilic Esophagitis: Systematic Review and Meta-analysis of Placebo Controlled Randomized Clinical Trials. J Gastroenterol Hepatol. 2016;31(6):1111-9.

31. Helou EF et al. 3-yr-follow-up of topical corticosteroid treatment for eosinophilic esophagitis in adults. Am J Gastroenterol. 2008;103(9):2194-9.

32. Furuta GT, Katzka DA. Eosinophilic Esophagitis. N Engl J Med. 2015;373(17): 1640-8.

33. Attwood SE et al. Eosinophilic oesophagitis: A novel treatment using Montelukast. Gut. 2003;52(2):181-5.

34. Lucendo AJ et al. Montelukast was inefficient in maintaining steroid-induced remission in adult eosinophilic esophagitis. Dig Dis Sci. 2011;56(12): 3551-8.

35. Corren J. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. Discov Med. 2012;13(71):305-12.

36. Straumann A et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. Gut. 2010;59(1):21-30.

37. Assa'ad AH et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. Gastroenterology. 2011;141(5):1593-604.

38. Spergel JM et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol. 2012;129(2):456-63, 463.e1-3.

39. Straumann A et al. Anti-TNF-alpha (infliximab) therapy for severe adult eosinophilic esophagitis. J Allergy Clin Immunol. 2008;122(2):425-7.

40. Netzer P et al. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. Eur J Gastroenterol Hepatol. 2007;19(10): 865-9.

41. Rocha R et al. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. Eur J Pediatr. 2011;170(11): 1471-4.

42. Cheng E et al. JAK-STAT6 pathway inhibitors block eotaxin-3 secretion by epithelial cells and fibroblasts from esophageal eosinophilia patients: Promising agents to improve inflammation and prevent fibrosis in EoE. Plos One. 2016; 11(6):e0157376.

43. Straumann A et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. Allergy. 2013;68(3):375-85.

44. Arias A et al. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: A systematic review and meta-analysis. Gastroenterology. 2014;146(7): 1639-48.

45. Markowitz JE et al. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents.

Am J Gastroenterol. 2003;98(4):777-82.

46. Liacouras CA et al. Eosinophilic esophagitis: A 10-year experience in 381 children. Clin Gastroenterol Hepatol. 2005; 3(12):1198-206.

47. Peterson KA et al. Elemental diet induces histologic response in adult eosinophilic esophagitis. Am J Gastroenterol. 2013;108(5):759-66.

48. Abu-Sultaneh SM et al. Fluticasone and food allergen elimination reverse sub-epithelial fibrosis in children with eosinophilic esophagitis. Dig Dis Sci. 2011; 56(1):97-102.

49. Spergel JM et al. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. Ann Allergy Asthma Immunol. 2005;95(4): 336-43.

50. Molina-Infante J et al. Selective elimination diet based on skin testing has suboptimal efficacy for adult eosinophilic esophagitis. J Allergy Clin Immunol. 2012; 130(5):1200-2.

51. Gonsalves N et al. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. Gastroenterology. 2012; 142(7):1451-9.

52. Kliewer KL et al. Should wheat, barley, rye, and/or gluten be avoided in a 6-food elimination diet? J Allergy Clin Immunol. 2016;137(4):1011-4.

53. Doerfler B et al. Practical approach to implementing dietary therapy in adults with eosinophilic esophagitis: The Chicago experience. Dis Esophagus. 2015;28(1): 42-58.

54. Kagalwalla AF et al. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2006;4(9): 1097-102.

55. Lucendo AJ et al. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: A prospective study on the food cause of the disease. J Allergy Clin Immunol. 2013;131(3): 797-804.

56. Molina-Infante J et al. Four-food group elimination diet for adult eosinophilic esophagitis: A prospective multicenter study. J Allergy Clin Immunol. 2014;134(5): 1093-9.e1.

57. Standards of Practice C. Egan JV et al. Esophageal dilation. Gastrointest Endosc. 2006;63(6):755-60.

58. Richter JE. Esophageal dilation in eosinophilic esophagitis. Best Pract Res Clin Gastroenterol. 2015;29(5):815-28.

59. Moawad FJ et al. Meta-analysis: The safety and efficacy of dilation in eosinophilic oesophagitis. Aliment Pharmacol Ther. 2013;38(7):713-20.

60. Jacobs JW Jr, Spechler SJ. A systematic review of the risk of perforation during esophageal dilation for patients with eosinophilic esophagitis. Dig Dis Sci. 2010; 55(6):1512-5.

61. Schoepfer AM et al. Esophageal dilation in eosinophilic esophagitis: Effectiveness, safety, and impact on the underlying inflammation. Am J Gastroenterol. 2010;105(5):1062-70.

62. Dellon ES et al. Esophageal dilation in eosinophilic esophagitis: Safety and predictors of clinical response and complications. Gastrointest Endosc. 2010; 71(4):706-12.

63. Kavitt RT et al. Randomized controlled trial comparing esophageal dilation to no dilation among adults with esophageal eosinophilia and dysphagia. Dis Esophagus. 2015 Jul 30. [Epub ahead of print].

64. Saligram S, McGrath K. The safety of a strict wire-guided dilation protocol for eosinophilic esophagitis. Eur J Gastroenterol Hepatol. 2014;26(7): 699-703.

65. Lipka S et al. The natural history of steroid-naive eosinophilic esophagitis in adults treated with endoscopic dilation and proton pump inhibitor therapy over a mean duration of nearly 14 years. Gastrointest Endosc. 2014;80(4):592-8.

66. Bohm M et al. Esophageal dilation: Simple and effective treatment for adults with eosinophilic esophagitis and esophageal rings and narrowing. Dis Esophagus. 2010;23(5):377-85.

67. Kavitt RT et al. Diagnosis and treatment of eosinophlic esophagitis in adults. Am J Med. 2016. [Epub ahead of print].

ASTHMA PHENOTYPES AND THE MICROBIOME

Ogechukwu Ndum,[1] *Yvonne J. Huang[2]

1. Division of Allergy and Immunology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA
2. Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA
**Correspondence to yvjhuang@umich.edu*

Disclosure: The authors have declared no conflicts of interest.
Received: 01.04.16 **Accepted:** 28.06.16
Citation: EMJ Allergy Immunol. 2016;1[1]:82-90.

ABSTRACT

Asthma is characterised by episodic bronchospasm, airway hyperreactivity, and airway inflammation. Current treatment is aimed at reversing bronchospasm with bronchodilators and decreasing airway inflammation with corticosteroids. Asthma patients as a collective group, however, have variable responses to treatment, and our understanding and view of asthma as a single pathologic process has evolved substantially. We now recognise that asthma is a heterogeneous disease with many phenotypes, as reflected by differences in natural history, complexity, severity, and responses to treatment. The underlying aetiologies for many phenotypes are poorly understood and likely multifactorial. Recent evidence increasingly supports an important role for microbial exposures and our microbiota as factors mediating asthma pathogenesis. However, given the phenotypic heterogeneity of asthma, we further propose that microbiota may play an additional role in shaping asthma phenotype. Beginning with a brief overview of concepts of asthma phenotypes and endotypes, the intent of this article is to summarise current knowledge of the microbiome in asthma, highlighting recent studies that have examined relationships between microbiota and phenotypic features of asthma. We conclude with a discussion of future research directions, considering important issues and challenges in this area of investigation.

Keywords: Asthma, endotype, Type 2 inflammation, microbiota, *16S* rRNA, metagenomics.

FROM PHENOTYPE TO ENDOTYPE: ASTHMA IS NOT ONE SIZE FITS ALL

Multiple asthma phenotypes have been described[1,2] using frameworks that incorporate demographic, clinical, and inflammatory features. To describe known biology and more precisely define phenotypes, the term 'asthma endotype' was proposed in a 2011 consensus report published by the European Academy of Allergy and Clinical Immunology (EAACI) and the American Academy of Allergy, Asthma and Immunology (AAAAI).[3] Fundamentally, an 'endotype' is a subtype of a condition defined by a distinct functional or pathophysiologic mechanism.[3] It also has been proposed that asthma endotypes should be distinct in several parameters, including clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and treatment response.[3]

Recent molecular studies of airway gene expression patterns have helped define at least one asthma endotype based on markers of T helper Type 2 (Th2) inflammation. Two of these studies examined bronchial epithelial cell gene expression patterns in asthmatic subjects,[4,5] identifying sets of interleukin (IL)-13-inducible epithelial genes (*POSTN*, *CLCA1*, and *SERPINB2*) that characterised subjects who responded favourably to inhaled corticosteroids. A similar approach was adopted in a recent study of sputum cell expression of Type 2 and non-Type 2-related genes.[6] Sputum-based gene expression profiling also molecularly identified subgroups of 'Type 2 high' versus 'Type 2 low' asthma, in particular expression of IL-4, IL-5, and IL-13. These studies, in conjunction with earlier data on the high prevalence of asthmatic subgroups with non-eosinophilic inflammation, have helped establish Type 2 high asthma as an endotype

that characterises only ~50% of patients.[7,8] A component of the definition of this endotype is the greater likelihood of responsiveness to corticosteroid therapy.[5]

However, not all Type 2 high asthma is responsive to steroid therapy alone. For instance, some patients with severe asthma have ongoing poor asthma control and airway eosinophilia despite high doses of inhaled corticosteroids.[9] It is now known that Type 2 cytokines derive from other cellular sources besides classical Type 2 lymphocytes (e.g. innate lymphoid cells),[9,10] and thus not all Type 2 immune responses may be steroid-sensitive. In this setting, new immunotherapeutics targeting specific cytokines that drive Type 2 inflammation (e.g. anti-IL-5, anti-IL-13, and anti-IL-4Rα) will become important adjunctive therapies in patients with evidence of ongoing Type 2 or eosinophilic inflammation despite steroid therapy.[11]

In contrast, Type 2 low asthma likely encompasses several sub-types of asthma with different underlying mechanisms, though these remain poorly understood. Thus the term Type 2 low asthma in itself does not define a particular endotype, but a common feature is poorer responses to corticosteroid therapy. Understanding mechanisms of Type 2 low asthma has been identified as a research priority.[12] Although markers of Type 2 inflammation run a continuum, classifying asthma as Type 2 high versus Type 2 low is useful both clinically and in research. While the best approach to identify such patients in clinical practice remains undetermined, the distinction facilitates 1) appropriate tailoring of available treatments that largely target Type 2 inflammation,[8,11] and 2) focussing of research efforts on mechanisms and treatment targets for Type 2 low phenotypes of asthma.

Despite the goal of asthma endotyping schemes, overlap among endotypes in clinical and inflammatory features is certain. Broad characteristics like timing of disease onset, gender, the presence of atopy, and level of lung function are common across describedphenotypes with different underlying mechanisms.[13-16] For example 'late-onset asthma' includes several phenotypes.[17] Among these, aspirin-exacerbated respiratory disease represents one of the best studied endotypes,[18] characterised by later onset of asthma, chronic rhinosinusitis with nasal polyps, and acute respiratory reactions following the ingestion of non-steroidal anti-inflammatory drugs. Although eosinophilic sinus disease is prominent, the pathogenesis of aspirin-exacerbated respiratory disease is thought to involve inflammatory lipid mediators and cyclo-oxygenase pathways of arachidonic acid metabolism,[18] rather than Type 2 immune responses specifically.

Other phenotypes of late-onset asthma are little understood in comparison.[1,17] A distinct subgroup of adult-onset asthma patients have recurrent severe exacerbations with pulmonary function often lower than in allergic asthmatic patients, and the presence of sputum eosinophilia despite atopy being less common.[19,20] Obesity-associated asthma is also a significant problem. However, adult-onset asthma complicating obesity may result from a different interplay of mechanisms versus childhood asthma, complicated by obesity. Studies of adult and paediatric cohorts have implicated both non-Type 2 and Type 2 pathways in subsets of obese asthma patients.[21-23] Additional phenotypes characterised by low or absent Type 2 inflammation include those with neutrophil-predominant (non-eosinophilic) airway inflammation that is not solely attributable to concurrent use of inhaled corticosteroids.[7,24] Potential aetiologies include chronic infection by bacteria and smoking-induced airway inflammation. Moreover, among patients on corticosteroids, it is quite possible that this superimposed therapy further shapes both the inflammatory and microbial milieu of the airways,[25-28] complicating the understanding of potential asthma endotypes at this end of the disease spectrum.

In summary, many asthma phenotypes and potential endotypes exist but the underlying mechanisms for many of these remain unclear, especially in Type 2 low disease. It is likely that multiple factors shape the development of asthma phenotypes, such that the ultimate definition of any one endotype will contain clinical and/or inflammatory criteria that overlap with other endotypes. In addition, links between patterns of microbiota composition and specific features of asthma have been reported.[26-29] Table 1 presents a non-comprehensive list of recent literature on asthma phenotypes, including studies that have focussed on the lower respiratory microbiome in asthma. We believe that as knowledge advances on how microbial factors shape asthma, the microbiome will become an additional domain necessary to consider and incorporate into efforts to define asthma endotypes.

This parallels a recent editorial by Stappenbeck and Virgin[30] who argue that we can no longer ignore the fact that "mammals are defined by their metagenome, a combination of host and microbiome genes," and that this knowledge must be incorporated into efforts to understand human disease pathogenesis and disease phenotypes.

THE MICROBIOME AND ITS POSSIBLE ROLE IN ASTHMA PHENOTYPE

Study of the role of microbes in asthma has been of long-standing interest and is of increasing importance, as newer techniques to molecularly identify microbes advance knowledge in this area. Interactions between the microbial environment and the naïve immune system are key in the development of immune defences and determine the repertoire of the immune system.[31] An emerging body of evidence shows that the microbial environment plays a role in the pathogenesis of atopic diseases, including asthma.[32] Asthma has been associated with distinct differences in the composition of gut and respiratory microbiota, compared with healthy individuals.[33-37] Moreover, different patterns of respiratory microbiota composition are associated with different phenotypic features of asthma,[26-29,38] suggesting that microbial dysbiosis could play a role in asthma endotype(s). Specifically, features like airway hyperresponsiveness, airflow obstruction, obesity, and level of asthma control have been found to correlate with particular features of airway microbiota composition.[26,27,29]

Environmental Microbial Exposures and Asthma in Children

The association between a deficiency in environmental microbial exposure and increased prevalence of atopic disease is the basis of one of the earliest prevailing hypotheses on the pathogenesis of allergic disease. The 'hygiene hypothesis' is based on the principle that lack of early exposure to infectious agents or microbes that stimulate protective immune responses increase susceptibility to allergic diseases. Children raised in homes with multiple siblings[39] or pets have decreased prevalence of atopic disease.[40,41] Increased family size, exposure to day care, and increased crowding have also been associated with decreased rates of allergy or asthma.[42-44] Asthma prevalence is greater among urban children, compared with their counterparts raised in rural areas and farming environments[42-44] who may more likely be exposed to domesticated animals, outdoor microbes, and additional environmental exposures that shape immune responses. Though exact mechanisms remain incompletely understood, recent molecular insights have implicated endotoxin-induced modification of communication between airway epithelial and dendritic cells.[45]

Connections Between the Gut Microbiome and Asthma Development

It is estimated that 500–1,000 different bacterial species inhabit the mature gastrointestinal tract, with bacterial cells outnumbering host cells 10-fold.[46] The micro-organisms colonising the gut perform different functions vital for human health, including processing of dietary constituents, regulation of host metabolism,[47] and immune system maturation, including development of immune tolerance.[31] Differences in birth mode and antibiotic exposures have been linked to alterations in gut microbial ecology and immune development.[46] Studies utilising high-throughput molecular platforms have reported strong associations between gut microbiome features, immune responses, and the tendency to develop allergy or asthma. A comprehensive overview of these studies is beyond the scope of this article, and we refer readers to other reviews on this topic.[33]

In a study by Abrahamsson et al.,[35] 47 infants had stool samples collected at 1 week, 1 month, and 12 months of age. Seven years later, the children were assessed for allergic disease and skin-prick test reactivity. Lower microbiota diversity was found at 1 week and 1 month in children who went on to develop asthma by 7 years of age. A more recent study by Arrieta et al.[36] similarly found that differences in gut microbiota composition in very early life were associated with greater risk of having atopic wheeze later in childhood. Decreased relative abundances of *Lachnospira, Veillonella, Rothia,* and *Faecalibacterium* in the first 100 days of life were seen among infants at high-risk of developing asthma in childhood. Another study showed that infants colonised at 3 weeks of age with *Bacteroides fragilis* group and/or *Clostridium coccoides* subcluster XIVa, had increased risk of asthma at 3 years of age.[37]

In addition to their immunostimulatory effects, gut bacteria also express crucial enzymes that permit metabolism of otherwise indigestible polysaccharides and dietary starches, leading to

the production of short-chain fatty acids (SCFA), such as butyrate, propionate, and acetate that can be used for host ATP synthesis or synthesis of other energy substrates in the liver.[47] SCFA are known to be important to colonic epithelial and mucosal health.[47,48] Recent studies also suggest a role for gut-derived SCFA in modulating allergic airway inflammation.[49]

In contrast to paediatric studies or animal models of asthma pathogenesis, the potential role of the gut microbiome in adults with asthma has not been directly studied. Obesity is an important comorbidity of asthma and has been associated with increased asthma severity that is less steroid-responsive. Independent of asthma, the distal gut microbiota in obese humans or mice appears to be altered compared with lean states.[50,51] Specifically, obesity has been associated with less diverse gut bacterial communities, with reported alterations in the ratio of *Firmicutes* to *Bacteroidetes*, the two most common bacterial phyla present in the gut.[51]

Table 1: Abbreviated list of publications highlighting the heterogeneity of asthma, Type 2 high versus Type 2 low phenotypes, and recent studies of the lower respiratory microbiome in asthma.

	Author	Year	Significant findings
Heterogeneity of asthma phenotypes	Wenzel.[1]	2012	Review of clinical and molecular phenotypes of asthma, and advances in identifying specific molecular markers particularly in Type 2 high asthma.
	Lotvall et al.[3]	2011	Proposes classification of asthma by 'endotype', defined as a subtype of a condition that is defined by a specific pathophysiologic mechanism.
	Haldar et al.[13]	2008	Unbiased cluster analysis approach to phenotype asthma patients seen in primary (n=184) and secondary care (n=187). Identified clusters included early-onset atopic/obese asthma, non-eosinophilic asthma, and clusters with discordant symptoms and airway inflammation.
	Moore et al.[14]	2010	Unsupervised hierarchical cluster analysis of mild, moderate, and severe asthma subjects in the NIH Severe Asthma Research Program (n=726) revealed five predominant clusters, differing in asthma onset, symptoms, lung function, medication needs, and healthcare utilisation.
	Modena et al.[59]	2014	Bronchial airway epithelial genes correlating with FeNO were first clustered to identify 'subject clusters'. Subsequent analysis for differences in gene expression between the FeNO-related subject clusters revealed novel biological pathways in addition to Type 2 inflammation.
	Yan et al.[15]	2015	Analysis of the sputum transcriptome using cluster analysis to identify gene expression profiles associated with clinical phenotypic features of asthma. Three distinct transcriptomic endotypes were identified, each associated with distinct clinical features.
Type 2 driven asthma (Type 2 high)	Woodruff et al.[4]	2007	Differential gene expression analysis of bronchial airway epithelial cells identified IL-13 inducible epithelial genes (*CLCA1, POSTN,* and *serpinB2*) associated with asthma and also response to corticosteroids (*FKBP51*).
	Woodruff et al.[5]	2009	Expression of IL-13-inducible epithelial genes (*CLCA1, POSTN,* and *serpinB2*) used to molecularly phenotype asthmatic and healthy subjects. In addition to other features of atopic asthma, Type 2 high subjects showed greater lung function improvement in response to inhaled corticosteroids.
	Smith et al.[9]	2016	Increased detection of ILC2 in blood and sputum of patients with severe asthma and persistent eosinophilia, compared to mild asthma.

Table 1 continued.

	Author	Year	Significant findings
Non-Type 2 asthma phenotypes (Type 2 low)	Berry et al.[60]	2007	Asthmatics with decreased eosinophilic airway inflammation have less subepithelial thickening, increased mast cells in airway smooth muscle, and decreased response to mometasone.
	Amelink et al.[19]	2013	Study of phenotypic features of 176 patients with adult-onset asthma (age >20 years). Severe adult-onset asthma was associated with absence of atopy, greater nasal polyposis, higher exhaled nitric oxide, blood neutrophils, and sputum eosinophils.
	Holguin et al.[23]	2011	Analysis of 1,049 patients showing that asthma is differentially affected by obesity depending on whether asthma onset was early (<12 years) or late (≥12 years).
Asthma features and the lower respiratory microbiome	Hilty et al.[25]	2010	Bronchial brushings and BAL from 11 adults with asthma, 5 with COPD, and 8 healthy control subjects analysed by traditional *16S* rRNA clone library/Sanger sequencing. Asthmatic patients (all on inhaled steroids) demonstrated greater prevalence of potentially pathogenic members of the Proteobacteria phylum (e.g. *Haemophilus* spp.), compared with healthy controls.
Asthma features and the lower respiratory microbiome	Huang et al.[26]	2011	Prospective analysis of protected bronchial brushings from 42 asthmatic and 10 healthy subjects using *16S* rRNA-based microarray. Asthmatic subjects (on standardised doses of fluticasone) possessed a significantly different bacterial microbiota composition from healthy controls. The relative abundance of specific bacterial groups correlated with bronchial hyperresponsiveness.
	Marri et al.[34]	2013	Banked induced sputum supernatants from 10 healthy and 10 mild asthma subjects (8/10 not on inhaled steroids) analysed by *16S* rRNA sequencing (454-Roche Platform). Members of the Proteobacteria phylum are more commonly found in the asthmatic group.
	Green et al.[38]	2014	Induced sputum from 28 treatment-resistant severe asthmatics analysed by terminal restriction fragment length polymorphism profiling for bacterial community analysis. Dominant species in most patients were *Moraxella catarrhalis* or a member of the *Haemophilus* or *Streptococcus* genera, which were associated with worse FEV_1 and higher sputum neutrophil.
	Huang et al.[29]	2015	Protected bronchial brushings from 30 severe asthma subjects analysed by *16S* rRNA-based microarray. Differences in bronchial bacterial community composition were associated with features of severe asthma including obesity, asthma control scores, and epithelial gene expression profiles. Significant differences in airway bacterial community composition were also found between subjects with severe versus moderate asthma.
	Denner et al.[27]	2016	Banked endobronchial brushings and BAL from 39 asthmatic and 19 control subjects analysed by *16S* rRNA sequencing. Specimen types differed in bacterial diversity but differences between the subject groups were more robust in brush samples. Oral steroid use and FEV_1 measures were associated with differences in bronchial bacterial community composition.
	Simpson et al.[28]	2016	Induced sputum from 30 poorly controlled asthmatic patients analysed by *16S* rRNA pyrosequencing. Neutrophilic asthma subjects had reduced bacterial diversity along with high prevalence of *Haemophilus influenzae*, while *Tropheryma whipplei* was identified in 12 participants and associated with eosinophilic asthma.

NIH: National Institutes of Health; COPD: chronic obstructive pulmonary disease; BAL: bronchoalveolar lavage; IL: interleukin; FeNO: fractional exhaled nitric oxide; ILC2: Type 2 cytokine-producing innate lymphoid cells; FEV_1: forced expiratory volume in 1 second.

Feeding mice a diet high in fermentable fibre content altered the ratio of these two bacterial phyla, increased levels of circulating SCFA, and through their alteration of dendritic cell capacity to promote Type 2 cell effector function, protected against allergic inflammation in the lung.[49] These *in vivo* studies provide a framework for understanding possible links between the gut microbiome and allergic inflammation in the lung.

THE AIRWAY MICROBIOME AND ASTHMA

The properties of the lower airway microbiome are distinct. The bacterial make-up of the lower airway is different from the upper airway and does not completely reflect oropharyngeal flora.[52,53] While there is variability in the composition of lower airway microbiota from person to person, several studies have shown that the lower airway microbiome differs in the setting of chronic airway disease, compared with healthy state. For example, *Proteobacteria*, a large phylum that represents many known potential respiratory pathogens, are relatively more abundant in subjects with asthma and other obstructive airway diseases, compared with healthy controls.[54] Members of the *Proteobacteria* more prevalent in airways of asthmatic subjects include the genera *Haemophilus, Moraxella, Neisseria*, and members of the Enterobacteriaceae family.[25-29,34,38] Differences in respiratory microbiota composition have also been observed as early as the first year of life, mostly with nasopharyngeal sample analysis. In a prospective cohort of 234 children in Australia,[55] nasopharyngeal samples collected from infants at age 2 months, 6 months, and 12 months were analysed by *16S* rRNA gene sequencing to evaluate nasal bacterial microbiota composition. During this time, all incidents of acute respiratory infection in the children were documented. The infant nasopharyngeal microbiomes were dominated by six genera: *Moraxella* (31.2%), *Streptococcus* (15.5%), *Corynebacterium* (13.5%), *Staphylococcus* (10.3%), *Haemophilus* (9.7%), and *Alloiococcus* (8.8%). During acute respiratory viral infections, known to be associated with increased propensity to develop asthma,[56] *Moraxella, Streptococcus,* and *Haemophilus* were more prevalent. In adjusted multivariate analyses, early asymptomatic colonisation with *Streptococcus* was found to be a strong predictor for current wheeze at 5 years of age as well as subsequent chronic wheeze at 10 years of age. These findings suggest that important viral-bacterial-host interactions occur that likely impact immune responses leading to asthma.

Microbiome diversity has been found to differ in paediatric asthmatic patients compared with healthy controls. In a single-centre observational study, nasal epithelial cells were collected from eight children with asthma and six healthy controls ranging ages of 6–20 years. RNA sequencing was used to pursue microbial identification.[57] The nasal microbiome of asthmatic patients was less diverse when compared with healthy controls. Furthermore, there were higher levels of *Moraxella* in asthmatics. *Escherichia* and *Psychrobacter* (a member of the Moraxellaceae family) were found in greater abundance in nasal epithelial cells of asthmatic children compared with controls. *Haemophilus influenzae*, *Streptococcus* spp., and *Staphylococcus* spp. were detected in samples from asthmatic patients, although the relative abundance was not statistically significantly different compared with healthy controls.

Differences in the airway microbiome have also been observed across asthma of different severities.[29] Phenotypic characteristics such as body mass index, measures of airway hyperresponsiveness, and asthma control have also been found to correlate with different patterns of lower airway bacterial microbiota composition.[26,27,29] Moreover, a subset of asthma patients may harbour particular bacterial microbiota and be more likely to respond to treatment with macrolide antibiotic therapy. In a *post hoc* analysis of moderate asthma subjects randomised to treatment with clarithromycin for 16 weeks, those demonstrating an improvement in airway hyperresponsiveness after macrolide treatment had higher bacterial diversity at baseline.[26] Studies have taken a closer look at which organisms are associated with greater asthma severity.[24,25] A study of sputum bacterial composition in 28 treatment (steroid)-resistant asthmatic patients found that the relative abundance of *Moraxella catarrhalis, Haemophilus* spp., or *Streptococcus* spp. correlated with worse lung function, higher sputum neutrophil counts, and IL-8 concentrations.[38] In a study that analysed protected bronchial airway brushings,[29] similar patterns of increased proteobacteria were seen with greater asthma severity, with a subset of severe asthma patients demonstrating significantly greater relative abundance of *Klebsiella (pneumoniae* or *oxytoca* species).

Concurrent steroid treatment may influence these findings, and further studies are needed. Taken together, however, existing evidence suggests that patterns of microbiome diversity or the relative abundance of particular groups of organisms, may serve as a microbial marker of patients more likely to respond to antimicrobial or other microbiome-targeted therapies in the future.

CONCLUSION AND FUTURE DIRECTIONS

Recent studies have elucidated the existence and complexity of the upper and lower respiratory microbiomes, and their associations with asthma including phenotypic clinical and inflammatory features. These extend earlier studies that focussed on the role of environmental microbial exposures or gut microbiota in asthma development. There are unique challenges to studying the lower respiratory microbiome, however. Direct sampling requires invasive bronchoscopy that is burdensome, costly, poses some risks, and hampers abilities to conduct large clinical studies in the outpatient setting. Recent studies have clarified that the upper and lower respiratory tracts harbour distinct compositions of bacterial microbiota,[52,53] a difference magnified in the setting of chronic obstructive airway diseases.[54] Nonetheless, studies of larger numbers of patients are important because significant heterogeneity between individuals in microbiota composition has been observed, regardless of health or disease status and even among those with advanced lung disease due to cystic fibrosis or chronic obstructive pulmonary disease.[54,58] Longitudinal studies are also needed to understand the intrinsic stability or variability of the respiratory microbiome, both compositionally and functionally. We view this as a necessary foundation to link microbiome relationships to clinical outcomes and events (e.g. exacerbations) and identify potential microbial markers of disease endotype. Therefore, microbiome studies of other specimen types will be useful to gauge their utility, and potentially even their advantages over bronchoscopy-collected samples, to understand how respiratory microbiota influence asthma. There is precedence as quality-controlled sputum has been used for decades to understand inflammatory patterns in asthma, and recent analyses of nasopharyngeal samples have generated new hypotheses about microbiota relationships to asthma in children.[55]

We believe the following topics also merit further attention: 1) determining relationships between respiratory microbiota and patterns of host inflammation, which may help to define particular endotypes; 2) exploring whether alterations in the gut microbiome are associated with asthma in those with established disease; and 3) understanding what functions are elaborated by respiratory microbiota to shape host responses in asthma. Moreover, studies of the respiratory microbiome to date have largely focussed on bacteria due to well-established methods and the accessibility of techniques to study mixed populations of bacteria. Fungal and viral communities also are of interest, but molecular methods to analyse the mycobiome and virome, respectively, are more challenging than for bacteria. Other -omic approaches such as metagenomics (shotgun DNA sequencing), metatranscriptomics, and metabolomics can be leveraged to investigate the functional potential of members of the microbiota. However, applying these methods to study human respiratory samples, often of limited material volume, presents some challenges. Important choices may need to be made upfront regarding sample handling and processing for such analyses.

Asthma is a complex and heterogeneous disease whose pathogenesis, including that of different phenotypes, likely involves multiple components and pathways. The microbiome in itself is also complex, and its relationships with the host may be viewed as a network of interactions. More research is clearly needed, but emerging evidence suggests that microbial dysbiosis of the respiratory tract has the potential to shape manifestations of asthma and may underline particular disease endotypes.

Acknowledgements

Yvonne J. Huang is supported by grants from the National Heart, Lung and Blood Institute (HL105572) and the Michigan Institute for Clinical and Health Research.

REFERENCES

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25.

2. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. Discov Med. 2013;15(83): 243-9.

3. Lotvall J et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol. 2011;127(2):355-60.

4. Woodruff PG et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A. 2007;104(40):15858-63.

5. Woodruff PG et al. T-helper Type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med. 2009;180(5):388-95.

6. Peters MC et al. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. J Allergy Clin Immunol. 2014; 133(2):388-94.

7. Douwes J et al. Non-eosinophilic asthma: importance and possible mechanisms. Thorax. 2002;57(7):643-8.

8. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol. 2015;15(1):57-65.

9. Smith SG et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. J Allergy Clin Immunol. 2016;137(1):75-86.

10. Saglani S, Lloyd CM. Eosinophils in the pathogenesis of paediatric severe asthma. Curr Opin Allergy Clin Immunol. 2014;14(2):143-8.

11. Chung KF. Targeting the interleukin pathway in the treatment of asthma. Lancet. 2015;386(9998):1086-96.

12. Levy BD et al. Future Research Directions in Asthma. An NHLBI Working Group Report. Am J Respir Crit Care Med. 2015;192(11):1366-72.

13. Haldar P et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178(3):218-24.

14. Moore WC et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315-23.

15. Yan X et al. Noninvasive analysis of sputum transciptome discriminates clinical phenotypes of asthma. Am J Respir Crit Care Med. 2015;191(10):1116-25.

16. Wu W. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. J Allergy Clin Immunol. 2014;133(5):1280-8.

17. Ilmarinen P et al. Phenotypes, Risk Factors, and Mechanisms of Adult-Onset Asthma. Mediators Inflamm. 2015;2015: 514868.

18. Laidlaw TM, Boyce JA. Pathogenesis of aspirin-exacerbated respiratory disease and reactions. Immunol Allergy Clin North Am. 2013;33(2):195-210.

19. Amelink M et al. Severe adult-onset asthma: a distinct phenotype. J Allergy Clin Immunol. 2013;132:336-41.

20. Miranda C et al. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol. 2004;113(1):101-8.

21. Sutherland ER et al. Cluster analysis of obesity and asthma phenotypes. PLoS ONE. 2012;7:e36631.

22. Rastogi D et al. Obesity-associated asthma in children: a distinct entity. Chest. 2012;141(4):895-905.

23. Holguin F et al. Obesity and asthma: an association modified by age of asthma onset. J Allergy Clin Immunol. 2011;127(6): 1486-93.e2.

24. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. Proc Am Thorac Soc. 2009;6(3):256-9.

25. Hilty M et al. Disordered microbial communities in asthmatic airways. PLoS One. 2010;5(1):e8578.

26. Huang YJ et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol. 2011;127(2): 372-381.e1-3.

27. Denner DR et al. Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways. J Allergy Clin Immunol. 2016;137(5):1398-1405.e3.

28. Simpson JL et al. Airway dysbiosis: Haemophilus influenzae and Tropheryma in poorly controlled asthma. Eur Respir J. 2016;47(3):792-800.

29. Huang YJ et al. The airway microbiome in patients with severe asthma: Associations with disease features and severity. J Allergy Clin Immunol. 2015; 136(4):874-84.

30. Stappenbeck TS, Virgin HW. Accounting for reciprocal host-microbiom e interactions in experimental science. Nature. 2016;534(7606):191-9.

31. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41.

32. Lynch SV, Boushey HA. The microbiome and development of allergic disease. Curr Opin Allergy Clin Immunol. 2016;16(2): 165-71.

33. Fujimura KE, Lynch SV. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. Cell Host Microbe. 2015;17(5):592-602.

34. Marri PR et al. Asthma-associated differences in microbial composition of induced sputum. J Allergy Clin Immunol. 2013;131(2):346-52.e1-3.

35. Abrahamsson TR et al. Low gut microbiota diversity in early infancy precedes asthma at school age. Clin Exp Allergy. 2014;44(6):842-50.

36. Arrieta MC et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015;7(307):307ra152.

37. Vael C et al. Denaturing gradient gel electrophoresis of neonatal intestinal microbiota in relation to the development of asthma. BMC Microbiol. 2011;11:68.

38. Green BJ et al. Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. PLoS One. 2014;9(6):e100645.

39. Strachan DP. Hay fever, hygiene and household size. BMJ. 1989;299(6710): 1259-60.

40. Anyo G et al. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. Clin Exp Allergy. 2002;32:361-6.

41. Almqvist C et al. Effects of early cat or dog ownership on sensitisation and asthma in a high-risk cohort without disease-related modification of exposure. Paediatr Perinat Epidemiol. 2010;24:171-8.

42. Gern JE. The Urban Environment and Childhood Asthma Study. J Allergy Clin Immunol. 2010;125(3):545-9.

43. Naleway AL. Asthma and Atopy in Rural Children: Is Farming Protective? Clinical Medicine and Research. 2004; 2(1):5-12.

44. von Mutius E. The microbial environment and its influence on asthma prevention in early life. J Allergy Clin Immunol. 2016;137(3):680-9.

45. Schuijs MJ et al. 2015. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science. 2015;349(6252):1106-10.

46. Sommer F, Backhed F. The gut microbiota—masters of host development and physiology. Nat Rev Microbiol. 2013; 11(4):227-38.

47. Kasubuchi M et al. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. Nutrients. 2015; 7(4):2839-49.

48. Tremaroli V, Backhed F. Functional

interactions between the gut microbiota and host metabolism. Nature. 2012; 489(7415):242-9.

49. Trompette A et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20(2):159-66.

50. Ley RE et al. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102(31):11070-5.

51. Turnbaugh PJ et al. A core gut microbiome in obese and lean twins. Nature. 2009;457(7228):480-4.

52. Bassis CM et al. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. MBio. 2015;6(2): e00037.

53. Venkataraman A et al. Application of a neutral community model to assess structuring of the human lung microbiome. MBio. 2015;6(1):e02284-14.

54. Han MK et al. Significance of the microbiome in obstructive lung disease. Thorax. 2012;67(5):456-63.

55. Teo SM et al. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe. 2015; 17(5):704-15.

56. Feldman AS et al. Toward primary prevention of asthma. Reviewing the evidence for early-life respiratory viral infections as modifiable risk factors to prevent childhood asthma. Am J Respir Crit Care Med. 2015;191(1):34-44.

57. Castro-Nallar E et al. Integrating microbial and host transcriptomics to characterize asthma-associated microbial communities. BMC Medical Genomics. 2015;8:50.

58. Huang YJ, LiPuma JJ. The microbiome in cystic fibrosis. Clin Chest Med. 2016;37(1): 59-67.

59. Modena BD et al. Gene expression in relation to exhaled nitric oxide identifies novel asthma phenotypes with unique biomolecular pathways. Am J Respir Crit Care Med. 2014;190(12):1363-72.

60. Berry M et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62(12):1043-9.

EMJ EUROPEAN MEDICAL JOURNAL

CALL for PAPERS

If you are interested in submitting a paper to **EMJ**, contact: editor@emjreviews.com

Follow us:

www.emjreviews.com

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

DRUG ALLERGY: DELAYED CUTANEOUS HYPERSENSITIVITY REACTIONS TO DRUGS

***Rose L. Hamm**

University of Southern California, Los Angeles, California, USA
**Correspondence to rhammpt@msn.com*

Disclosure: The author has declared no conflicts of interest.
Received: 18.03.16 **Accepted:** 20.07.16
Citation: EMJ Allergy Immunol. 2016;1[1]:92-101.

ABSTRACT

Drug allergies, also termed adverse drug reactions (ADRs), are a problem for individuals of all ages, from paediatric to geriatric, and in all medical settings. They may be a predictable reaction to a specific drug (termed Type A) or particular to the individual (termed Type B). Health professionals, especially those caring for patients at the point of entry into the medical system, have a very important role in determining if and when a patient is having an ADR. The purpose of this article is to review the pathophysiology of ADRs, describe the signs and symptoms of different classifications of ADRs, and present the medical and wound treatment for patients with systemic and cutaneous reactions to drug allergies.

Keywords: Drug allergy, adverse drug reaction (ADR), drug hypersensitivity syndrome (DHS), severe cutaneous adverse reactions.

INTRODUCTION

An adverse drug reaction (ADR) is defined by the World Health Organization (WHO) as "a response to a medicine which is noxious and unintended, and which occurs at doses normally used in man." ADRs account for 3–6% of all hospital admissions and occur in 10–15% of hospitalised patients.[1] Drug allergies are one type of ADR and are defined by the Joint Task Force on Practice Parameters (representing the American Academy of Allergy, Asthma and Immunology [AAAAI]; the American College of Allergy, Asthma and Immunology [ACAAI]; and the Joint Council of Allergy, Asthma and Immunology [JCAAI]) as "an immunologically mediated response to a pharmaceutical and/or formulation (excipient) agent in a sensitized person."[2] This definition implies that the reaction can have both immunoglobulin (Ig)E-mediated and non-IgE-mediated mechanisms.

Incidence and prevalence of ADRs is uncertain because of the variability in studies. For example, the setting, patient population, age, and case verification all differ in the following reported studies. The Boston Collaborative Drug Surveillance Program, established in 1966, is a pharmacoepidemiologic research programme that continues to conduct studies on pharmaceutical drug reactions in a variety of settings and patient populations. An extensive list of their publications can be accessed at http://www.bu.edu/bcdsppublications.[3] Early studies of 15,438 in-patients reported an overall 2.2% reaction rate, with the highest rates causes by amoxicillin, trimethoprim-sulfamethoxazole, and ampicillin (51.4/1,000, 33.8/1,000, and 33.2/1,000, respectively).[4] A 6-month French study of cutaneous allergic reactions from systemic drugs occurring in a hospital setting found a rate of 3.6 per 1,000 patients had reactions.[5] A similar 10-month study in Mexico found a prevalence of 7 per 1,000 patients.[6] Two studies have reported both systemic and cutaneous reactions that were confirmed by allergists in an in-patient setting. One study in Singapore reported 366 cases from 90,910 admissions, and one in Korea reported 2,682 cases among 55,432 admissions.[1] Numerous other studies and meta-analyses have been reported in the literature on drug allergies for patients in various settings, on both adults and children, and on various individual medications.[1,7-9]

For example, the incidence of drug hypersensitivity syndrome (DHS) with anticonvulsants has been estimated at 1 in 10,000 exposures.[10] Table 1 shows

a list of the most common drugs that have been found to induce drug allergies.[2,11-13]

Multiple DHS (MDH) was first described by Sullivan et al.[14] in 1989 and is defined as a drug allergy to two or more chemically different drugs, mainly antibiotics. Chiriac and Demoly[15] further described the multiple drug intolerance syndrome in which patients reported "various adverse drug reactions to three or more chemically, pharmacologically, and immunogenically unrelated drugs, taken independently, and who display certain negative allergological tests." Two subtypes of MDH syndrome were proposed by Gex-Collet et al.,[16] one which develops against different drugs given simultaneously and a second which develops when sensitisations appear sequentially, sometimes years apart. Using both a skin patch test and the lymphocyte transformation test, they found sensitivity to antibiotics as well as anti-epileptics, hypnotics, antidepressants, local anaesthetics, and corticosteroids.[16] Studies have shown that 1–5% of all patients with drug allergies have MDH syndrome.[17]

Risk factors that have been associated with ADRs can be host-related or drug-related. Host-related factors include female sex,[18] concomitant diseases (such as HIV, reactivation of herpes virus, and renal or liver disease), ethnicity, polypharmacy, alcoholism, and genetic predisposition.[1,19] Certain classes of drugs tend to be associated with a higher incidence of drug allergies, based upon their ability to act as a hapten, prohapten, or covalent binder to immune receptors.[1] The method of drug administration can also affect the frequency of ADRs; topical, intramuscular, and intravenous (IV) methods are more likely to cause hypersensitivity reactions than oral medications.[19]

PATHOPHYSIOLOGY OF DRUG ALLERGIES

The pathophysiology of drug allergies is not fully understood; however, the cell-mediated immune reaction and the activation of T cells is proposed to occur through three different mechanisms:

- The hapten/prohapten hypothesis,
- The pharmacologic interaction with immune receptor (p-i) model
- The altered peptide repertoire hypothesis[8]

According to the hapten/prohapten hypothesis, the causative drug acts as either a hapten (a small chemical molecule that forms covalent attachment to a protein), prohapten (a chemical that can be converted to a hapten), an antigen, a co-stimulatory agent, an immunogen, or a sensitogen (a chemical that can elicit hypersensitivity in humans).[20,21]

The drug acting as a hapten binds covalently to serum or cell-bound proteins, including peptides embedded in major histocompatibility complex (MHC) molecules.[22] The chemical reaction activates the T cell, thereby initiating an immune response that can cause systemic or cutaneous reactions, and immediate or delayed side effects. Although drug–hapten complexes have been detected *in vivo* for a number of drugs, the exact mechanism of MHC molecule binding and T cell activation has not yet been defined.[20]

The p-i model was proposed by Pichler[23] and is based on the direct binding of the parent drug to the T cell receptor or the human leukocyte antigen (HLA) which results in T cell activation and a subsequent immune response.

The altered peptide repertoire hypothesis states that low-molecular-weight drugs bind non-covalently to parts of the HLA molecules within the antigen-binding cleft, thereby altering the shape of the cleft and the repertoire of peptides that are presented. If the subject is not tolerant to the new peptides presented, a T cell response is initiated with an immune response via interaction with a MHC.[8] No sensitisation is required in this case because there is direct stimulation of memory and effector T cells.[2]

A study by Bellón et al.[24] supported the T cell-mediated hypothesis by identifying 85 genes that were differentially expressed during the acute phase of drug-induced hypersensitivity syndrome (DIHS). Most of the genes upregulated in the acute phase were encoding proteins involved in the cell cycle, apoptosis, and cell growth functions; nine were involved in immune response and inflammation. They also found that histone messenger RNA levels were statistically significantly increased in severe and moderate reactions. Genes that were strongly upregulated in syndromes that include both cutaneous and mucosal involvement were those involved in inflammation, now termed alarmins or endogenous damage-associated molecular patterns.[24]

Associations have been discovered between HLA alleles and many of the serious cutaneous adverse reaction syndromes. This includes abacavir

hypersensitivity reaction; allopurinol drug reaction, eosinophilia, and systemic symptoms (DRESS)/DIHS; and Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) associated with aromatic amine anticonvulsants.[9] Two studies (PREDICT-1 and SHAPE) have shown 100% negative predictive value of *HLA-B*5701* for abacavir hypersensitivity across both Caucasian and African-American populations. Other specific correlations that have been found include nevirapine reactions with *HLA-B*3505/01* and *HLA-DRB1*0101*; allopurinol with *HLA-B*5801*, carbamazepine with *HLA-B*1502* and *HLA-B*5701*; abacavir, flucloxacillin, and neviraine with *HLA-B*3505/01*.[9]

Table 1: Most common drugs that induce drug hypersensitivity reactions.

Drug class	Specific drug	Latent period
Angiotensin converting enzyme inhibitors	Captopril	At any time
Xanthine oxidase inhibitor	Allipurinol	2–6 weeks[53]
Antibiotics	Beta-lactams (paediatrics)[54]	Immediate: 1 hour Non-immediate: ≥1 hour[55]
	Ceftriaxone	72 hours[56]
	Cyclosporine	
	Dapsone	Few days to weeks[57]
	Isoniazid	
	Levofloxacin	
	Minocycline	
	Penicillin	
	Sulfonamides	
	Trimethoprim	
Anticonvulsants	Carbamezapine	Usually 2–4 weeks; may be up to 3 months
	Lamotrigine	
	Phenobarbitone	
	Phenytoin	
	Primidone	
Antidepressants	Clomipramine (anafranil)	
Antifungals	Terbinafine	2–3 days
Antiretrovirals	Abacavir	
	Nevirapine	
Beta-blocker	Atenolol	
Biologic modifiers	Infliximab	
	Murine and humanised monoclonal antibodies	
	Recombinant interferons	
Drug colouring agents	Blue dyes	
Calcium channel blockers	Diltiazem	2–3 Days
Gold salts		
Antihypertensive	Hydralazine (apresoline)	
Immunosuppressants	Azathiprine	
Non-steroidal anti-inflammatory drugs	Aspirin	
Antiarrhythmic	Procainamide	
Sodium channel blockers	Mexiletine	
Disease-modifying anti-rheumatic drugs	Sulfasalazine	

Table 2: The Gell and Coombs classification system for drug hypersensitivity.

Classification	Mechanism	Clinical symptoms	Examples
Type I - IgE-mediated	Drug-IgE complex attachment to mast cells and subsequent release of histamine and other inflammatory mediators	Urticaria, angioedema, bronchospasm, wheezing, pruritus, vomiting, diarrhoea, anaphylaxis	Hay fever, asthma, eczema, bee stings, food allergies
Type II - Cytotoxic	IgG or IgM antibodies are directed to drug-hapten coated cells; RBC lysis	Haemolytic anaemia, neutropenia, thrombocytopenia	Rh factor incompatibility, AGEP
Type III - Immune complex	Antigen and antibody complexes deposit in blood; subsequent localised inflammatory response	Serum sickness, fever, rash, arthralgias, lymphadenopathy, urticaria, glomerulonephritis, vasculitis	DRESS, SJS/TEN
Type IV - Cellular mediated			
Type IVa	Activation and recruitment of monocytes	Maculopapular drug rash, red, fluid-filled lesions, granuloma formation, (chronic exposure)	Contact dermatitis, Poison ivy/oak, Latex allergy
Type IVb	Activation and recruitment of eosinophils		
Type IVc	Activation and recruitment of CD4+ or CD8+ T cells		
Type IVd	Activation and recruitment of neutrophils[2,19,52]		

RBC: red blood cell; DRESS: drug rash with eosinophilia and systemic symptoms; SJS/TEN: Stevens-Johnson syndrome/toxic epidermal necrolysis; Ig: immunoglobulin; Rh: rhesus; AGEP: acute generalised exanthematous pustulosis.

A study by Picard et al.[25] also detected Epstein-Barr virus (EBV), human herpes virus 6, or human herpes virus 7, reactivation in 76% of the patients with DRESS in response to carbamazepine, allopurinol, or sulfamethoxazole. Circulating CD8+ T lymphocytes were activated in all of these patients and nearly half of the expanded blood CD8+ T lymphocytes sharing the same T cell receptor repertoire detected in the blood, skin, liver, and lungs, recognised one of several EBV epitopes. They concluded that "cutaneous and visceral symptoms of DRESS are mediated by activated CD8+ T lymphocytes, which are largely directed against herpes viruses such as EBV."[25]

The reaction to anticonvulsant medications, termed anticonvulsant hypersensitivity syndrome (AHS), has been linked to the presence of arene oxides. Arene oxides are intermediate metabolites produced by the metabolisation of the drugs by cytochrome P-450 and usually detoxified by epoxide hydroxylase; however, there is evidence that individuals who develop AHS are unable to detoxify arene oxides.[26,27]

ADRs have been classified as Type A: those that are predictable and dose dependent reactions, including overdose, side effects, and drug interactions (e.g. a gastrointestinal bleed following treatment with non-steroidal anti-inflammatory drugs [NSAIDs]); and Type B, those that are unpredictable, more likely to be dose independent, and may include immunologically mediated drug hypersensitivity or non-immune mediated reactions, thus being considered allergic reactions.[1,8] A more extensive classification, the Gell and Coombs system, describes the predominant immune mechanisms that lead to the clinical symptoms of hypersensitivity and is presented in Table 2.[19] The table also reflects recent modifications based upon the cells recruited and activated in Type IV reactions.

Clinical Presentation

Signs and symptoms of allergic reactions usually occur 1–3 weeks after the first exposure or ingestion of the causative medication and can either be local (caused by contact dermatitis), systemic, or cutaneous. The local erythema, rash, and pruritis

associated with contact dermatitis (Type IV) will follow the pattern of contact with the offending substance, e.g. an allergic reaction to a silver dressing or elastic wrap (Figure 1). This type of reaction is more easily identified than an ingested drug, and is effectively treated with discontinuation of the causative solution or material and the application of a topical anti-inflammatory cream.

Typically, the erythematous, maculopapular rash that appears within 1–3 weeks after drug exposure will occur first on the trunk and then spread to the extremities. Urticaria is typically a manifestation of a Type I allergic reaction; however, it may also appear with Type III or pseudo-allergic reactions.[19]

Systemic symptoms vary from mild to severe, and can involve the liver, kidneys, lungs, bone marrow, and other autoimmune phenomena.[20,22] The most common syndromes involving both systemic and cutaneous events are listed in Table 3. Warning signs of a severe life-threatening reaction due to cardiovascular collapse include urticaria, laryngeal or upper airway oedema, wheezing, and hypotension.[19] Fever, mucous membrane lesions, lymphadenopathy, joint tenderness and swelling, and abnormal respiratory examination are also signs of serious systemic reactions.

Cutaneous reactions can also vary from mild itching to severe syndromes such as SJS, TEN, DRESS, or acute generalised exanthematous pustulosis (AGEP). Serious cutaneous adverse reactions are associated with a high rate of morbidity and mortality (Table 4).[1]

SJS and TEN begin with a fever, sore throat, and stinging eyes for 1–3 days, followed by mucosal lesions involving conjunctive, oral and genital mucosa, trachea, bronchi, and gastrointestinal tract. Cutaneous lesions develop next with erythematous macules, progressing to flaccid blisters that tear easily (Figure 2).[28] Because of the target appearance and two zones of colour, these initial lesions are referred to as targetoid lesions;[29] they involve almost all of the body, including the head, anterior and posterior trunk, upper and lower extremities, and may also progress to the lower back and gluteal region. Signs of impending severe cutaneous reactions are skin pain, epidermolysis, and a positive Nikolsky sign (slight rubbing of the skin causing epidermal/dermal separation).[30]

AGEP is characterised by numerous small, primarily non-follicular, sterile pustules that present within large areas of oedematous erythema; however, unlike SJS they do not occur in the mouth and vagina. AGEP is also accompanied by fever, neutrophilia, and sometimes by facial oedema, hepatitis, and eosinophilia.[31]

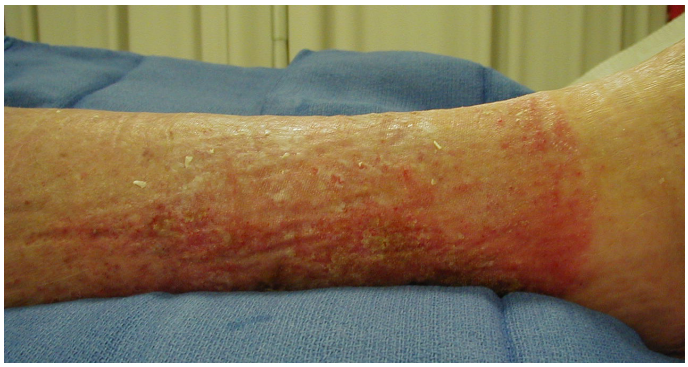


Figure 1: Contact dermatitis. The distinct line of erythema at the distal leg is indicative of an allergic reaction to the elastic compression used to manage chronic oedema.

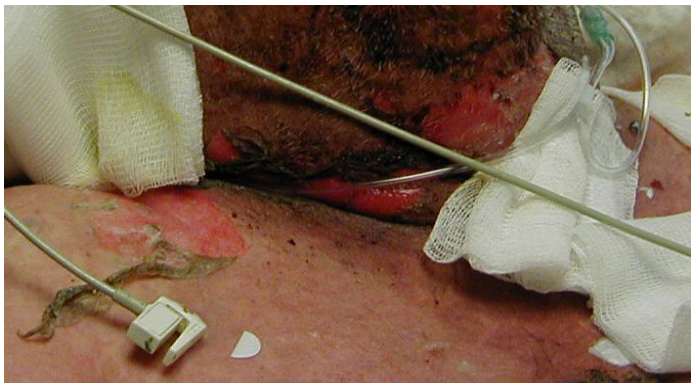


Figure 2A: Sloughing blisters on the skin of a patient with toxic epidermal necrolysis.

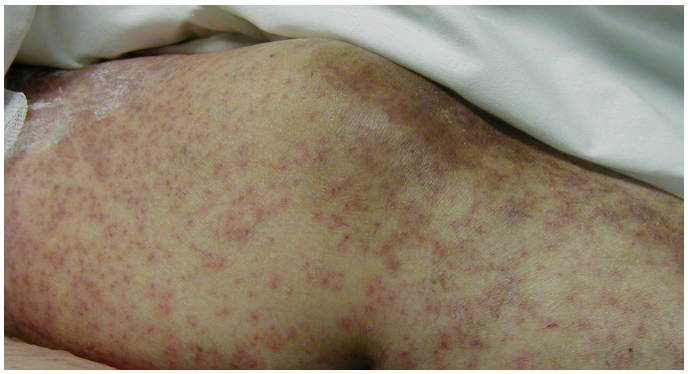


Figure 2B: Vasculitic cutaneous reaction on the lower extremities in response to ingestion of an antibiotic.

Table 3: Drug hypersensitivity reactions based on severity of symptoms.

Name	Identifying characteristics
MPE	Generalised, widespread rash with red macular (not elevated) or papular (elevated) skin eruptions.
EM minor	Localised skin eruptions, usually on the lower extremities, that begin to heal within 7 days.
FDE	One or more local annular or oval erythematous patches; resolve with hyperpigmentation; recur at the same location.
DRESS	Three of the following: fever, exanthema, eosinophilia, atypical circulating lymphocytes, lymphadenopathy, hepatitis. May also have facial oedema; can occur up to 12 weeks after initiation of drug or after a dose increase. May be associated with reactivation of the human herpes virus.
Haemolytic anaemia	Fatigue, shortness of breath, pallor; failure to thrive in paediatric cases.
Serum sickness	Fever, arthralgias, rash, lasting 1–2 weeks. May also involve arthritis, oedema, or GI symptoms.
SJS; also known as EM	Cutaneous lesions of erythematous papules, vesicles, bullae, or iris lesions covering <10% of the body surface area; mucosal lesions or conjunctivitis.
TEN	Cutaneous lesions of erythematous papules, vesicles, bullae, or iris lesions covering >30% of the body surface area; mucosal lesions or conjunctivitis.
Chemotherapy-induced acral erythema	Painful, symmetrical swelling and erythema of the palms and soles of patients on high doses of chemotherapy.
SDRIFE; also called Baboon's syndrome	Bright red, well-demarcated, anogenital lesions associated with a symmetrical eczematous eruption involving axillae, antecubital fossae, eyelids, and the sides of the neck.
Drug-induced lupus erythematosus	Typical lupus-like symptoms, including skin signs associated with long-term use of the putative drug; symptoms resolve with the withdrawal of the drug.

MPE: maculopapular exanthemas; SJS: Stevens-Johnson syndrome; EM: erythema multiforme; FDE: fixed drug eruption; DRESS: drug rash with eosinophilia and systemic symptoms; TEN: toxic epidermal necrolysis; SDRIFE: symmetrical drug related intertriginous and flexural exanthema; GI: gastrointestinal.

The SCORTEN scale (SCORe of Toxic Epidermal Necrosis) is a severity-of-illness scale that can be used to determine the mortality risk of an individual patient.[32] Although it was initially developed for patients with SJS and TEN, it has been validated and used for patients with burns and other exfoliative disorders. Calculations should be performed within the first 24 hours after admission and on Day 3.[32] Table 5 and Table 6 list the risk factors and mortality scores, showing that more risk factors result in a higher score on the SCORTEN scale, thereby indicating a higher mortality rate.

DIAGNOSIS

Any patient who exhibits the signs of drug allergy should first have an extensive review of their subjective medical history and be evaluated for a differential diagnosis which would include close scrutiny of all medications, both prescribed and over-the-counter. Special attention is advised to the temporal relationship between initiating a drug and the onset of clinical symptoms. Any report of previous allergies may be an indicator of risk for a newly-observed allergic reaction. For example, this author found that patients who reported allergies to latex may be more likely to develop allergic reactions to dressings with topical antimicrobials. The medical history is accompanied by an intensive integumentary examination for any skin changes; the type of skin reaction is critical for providing clues to the immune-mediated mechanism of the drug reaction.[19]

Patch or skin tests to detect antigen-specific IgE are useful in most forms of DIHS, specifically for Types I and IV, but not for SJS/TEN and vasculitis. Type II cytotoxic reactions can be detected by a complete red blood count, as haemolytic anaemia, thrombocytopenia, or neutropenia will be evident.[19]

Table 4: Severe cutaneous adverse reactions.

Stevens-Johnson syndrome (SJS)
Toxic epidermal necrolysis (TEN)
Drug-induced hypersensitivity syndrome (DIHS)
Drug rash with eosinophilia and systemic symptoms (DRESS)
Acute generalised exanthematous pustulosis (AGEP)

Table 5: SCORTEN (SCORe of Toxic Epidermal Necrosis) Scale risk factors for determining mortality rates of patients with toxic epidermal necrolysis or Stevens-Johnson syndrome.

Risk factor	0	1
Age	<40 years	>40 years
Associated malignancy	No	Yes
Heart rate (beats per minute)	<120	>120
Serum urea nitrogen (mg/dL)	<27	>27
Detached or compromised body surface	<10%	>10%
Serum bicarbonate (mEq/L)	>20	<20
Serum glucose (mg/dL)	<250	>250

Table 6: Interpretation of the SCORTEN (SCORe of Toxic Epidermal Necrosis) Scale.

No. of risk factors	Mortality rate (%)
0-1	3.2
2	12.1
3	35.3
4	58.3
5 or more	>90

Diagnostic laboratory values can play a role in prognosis of the disease, especially TEN and SJS. Neutropenia and lymphopenia can occur and may be a negative prognostic factor.[33] The use of granulocyte-colony-stimulating factor in the treatment of TEN has been shown to reverse the neutropenia with a corresponding increase in re-epithelialisation.[30] Hyperferritinemia as a result of acute liver failure can be a useful marker for the severity of DIHS.[34] Fujita et al.[35] developed a rapid immunochromatographic test for detection of granulysin, a cytotoxic lipid-binding protein that causes apoptosis and is present in the blister fluid of patients with SJS/TEN. The granulysin was found to be elevated before skin and mucosal detachment occurred, suggesting that it may be a useful marker for detection of SJS/TEN in the early stages.

A retrospective study by Watanabe et al.[36] suggested distinct differences between SJS/TEN and erythema multiforme major (EMM) which can be helpful in making a definitive diagnosis. SJS/TEN patients were more likely to have mucous membrane involvement, higher C-reactive protein levels, and hepatic dysfunction. EMM patients had stronger mononuclear cell infiltration and required lower doses of systemic corticosteroids.

Sun et al.[37] studied the potential of the drug-induced lymphocyte test in patients with tuberculosis, and found that it has high specificity and limited sensitivity in the diagnosis of anti-tuberculosis drug-induced ADRs, suggesting that it may have predictive validity for ADRs, especially when the result is positive. The basophil activation test (BAT) has been used to detect an immediate reaction to pristinamycin[38] as well as neuromuscular blocking agents, antibiotics, NSAIDs, and iodinated radiocontrast media.[39] Both authors concluded that more large-scale multicentre studies are needed to validate the use of BAT as a diagnostic test in drug allergies.

The drug provocation test (DPT) is used to detect immediate hypersensitivity reactions, and can be beneficial in predicting immediate reactions in children who have a history of non-immediate reactions to amoxicillin.[40] Alvarez-Cuesta et al.[41] studied the use of DPT with patients who were on anti-neoplastic or biological agents. They found that the DPT was helpful in excluding hypersensitivity in 36% of referred patients and avoided unnecessary desensitisation in non-hypersensitive patients in 30–56% of the subjects tested, depending upon the culprit drug.

TREATMENT OF DRUG ALLERGIES

The first and foremost medical strategy is identification and cessation of the causative agent, usually the last one the patient initiated 1–3 weeks prior to onset of symptoms. An exception is DRESS which can occur after a longer latent period (1–8 weeks), in these cases it is recommended to consider medications started within the 6 months prior to onset of symptoms.[42] Thereafter treatment is predicated upon the

severity of the symptoms, both cutaneous and systemic. Reactions that cause drug fever, a non-pruritic rash, or mild organ system reactions may require no treatment other than discontinuance of the medication.[22] Corticosteroids are used for both treatment of symptoms and prevention of progression. Recommended systemic corticosteroids dosing begins at 0.5–1 mg/kg/day and is tapered over 6–8 weeks; for SJS, 1 mg/kg/day of prednisolone or 1–2 mg/kg/day of methylprednisolone.[43] Topical steroid ointments and oral histamines are also beneficial for dermatologic symptoms, and may be sufficient in milder cases. Steroid therapy for TEN is reported as both controversial and no longer recommended; if used, it should be within the first 48 hours of treatment due to the increased risk of septic complications with an anti-inflammatory agent. Strict control of blood glucose levels is needed for patients with history of diabetes or on corticosteroids.[44]

For patients with extensive skin involvement, supportive care in an acute burn or intensive care unit is recommended for prevention of infection, life support measures, and pain management.[45] Mechanical ventilation, fluid resuscitation with IV fluids such as Ringer's solution for electrolyte balance, anti-coagulation with heparin to prevent thromboembolism, and supplemental nutrition via a nasogastric tube may be needed in severe cases.[12,46] Antibiotic therapy is not given as a prophylactic measure but dependent upon clinical symptoms, including positive skin cultures, sudden drop in temperature, or deterioration of the patient's medical condition.[2,45] In order to prevent caloric loss and an increase in metabolic rate, a room temperature of 30–32°C is also recommended.[46]

Clinical studies on the use of IV Ig for patients with SJS and TEN have shown mixed results. Successful treatment appears to be dose dependent (1 g/kg/day for 3 days with a total of 3 g/kg over 3 consecutive days) with early treatment recommended.[47] Other medications which have been studied and found beneficial include IV infleximab, cyclosporin, and IV N-acetylcysteine.[28] Aciclovir has been suggested for lesions that occur in the oral cavity of patients with TEN.[48]

Desensitisation is a possible treatment strategy, especially for patients with IgE-mediated drug allergies (e.g. certain antibiotics, platinum salts, monoclonal antibodies), as well as those on taxanes or chemotherapy. Desensitisation involves administering a low dose of the drug and gradually increasing the dose every 15–30 minutes until the therapeutic dose is reached. The dose is then administered at regular intervals for the duration of treatment which also maintains the desensitised state.[8,49] All patients with known drug allergies require education regarding future episodes, cross-reacting drugs to avoid, and the benefit of wearing a medical-alert device.

WOUND MANAGEMENT

For severe cases involving loss of epidermis, wound management goals are to prevent fluid loss, prevent infection, and facilitate re-epithelialisation. Although patients with SJS/TEN are best treated in an acute burn centre, there are some definite differences in their clinical presentation that affects treatment. For example, SJS/TEN epidermal involvement may continue to spread after admission; subcutaneous necrosis is deeper in burns, thereby creating subcutaneous oedema that is not observed in SJS/TEN; fluid requirements for SJS/TEN are usually two-thirds to three-quarters those of burn patients with the same area involvement; and re-epithelialisation is usually faster in SJS/TEN due to more sparing of the hair follicles in the dermal layer.[46] Skin lesions can be expected to heal in an average of 15 days; oral and pharyngeal lesions may take approximately 4 weeks longer.[47]

Debridement of detached epidermal tissue is controversial and usually not advisable in patients who have a positive Nikolsky sign.[46] Collagen sheet dressings,[29] Biobrane® (Dow B. Hickam, Inc., Sugarland, Texas, USA),[48] and other occlusive non-adhesive wound coverings that prevent fluid loss and minimise pain with dressing changes have been recommended. These biological dressings create a physiological interface between the wound surface and the environment that is impermeable to bacteria, thus helping to prevent local wound infection.[50] In addition, the collagen sheets are non-inflammatory, facilitate fibroblast migration to the wound site, assist in extracellular matrix synthesis, are non-toxic, and minimise scarring.[26]

Oral topical anaesthetic gel (lignocaine 2%) and chlorhexidine mouth rinse have been used for oral lesions, and dexamethasone (0.1%) eye drops for ocular lesions.[47] Post-healing, artificial tears and lubricants may be needed.[46]

Skin care after full closure includes use of sun screens and/or avoidance of sun exposure. Re-administration of the causative medication should also be avoided. A second episode due to the same drug may have a shorter onset than after the first episode; however, the symptoms may be more severe.[51]

SUMMARY

In summary, ADRs can be immediate or non-immediate, in which case symptoms will appear 1–3 weeks after the first exposure to the causative medication. The most immediate and important medical treatment involves identification and cessation of that medication. Appropriate medical and wound care is dependent upon the severity of the cutaneous and systemic reactions, and in severe cases may involve hospitalisation. The morbidity, mortality, and socioeconomic costs of ADRs are significant and further research on pathology, diagnosis, and prevention is warranted.

REFERENCES

1. Thong BY, Tan TC. Epidemiology and risk factors for drug allergy. Br J Clin Pharmacol. 2011;71(5):684-700.

2. Solensky R, Khan DA. An up-dated practice parameter. Annals of Allergy, Asthma, and Immunology. 2010;105(4): 259-73.e78.

3. Boston University School of Public Health. Boston Collaborative Drug Surveillance Program. 1992-present. Available at: http://www.bu.edu/bcdsp/publications. Last accessed: 18 March 2016.

4. Bigby M et al. Drug-induced cutaneous reactions: A report from the Boston Collaborative Drug Surveillance Program on 15,438 consecutive inpatients, 197 to 1982. JAMA. 1986;256(24):3358-63.

5. Fiszenson-Albala F et al. A 6-month prospective survey of cutaneous drug reactions in a hospital setting. Br J Dermatol. 2003;149(5):1018-22.

6. Hernandez-Salazar A et al. Epidemiology of adverse cutaneous drug reactions. A prospective study in hospitalized patients. Arch Med Res. 2006; 37(7):899-902.

7. Gomes ER, Demoly P. Epidemiology of hypersensitivity drug reactions. Curr Opin Allergy Clin Immunol. 2005;5(4):309-16.

8. Wheatley LM et al. Report from the National Institute of Allergy and Infectious Deseases workshop on drug allergy. J Allergy Clin Immunol. 2015;136(2): 262-71.e2.

9. Phillips EJ et al. Drug hypersensitivity: Pharmacogenetics and clinical symptoms. J Allergy Clin Immunol. 2011;127(3 Suppl): S60-6.

10. Wolkenstein P, Ruvuz J. Drug-induced severe skin reactions – incidence, management and prevention. Drug Safety. 1995;13(1):56-68.

11. Rademaker M, Maling T. Drug hypersensitivity syndrome. Medsafe, Prescriber Update. 2003;24(2):22-23. Available at: http://medsafe.govt.nz/profs/puarticles/dhs.htm. Last accessed: 18 March 2016.

12. Hamm RL. Drug-hypersensitivity syndrome: Diagnosis and treatment. J Am Coll Clin Wound Spec. 2012;3(4):77-81.

13. Kumari R et al. Drug hypersensitivity syndrome. Indian J Dermatol Venereol Leprol. 2011;77(1):7-15.

14. Sullivan TJ et al. Studies of the multiple drug allergy syndrome. J Allergy Clin Immunol. 1989;83:270.

15. Chiriac AM, Demoly P. Multiple drug hypersensitivity syndrome. Curr Opin Allergy Clin Immunol. 2013;13(4):323-9.

16. Gex-Collet C et al. Multiple drug hypersensitivity – proof of multiple drug hypersensitivity by patch and lymphocyte transformation tests. J Investig Allergol Clin Immunol. 2005;15(4):293-6.

17. Asero R. Multiple drug allergy syndrome: A distinct clinical entity. Current Allergy and Asthma Reports. 2001; 1(1):18-22.

18. Buhari GK et al. Immediate-type hypersensitivity reactions due to antituberculosis drugs: a successful readministration protocol. Ann Allergy, Asthma Immunol. 2015;115(1):39-44.

19. Reidl M, Casillas AM. Adverse drug reactions: Types and treatment options. Am Fam Physician. 2003;68(9):1781-90.

20. Pirmohamed M et al. New genetic findings lead the way to a better understanding of fundamental mechanisms of drug hypersensitivity. J Allergy Clin Immunol. 2015;136(2):236-44.

21. Pavlos R et al. T Cell-mediated hypersensitivity reactions to drugs. Annu Rev Med. 2015;66:439-54.

22. Delves PJ. Drug hypersensitivity. Available at: http://www.merckmanuals.com/professional/immunology-allergic-disorders/allergic,-autoimmune,-and-other-hypersensitivity-disorders/drug-hypersensitivity. Last accessed: 18 March 2016.

23. Pichler WJ. Delayed drug hypersensitivity reactions. Ann Intern Med. 2003;139(8):683-93.

24. Bellón T et al. Differential gene expression in drug hypersensitivity reactions: induction of alarmins in severe bullous diseases. Br J Dermatol. 2010; 162(5):1014-22.

25. Picard D et al. Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS): A Multiorgan Antiviral T Cell Response. Sci Transl Med. 2010;2(46): 46ra62

26. Havill S. Anticonvulsant hypersensitivity syndrome. Medsafe Publications. Available at: www.medsafe.govt.nz/Profs/PUarticles/6.htm. Last accessed: 6 July 2016.

27. Vittorio CC, Muglia JJ. Anticonvulsant hypersensitivity syndrome. Arch Intern Med. 1995;155(21):2285-90.

28. Chia FL, Leong KP. Severe cutaneous adverse reactions to drugs. Curr Opin Allergy Clin Immunol. 2007;7(4):304-9.

29. Bhattacharya S et al. Collagen sheet dressings for cutaneous lesions of toxic epidermal necrolysis. Indian J Plast Surg. 2011;44(3):474-7.

30. Bircher AJ. Symptoms and danger signs in drug hypersensitivity. Toxicology. 2005; 209(2):201-7.

31. Acute generalized exanthematous pustulosis. Available at: https://en.wikipedia.org/wiki/Acute_generalized_exanthematous_pustulosis. Last accessed: 6 July 2016.

32. Bastuji-Garin S et al. SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol. 2000;115(2): 149-53.

33. Ang CC, Tay YK. Hemtological abnormalities and the use of granulocyte-colony-stimulating factor in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis. Int J D. 2011;50(12): 1570-8.

34. Miyazaki M et al. Acute liver failure caused by drug-induced hypersensitivity syndrome associated with hyperferritinemia. Word J Gastroenterol. 2011;17(44):4928-31.

35. Fujita Y et al. Rapid immunochromatographic test for serum granulysin is useful for the prediction of Stevens-Johnson Syndrome and toxic epidermal necrolysis. J Am Acad Dermatol. 2011;65(1):65-8.

36. Watanabe R et al. Critical factors differentiating erythema multiforme majus from Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). Eur J Dermatol. 2011;21(6):889-94.

37. Sun Q et al. Drug-induced lymphocyte stimulation test in the prediction of drug-induced hypersensitivity to antituberculosis drugs. Diagn Microbiol Infect Dis. 2015;82(2):172-6.

38. Viel S et al. The basophil activation test: A sensitive test in the diagnosis of allergic immediate hypersensitivity to pristinamycin. Int Arch Allergy Immunol. 2015;167(2):94-8.

39. Mangodt EA et al. In vitro diagnosis of immediate drug hypersensitivity: Should we go with the flow. Int Arch Allergy Immunol. 2015;168(1):3-12.

40. Mori F et al. Amoxicillin allergy in children: five-day drug provocation test in the diagnosis of nonimmediate reactions. J Allergy Clin Immunol Pract. 2015;3(3): 375-80.

41. Alvarez-Cuesta E et al. Delving into cornerstones of hypersensitivity to antineoplastic and biological agents: Value of diagnostic tools prior to desensitization. Allergy. 2015;70(7): 784-94.

42. Sullivan JR, Shear NH. The drug hypersensitivity syndrome: What is the pathogenesis? Arch Dermatol. 2001;137: 357-64.

43. Thong BY. Update on the management of antibiotic allergy. Allergy Asthma Immunol Res. 2010;2(2):77-86.

44. Chave TA et al. Toxic epidermal necrolysis: Current evidence, practical management, and future directions. Br J Dermatol. 2005;153(2):241-53.

45. Chowta NK et al. Carbamzepine-induced toxic epidermal necrolysis. Indian J Crit Care Med. 2011;15(2):123-5.

46. Ghislain P, Roujeau J. Treatment of severe drug reactions: Stevens-Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity syndrome. Dermatology Online Journal. 2002;8(1):5. Available at: http://escholarship.org/uc/item/97d8t291 Last accessed: 18 March 2016.

47. Mittmann N et al. IVIG for the treatment of toxic epidermal necrolysis. Skin Therapy Lett. 2007;12(1):7-9.

48. Digwood-Lettieri S et al. Levofloxacin-induced toxic epidermal necrolysis in an elderly patient. Pharmacotherapy. 2002; 22(6):789-93.

49. Thien FC. Drug hypersensitivity. Med J Aust. 2006;185(6):333-8.

50. Park SN et al. Biological characterization of EDC-crosslinked collagen-hyaluronic acid matrix in dermal tissue restoration. Biomaterials. 2003; 24(9):1631-41.

51. Grammer LC. Stevens-Johnson syndrome: affects more than just skin. Medscape General Medicine. 1997;1(2). Available at: http://www.medscape.com/viewarticle/719223. Last accessed: 18 March 2016.

52. Beatty R. Hypersensitivity. Available at: www.//mcb.berkeley.edu/course/mcb/SO/:ecture 20./Lecture20(6)pdf. Last accessed: 3 January 2016.

53. Allopurinol Drug report. Available at: http://livertox.nih.gov/Allopurinol.htm. Last accessed: 11 July 2016.

54. Ponvert C et al. Allergy to betalactam antibiotics in children: results of a 20-year study based on clinical history, skin and challenge tests. Pediatr Allergy Immunol. 2011;22(4):411-8.

55. Blanca M et al. Update on the evaluation of hypersensitivity reactions to betalactams. Allergy. 2009;64:183-93.

56. Kumar AA et al. A case report on Cefrtiazone induced hypersensitivity reactions (uticaria). Int J All Med Sci Clin Res. 2015;3(2):82-4.

57. Dapsone drug report. Available at: http://livertox.nih.gov/Dapsone.htm. Last accessed: 11 July 2016.

Sibling Screening May be Unnecessary in Food Allergies

SCREENING children for a food allergy that a sibling has may be an unwarranted practice that negatively impacts the nutrition and quality of life of the child. This is according to a team of researchers who tested and examined the status of food allergy among a cohort of 1,120 food allergic children and their biological siblings.

The team found that a food allergy in a child did not necessarily determine whether their sibling would also have the same allergy. "Our data suggest that the risk of food allergy in siblings of an affected child is only minimally higher than in the general population," said the lead author of the study, Dr Ruchi Gupta, Associate Professor in General Pediatrics and Primary Care, Department of Medicine, Northwestern University Feinberg School of Medicine, Evanston, Illinois, USA.

The food allergy status of the children was determined by applying stringent clinical criteria to data gathered from questionnaire-based interviews. Specific immunoglobulin E blood tests and skin-prick tests were also performed. The results of the study showed that a narrow majority (53%) of the siblings of food allergic children showed food sensitisation after testing, but they did not experience food allergy symptoms. This led the researchers to observe that testing might show a sensitisation to a particular food in a child who has never previously been exposed to it but that this might not mean the exposure will provoke allergic symptoms. "Our findings help support the National Institute of Allergy and Infectious Disease practice guidelines to not screen siblings before the child's initial exposure to a food," Dr Gupta said.

The study concluded that: "Routine screening without a history of allergic food reactions might lead to unnecessary food avoidance in kids who can actually tolerate that food, which impacts quality of life and nutrition. Food avoidance also increases the risk of developing an allergy to that food."

"Routine screening without a history of allergic food reactions might lead to unnecessary food avoidance in kids who can actually tolerate that food, which impacts quality of life and nutrition. Food avoidance also increases the risk of developing an allergy to that food."

ADAM Gene Involvement in Asthma Pathogenesis

A NEW study has undermined the common understanding that allergic reactions are a significant cause of airway remodelling in asthma patients.

Researchers from the University of Southampton, UK have found that allergic inflammation is promoted by the human gene *ADAM33*. *ADAM33* produces a metalloprotease enzyme that attaches to cells in the airway muscles. When the enzyme loses its anchor to the cell surface, it can be found in the airways and lungs and causes poorer lung function in people who have asthma. The team found that once the *ADAM33* gene is in these areas it initiates airway remodelling in asthma patients, causing more muscle and blood vessels to develop around the airways. "This finding radically alters our understanding of the field, to say the least," explained the lead researcher, Dr Hans Michael Haitchi, Associate Professor in Respiratory Medicine, Faculty of Medicine, University of Southampton, Southampton, UK. "For years we have thought that airway remodelling is the result of the inflammation caused by an allergic reaction, but our research tells us otherwise," Dr Haitchi said.

> "For years we have thought that airway remodelling is the result of the inflammation caused by an allergic reaction, but our research tells us otherwise."

The research team sought to analyse the impact of the *ADAM33* gene in asthma by studying human tissue samples and mice. Dr Haitchi and his team found that switching off *ADAM33* or inhibiting its enzymatic reaction when it is in the airways reduced the features of asthma, including airway remodelling, twitchiness, and inflammation.

Previous research has found that some *ADAM33* gene alleles result in airway remodelling but do not cause inflammation. The introduction of the house dust mite allergen was shown to enhance both remodelling and airway inflammation. Dr Haitchi and his team also studied the impact of the house dust mite allergen on asthma features, by examining mice that had the *ADAM33* gene removed. They found that airway remodelling and twitchiness was reduced by 50% and airway inflammation was reduced by 35% in the mice without the gene.

The team concluded that switching off of ADAM33 or inhibiting the enzymatic activity of its associated metalloprotease could prevent the development of asthma and that their findings have identified a novel target for disease modifying therapy. They also pointed to failure of current anti-inflammatory steroid therapy to prevent the airway remodelling caused by the *ADAM33* gene, adding greater significance to the study.

Sucking Thumbs and Biting Nails: Not So Bad After All

BITING nails and sucking thumbs may not be so bad after all. A study has found that children who either bite their nails or suck their thumbs are less likely to develop allergies as they get older.

Prof Bob Hancox, Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand, who led the study suggested that because of the exposure to an increased amount of microbes that both habits brought, it altered their immune function which in turn made them less prone to developing allergies. He commented that: "The findings support the 'hygiene hypothesis' which suggests that being exposed to microbes as a child reduces your risk of developing allergies."

Participants in the study (N=1,037) were taken from the Dunedin Multidisciplinary Study which has been following the participants from birth to adulthood for >40 years. As part of this study, the parents of the participants were asked about the habits of their children at ages 5, 7, 9, and 11 years old.

> "The findings support the 'hygiene hypothesis' which suggests that being exposed to microbes as a child reduces your risk of developing allergies."

Each participant underwent a skin-prick test (at both 13 and 32 years of age) that would test positive if any allergies were present. The study found that surprisingly, there was a reduction in the amount of allergens present in participants who either sucked their thumb, bit their nails, or both, in comparison with those who had none of these habits. Results showed that at 13 years old, 38% who had one habit or the other and 31% who had both habits tested positive for at least one allergen. Of those children who displayed neither habit, 49% tested positive for at least one allergen.

The Dunedin study also went on to show that these results were still true at 32 years old even including external factors such as gender, history, pet ownership, smoking status, and a history of allergy in the family. However, when performing skin-prick tests for allergic diseases such as asthma or hay fever, no such link was found. Researchers warn that despite this, parents should not actively be encouraging their children to take up these habits. If their children are already doing so however, it should be nothing of concern.

Precision Medicine Vital for Insect Venom Allergy Sufferers

INSECT allergy sufferers can still be at risk of severe allergic reactions even after immunotherapy, according to research currently being carried out. The genetic profiles of individuals most at-risk have been discovered in a study looking at the development of personalised medicine for high-risk patients, the majority of whom may not even realise that they have such a severe allergy.

Current treatment for insect venom allergy consists of a series of injections and long-term treatment with an allergen vaccine. However, it is not always the same allergens causing the reactions in every patient, meaning that this standardised treatment is often suboptimal, and in extreme cases can be detrimental to the patient's health, even causing death.

The team behind the recent study at Aarhus University have therefore been developing artificial allergens that mirror the allergens in the venom, as well as artificial human antibodies in the hope of finding out how they bind together and thus, what drives the allergic reaction in different patients. Particular interest is being focussed on the antibody immunoglobulin (Ig)E, as explained by Prof Spillner, Aarhus University, Aarhus, Denkmark: "We can isolate and rebuild IgE from the patient's blood and identify the target structure it reacts with. This way we can analyse how it behaves together with the allergens. The better we understand the molecular mechanisms of action, the greater our chances are of developing new concepts in allergy treatment."

> "The better we understand the molecular mechanisms of action, the greater our chances are of developing new concepts in allergy treatment."

The group used a small group of insect venom allergens to create detailed models of the antibodies in patients that can cause anaphylaxis, paving the way to a much greater understanding of how allergies work and thus providing the groundwork for more extensive diagnostic practice in the future. Prof Spillner commented: "The component resolved approach is a major breakthrough in allergy and, in particular, in insect venom allergy and could be a benchmark for individualised immunotherapeutic treatment." Therefore, allergy sufferers who are at the greatest risk of immunotherapy failure could be identified and more effective, personalised treatment offered instead in the future.

Special News Feature

Allergy & Immunology 1.1 is the first Journal in this field that the European Medical Journal has published. Because of this our editorial team put together this feature specifically for its release.

If you have enjoyed this be sure to subscribe to our newsletter by clicking here, and receive even more great content in the future.

UPCOMING EVENTS

17th Biennial Meeting of the European Society for Immunodeficiencies (ESID) 2016

21st–24th September 2016

Barcelona, Spain

This scientific programme will draw together international experts to discuss the latest advances in the immunodeficiency field. The areas of research featured include diagnostic immunology, genetics and immunobiology of human diseases, as well as new insights into stem cell and cellular therapies. The content will be delivered through keynote lectures, symposia, educational workshops, and meet-the-professor sessions.

46th Annual Meeting of the German Society for Immunology (DGfI)

27th–30th September 2016

Hamburg, Germany

Contemporary topics of basic and translational immunological research will be presented in plenary sessions, symposia, and a series of workshops. The plenary lectures will hold informative discussions on immunology of infection, autoimmunity, and regulation of the immune response. The main symposia will be exploring a varied selection of topics, with tolerance and pregnancy, organ-specific immunity, and innate immunity among them.

The British Society for Allergy and Clinical Immunology (BSACI) Annual Meeting 2016

29th September–1st October 2016

Telford, UK

This annual meeting will provide a scientific programme catered towards doctors, nurses, dieticians, psychologists, and students. It will have a focus on immunotherapy, respiratory allergy, and allergic manifestations of the skin. Sessions will cover an array of engaging topics, including venom allergy, anaesthetic allergy, and food allergy prevention. The event will also offer workshops and interactive sessions for managing health beliefs and living with food allergy.

11th German Allergy Congress (DAK)

29th September–1st October 2016

Berlin, Germany

This event offers a programme developed in coalition with a number of distinguished German organisations working in the field of allergy medicine. The motto of the congress is 'Modern Allergology - Concepts for the Future'. This will be reflected in the informative and insightful discussions on the latest research results contributing to the improvement of diagnosis and treatment of allergic diseases, including allergic rhinoconjunctivitis and atopic eczema.

4th Food Allergy and Anaphylaxis Meeting (FAAM) 2016

13th–15th October 2016

Rome, Italy

FAAM 2016 will provide a forum for the exchange of the latest science and medicine relating to food allergy biology, nutritional support, and therapeutic approaches for patient care and safety. Its multifaceted approach will bring together the many stakeholders in the field, including food technologists, policy makers, and patient organisations. Highlighted at the congress include identifying patients at risk of severe reactions in public spaces and discussions about hypoallergenic food.

10th European Mucosal Immunology Group Meeting (EMIG) 2016

19th–21st October 2016

Copenhagen, Denmark

The EMIG 2016 congress will cover the key and current aspects of immune responses of mucosal surfaces. Its aim is to share the most recent developments in the understanding of immune homeostasis and in inflammation and infection at mucosal surfaces. The themes of research presented at the congress will include intercellular communication, immune cell differentiation, and immune disorders.

The Annual Meeting of the Austrian Society for Allergology and Immunology (ÖGAI) 2016

16th–19th November 2016

Innsbruck, Austria

The meeting will host up to 300 basic and translational researchers and physicians from all over Austria to discuss their most recent findings. A range of topics will be explored in the sessions, including clinical allergology, adaptive immune regulation, and tumour immunology. The ÖGAI is also keen to emphasise its commitment to supporting early-career researchers who will be provided with the opportunity to discuss and present their work in oral and poster presentations.

European Academy of Allergy and Clinical Immunology (EAACI) Congress 2017

17th–21st June 2017

Helsinki, Finland

The EAACI congress 2017 attracts around 8,000 international clinicians, researchers, and health professionals from around the world. Next year's theme is 'On the road to prevention and healthy living' which will provide the optimal setting for scientific exchange to be translated into concrete initiatives for the benefit of allergy sufferers everywhere. The latest advances in allergy, asthma, and clinical immunology will be presented at the congress, including discussions on allergy diagnostics, dermatology, genomics, and immunotherapy. EAACI 2017 offers itself as the most important professional meeting of the year for allergy research, treatment, and prevention, and it is an event devoted to ensuring the lives of all individuals with allergies remain healthy.

EMJ EUROPEAN MEDICAL JOURNAL

INTERACT WITH US ON SOCIAL MEDIA

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

LATEST **NEWS** • **CONGRESS** UPDATES • JOIN **DISCUSSIONS** • **AWARENESS** CAMPAIGNS

Buyer's Guide

- ABIONIC SA
- AEROCRINE AB
- AIMMUNE THERAPEUTICS
- ALK
- ALLERGOPHARMA GMBH & CO. KG
- ALLERGY THERAPEUTICS PLC.
- ANALLERGO S.P.A.
- ASTRAZENECA
- BAUSCH & LOMB, INC.
- BOEHRINGER INGELHEIM GMBH
- BOSTON SCIENTIFIC CORP.
- BÜHLMANN FCAL
- CSL BEHRING
- DBV TECHNOLOGIES
- DST DIAGNOSTISCHE SYSTEME & TECHNOLOGIEN GMBH
- DIATER S.P.A.
- DR FOOKE-ACHTERRATH LABORATORIEN GMBH
- EUROIMMUN AG
- EXBIO
- FAES FARMA
- FRAUNHOFER ITEM
- FRESENIUS MEDICAL CARE AG & CO.
- GLAXOSMITHKLINE PLC
- HAL ALLERGY GROUP
- HITACHI CHEMICAL DIAGNOSTICS, INC.
- HYCOR BIOMEDICAL
- INDOOR BIOTECHNOLOGIES, INC.
- INFLAMAX RESEARCH, INC.
- IMMUNOTEK
- LABORATORIOS LETI, S.L. UNIPERSONAL
- LOFARMA S.P.A.
- MEDA AB
- MENARINI GROUP
- MOBILE CHAMBER EXPERTS GMBH
- NESTLÉ HEALTH SCIENCE
- NOVARTIS AG
- OMEGA DIAGNOSTICS GROUP PLC.
- PECTOLITE GMBH
- RED MAPLE TRIALS, INC.
- REFLAB APS/R-BIOPHARM AG
- REGENERON PHARMACEUTICALS, INC.
- SANOFI GENZYME CORP.
- SHIRE INTERNATIONAL GMBH
- SIEMENS HEALTHCARE GMBH
- SMARTPRACTICE
- STALLERGENES GREER
- SYSMEX EUROPE GMBH
- TEVA PHARMACEUTICAL INDUSTRIES, LTD.
- THERMO FISHER SCIENTIFIC, INC.
- URIACH