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CONGRESS ISSUE

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AVAMYS, once daily, delivers relief from nasal and ocular symptoms of allergic rhinitis in an easy-to-use device.[1,2,3,4]

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Medical Design Excellence Award 2008 [5]

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Special precautions: Pharmacokinetics of intranasal fluticasone furoate in patients with severe liver disease may be altered. Systemic effects of nasal corticosteroid may occur, particularly at high doses prescribed for prolonged periods. These effects vary between patients and different corticosteroids. The dose of intranasal fluticasone furoate should be reduced to the lowest dose at which effective control of the symptoms of rhinitis are maintained.[2]

References: 1. Berger WE, Godfrey JW, Slater AL. Intranasal corticosteroids: the development of a drug delivery device for fluticasone furoate as a potential step toward improved compliance. *Expert Opin Drug Deliv* 2007;**4**(6):689-701. **2.** AVAMYS Nasal Spray (Suspension) Approved Package Insert. GlaxoSmithkline South Africa (Pty) Ltd. 4 December 2008. **3.** Fokkens WJ, Jogi R, Reinartz S, *et al.* Once daily fluticasone furoate nasal spray is effective in seasonal allergic rhinitis caused by grass pollen. *Allergy*. 2007;**62**:1078-1084. **4.** Vasar M, Houle PA, Douglass JA, *et al.* Fluticasone furoate nasal spray: Effective monotherapy for symptoms of perennial allergic rhinitis in adults/adolescents. *Allergy Asthma Proc.* 2008; **29**(3): 313-321. **5.** Medical Design Excellence Awards. 2008 Winners and their Suppliers. Available from: http://www.devicelink.com/expo/awards/awards/index.php?catId=-1&year=2008&view=View. Cited 2017 March 26.

 S4 **AVAMYS** Nasal Spray (Suspension). **REGISTRATION NUMBER:** 41/21.5.1/0968. Each 50 µl spray contains 27,5 µg of fluticasone furoate. Preservative: Benzalkonium chloride 0,015 % m/m. **PHARMACOLOGICAL CLASSIFICATION:** A.21.5.1 Corticosteroids and analogues. **INDICATIONS: Adults/Adolescents (12 years and over): Treatment of the symptoms of seasonal allergic rhinitis:** Indicated in the treatment of nasal symptoms and ocular symptoms. **Treatment of the symptoms of perennial allergic rhinitis:** Indicated in the treatment of nasal symptoms. **Children (2 to 11 years):** Treatment of the symptoms of seasonal allergic rhinitis: Indicated in the treatment of nasal symptoms. Treatment of the symptoms of perennial allergic rhinitis: Indicated in the treatment of nasal symptoms. **CONTRA-INDICATIONS:** Hypersensitivity to the active substance or to any of the excipients of AVAMYS nasal spray. **WARNINGS:** If there is any reason to suppose that adrenal function is impaired, care must be taken when transferring patients from systemic steroid treatment to fluticasone furoate. **PREGNANCY AND LACTATION:** Safety and efficacy in pregnancy and lactation has not been established. **DOSAGE AND DIRECTIONS FOR USE:** For administration by the intranasal route only. Shake the nasal spray before use. For full therapeutic benefit regular scheduled usage is recommended. Adults/Adolescents (12 years and over): Recommended starting dosage is two sprays in each nostril once daily (total daily dose: 110 µg). Once adequate control of symptoms is achieved, dose reduction to one spray in each nostril (total daily dose: 55 µg) may be effective for maintenance. Children (2 to 11 years of age): The recommended starting dosage is one spray in each nostril once daily (total daily dose: 55 µg). Patients not adequately responding to one spray in each nostril once daily (total daily dose: 55 µg) may use two sprays in each nostril once daily (total daily dose: 110 µg). Once adequate control of symptoms is achieved, dose reduction to one spray in each nostril once daily (total daily dose: 55 µg) is recommended. Children under 2 years of age: There is no experience in **children under the age of 2 years. Hepatic Patients:** The normal adult dosage is applicable in mild to moderate hepatic impairment. There are no data in patients with severe hepatic impairment. **SIDE-EFFECTS AND SPECIAL PRECAUTIONS:** ***Side-effects:*** Clinical Trial Data: ***Respiratory, thoracic and mediastinal disorders:*** Very common: Epistaxis. Common: Nasal ulceration. **Special precautions:** Pharmacokinetics of intranasal fluticasone furoate in patients with severe liver disease may be altered. Fluticasone furoate has a negligible (0,50 %) systemic bioavailability at intranasal doses of up to 24 times the recommended adult daily dose. Systemic effects of nasal corticosteroid may occur, particularly at high doses prescribed for prolonged periods. These effects vary between patients and different corticosteroids. Treatment with higher than recommended doses of nasal corticosteroids may result in clinically significant adrenal suppression. If there is evidence for higher than recommended doses being used, then additional systemic corticosteroid cover should be considered during periods of stress or elective surgery. Fluticasone furoate 110 µg once daily was not associated with HPA axis suppression in adult, adolescent or paediatric subjects. However the dose of intranasal fluticasone furoate should be reduced to the lowest dose at which effective control of the symptoms of rhinitis are maintained. It is recommended that the height of children receiving prolonged treatment with nasal corticosteroids is regularly monitored. **KNOWN SYMPTOMS OF OVERDOSAGE AND PARTICULARS OF ITS TREATMENT:** Intranasal doses of up to 2 640 µg per day were administered over three days with no adverse systemic effects observed. Acute overdose is unlikely to require any therapy other than observation. All adverse events should be reported by calling the Aspen Medical Hotline number or directly to GlaxoSmithKline on +27117456000. For full prescribing information refer to the package insert approved by the medicines regulatory authority. **HCR:** GlaxoSmithKline South Africa (Pty) Ltd. (Co. reg. no. 1948/030135/07). 39 Hawkins Avenue, Epping Industria 1, Cape Town, 7460. ZAF/FF/0001/15a(1)a 07/18

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GUEST EDITORIAL

It has been my pleasure and a privilege to edit this congress edition of *Current Allergy and Clinical Immunology*.

The journal will be published to coincide with the 2018 annual ALLSA Congress. This year the congress is held jointly with the South African Paediatric Association for an even more jam-packed and exciting programme.

This edition of the journal contains contributions from many different countries including Japan, Sweden and the United Kingdom and, of course, our own home-grown authors.

Professor Andrew Bush from Imperial College, London, has provided an excellent practical approach on the management of difficult asthma in children, a topic that is always relevant, especially in South Africa with our increasingly high rates of asthma-related mortality. Prof Bush will also be speaking at the congress and we welcome his return visit to Cape Town.

Professor Adnan Custovic, also from Imperial College, and his research fellow, Toshinori Nakamura, have contributed a review on the heterogenicity of atopic eczema. We look forward with anticipation to Prof Custovic's ever-entertaining lectures during this year's conference.

Component-resolved diagnostics is a 'hot topic' for many of us in the allergy field. One of the experts in this department, Professor Magnus Borres from Uppsala University in Sweden has summarised the way in which we can utilise components in everyday clinical practice. Be sure not to miss his lectures at the congress on this topic.

Dr Sakura Sato from Japan is one of the leading clinicians and researchers in the food-allergy field in East Asia. Her department, under the leadership of Professor Motohiro Ebisawa at Sagamihara National Hospital, is an official WAO Centre of Excellence. She summarises recent trends in the diagnosis and management of food allergy.

Recent advances in the diagnosis of paediatric coeliac disease are discussed in a contribution by Dr Mark Furman, the lead paediatric gastroenterologist at London's Royal Free Hospital. Our own Prof Lehloenya from the Dermatology division at UCT discusses the different phenotypical presentations that can occur with atopic eczema, while Prof Jonny Peter, Head of Department of Allergy and Clinical Immunology at UCT reviews the importance of the mast cell and basophil and their complex immune interactions in allergic disease. From the Division of Paediatric Rheumatology at UCT, Prof Chris Scott discusses autoinflammatory conditions and their management based on a case review of a complicated clinical case that required multidisciplinary involvement in order to plan a way forward for the patient and her family.

An interesting case report on allergic dermatitis has been submitted by Dr Ntatamala from the Division of Occupational Medicine, University of Cape Town, and from Ghana we have an article on ocular allergy by Dr Samuel Kyei. Aeroallergens in Nigeria are discussed by Dr Tiwalade Adeniyi.

As always, we have our regular features including an ethics topic by Professor Sharon Kling, as well as the latest installments of the ever-popular "ABC of Allergies" and "PID123" series.

Don't forget to complete the CPD questionnaire to obtain your CPD points. The questionnaire can also be completed online via our all-new ALLSA website, which can be found at www.allsa.org. Please visit the website, update your details and obtain your new login and password.

We look forward to seeing you all at the congress!

Dr Sarah Karabus

The ALLSA abstracts from the ALLPAEDS Congress will be published with the congress report in the Vol 31 No 4 (2018) issue of the *Current Allergy and Clinical Immunology Journal*.

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Review Article

DIFFICULT ASTHMA

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ABSTRACT

This article describes a new approach to children with asthma who do not respond to low-dose asthma treatment. The answer is not to give more pharmacotherapy but to re-evaluate the whole situation, making objective measurements rather than relying merely on history. First, the diagnosis of asthma must be probed, because it is frequently incorrect. Next, the airway disease must be seen in the context of co-morbidities (exercise-induced laryngeal obstruction, obesity, allergic rhinitis) and social and environmental factors (adherence, adverse environmental factors, psychosocial issues) before any invasive testing for severe, therapy-resistant asthma are undertaken. Furthermore, asthma attacks (which should not be considered using the feeble word 'exacerbations') should be considered never-events with serious implications. There should be objective measurements of severity, an end to the stereotypical three- or five-day course of prednisolone without ensuring that the attack is over, and a detailed evaluation of what went wrong and how a further attack can be prevented must be undertaken. If asthma outcomes are to start improving after a decade of stagnation, we need to change our approach, make measurements, and cease to escalate pharmacotherapy uncritically when the real problem of difficult asthma lies outwith the airway.

Keywords: atopy, eosinophil, nitric oxide, obesity, adherence, exercise-induced laryngeal obstruction

By tradition, severe asthma is defined by the level of prescribed medication,[1] but such a simple approach fails to capture the complexity of difficult asthma. Indeed, 60% of asthma deaths in the United Kingdom[2] involved people who did not meet the definition of severe. All respiratory paediatricians acknowledge that a child is more than just a pair of lungs, and indeed the airway disease must be seen in the context of extra-pulmonary comorbidities and social and environmental factors.[3] Asthma is typically a disease which everyone thinks they can diagnose and treat, often without needing any tests. This culture of complacency and no measurement has contributed to the fact that for a decade asthma outcomes have stalled.[4] This needs to change at all levels.

TWO IMPORTANT STUDIES

Two North American studies[5,6] challenge the assumption that the answer to asthma not responding to treatment is to give more pharmacotherapy. The first study attempted to answer the question as to whether children symptomatic when prescribed at least 800 mcg budesonide per day plus salmeterol should be treated with azithromycin or a leukotriene receptor antagonist. They evaluated 292 children of whom only 55 could be randomised, and the study ended in futility. Most of those who were evaluated but not recruited either did not have asthma or were not taking treatment.[5] The Best Add-on Therapy Giving Effective Responses (BADGER) trial study aimed to determine, in a three-way crossover design in children symptomatic on fluticasone 200 mcg/day, whether increasing the fluticasone dose to 500 mcg/day or adding salmeterol or montelukast was the best strategy. Adding salmeterol was more likely to be beneficial, but the most important lesson was that very few gained any benefit from the larger dose of fluticasone.[6] The message of these two studies is clear; a child with asthma apparently not responding to treatment with the equivalent of 100 mcg twice daily of fluticasone and additional salmeterol does not need further treatment, as suggested by so many 'evidence-based' stepwise guidelines.[7,8] The patient instead needs a full evaluation of the problem. Sadly, we as a profession have been seduced into inappropriate prescribing, to the detriment of our patients and health-service budgets, and to the benefit of Pharma.

DIFFICULT ASTHMA: WHAT IS THE CONTEXT?

Given that most children with atopic, eosinophilic asthma respond to low-dose therapy if regularly and properly administered,[6] in other words, the primary airway pathology is not difficult. Hence, either the child does not have asthma at all, or the problem lies either with comorbidities or in social/environmental factors.[3]

IS IT ASTHMA AT ALL?

The diagnosis of asthma is notoriously poorly done, largely because a smug, no-measurement culture is pervasive. All children cough from time to time, and if the parent complains

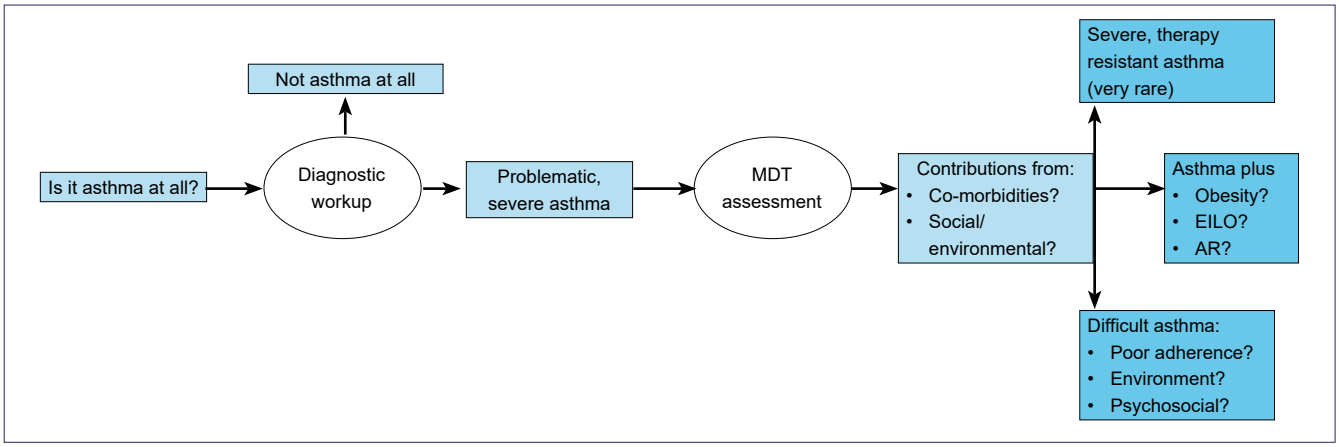

Figure 1: Schema for investigating asthma not responsive to simple therapies. Abbreviations: AR, allergic rhinitis; EILO, exercise induced laryngeal obstruction; MDT, multi-disciplinary team

enough, disregard of the duration of respiratory symptoms in normal children with colds[9] will lead to an erroneous diagnosis of asthma. Test before diagnosis was one of the themes of the recent Lancet commission.[4] In a series from academic primary care in the Netherlands (GP) of 652 children age 6–16 years coded 'asthma' or being prescribed asthma medications, only 21% had any objective evidence to support an asthma diagnosis; more than half were wrongly diagnosed, many having been prescribed quite high dose treatment.[10] The situation in adults is not much better.[11] The first step is to re-look at the diagnosis, with a detailed history and physical examination. This is followed by garnering objective evidence of the presence (or otherwise) of variable airflow obstruction (acute response to β-2 agonist if low peak flow, acute bronchoconstriction to (field) exercise testing, and home peak-flow monitoring), atopy (history of food allergy, eczema, perform skin-prick tests (SPT)) and airway inflammation (exhaled nitric oxide (FeNO), and a raised peripheral blood eosinophil count).[12]

The over-diagnosis of asthma has serious consequences; children are given medications they do not need, money is wasted, but most importantly the diagnosis is trivialised, we lose sight of the fact that asthma kills people, and we do not risk stratify and focus on those at risk of bad outcomes (see below). We must do better. Our pathway for investigating such children is given in the Figure 1, and discussed in detail below.

ASTHMA PLUS: ARE CO-MORBIDITIES CONTRIBUTING TO POOR CONTROL?

These are obesity, exercise-induced laryngeal obstruction (EILO) and allergic rhinitis (AR); gastro-oesophageal reflux, at least in my hands, is not a factor.

EXERCISE-INDUCED LARYNGEAL OBSTRUCTION

This typically presents with noisy breathing towards the end of and after exercise due to adduction of the vocal cords, usually in inspiration leading to stridor, occasionally in expiration.[14] A history of laryngomalacia may be present in infancy.[15] By contrast, exercise-induced bronchoconstriction causes dyspnoea after exercise. The easiest way of making a diagnosis is by asking for an episode to be recorded on a smartphone. Treatment is by physiotherapy, speech therapy or sports psychology.[14]

OBESITY

The first question is: Does the obese child have an airway disease at all? In a Norwegian study,[16] around half of teenagers reporting breathlessness had no objective evidence of either exercise-induced bronchoconstriction or EILO, yet many were being treated with asthma medications. These are not good treatments for deconditioning. And this is another manifestation of the current no-measurement culture in this disease. If any doubt exists, an exercise test should be performed.

If the obese child truly has an airway disease, there is evidence that this may be different from 'lean' asthma. The evidence about the role of atopy is conflicting.[17,18] Obesity may lead to dysanapsis, defined as normal flows in large lungs; the FEV_1 is normal or high, FVC high, and the FEV_1 : FVC ratio is reduced, and this is associated with worse outcomes.[19] In obese asthma, the airway, rather than being the source of inflammation may be the target of systemic inflammation via an IL-6-mediated pathway, independent of IgE and sputum eosinophilia.[20] In the obese child with a genuine airway disease, caution therefore should be exercised in escalating inhaled corticosteroid therapy. Weight loss works, however.[21]

ALLERGIC RHINITIS

The relationship between upper and lower airway disease, and the 'unified airway' is controversial; and I for one have never seen remodelling in the nose. However, there are good quality studies which show that treatment of AR (which should of course be treated on its merits anyway even if asthma is not present) improves concomitant asthma.[22]

WHAT IS THE CONTRIBUTION OF SOCIAL AND ENVIRONMENTAL FACTORS?

ADHERENCE

This is probably the biggest factor in poorly controlled asthma. Currently, no uniformly satisfactory method proves adherence is good, only showing that it is not. If prescriptions are not being picked up, then poor adherence is guaranteed, but if they are, there is still no guarantee they are being administered.[23] We use electronic monitoring, telling the family we are recording activation of the inhaler. This results in placing the patient in one of four categories:[24]

- *Adherence good*, symptoms and FEV_1 improve, FeNO falls; these children were previously non-adherent but became adherent during monitoring and as a result get better.
- *Adherence poor*, no improvement in asthma; either a hard-core non-adherer or someone with genuine severe, therapy resistant asthma who is tired of taking treatments which do not work. The next step is directly observed therapy to see what happens when we know treatment is being given.
- *Adherence good*, but no change in asthma parameters, control or attacks; this is the group with true severe, therapy resistant asthma who should be referred for detailed evaluation.
- *Poor adherence*, good control; this is the group who are being over-treated, treatment is reduced.

Even electronic monitoring is not enough; when this is combined with monitoring of inhalation, it is clear that electronic monitoring alone overestimates adherence by 20%.[25] Even when poor adherence is identified, dealing with it is another matter. Some of these children may merit a trial of omalizumab, if only to keep them alive; we cannot penalise children for parental inadequacy.[26]

ADVERSE ENVIRONMENTAL ISSUES

Steroid resistance results from high exposure to allergens to which the child is sensitised, an all too common situation in my practice. Exposure to cigarettes and e-cigarettes is also a common adverse factor. As with adherence, dealing with it is harder than identifying it.

PSYCHOSOCIAL FACTORS

Our clinical psychologists are kept very busy in the difficult asthma clinic. Depression and denial is very common.[25] It is pointless to try to determine whether asthma caused depression or depression led to asthma; both should be treated on their merits. We have safeguarding concerns in around 10% of our really severe asthma children, most often around exaggeration of symptoms, but also administration of medicines and adverse environmental circumstances. There are financial benefits to having a child with 'difficult asthma' at least in the United Kingdom, in the child with apparently very normal spirometry and a low FeNO, but complaining of a multiplicity of symptoms. Steps to be taken include a methacholine challenge – if this is negative, then symptomatic asthma is unlikely. Another useful strategy is a discussion with the school – in one instance, an allegedly steroid dependent asthmatic was in fact captain of sport and never used an inhaler in school. Sometimes an admission to hospital is illuminating;[27] frequently, as medication is taken under supervision, spirometry normalises, FeNO falls to normal, and the child becomes fully active with little or no salbutamol use, meaning that either they are not taking treatment at home, or adverse environmental factors are nullifying treatment, or both.

GENUINE SEVERE, THERAPY-RESISTANT ASTHMA

A detailed discussion of this rare entity is beyond the scope of this article. Our detailed protocols have been described elsewhere.[26,28,29] A multidisciplinary team is essential. We phenotype the airway disease using bronchoscopy, and assess steroid responsiveness using a single dose of intramuscular triamcinolone.[30] These patients are usually atopic, and have eosinophilic airway disease or are paucigranulocytic.[31] Unlike adult physicians, we do not see neutrophilic asthma; indeed, intra-epithelial neutrophils are associated with better asthma outcomes.[32] Increasing evidence implicates the innate epithelial cytokines IL-25, IL-33, and TSLP[33] in severe paediatric asthma.[34,35] Therapeutic options have been reviewed elsewhere.[36]

MANAGEMENT OF ACUTE ATTACKS OF ASTHMA

The pharmacotherapy of acute asthma is well described elsewhere,[7,8] and will not be recapitulated here. The first necessity is to get rid of the feeble word 'exacerbation' and understand that an asthma attack should be a 'never-event' such as amputating the wrong leg.[4] Exacerbation implies a trivial and reversible inconvenience, whereas an attack is the single greatest predictor of further attacks and death,[2,37,38] and a marker of worse long-term outcomes.[39,40] The culture of making no measurements, giving a three- or five-day course of prednisolone with no follow up, has to cease.

HOW BAD IS THE ATTACK?

Objective measurements of oxygen saturation and peak flow must be combined with accurate counting of respiratory and heart rate and documentation of work of breathing, wheeze and other physical signs. It is all too common for prednisolone and sometimes even intravenous treatments to be given without this happening. So when I see a child with 'difficult asthma' who has had multiple prednisolone courses, the first thing I want to know is: What was the evidence that these courses were indicated? If the child has a genuine asthma attack, meriting prednisolone, that child should not have prednisolone stopped without a review determining that the attack has truly resolved.

The next step is a detailed review of what went wrong:[2]

- Why was the child's condition allowed to get to the point of needing prednisolone?
- Does the child have an asthma plan, was it followed, and should it be changed?
- Is the child adherent to medications, does the family know how to use the inhaler?
- Does the family bring the child for regular check-ups or instead have a pattern of multiple emergency attendances (a marker for asthma death risk)?
- Can the environment be changed to reduce risk?

This last question has been thrown into sharp focus by a recent study. From a previous study,[41] it is known that allergen sensitisation, allergen exposure and viral infection in combination are strongly predictive of risk of an asthma attacks; however, only allergen exposure can be changed. They therefore randomised children age 3–17 years who, first, had been admitted to hospital with an asthma attack and, secondly, were house-dust mite (HDM) sensitised, to receive mite-impermeable

bedding covers or placebo covers.[42] Over a 12-month period, 434 consented to the study, of whom 284 were mite-sensitised (mean age 7.7 years, 66% male); 146 to active covers, 138 to placebo. There was a significant risk reduction of a further attack (hazard ratio 0.55, (95% CI 0.36–0.85)), albeit with no difference in oral steroid use. This is proof of concept that environmental manipulation can reduce the risk of asthma attacks, but perhaps the intervention was too narrow. An older study,[43] using a multi-pronged environmental intervention addressing multiple allergens, air quality and other pollutants, and education led to improvement in asthma control which were sustained beyond the duration of the intervention. This approach needs further exploration.

FUTURE RISK

This is another area needing work – we simply must risk stratify our patients. Markers of risk include a previous bad acute attack of asthma, prescription of more than six canisters of salbutamol/year, history of multiple emergency attendances with failure to attend routine reviews, and recent discharge from hospital.[2] These children need the closest possible monitoring, and further work is needed to try to find biomarkers of risk. Adult studies[44–46] suggest persistent induced sputum or blood eosinophilia are markers of risk; these studies need extending into children. This subject has been recently reviewed.[47,48]

SUMMARY AND CONCLUSIONS

Difficult asthma is usually not due to difficult airway pathology, but to difficult co-morbidities and social and environmental features. We do not need more 'evidence-based' guidelines, most recommendations of which are not evidence based, but a new approach if asthma outcomes are to be improved. The response to a child with asthma not responding to simple treatments is not to give more treatments, but to re-evaluate the entire situation. To do this, we need to move to a measurement based culture; no-one would treat hypertension without knowing the blood pressure, or give insulin without measuring the blood sugar. We should be making measurements of physiology and inflammation to make an objective diagnosis of asthma and monitor treatment. We should not assume that breathlessness means the child needs inhaled corticosteroids. We must take a measurement culture into the management of asthma attacks, and regard them as the catastrophic, never-events that they are, rather than minor inconveniences which just need a short course of oral steroids. Unrealistic, ivory tower aspirations? Not really; something has to happen if asthma outcomes are to improve.

DECLARATION OF CONFLICT OF INTEREST

Andrew Bush is an NIHR Senior Investigator and additionally was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College, London.

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Review Article

UNDERSTANDING THE HETEROGENEITY OF ATOPIC DERMATITIS IN CHILDHOOD

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ABSTRACT

No universally accepted definition of atopic dermatitis (AD) exists in epidemiological studies, and no objective test that can unequivocally confirm the diagnosis. The heterogeneity of AD is now broadly accepted, but there is no consensus on what the best approach is to disaggregate this complex condition, and how best to identify different AD endotypes. One approach is to use data-driven techniques to uncover the unobserved (ie latent) structure in the data set(s) to identify homogenous groups of individuals in the heterogeneous AD population. Since AD usually starts early in life, and its clinical features may progress, remit or relapse over time, analyses assessing trajectories related to their presence or absence over time may help us understand AD heterogeneity. However, studies that used unsupervised methods such as latent class analysis to discover AD 'phenotypes' have reported inconsistent findings. If we are to use data-driven analyses to uncover patterns of AD with different long-term consequences, then the discovered 'phenotypes' must be consistent and reproducible. We therefore need to understand the reasons for the inconsistencies between different studies. We suggest that in addition to capturing a simple presence or absence of symptoms, more detailed clinical features, such as the severity and medication used, should be assessed in longitudinal studies. Ultimately, the objective is to move from 'deep phenotyping' to more informative genetic studies, followed by functional studies to understand mechanisms. In this way, the identification of novel therapeutic targets could be facilitated.

INTRODUCTION

Atopic dermatitis (AD) is a non-contagious inflammatory skin disease that chronically relapses and is intensely pruritic.[1] In contrast to high-income countries where AD is very common,[2] in the rural areas of Africa it is a rare disease. According to the International Study of Asthma and Allergies in Childhood,[3] the lifetime prevalence by age 13 years was reported to be >20% in developed countries, 16% in Cape Town and 6% in Addis Ababa. A questionnaire-based population survey in Ethiopia reported the lifetime prevalence of AD to be as low as 0.3% in the rural areas.[4] A population-based South African study of rural, peri-urban and urban Xhosa children aged 3–11 years reported a prevalence of visible flexural eczema, according to the UK diagnostic criteria, of 1.8% and a point prevalence of AD, according to a dermatologist, of 1.0%.[5]

Traditionally, AD has been considered to start in early infancy, with a significant proportion of affected children remitting in later childhood.[6] However, recent studies have shown that a substantial number of children with AD can suffer symptoms beyond childhood,[7] and some patients develop the disease for the first time in adolescence or adulthood.[8] AD which develops in childhood appears to differ in clinical presentation (such as distribution of skin lesions) and associated risk factors (e.g. allergic sensitisation[9] and *Filaggrin* (*FLG*) loss-of-function mutations[8]) from that which starts in adulthood. AD is often considered to be a first step in the sequential development of allergic disease (the so-called 'atopic march'), with a progression to asthma and rhinitis.[10] However, a recent study which investigated the development of AD, wheeze and rhinitis at the individual rather than a population level has suggested that the conceptual framework of the atopic march may represent an oversimplification of the developmental pathways, with more than 6% of children with any of these clinical manifestations following the sequence resembling the 'atopic march'.[11]

Although the first diagnostic criteria for AD were proposed by Hanifin and Rajka in 1980,[12] currently there is no uniform clinical definition, and no objective test that can unequivocally confirm the diagnosis. Furthermore, the use of the different terms, such as atopic dermatitis, eczema or atopic eczema, varies geographically.[13] Consequently, numerous different definitions have been used in epidemiological and genetic studies.[14,15] Of note is that one of the most common definitions for epidemiological studies (UK working party criteria)[14] has low positive predictive values in African populations,[5,16] which was only 18.4% in a general population study in South Africa. This

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lack of a standard definition makes the extrapolation of findings unreliable; consequently, projecting the results from European populations to African populations is not appropriate. We urgently need studies based on globally harmonised outcomes and designed explicitly for AD to help understand this emerging global problem and to ascertain differences in AD across different populations.

HETEROGENEITY OF ATOPIC DISEASES

One of the most important paradigm shifts in the field of allergy in recent years has been the increasing awareness of the heterogeneity of allergic sensitisation[17] and the clinical manifestations of 'atopic diseases'.[18–21] This has led to the view that most atopic diseases (including AD) are not single conditions, but umbrella terms for several diseases with similar clinical presentations, but distinct pathophysiological mechanisms.[22] Although the heterogeneity of AD is now broadly accepted,[23,24] there is no consensus on what the best approach is for understanding the complexity of its subtypes and discovering their underlying mechanisms, which is a prerequisite for identifying novel therapeutic targets. As yet there have been no reliable indicators (e.g. clinical features, genes, cytokines or comorbidities) that allow the disaggregation of AD or the prediction of future risk. The heterogeneity of AD may contribute to the inconsistencies in findings between different populations, and may dilute the strength of the association with genetic variants and biomarkers.[23] Ultimately, if we are to achieve precision medicine (ie the management of a disease with the precise selection of treatment or risk-prediction for individual patients[25]), we need to understand the mechanisms responsible for generating heterogeneity. In this article, we focus on how data-driven approaches may help us to understand the heterogeneity of AD. First, we clarify three keywords to build the concept (phenotype, endotype and biomarker). We then proceed to discuss how better phenotyping in longitudinal studies may improve genetic studies and contribute to an understanding of the underlying pathophysiology. Finally, we review the AD trajectories through childhood, and the limitations of the identified 'phenotypes'.

PHENOTYPE, ENDOTYPE AND BIOMARKER

It is important to make a distinction between the terms 'phenotype', 'endotype' and 'biomarker' (see Table I).[25] A phenotype is an observable characteristic – clinical, physiological, morphologic, biochemical – and/or the response to different treatment without any implication of a mechanism.[26] An endotype can be referred to as a subtype of a complex condition which is characterised by a distinct pathophysiological mechanism. It is worth emphasising that the same pathophysiological mechanism (ie endotype) may lead to different clinical presentations (ie phenotypes), and vice versa, and that the same phenotype may arise in response to different mechanisms. We wish to emphasise that latent 'phenotypes' discovered using data-driven methods are not observed, but latent by nature, and ideally should not be referred to as phenotypes (ie observable characteristics); a more appropriate term is the latent class. However, as the term 'phenotype' has been used in this context for more than a decade,[27] we will maintain this nomenclature while accepting that it is not optimal. Furthermore, latent phenotypes are not clinically relevant and cannot be considered as endotypes until their underlying mechanisms have been discovered and confirmed. Like asthma,[22] AD 'endotypes' are for the time being hypothetical constructs that may in the long term help us to understand better the mechanisms of AD-related conditions and may help identify more effective stratified treatment approaches.

TABLE I: DESCRIPTION OF KEYWORDS RELEVANT TO PRECISION MEDICINE

KEYWORDS	DESCRIPTION
Phenotype	An observable characteristic – clinical, physiological, morphologic, biochemical – and the response to different treatments without any implication of a mechanism.
Endotype	A subtype of a condition defined as having a distinct functional or pathophysiological underlying mechanism.
Biomarker	A measurable indicator for examining any aspects of health or disease. It is used to identify the characteristics of a given cohort that will examine or predict normal biological processes, pathogenic processes or a treatment response.
Precision medicine	Management of a disease with a precise selection of treatment or risk prediction for an individual patient.
Latent subtype	Identified subgroups of patients based on unobserved (latent) patterns within observed data using statistical methods, for example latent variable modelling techniques.

A biomarker is a measurable indicator for examining any aspects of health or disease. It is used to identify the characteristics of a given cohort that will examine or predict normal biological processes, pathogenic processes or a treatment response.[26]

WHAT DOES GENETICS TELL US ABOUT THE HETEROGENEITY OF AD?

The relationship between AD and genetic factors illustrates the complexity and heterogeneity of AD.[28] Immune dysregulation, skin-barrier dysfunction and their interaction are important factors contributing to the development and pathogenesis of AD.[29,30] Filaggrin is a filament-aggregating protein which plays an important role in maintaining barrier integrity,[30] and a number of studies have linked AD with *FLG* loss-of-function mutations.[31–33] These genetic variants are now considered to be important risk factors for the development and persistence of AD.[34] However, a population-based UK birth-cohort study showed that *FLG* loss-of-functional mutations were present in 8.8% of the population, only 46% of whom had AD.[32] Moreover, case-control studies of AD in South Africa and Ethiopia found that no children with AD had the FLG mutations which are found in European and Asian populations.[35,36]

The strength of the association of AD with *FLG* mutations may vary throughout childhood, even in a single population. A population-based UK birth cohort study, which followed children until age 11 years, performed a survival analysis to

determine the period of the persistence of AD defined by the parent-reported flexural dermatitis among children having the onset by 42 months of age.[32] In this study, AD children with *FLG* mutations experienced longer persistence than those without the mutations (the mean persistence: 76.7 vs 65.6 months), but more than 75% of them 'outgrew' their AD within the observational period. This finding implies that the skin-barrier dysfunction in some children with AD and *FLG* mutations may be compensated for by as yet unknown mechanisms. Given that in some of these children AD resolves with increasing age, the association of AD with *FLG* mutations obtained by cross-sectional analyses may differ, depending on age. For example, a population study of adolescent AD showed no AD association with *FLG* mutations.[8] In addition to *FLG*, several other genes and loci related to skin barrier have been associated with AD, with a relatively small effect size.[37–39]

The above findings suggest that the association of *FLG* loss-of-function mutations (and other genetic variants) with AD may significantly vary with increasing age, indicating that approaches to capturing the time-varying features (and subtyping AD based on this) may help to understand AD heterogeneity. This may, in turn, lead to a more thorough understanding of the genetic risk factors. Within this framework, using better ('deep') phenotyping may allow us to identify a clearer contribution of each genetic variant to the development of specific AD phenotypes. One example of such an approach in asthma, is the discovery of the association of a specific asthma phenotype (early-life onset with recurrent, severe exacerbations) with a functional variant in a novel susceptibility gene CDHR3.[40] This approach followed the sequence from better phenotyping and more informative genetic studies to functional studies in order to understand the mechanisms and the therapeutic target identification. Subsequent studies have shown that the expression of the risk variant in CDHR3 (rs6967330 AA) mediates enhanced rhinovirus-C binding and increased progeny yields in vitro.[41] In the process, CDHR3 was identified as a receptor for rhinovirus C and a potential therapeutic target.

APPROACHES TO IMPROVING OUR UNDERSTANDING OF AD HETEROGENEITY

AD usually starts early on in life, and clinical features such as symptoms or signs may progress, remit or relapse over time. We propose that an approach based on the understanding of the individual temporal trajectories of clinical features over the life course may contribute to our understanding of the complexity of AD. The conventional cross-sectional studies are unable to capture changes adequately over time. Moreover, the preferred study design for investigating this type of disease development is a population-based birth cohort.[22] We propose, further, that in addition to capturing a simple presence or absence of symptoms or signs, more detailed clinical features should be assessed in long-term studies,[42] and that the time frame should optimally be extended to adulthood. For example, a birth-cohort study which followed participants to young adulthood described that, of the children who had the onset by age seven and were cleared of AD symptoms or signs at age 11, 10.7% experienced a recurrence at age 16.[43]

In the following section, we review the two most common approaches to defining phenotypes of AD based on symptom temporality: subjective approach and data-driven (see Table II).[23, 44] Subjective approaches are usually based on clinical insights about age of onset, progression and remission, which are then applied to and tested in the longitudinal dataset(s).[45] A typical example of this approach is the description of three wheezing phenotypes (transient early, late onset and persistent) in the Tucson Children's Respiratory Study.[46] In epidemiological studies of AD, the subjective approach has been used to describe the natural course and investigate the association of AD with risk factors.[9,47–49] Most studies have used similar, although slightly different, classifications. A recent review has suggested four natural courses of the disease in childhood, depending on the timing of the onset: very early onset (3 months–2 years), early (2–6 years), childhood (6–14 years), and adolescent type (14–18 years).[23] Very early onset AD accounts for 60–80% of all forms of AD. These patients have a high probability of the remission in early childhood (although up to 40% may experience the persistent course) and tend to have the highest risk of

TABLE II: DESCRIPTION OF SUBJECTIVE AND DATA-DRIVEN APPROACHES[45,51]

	SUBJECTIVE APPROACH	DATA-DRIVEN APPROACH
Description	Subtyping based on clinical insights about the age of onset, progression and remission.	Subtyping based on statistical modelling that focuses on the unobserved structure (latent variable) in the dataset.
Method	1. Investigates individual changes in the profile of clinical features of atopic dermatitis (e.g. the presence and/or severity of symptoms). 2. Classifies children into different phenotypes based on common observed patterns of clinical features over time, usually based on clinical experience.	1. Assumes that observable characteristics are imperfect indicators of an underlying (latent) construct. 2. Uses a series of latent variable modelling techniques to look for structure in the data without having a specific research hypothesis.
Characteristics	1. Proposed phenotypes are predominantly based on expert opinions and consensus on a single dimension of a disease. 2. The identified phenotype is often clinically meaningful, but rarely associates with an endotype.	1. By using model-fit parameters, phenotypes can be identified objectively from an array of data. 2. Proposed phenotypes are assumed to be a reflection of different underlying aetiologies. 3. Proposed phenotypes need careful interpretation with scientific rigour and validation by testing hypotheses which arise from the process.

developing the other atopic diseases. The relative proportions of the early onset, childhood, and adolescent phenotypes are 10% or less, respectively.[23] A systematic review of AD persistence reported that patients with childhood onset (age 6–11 years) had the highest risk of persistence among those with childhood AD.[50] However, compared to the very early onset of AD, the long-term course of early onset, childhood, and adolescent AD has not been well investigated.

Phenotype discovery can be extended by using data-driven approaches that use a series of statistical models to shed light on the unobserved (ie latent) structure in the dataset to identify homogenous groups of individuals within the population. One such approach is the latent class analysis (LCA), one of the most popular methods to model trajectories in cohort studies.[45,51] LCA relies on the assumption that observable characteristics are imperfect indicators of an underlying (latent) construct.[52] One of its advantages is that it enables objective assignment of children to different latent phenotypes by using model-fit parameters.[45]

To date, three birth cohorts have reported trajectories of AD using LCA (see Table III). The Protection Against Allergy Study in Rural Environments (PASTURE, a birth cohort of farmers' and non-farmers' children from rural areas in five European countries) was the first study to describe AD phenotypes using LCA.[53] This study assigned children to four different phenotypes based on longitudinal patterns of flexural rash within the first six years of life:

TABLE III: CHARACTERISTICS OF COHORTS AND ASSOCIATIONS OF EACH PHENOTYPE WITH RISK FACTORS

	PASTURE	ALSPAC	PIAMA
Cohorts	A selected birth cohort*1	A population-based birth cohort	A selected birth cohort*2
Locations	Five European countries*3	United Kingdom.	The Netherlands
No of subjects	1 038	9 894	3 652
Observation period (years)	6	16	11
Time points	7	12	10
Definition of AD	ISAAC*4	Parent-reported questions*5	ISAAC*4
ESTIMATED PREVALENCE OF EACH PHENOTYPE*6 (%)			
Phenotype 1	6.5 (67/1 038)*7	7.3	4.9
Phenotype 2	9.2 (96/1 038) *7	12.9	15.4
Phenotype 3	NA	7.0	3.8
Phenotype 4	4.8 (50/1 038) *7	7.0	6.5
Phenotype 5	NA	7.9	6.5
Phenotype 6	79.5 (825/1 038) *7	58.0	62.9
FILAGGRIN LOSS-OF-FUNCTION MUTATIONS, ORS 95% CI COMPARED TO PHENOTYPE 6			
Phenotype 1	NA	4.3 (3.3–5.6)	1.3 (0.5–3.7)
Phenotype 2	NA	2.1 (1.5–3.0)	0.87 (0.3–3.0)
Phenotype 3	NA	2.2 (1.5–3.3)	5.6 (2.7–11.9)
Phenotype 4	NA	1.5 (0.9–2.4)	0.95 (0.3–3.5)
Phenotype 5	NA	2.3 (1.6–3.4)	1.9 (0.98–1.2)
ASTHMA*8, ORS 95% CI COMPARED TO PHENOTYPE 6			
Phenotype 1	2.6 (1.3–5.5)	5.5 (4.3–7.1)	14.3 (7.3–27.8)
Phenotype 2	1.6 (0.8–3.3)	1.6 (1.1–2.2)	3.0 (1.1–8.5)
Phenotype 3	NA	3.1 (2.2–4.3)	5.9 (2.3–15.2)
Phenotype 4	0.8 (0.3–2.7)	2.2 (1.5–3.3)	0.6 (0.03–13.6)
Phenotype 5	NA	1.9 (1.3–2.8)	1.7 (0.4–7.9)

Phenotype 1 stands for 'Early-onset-persistent', 2 for 'Early-onset-early-resolving', 3 for 'Early onset-late-resolving', 4 for 'Mid-onset-resolving', 5 for 'Late-onset-resolving' and 6 for 'Unaffected/transient'. Early-transient class and late-onset class in PASTURE were assigned to early-onset-early-resolving and mid-onset-resolving class, respectively. NA = 'not applicable'.

**1 A selected birth cohort of farmers' and non-farmers' children from rural areas in five European countries.*
**2 A selected birth cohort from allergic and non-allergic mothers.*
**3 Austria, Finland, France, Germany and Switzerland.*
**4 Core question 3 of International Study of Asthma and Allergies in Childhood (ISAAC).*
**5 Questions consisted of itchy skin rash affecting age-specific skin regions.*
**6 Prevalence estimates show the prevalence of each class in the cohorts.*
**7 In PASTURE, individuals were assigned to each latent class with their probability of the membership being more than 0.5. Prevalence based on the number of children in each latent class was reported. In ALSPAC and PIAMA, only the estimated prevalence was reported.*
**8 Definitions of asthma: Physician-confirmed asthma at age six (PASTURE) and parent-reported asthma at age seven (ALSPAC and PIAMA).*

1. 'unaffected or transient' (children had less than 0.2 of probability of having the rash throughout childhood);
2. 'early onset and persistent';
3. 'early onset and transient', and
4. 'late onset' (onset after age two years of age).

The Avon Longitudinal Study of Parents and Children (ALSPAC), and the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) studies used LCA to identify AD phenotypes throughout childhood (birth to age 16 in ALSPAC and birth to age 11 years in PIAMA).[54] They found four phenotypes similar to those identified in PASTURE, and in addition two further novel phenotypes:

- 'early-onset-late-resolving', where children had a high probability of AD from birth to age 30 months, which declined to less than 10% in late childhood, and
- late-onset-resolving', where the risk of AD rose steeply after age 5–6 years, and then declined gradually from age ten years.

The longer observation period of these two cohorts compared to PASTURE may have contributed to the identification of these new phenotypes. Table III shows the prevalence of each phenotype and their association with different risk factors in each of the cohorts. Although the four common phenotypes in the three cohorts shared similar profiles, there were differences with regard to associations with risk factors. For example, the 'early-onset persistent' phenotype, which accounted for 5–7% of the population, had the strongest association with asthma in all cohorts; however, the association with *FLG* loss-of-function mutations was significant in ALSPAC but not in PIAMA (in which this association was the strongest with 'early-onset-late-resolving' AD).

The inconsistent findings may have arisen for several reasons. First, all three studies defined AD using the simple presence of parentally reported current AD symptoms, which introduces heterogeneity since this definition is unable to differentiate completely between other pruritic skin conditions.[55] Secondly, there were some differences in the study design (ALSPAC is a population-based cohort, but PASTURE and PIAMA recruited selected populations). Furthermore, only a single dimension of AD was analysed, which may be insufficient to capture disease heterogeneity; and it is possible that using several dimensions of the disease may offer better insights[56] (e.g. severity and medication-use, allergic sensitisation, etc).

The international Harmonising Outcome Measures for Eczema (HOME) initiative (a global initiative of patients, healthcare professionals, journal editors, regulatory authorities and the pharmaceutical industry), which aims to develop consensus-based core outcomes for clinical trials, suggested the use of four outcomes: clinical signs, symptoms, long-term control and quality of life (QoL).[57]

Finally, these inconsistencies may suggest that there remains substantial heterogeneity within phenotypes discovered by LCA. Despite the widespread use of LCA, little is known about how factors such as the sample size and the timing and the frequency of data collection influence the outcomes of models or phenotype identification. It is possible that not all time points carry useful information and therefore some might be redundant, or even cause uncertainty in the results.

CONCLUSIONS

If we are to use data-driven analyses to understand the factors associated with patterns of AD with different long-term consequences, then the discovered 'phenotypes' must be consistent and reproducible. The inconsistencies between studies may be partly attributed to differences in study design or could be due to true differences between different populations. However, the inconsistent findings may also arise as an artefact of the analysis. Therefore, we may need other methods of analysis that allow us to have more explicit discrimination than LCA.

DECLARATION OF CONFLICT ON INTEREST
The authors declare no conflict of interest.

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Review Article

WHAT IS NEW IN ALLERGY AND COMPONENT TESTING?

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INTRODUCTION

Considerable progress has been made in the area of molecular allergology over the past few years. Molecular or component-resolved diagnostic (CRD) tests are promising and studies continue to emerge regarding their utility. A better understanding of allergen components and their role in the mechanisms of sensitisation and tolerance induction leads to a more targeted management of inhalant and food allergy.

The impact of molecular spreading of furry-animal components on clinical disease has been documented. The first case report is now published confirming that a dog-allergic patient mono-sensitised to Can f 5, prostatic kallikrein, was allergic to male dogs only. It has been shown that used together, Ara h 2 and Ara h 6 – both 2S albumins – can provide the highest diagnostic accuracy. New data shows that the measurement of serum tryptase is of value in the diagnosis of food-allergic reactions and especially in severe anaphylactic reactions. Measuring serum IgE (sIgE) to Der p 23 adds important information on disease progression, and may serve as a severity marker for asthma in patients allergic to house-dust mite (HDM).

This article focuses on recent publications but also provides general information that may be useful to those embarking on understanding the diagnosis and management of allergy and asthma.

INHALANT ALLERGENS

PREDICTING ASTHMA AND ALLERGY

Sensitisation in early childhood can precede respiratory allergy in adolescence.[1] Wickman et al used micro-array component data from a random sample of 786 children at ages four, eight and 16 years from their prospective birth cohort. The purpose was to identify markers that could predict the risk of future respiratory allergy. An English birth cohort was used as a comparison. They concluded that Bet v 1 (birch pollen), Fel d 1 (cat), Phl p 1 (grass pollen) and Ara h 1 (peanut) were predictive for the Swedish population. Relevant components in the group from the United Kingdom included Der p 1 & Der f 2 (HDM), Phl p 1 and Phl p 5 (grass pollen) and Fel d 1 (cat). The authors concluded that IgE-reactivity early in life to these allergen molecules identifies children with a high risk of asthma and/or rhinitis at 16 years.

Cipriani et al have studied the diagnostic relevance of IgE-sensitisation profiles to eight recombinant *Phleum pratense* molecules in 1 120 children (aged 4–18 years) with seasonal allergic rhinoconjunctivitis.[2] Sensitisation to Phl p 7 was a reliable biomarker of asthma, whereas Phl p 12 was useful as a marker of oral-allergy syndrome (OAS). Sensitisation to Phl p 7 was associated with a more severe form of seasonal allergic rhinitis (AR) and complex component profiles were associated with longer disease duration.

HOUSE-DUST MITE COMPONENTS

Twenty allergens in *D pteronyssius* have been identified thus far. The major allergens, Der p 1 and Der p 2, will identify between 63 and 97% of patients sensitised to *D pteronyssius* extracts, as shown in studies from Europe, North America and Japan.[3] A significant proportion of HDM-sensitised patients may be missed if using only major allergens in the diagnostic work-up.

Up to 74% of *D pteronyssius*-allergic patients are sensitised to Der p 23. This is similar to the frequency of sensitisation to that of Der p 1 and Der p 2.[4] Although levels of sIgE to Der p 23 are on average five times lower than to Der p 1 and Der p 2 in HDM-allergic patients, Der p 23 appears to be ten times more potent than Der p 1 in activating mast cells.[3]

Matricardi et al have shown that the number of HDM component-specific sensitisations increased with an increase in disease severity, as well as with age of onset. Importantly, sensitisation to Der p 1 and Der p 23 before the age of five was predictive of asthma at school-going age.[5]

Children with both HDM allergy and asthma were shown to be sensitised to a higher number of HDM allergen components than non-asthmatic children with HDM allergy. Furthermore, the IgE levels to Der p 1, Der p 2 and Der p 23 were higher in the asthmatic children than in non-asthmatic children.[6]

Specific IgE (s-IgE) to Der p 23 can add important information on the disease progression, and may serve as a severity marker for asthma in HDM allergy.

FURRY ANIMALS

Sensitisation to dog and/or cat in early life is a strong predictor of the development of childhood asthma.[7] Sensitisation to furry

animals appears to be on the increase globally.[8,9] Aranda et al have shown that sensitisation to dog increased from 8 to 40% in Brazilian children over a 12-year period (2004–2016). More than 40% of the households have at least one dog. Sensitisation to dog seems to start early in life and Nagao et al found that dog was the most common inhalant sensitiser in young Japanese children at risk of developing respiratory allergies.[10] Schoos et al examined sensitisation trajectories in childhood by using a cluster analysis during the first six years of life.[11] They found that asthma was associated with a specific sensitisation pattern to dog, cat and horse in early childhood.

Davila et al have recently published a consensus document on dog and cat allergy in which they state that measurement of IgE antibodies to animal-dander extract is an established diagnostic tool, however, this has several limitations. In some cases, especially in polysensitised patients, molecular diagnosis is strongly recommended.[12] Molecular diagnosis is recommended in order to distinguish between co-sensitisation and cross-reactivity between various different animals. It is useful in polysensitised patients, in order to provide recommendations for avoidance as well as to identify allergens that are significant components in immunotherapy. Finally, molecular diagnosis may be useful for attempting to predict symptoms and disease severity, especially in patients with asthma and particularly in severe asthma.

DOG COMPONENTS

There are six known dog allergens. Four of these, Can f 1, 2, 4 and 6 belong to the lipocalin family and are present in dog dander, saliva and urine.[13] Can f 1 is a major allergen and is detected in 50–90% of patients sensitised to dog. Sensitisation to Can f 2 and to the more recently characterised Can f 4 and Can f 6 is less common. These allergens can be detected in 15–35% of dog-sensitised patients and have been shown to cross-react with cat and horse lipocalins. Can f 3, the dog serum albumin, is a highly cross-reactive minor allergen detected in 15–35 % of dog-sensitised patients. IgE reactivity to Can f 5, the prostatic kallikrein from a male dog, has been found in up to 70% of dog-sensitised individuals among whom 38% were not sensitised to Can f 1, 2 or 3. This suggests that there may be individuals who are allergic specifically to male dogs.

TABLE I: ANIMAL COMPONENTS

ANIMAL	COMPONENT		CLINICAL RELEVANCE
Cat	Fel d 1	Uteroglobin	True sensitisation Major cat allergen Risk factor for asthma
	Fel d 2	Serum albumin	Cross-reacts with other animal albumins Minor cat allergen
	Fel d 4	Lipocalin	Cross-reacts with other animals
Dog	Can f 1	Lipocalins	True sensitisation, major dog allergen
	Can f 2		Minor allergens, cross-react with cat and horse allergens
	Can f 4		
	Can f 6		
	Can f 5	Prostatic kallikrein	True sensitisation to male dogs
	Can f 3	Serum albumin	Cross-reacts with other animals

Photo credit: Polka100

The first case report has recently been published confirming that a dog-allergic patient mono-sensitised to Can f 5 was allergic to only male dogs. This individual had a negative skin-prick test (SPT) and no reaction at ocular provocation test using female dog-dander extract.[14]

Sensitisation to the lipocalins in general has been shown to be associated with asthma.[15] Konradsen et al found that sensitisation to Can f 2 was more common in patients with severe asthma.[16] Ukleja-Sokolowska et al have been able to document the association between Can f 2 sensitisation and dyspnoea in pet-allergic patients.[17]

A number of studies have now shown the impact of molecular spreading of furry animal components on clinical disease. Käck et al show that polysensitisation to all six dog components conferred the overall highest risk for a positive reaction to dog nasal challenge.[18] Sensitisation to more than three single lipocalins, prostatic kallikrein and secretoglobin components from furry animals has been associated with asthma severity.[19] Moreover, sensitisation to several components from the same species has shown to be a marker for clinical pet allergy.[20,21] Käck et al also found that the presence of IgE to Can f 4 and 6, together with the serum albumin Can f 3, conferred the highest risk for a positive nasal challenge. [24]

CAT COMPONENTS

The secretoglobin Fel d 1 is the dominant allergen in cat-dander extract.[13] Sensitisation to Fel d 1 often starts early in life and is most likely to precede the appearance of allergic symptoms. The first described cat lipocalin, Fel d 4 was reported to bind IgE antibodies in a high proportion (>60%) of cat-allergic subjects. Furthermore, the cat lipocalin Fel d 7 was recently identified and shown to bind IgE in 38% of a cat-allergic population. The minor cat allergen Fel d 2 (serum albumin) cross-reacts with other albumins, but its clinical significance is not yet fully


Cat: photo credit Elena Kouptsovasic

understood. Tsolakis et al found that sensitisation to Fel d 2 and 4 were independently associated with type-2 biomarkers, such as exhaled nitric oxide and blood eosinophils.[22] Uriarte and Sastre found that sensitisation to albumins was associated with severe respiratory symptoms and with high odds ratios for asthma diagnosis.[23]

FOOD ALLERGY

HEN'S EGG COMPONENTS

The most important allergens in egg white are Ovomucoid (Gal d 1), Ovalbumin (Gal d 2), Ovotransferrin (Gal d 3) and Lysozyme (Gal d 4). Ovomucoid is resistant to heat and digestion and it has been shown that children with persistent egg allergy have significantly higher levels of ovomucoid than children who outgrow their egg allergy. Modification of allergens is an important strategy in order to attempt to reduce allergenicity in food allergy. Gal d 2, 3 and 4 are heat-labile and thus hydrolysed egg is usually well tolerated by this group of egg-allergic patients.[25] Children with IgE-mediated egg allergy can often also tolerate baked egg within a wheat matrix.[26]

COW'S MILK COMPONENTS

The main allergens in cow's milk are α-lactalbumin (Bos d 4), β-lactoglobulin (Bos d 5), Bovine serum albumin (Bos d 6) and casein (Bos d 8). Bos d 4, 5 and 6 are whey proteins and are heat-labile. Casein remains stable when exposed to heat and digestive enzymes. Petersen et al studied whether CRD could predict duration and severity of allergy in cow's milk protein-allergic (CMPA) children. They found a correlation between sIgE level to cow's milk and casein and the severity of the allergic reaction elicited by food challenges.[27] They documented that high levels of sIgE to milk and its components, especially casein , were predictive of persisting CMPA. The levels of casein IgE tend to reflect the severity of the milk allergy, with children with higher levels being at risk of reacting to both fresh and baked milk as well as being less likely to outgrow their allergy. Children with higher levels of IgE to predominantly whey protein, are often able to tolerate baked-milk products.

WHEAT SENSITISATION AND GRASS POLLEN

Wheat sensitisation is common in grass pollen-allergic individuals and Nilsson et al recently found that 60%t of the grass-pollen subjects were sensitised to wheat with a median of 0.5 kUA/L. Wheat-sensitised subjects were more often sensitised to the two allergens – Phl p 12 and CCD – known to be cross-reactive between grass and wheat.[28] It is therefore a risk that grass-pollen allergy is misdiagnosed as wheat allergy. This could be avoided to a degree with the usage of the specific allergen components in order to diagnose a genuine sensitisation to wheat.

Beware of a patient with pollen allergy who tests positive to wheat, peanut and soy. This most likely represents cross-reactivity due to CCD, LTP, PR-10 protein or profilin, rather than true food allergy and may not be clinically significant.

TREE-NUT COMPONENTS

Cashew has been shown to cross-react with pistachio and pecan with walnut.[29] Brandstrom found that cashew-allergic children had IgE against novel citrus-seed allergens of lemon, orange and tangerine.[30] The individuals described in the report were all cashew-allergic and known to tolerate the fruit pulp. When ingesting foods that contained the seeds of these fruits, they developed anaphylaxis.

TABLE II: HEN'S EGG COMPONENTS

COMPONENT			CLINICAL RELEVANCE
Gal d 1	Egg white	Ovomucoid	Heat-stable React to all forms of egg High levels correlate with severe and persistent allergy
Gal d 2		Ovalbumin	Heat-labile Risk of reaction to raw and lightly cooked egg
Gal d 3		Ovotransferrin (Conalbumin)	Heat-labile Risk of reaction to raw and lightly cooked egg
Gal d 4		Lysozyme	Heat-labile Risk of reaction to raw and lightly cooked egg
Gal d 5	Egg yolk	Serum albumin	Found in yolk, chicken meat and feathers Associated with bird-egg syndrome

TABLE III: COW'S MILK COMPONENTS

COMPONENT		CLINICAL RELEVANCE
Bos d 4	α -lactalbumin	Heat-labile React to fresh milk May tolerate baked milk
Bos d 5	β -lactoglobulin	Heat-labile React to fresh milk May tolerate baked milk
Bos d 6	Bovine serum albumin	Heat-labile React to fresh milk May tolerate baked milk The main allergen in beef
Bos d 8	Casein	Heat-stable React to all forms of milk High levels correlate with severe and persistent allergy Cross react with other mammalian milks
Bos d lactoferrin	Lactoferrin	Heat-labile React to fresh milk

Birch tree forest: photo credit : Creative Commons Zero

Peanuts. Photo credit Stephen Firmender

The components Jug r 1 in walnut, Ana o 3 in cashew and Cor a 9 and 14 in hazelnut have been shown to be useful when diagnosing primary tree-nut allergy, rather than sensitisation due to cross-reactive components.

PEANUT COMPONENTS

Three peanut-specific storage proteins (Ara h 1, 2 and 3) and two cross-reactive proteins (Ara h 8 and Ara h 9) are used as diagnostic tools in clinical practice. The storage protein Ara h 2 appears to be the most useful in terms of both sensitisation frequency and in eliciting clinical symptoms in peanut-allergic patients.[31] Ara h 6 is a major peanut allergen and is similar to Ara h 2 in many respects. Both are storage proteins of the 2S albumin type and are heat-stable and resistant to digestion. They are both associated with potentially severe systemic reactions.[32] They are 58% homogenous at the amino-acid level, and are highly immunogenic and potent in functional assays such as histamine-release and basophil-activation tests.[33] Adding Ara h 6 to a diagnostic panel of tests can thus improve the diagnostic accuracy for true peanut allergy.

In a pan-European study of both children and adults, 85% of subjects with early-onset peanut allergy had elevated sIgE to any peanut-storage protein.[34] Of these, 93% and 87% were positive to Ara h 2 and Ara h 6 respectively. In adult-only studies, the frequency of Ara h 6 sensitisation among peanut-allergic subjects is shown to be between 50% and 80%.[35–37]

Although sensitisation to Ara h 2 and 6 usually occurs together, sensitisation to Ara h 6 alone has been detected in up to 4% of study subjects.[38] Ara h 6 sensitisation in the absence of Ara h 2 s-IgE was reported in five Dutch children.[39] Three of these children reacted during a peanut oral-food challenge. Another report from Sweden described a child with no detectable sIgE to Ara h 1–3, who developed anaphylaxis during an oral peanut challenge.[40]

The collective data so far demonstrate that Ara h 6 is an important marker of peanut allergy, with a diagnostic accuracy similar to that of Ara h 2. The sensitivity of Ara h 6 is reported to range from approximately 60 to 90%, while the specificity is reported to be above 95%. Several studies indicate that when used together, Ara h 2 and Ara h 6 can provide the highest diagnostic accuracy.[38,41] Monosensitisation to Ara h 6 is seen in an important minority of patients.

TABLE IV: TREE-NUT COMPONENTS

NUT		COMPONENT	CLINICAL RELEVANCE
Cashew	Storage protein	Ana o 3	Resistant to heat and digestion Associated with systemic reactions
Hazelnut	Storage protein	Cor a 9 Cor a 14	Resistant to heat and digestion Associated with severe systemic reactions
	PR-10 protein	Cor a 1	Heat-labile, may tolerate roasted hazelnut Associated with oral-allergy syndrome, birch and birch-related tree-pollen allergy
	LTP	Cor a 8	Resistant to heat and digestion Associated with severe systemic reactions and oral-allergy syndrome Associated with peach and peach-related fruit allergy
Walnut	Storage protein	Jug r 1	Primary walnut allergy Resistant to heat and digestion Associated with systemic reactions
	LTP	Jug r 3	Resistant to heat and digestion Associated with systemic and local reactions May cross-react with other LTP-containing foods

TABLE V: PEANUT COMPONENTS

COMPONENT		CLINICAL RELEVANCE
Ara h 1	Storage proteins	Stable to heat and digestion Risk of systemic reactions
Ara h 2		
Ara h 3		
Ara h 6		
Ara h 5	Profilin	Associated with oral-allergy syndrome
Ara h 8	PR-10 protein	Heat-stable Associated with oral-allergy syndrome, birch and birch-related tree-pollen allergy
Ara h 9	LTP	Heat-stable Risk of severe systemic and local reactions, associated with peach and peach-related fruit allergy

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Apis mellifera (Honeybee) photo credit Zhiwei Zhou

LIPID-TRANSFER PROTEIN

Non-specific lipid-transfer protein (LTP) is by far the most frequent cause of primary food allergy in the Mediterranean area where it also induces the largest number of food-dependent anaphylactic reactions.[42] LTP allergy is a fascinating food allergy and differs from other IgE-mediated food allergies such as egg, milk and peanut. China, Australia and central/northern Europe are starting to report LTP allergy and more reports are sure to come from other countries. LTP-sensitised patients may experience reactions upon the ingestion of a wide variety of plant foods. Pru p 3 – the peach LTP – is regarded as the 'LTP sensitiser'. This is thought to be because it seems to pass through the gut epithelium via fast transcellular route which is not used by other less allergenic LTPs. There are recent reports that the presence of birch pollen may be protective against LTP-allergic symptoms. In some areas, high levels of birch pollen are correlated with a low prevalence of LTP hypersensitivity.[43]

One of the most intriguing aspects of LTP hypersensitivity is the extreme variability of its clinical expression. LTP is probably the main cause of food-induced contact urticaria, at least in Spain. Peach-induced contact urticaria may be the only sign of LTP hypersensitivity in more than 60% of patients.

Co-factors are important in LTP hypersensitivity. In some cases, the co-factor is represented simply by the isolated ingestion of the offending food after fasting. It is possible that an empty gastrointestinal tract absorbs the allergen more rapidly. Other co-factors include exercise, alcohol and non-steroidal anti-inflammatory drugs (NSAIDs). It was recently shown that NSAIDs can enhance the IgE-mediated activation of basophils. In patients with otherwise mild or non-clinically significant allergy, taking an NSAID with that particular food may lead to a severe reaction, even anaphylaxis.[44]

TRYPTASE

Preventive measures to decrease the frequency and intensity of anaphylatic events are essential to provide optimal care for the allergic patient. Worm et al have examined factors that increase the risk for a severe reaction in anaphylaxis using the European Anaphylaxis Registry.[45] The most important risk factors for severe anaphylaxis were mastocytosis and older age. This is in line with the known association of mastocytosis with fatal Hymenoptera sting reactions. Gulen et al studied 122 consecutive patients with systemic mastocytosis in order to identify risk factors of anaphylaxis in these patients.[46] Factors that were predictive of anaphylaxis included male sex, absence of skin mastocytosis lesions, the presence of atopy, IgE >15 kU/L and baseline tryptase <40 ng/mL. It is therefore important to measure tryptase and in some cases perform mutation analysis of peripheral blood when evaluating patients with anaphylaxis.

Dua et al showed that serum tryptase rises in food-allergic reactions and is correlated with symptom severity. They also determined optimal sampling time points and a diagnostic cutoff for confirming reactions.[47] They performed a prospective study of 160 adult peanut-allergic patients who underwent oral-food challenges. They compared baseline tryptase levels with peak levels at two hours and showed that a rise in tryptase of 30% is associated with a clinical reaction. The rise in tryptase compared with the baseline occurred in 62% of all reactions and in 100% of anaphylatic reactions. The group is the first to demonstrate that moderate-to-severe respiratory symptoms during a challenge are associated with a higher tryptase. They failed to prove any correlation between baseline tryptase levels and reaction severity. The optimal time for sampling appears to be between 30 minutes and two hours after the onset of a reaction as tryptase may not be detectable during the first 15–30 minutes.

Thus tryptase is of value in food-allergic reactions and especially in severe anaphylaxis. It is important to measure tryptase between 30–120 minutes after the reaction and compare it with the baseline level. Mastocytosis patients are at higher risk of severe anaphylaxis. Tryptase measurement should be performed in all cases of anaphylaxis if possible.

CONCLUSION

CRD helps clinicians to discriminate between true IgE-mediated allergy and sensitisation without clinical relevance.

DECLARATION OF CONFLICT OF INTEREST

Magnus Borres is medical director at Thermo Fisher Scientific. Sarah Karabus declares no conflict of interest.

This article has been peer reviewed.

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Review Article

RECENT TRENDS IN THE MANAGEMENT OF FOOD ALLERGY

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ABSTRACT

This article focuses on recent studies in the management of food allergy. The specific Immunoglobulin E (sIgE) to allergen extract or component can predict reactivity to food. The predictive decision point of sIgE was estimated for hen's egg, cow's milk, wheat, peanut and cashew-nut allergy. Component-resolved diagnostics have been used for food-allergy diagnosis. Many studies focusing on peanut and tree nuts have been investigated. The storage proteins, such as Ara h 2 for peanut, Cor a 14 for hazelnut, Jug r 1 for walnut and Ana o 3 for cashew, showed better diagnostic markers than the extract for each allergy. These can be considered when deciding whether an oral food challenge is required to diagnose food allergy. Severe food-allergic patients are always at risk of anaphylaxis. More recent studies have indicated that even in patients with high levels of sIgE, oral food challenges at lower target doses were conducted relatively safely, and improved the patients' quality of life. In addition, oral immunotherapy at a lower target dose may lead to fewer adverse reactions. More approaches that are both safe and effective are needed to manage food allergy, especially in severe cases.

Keywords: antigen-specific IgE; component-resolved diagnostics; food allergy; oral food challenge; oral immunotherapy

INTRODUCTION

The prevalence of food allergies has increased in the past decade, and their standard management involves strict avoidance of these foods in the diet.[1,2] Molecular allergy diagnostics or component-resolved diagnosis (CRD) can be used to manage such allergies. This improves the accuracy of diagnosing food allergy,[3–19] while the number of oral food challenges (OFCs) used to diagnose food allergy can be reduced. In cases of severe food-allergic patients who are at risk of anaphylaxis, some studies have indicated that an OFC at a lower target dose may be administered safely and still improve the patients' quality of life (QoL).[20,21] In addition, oral immunotherapy (OIT) targeting a lower dose has led to a reduced adverse reaction compared with conventional OIT.[20] This article covers the management of food allergy with a focus on recent advances.

ANTIGEN-SPECIFIC IGE TO DIAGNOSE FOOD ALLERGY

Food allergy is diagnosed by considering both the patient's clinical history and their sensitisation to food allergens (see Figure 1).[22] OFCs are the most reliable test for investigating whether or not the existence of food allergies can be proved. However, they require resources and trained medical staff and introduce the risk of adverse reactions such as anaphylaxis.

Elevated specific IgE levels are risk factors for positive OFCs.[23] Antigen-sIgE values with more than 95% or 90% predictive decision points have been reported for egg, milk, wheat (omega-5 gliadine), peanuts (Ara h 2) and cashew nut (Ana o 3).[7,19,24–26] Although these decision points can help with investigating the reactivity to food, these were influenced by several factors, such as the severity of the patient's allergy, the challenge food, target doses and total IgE levels.[3,27–29]

MOLECULAR ALLERGY DIAGNOSTICS

Previous studies regarding allergen components are summarised in Tables I and II. The CRD for diagnosing food allergy was best demonstrated in studies of peanut allergy (see Table I), and 17 peanut allergens have been demonstrated. Ara h 2 is one of the storage proteins and immunodominant peanut allergens.[30] Sensitisation to non-specific lipid-transfer proteins (nsLTP; Ara h 9, 16 and 17), Bet v 1 homologs (Ara h 8) and profilins (Ara h 5) is caused by cross-reactions.[22] A 95% probability of a positive peanut OFC was estimated for an Ara h 2-sIgE.[8] Co-sensitisation to Ara h 2 and Ara h 6 was the best marker of severe reactions at low dose during a peanut OFC.[10] On the other hand, Ara h 8 was associated with either no symptoms or mild local symptoms.[31]

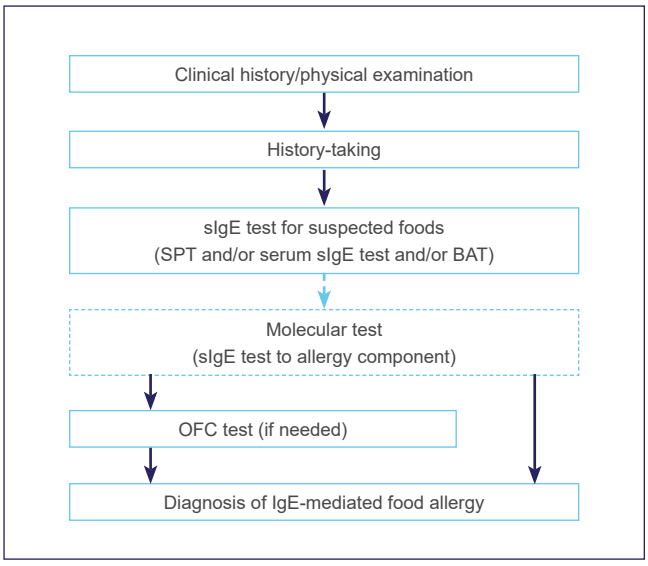

Figure 1: Flow chart showing the approach to diagnosing food allergy. The diagnostic approach for IgE-mediated food allergy is based on the combination of clinical history and/or physical examination, and the presence of sIgE antibodies. If food allergy is not diagnosed using this information, an OFC is required (Sato, et al Curr Opin Allergy Clin Immunol 2018)

BAT = basophil activation test, SPT = skin-prick test

The most important allergen to predict reactivity to tree nut and peanut is 2S albumin. For hazelnut, Cor a 9 and 14 can be useful for predicting reactivity to hazelnut compared to hazelnut extract (see Table II).[15,16] A recent systematic review showed that Cor a 14 indicated the best diagnostic accuracy for hazelnut allergy.[32] For cashew nut, Ana o 3-sIgE was highly predictive of cashew and pistachio allergy.[12] A 95% positive predictive value (PPV) for a positive cashew challenge was estimated for Ana o 3.[7] For walnut, Jug r 1-sIgE can improve the diagnostic accuracy of walnut allergy compared to walnut extract-sIgE in children,[13] whereas there was no improvement in adults.[14] Tree-nuts allergy is included as heterogeneous patients such as mild pollen-induced cross-allergy, as well as primary allergy with severe reactions. The diagnosis of tree-nut allergy using CRD can be applied to investigating those patients with or without cross-reactivity.

ORAL FOOD CHALLENGES

OFCs are accurate tests that are used to diagnose food allergies and examine the tolerance levels of causative foods. The methodology of OFC differs between countries: for example, target doses and time between doses.[21] Severe symptoms can be induced accidentally during OFC. Risk factors for severe reactions are associated with a past history of anaphylaxis, a lower threshold of induced symptoms, complications with bronchial asthma and high levels of sIgE.[33]

TABLE I: SUMMARY OF COMPONENT-RESOLVED DIAGNOSIS – PEANUT AND TREE NUTS

ANTIGEN	COMPONENT TO FOOD ALLERGENS	AUTHOR	PUBLISHED YEAR	RESULTS
	Ara h 2 (2S albumin)	Ebisawa et al	2012	Ara h 2 was predicting clinical reactivity to peanut allergy
		Klemans et al	2015	
		Beyer et al	2015	A 95% probability for a positive peanut challenge was estimated for an Ara h 2 sIgE
	Ara h 2 (2S albumin) Ara h 6 (2S albumin)	Kukkonen et al	2015	Co-sensitisation to Ara h 2 and Ara h 6 was associated with severe reactions to peanut
	Ara h 8 (Bet v 1 homolog)	Sicherer et al	2013	Ara h 8 was associated with either no or very mild local symptoms
Hazelnut	Cor a 9 (11S globulin) Cor a 14 (2S albumin)	Beyer et al	2015	Cor a 9 and Cor a 14 were associated with hazelnut allergy
	Cor a 14 (2S albumin)	Carraro et al	2016	Cor a 14 was a good predicting marker for hazelnut allergy
		Eller et al	2016	
Cashew nut	Ana o 3 (2S albumin)	Savvatianos et al	2015	Ana o 3 was highly predictive of cashew and pistachio allergy
		Lange et al	2017	A 95% probability for cashew-nut allergy was estimated for an Ana o 3 sIgE
Walnut	Jug r 1 (2S albumin)	Blankestijn et al	2017	Jug r 1 sIgE did not improve the diagnostic accuracy in adults
		Sato et al	2017	Jug r 1 sIgE has additional value to crude extract testing in children

TABLE II: EXAMPLES OF TOTAL CHALLENGE DOSES IN AN OFC TEST (OPEN METHOD)

DOSES	HEN'S EGG	COW'S MILK	WHEAT
Low dose	One cooked egg yolk, approximately 1/32 cooked whole egg	Approximately 3 mL	Udon noodle (50–75 mg wheat protein)
Medium dose	About 1/8–1/2 cooked whole egg	15–50 mL	Udon noodle (375–1 250 mg wheat protein)
Full dose	One cooked whole egg 50 g (corresponding to one egg)	200 mL	Udon noodle 200 g, one of six slices of 5 000 mg wheat protein

The full-challenge dose is assumed to be a dose to confirm the tolerance acquisition, assuming a single meal amount in elementary-school pupils. In infants, it is taken into consideration to reduce the total challenge dose when necessary. The low-challenge dose is set as a dose that may be mixed in case of accidental ingestion and in high-risk cases an initial challenge test is assumed. As for the administration interval, 20 minutes or longer is desirable (Allergol Int. 2017;66:248–264)

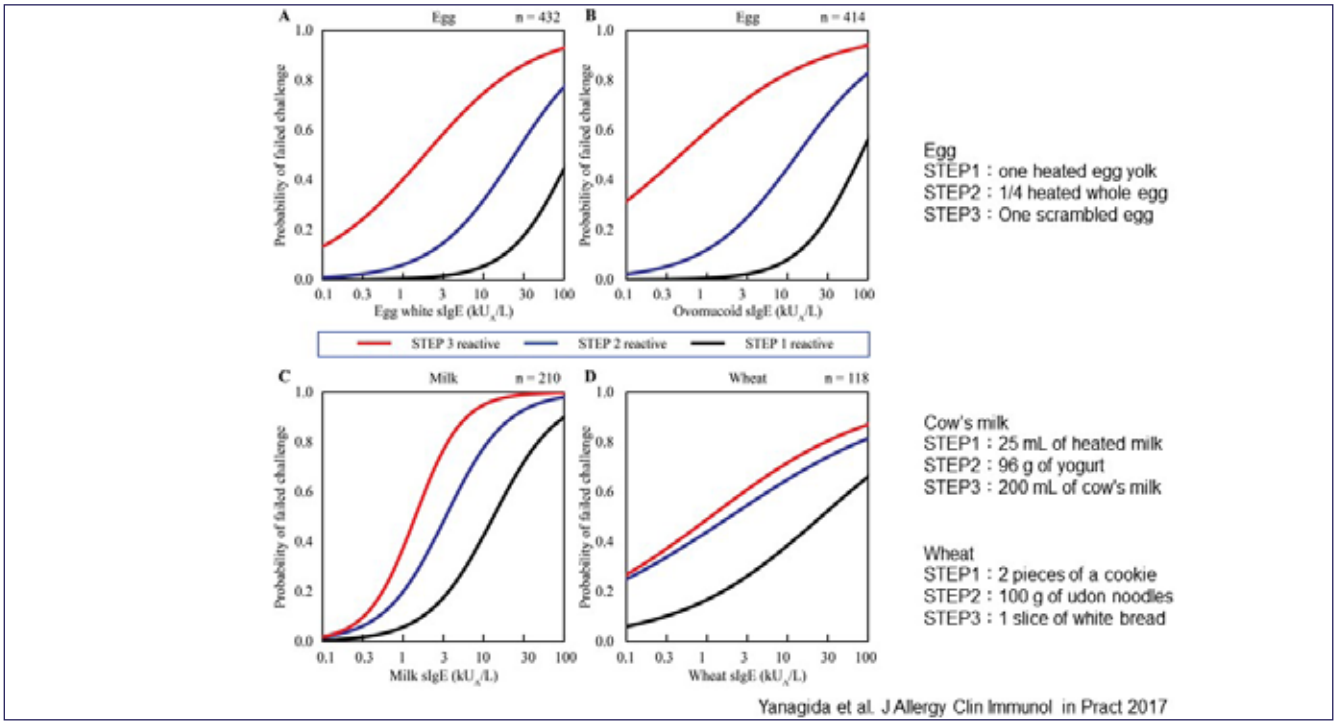

Figure 2. Probability of the 3-level stepwise OFC failing: A comparison of the probability curves. (A) egg white (n = 432), (B) ovomucoid (n = 414), (C) milk (n = 210) and (D) wheat-specific IgE (n = 118) are shown. A logistic regression model was used to evaluate the sIgE values necessary to predicting the probability of inducing symptoms. The black line indicates STEP 1, the blue line STEP 2, and the red line STEP 3 (Yanagida et al J Allergy Clin Immunol in Pract 2017)

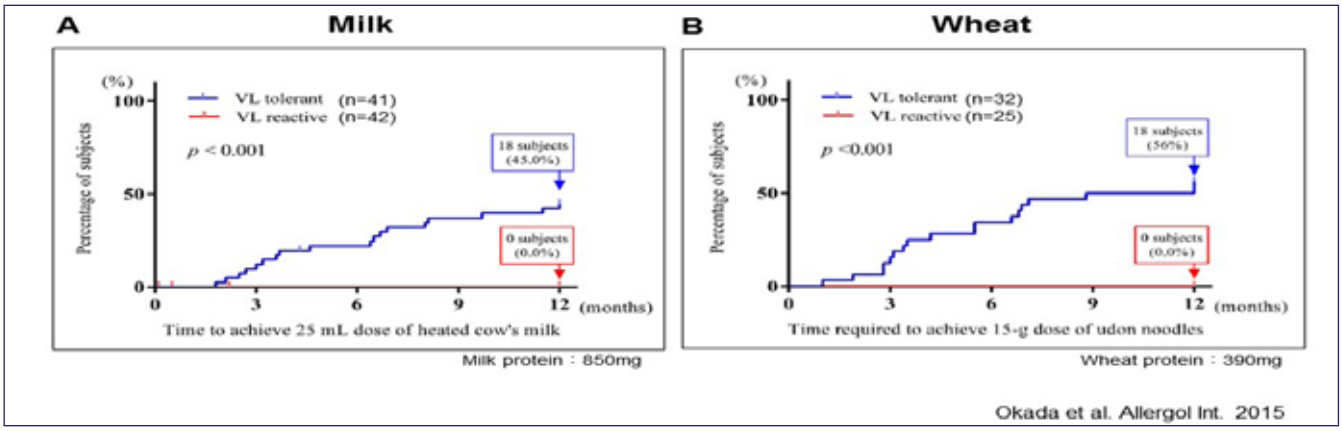

Figure 3. Better management of milk and wheat allergy using a very low-dose food challenge: A retrospective study. (A) milk (n = 83) and (B) wheat (n = 57) are shown. The blue line indicates VL-tolerant patients, the red line VL-reactive patients.
VL = very low dose

More recently, a study based on the results of OFCs showed that even if the sIgE value exceeds the 95% positive predictive decision point, the positive OFC rate decreased with a lower target dose (see Figure 2). We also found that approximately half of the children with milk or wheat allergy who were able to consume low doses of these foods have been able to consume moderate amounts after daily consumption of low doses for one year (see Figure 3).[3,4] Therefore, in the case of high-risk patients, it is better to consider performing OFC at a lower target dose.

ORAL IMMUNOTHERAPY

OIT appears to be effective in inducing desensitisation. However, adverse reactions, mostly mild to moderate, induced by OIT have occurred frequently, with some patients experiencing anaphylaxis. Some studies focused on reducing the severity of adverse reactions from OIT. Low-dose OIT, which is a lower target dose, is one of them; it may be a relatively safe and effective approach for persistent, severe food-allergic patients, as an immunological change may have been induced despite the small intake. Further studies on the use of OIT are needed.

CONCLUSION

The positive predictive decision points of sIgE and CRD improve diagnostic accuracy and the prediction of the severity of symptoms. They can help to reduce the OFC required to diagnose food allergy. Even in the case of severe food allergy, some studies have indicated that OFCs and OIT at lower target dose could be conducted relatively safely and effectively. More effective, safe approaches for severe food-allergic patients are needed.

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DECLARATION OF CONFLICT OF INTERESTS

M Ebisawa is on the DBV technologies Scientific Advisory Board. The two remaining authors declare that they have no relevant conflicts.

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Review Article

THE VALUE OF SINGLE AND COMBINATION SEROLOGICAL TESTS IN DIAGNOSING PAEDIATRIC COELIAC DISEASE IN THE ERA OF THE LATEST ESPGHAN GUIDELINES

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ABSTRACT

The incidence of coeliac disease is about 1% in most populations and the diagnosis is based on a combination of clinical, genetic, serological and histological features. Previously, endoscopy with duodenal biopsy was mandatory in establishing a conclusive diagnosis of coeliac disease, but the 2012 ESPGHAN guidelines confirmed that in the right clinical setting a diagnosis could be made without endoscopy or biopsy. This emphasised the crucial role of serological tests in the diagnostic process.

The serological diagnosis is based on the detection of class IgA anti-tissue transglutaminase (anti-tTG) and antiendomysial (anti-EMA) antibodies. In patients with IgA deficiency, IgG anti-tTG or IgG anti-deamidated gliadin peptide antibody (anti-DGP) is used with an excellent sensitivity and specificity. However, the cost of anti-EMA antibodies is high and their use is qualitative rather than quantitative. Various serology tests, either alone – such as anti-tTG IgA in two separate blood samples if levels are at least ten times the upper limit of normal (ULN) – or as combination tests – such as anti-DGP IgG and anti-tTG antibodies – have been reported. There is evidence that combination serology testing is more effective than single-screening and single-diagnosing paediatric coeliac disease. Although with the new guidelines the endoscopy can be avoided, it is still indicated when the criteria are not fulfilled.

INTRODUCTION

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten contained in wheat and similar prolamines present in barley and rye in certain genetically susceptible individuals. It is characterised by a combination of gluten-dependent clinical manifestations, a positive serology (e.g. anti-tissue transglutaminase TG2 (anti-tTG) antibodies) and enteropathy.[1,2] The prevalence in Caucasian populations is approximately 1%, with studies showing an increased incidence in the past few years, not only due to increased detection, but also as a result of a genuine rise in the incidence of CD.[3,4]

The clinical presentation in CD is variable, ranging from typical malabsorption signs to more atypical extraintestinal manifestations. Previously, the endoscopy with histological demonstration of villous atrophy (Marsh classification) was mandatory to establishing a diagnosis of CD in both adults and children;[5] however, in 2012 the European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) published new guidelines, stating that in children with typical symptoms the combination of high anti-transglutaminase type-2 (anti-tTG) antibody levels (at least ten times the upper limit of normal) with positive antiendomysial IgA antibodies (anti-EMA) and positive human leukocyte antigen (HLA) type DQ2 or DQ8 was sufficient to confirm the diagnosis of CD, making the endoscopy unnecessary in these cases.[1] The sensitivity and specificity for the anti-tTG test are 96% and 99% respectively, and for the IgA anti-EMA test between 95% and 100%.[6–8] These levels confirm the efficacy of serology as a diagnostic tool without the need to perform duodenal biopsies.

PATHOGENESIS

Three main factors are involved in the pathogenesis of CD: genetic predisposition, the ingestion of gluten, and other environmental influences. From the genetic point of view, 2 HLA class II phenotypes have been described: HLA-DQ2 (DQA1*05-DQB1*02) and HLA-DQ8 (DQA1*03-DQB1*0302).[9] Although around 30% of the general population are carriers for at least one of these alleles, the absence of either HLA-DQ2 or HLA-DQ8 has a negative predictive value of nearly 100%. New research is investigating whether other genes could be involved

in the development of CD.[10] There is a possibility that these may be located outside the coding part of the genome.[11,12]

CD is initiated by the ingestion of gluten (proteins contain both gliadins [monomeric proteins] and glutenins [polymeric aggregated proteins]).[13] These large fragments are incompletely digested by the gastric, pancreatic and brush border peptidases. Gluten is an excellent substrate for transglutaminase 2 (TG2, also known as tissue transglutaminase). This enzyme converts glutamine residues into negatively charged glutamate residues in a process termed 'deamidation'. This process facilitates the binding of gliadin peptides to HLA antigens of class II DQ2 or DQ8, which are expressed on antigen-presenting cells (APCs), including macrophages, dendritic cells and B cells. These APCs interact with gliadin-specific CD4+ T cells,[14] which produce inflammatory cytokines such as IFN-γ and represent the adaptive immune response.[15]

The innate immune response to gliadin occurs in the epithelial component of the intestinal mucosa; it involves an increased production of cytokines such as IL-15,[16] which are produced by monocytes/macrophages, and of dendritic and epithelial cells.[17] This results in the differentiation of intraepithelial lymphocytes into cytotoxic CD8+ TH cells that express the natural killer cell marker NK-G2D. The damage to the intestinal mucosa from this cascade of inflammatory mediators manifests as an enteropathy, with inflammation resulting in villous atrophy and crypt hyperplasia.

In addition to autoantibodies against TG2, CD patients also develop antibodies to gluten (antibodies against deamidated gliadin peptides[DGP]).[18]

Despite more than 30% of the population being HLA-DQ2/DQ8 carriers, gluten exposure alone is not sufficient to develop CD. This could also indicate the importance of other environmental factors. Another good example to support the importance of the environment is the high prevalence of CD in the Finnish Karelia population (> 2%) in contrast to its low prevalence in the Russian Republic of Karelia (0.2%), two neighbouring regions with genetically similar populations.[19]

While breastfeeding and delaying the age of gluten introduction (between 4 and 6 months) showed no protective effect for developing CD,[20] other environmental factors, such as gut microbiota,[21] infections of the gastrointestinal tract in infancy[22,23] and the amount of gluten consumed in early infancy,[24] have attracted increasing interest in the past few years.

CLINICAL MANIFESTATIONS AND WHEN TO TEST FOR COELIAC DISEASE

The symptoms in CD are categorised as typical (chronic diarrhoea, failure to thrive, distended abdomen, irritability, anorexia) and atypical (recurrent abdominal pain, constipation, short stature, vomiting, iron-deficiency anaemia, arthritis, aphthous stomatitis, dermatitis herpetiformis-like rash, pubertal delay, elevated liver enzymes, dental enamel defects and fatigue).

Many studies report a decrease in the 'typical' presentation, with higher rates of atypical manifestations, which suggests a shift in the clinical spectrum of the disease.[25,26]

In the 2012 ESPGHAN guidelines, children were classified into two different groups – those with symptoms suggestive of CD and those who are asymptomatic but who had an increased risk for CD. The new criteria were remarkable in three main respects: the non-biopsy approach, the major role of serological tests in the diagnostic process of symptomatic subjects and the introduction of genetic testing for HLA DQ2/DQ8.[1]

More recently, studies are adding even more value to the serological aspect, leading to the conclusion that HLA analysis is not really required for an accurate diagnosis of CD.[6]

SEROLOGICAL ASPECTS

The anti-tTG and anti-EMA antibodies of the IgA isotype have the highest diagnostic accuracy for CD; however, the IgG isotypes are also available and may play an important role in patients with IgA deficiency.

The ESPGHAN guidelines for symptomatic children recommend testing for anti-tTG IgA antibodies (high sensitivity and specificity, availability and low cost compared with EMA IgA) and total serum IgA to exclude IgA deficiency. They recommend that testing for IgG anti-DGP antibodies can be used as an alternative to total serum IgA or in patients with IgA deficiency. A second step in the diagnosis is a positive IgA-EMA test in a different blood sample if the test for anti-tTG IgA antibody is positive.[1]

Anti-tTG antibodies (IgA and IgG class) can be detected in blood samples of patients by various immunometric methods (enzyme-linked immunosorbent assay, radioimmunoassay, fluorimetric assays or others) using purified or recombinant TG2 antigens or tissue sections/fluids containing TG2. Anti-tTG tests offer quantitative results (expressed in arbitrary units and calculated on the basis of test-specific reference standards).[27]

The test for detecting anti-EMAs requires microscopic evaluation and may be subject to interobserver variability. Despite these limitations, the specificity of EMA test results is excellent in expert laboratories, and this test is considered the reference standard for CD-specific antibody detection.[28] Low-level positive anti-tTG may occur in some other conditions (autoimmune conditions, cancer, liver and infectious diseases), whereas this is not the case with EMA, which has a higher specificity and false positives are very unlikely.

Although anti-EMA IgA is considered the 'gold standard' serology test for CD, the majority of guidelines identify anti-tTG IgA as the test of choice for diagnostic evaluation. There is evidence, however, that the sensitivity and specificity of anti-tTG IgA is not significantly different from that of anti-EMA IgA.[29]

A meta-analysis published in 2010, in which eight studies were reviewed, demonstrated that the sensitivity and specificity of

anti-EMA IgA were 90% and 99% respectively and for anti-tTG IgA the pooled sensitivity was 89%, with a specificity of 98%, so the two are very similar.[30]

Tests for the detection of anti-DGP IgA and IgG, performed by semi-automated immunoasssays, are much more specific than native gliadin-based tests (anti-gliadin antibodies [AGA]). In a published investigation by Tonutti et al, it was suggested that the anti-DGP antibodies of the IgG class could identify CD in patients aged less than two or three years old who may test negative to IgA anti-tTG, because very young children can have a delayed anti-tTG response.[31] Wolf et al, in a study of 376 children, did not demonstrate the same age-related test variance, but noted a stronger anti-DGP IgG response in very young CD patients.[2]

In the 2017 paper by Wolf et al, it was concluded that a positive value of anti-tTG IgA antibodies more than ten times the normal (ULN) value (as per ESPGHAN guidelines) has such a high positive predictive value (PPV) that the second sample that looks for anti-EMA IgA positivity would be necessary only to exclude a sampling error (e.g. incorrect patient). Both studies concluded that confirmation through anti-EMA IgA or HLA-typing adds only negligible information to confirm the diagnosis.[2,32] Only if the patient does not have the confirmatory antibody levels could the genetic testing through HLA be helpful by ruling out false-positive results.[32]

Werkstetter et al drew similar conclusions regarding the optional need for genetic testing and suggested that if anti-tTG IgA is higher than ten times the ULN and anti-EMA IgA are positive in a second blood sample, the non-biopsy approach is reliable with a PPV > 99% without the need for HLA testing.[6]

Published investigations assessing the value of the anti-DGP demonstrated that anti-DGP IgA has 94.6% sensitivity and 99.1% specificity for CD, and the anti-DGP IgG antibody has been reported to have 92.4% sensitivity and 100% specificity for CD;[33] however, other investigations found lower sensitivity and specificity.[34,35]

In a meta-analysis, Lewis et al found that the pooled specificities of the anti-tTG IgA and anti-DGP IgA were not significantly different, at 96.5% and 94.1% respectively. However, at 93.0%, the sensitivity of anti-tTG IgA was much better than the anti-DGP IgA sensitivity, which was only 87.8%.[36]

It has been suggested that combining the anti-tTG and anti-DGP tests could improve their characteristics compared to either test in isolation.[37,38] Wolf et al[32] compared the use of total-IgA and anti-tTG IgA (group 1) with anti-DGP IgG and anti-tTG IgA (group 2) and concluded that CD was confirmed if anti-tTG was more than ten times the ULN or anti-DGP IgG was more than ten times the ULN; CD not confirmed if anti-tTG IgA and anti-DGP IgG were negative; otherwise, a biopsy is necessary. When compared, both procedures had comparable PPV, but regarding the NPV, combination testing both anti-DGP IgG and anti-tTG IgA (group 2) was found to be superior. In addition, this method could detect the anti-tTG IgA-negative patients (seronegative coeliac disease patients),[39,40] as described in other studies, although the PPV was found to be low.[40] The same conclusions were drawn in adults – a combination of anti-tTG IgA and anti-DGP IgG show the best performance from a clinical diagnostic point of view.[41]

Despite the fact that the combined detection of anti-DGP and anti-TTG had very good PPV when compared with anti-tTG alone, the PPV when compared with the classical anti-TTG and anti-EMA was lower (41.7% versus 100%). Strangely, even the combined detection of the three markers (anti-tTG + anti-EMA + anti-DGP) had a lower PPV than the classic combined detection of anti-tTG and anti-EMA antibodies (41.7% versus 100%) in large cohorts.[42]

A Swedish study assessing the screening of schoolchildren showed that if the anti-tTG IgA levels exceed ten times the upper limit of normal, it is safe to diagnose CD without the need for a biopsy,[43] even in asymptomatic children. Trovato et al drew the same conclusions in an Italian group.[44] Of course, more studies are needed to validate this.

CONLUSION

The ESPGHAN 2012 guidelines have been validated in many prospective and retrospective studies since their publication,[45,46] making the duodenal biopsy unnecessary if the criteria are fulfilled with subsequent reduction in risks, costs, endoscopy waiting times and delay of treatment. In addition, the guidelines have confirmed the value and reliability of serological tests for the diagnosis of CD.

But new studies are arising which indicate that the genetic typing of HLA-DQ2/DQ8 may be needed only in very specific cases – for example, to rule out false-positive results and the screening of asymptomatic high-risk children.

The excellent sensitivity and specificity of the serological assays, as discussed in the guidelines, lead to the use of anti-tTG and anti-EMA IgA antibodies being recommended; however, recent publications discuss omitting the more expensive EMA antibody if anti-tTG IgA is ten times the ULN in two separate blood samples (ruling out mixed samples).

Regarding the anti-DGP IgG antibodies, their use in combination with the anti-tTG is controversial – it has a good PPV but one that is lower when compared with the classical anti-TTG plus anti-EMA.

Endoscopy with biopsies is still indicated when the anti-tTG IgA levels do not exceed ten times the ULN. Importantly, meeting the ESPGHAN 2012 criteria does not contraindicate endoscopy but merely offers the option to avoid it.

Each paediatric gastroenterology centre will need to assess the cost-effectiveness of their choice of serological testing and the use of combination testing, which seems to be favourable as opposed to single antibody testing.

DECLARATION OF CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Review Article

ECZEMA: THE SPECTRUM OF CLINICAL PRESENTATIONS

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INTRODUCTION

In 1982, A Bernard Ackerman, considered by many to be the founding father of dermatopathology, published a plea to expunge the word 'eczema' from the lexicon of dermatology and dermatopathology. His reasoning was that the term has never been defined in a repeatable way and despite innumerable attempts at a definition, the word 'eczema' still lacks specific meaning.[1–3] The definition of eczema that is most acceptable to dermatologists is that it is a non-infectious papular or papulo-vesicular eruption characterised histologically by spongiosis (intercellular oedema within the epidermis). However, there are other dermatoses such as pityriasis rosea that fit this definition but are not considered eczematous. In the same breath, the prototype eczematous condition, atopic eczema, often does not exhibit spongiosis.[1] Despite Ackerman's attempts as well as those of many others, the terms 'eczema' and 'dermatitis' are used interchangeably both in clinical medicine and dermatopathology.[4] In this review, we use the term 'eczema' as a synonym for both terms.

The challenge in case definition, suggests that what is commonly referred to as eczema is a group of heterogeneous conditions that have a spectrum of overlapping clinical and histological features that do not always all manifest in one phenotype. This is possibly the reason why many non-dermatologists sometimes find it difficult to diagnose eczema. Even the most experienced dermatologists frequently rely on a correlation of clinical features and histology to make the diagnosis of eczema. Until we can determine the pathogenesis, develop diagnostic tests and protocols to differentiate the individual conditions broadly referred to as eczema, clinicians will have continue managing this constellation of disorders under this broad term.

Pattern recognition is an integral part of diagnosing any skin disease. To recognise these patterns, primary, secondary and tertiary features of the eruption need to be identified and described. Primary morphology refers to the single unit or lesion of the eruption, for example a patch, a plaque, a nodule, a blister, etc. Secondary morphology refers to how these single units relate to each other, for example: Are they grouped, like vesicles in herpes simplex, are they annular papules as seen in granuloma annulare or are they linear blisters as in herpes zoster? Tertiary morphology refers to the distribution of the lesions in the context of the whole body, for example grouped vesicles on the lower lip or dermatomal in herpes simplex and herpes zoster respectively, erythematous patches and plaques that are accentuated in the cubital and popliteal fossae in childhood atopic eczema. These, together with the history and the context help reach the diagnosis. It is also important to remember that a clinician usually encounters the eruption at one point in time and needs to make an inference on how the lesions appeared previously and how they will evolve. Using this approach, this article will describe briefly and pictorially the spectrum and evolution of conditions that are referred to as eczema. We will also briefly highlight important management considerations for each phenotype of eczema.

ATOPIC ECZEMA

Atopic eczema is a clinically defined chronic, relapsing, pruritic, inflammatory skin disease that clusters in families with atopic diseases (atopic eczema, bronchial asthma and/or allergic rhinoconjunctivitis). Prevalence of atopic eczema ranges from 2% in young adults and up to 20% in children. Atopic eczema is a result of a complex interaction of genetic and environmental factors. Barrier defect of the skin, characterised by increased transdermal water loss, is central to the pathogenesis. The skin in atopic eczema is more susceptible to irritation by soaps and other contact irritants, coarse fibres in clothes, high ambient temperature and weather changes among others. The morphology and distribution pattern of atopic eczema lesions varies, not only between individuals, but also with age and anatomical location. In infancy, the cheeks are usually the first to manifest a dry, scaly, erythematous rash that over time becomes generalised. In severe cases, the rash is wet and weepy, often sparing the central face (see Figure 1). Excoriations are a common feature at this age. As the children start to become mobile and up to preschool years, the lesions tend to become localised to extensor surfaces of joints and genitalia, lichenified and the excoriations deeper (see Figure 2). In later preschool years and school-going age, the lesions tend to be more accentuated in the flexor surfaces, particularly elbows and knees. Other body folds, for example eyelids, earlobes and the neck can also be affected (see Figure 3). In adulthood, the clinical presentation is more variable. The childhood pattern may be maintained or the lesions may localise to flexures, eyelids, nipples, lips and hands (see Figure 4). The adulthood lesions tend to be dry and lichenified.[5] People with atopic eczema are more susceptible to bacterial, viral and fungal infections, some of which play a role in triggering and maintaining the chronic inflammation (see Figure 5).[6,7]

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The natural history of atopic eczema in an individual cannot be predicted. Atopic eczema persists in less than 5% of 20 years olds who had the disease as children. However, predisposition to contact eczema and skin sensitivity persists lifelong.[5] Management of atopic eczema requires a multi-pronged approach to control the triggers and drivers of the disease as well as targeted treatment. The management strategies include avoidance of allergens, irritants, long hot baths, dry environmental conditions and coarse fibres in clothes. Emollients, wet wraps, topical steroids, topical calcineurin inhibitors, sedating antihistamines, antibiotics, phototherapy and systemic immunomodulators are used, depending on the severity of the disease.[4]

Figure 1: Weepy eczema on the cheeks of atopic children. (Note the sparing of the central face.)

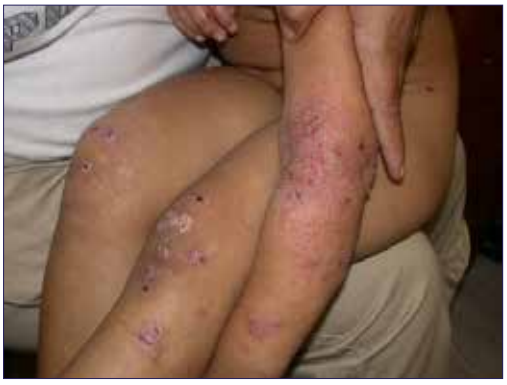


Figure 2: Eczema in an older child showing flexural accentuation as well as deep excoriations.

PITYRIASIS ALBA

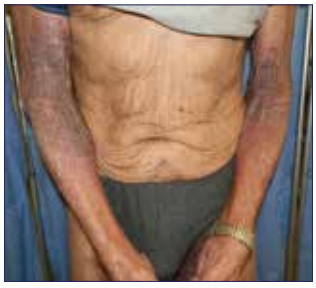
This is considered to be a low-grade form of eczema that predominantly affects children and adolescents. Pityriasis alba is characterised by round, scaly, hypopigmented patches that are usually found on the face. The lesions go through stages of being palpably scaly and erythematous; hypopigmented plaques with fine scale; hypopigmented macules; and then resolution. Itch is not a major feature of the condition. The pigmentary changes are more prominent in dark skin (see Figure 6). Emollients, mild topical corticosteroids and calcineurin inhibitors are effective treatments.[8]

SEBORRHEIC ECZEMA

Seborrheic eczema is a common, chronic and relapsing dermatosis that affects areas of the body that have large sebaceous glands with or without associated terminal hairs. These include the scalp, nasolabial folds, ears, eyebrows, the beard area, presternum, axillae and groin. Pseudonyms

Figure 3: Involvement of flexures in atopic eczema A) ear lobes, B) eyes, C) angle of the mouth, D) popliteal fossa, E) gluteal fold.

Figure 4 (right): Eczema in the cubital fossae of an adult.



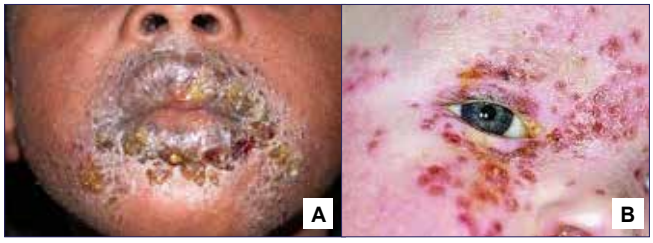


Figure 5: Secondarily infected atopic eczema, A) impetigo B) eczema herpeticum due to herpes simplex.

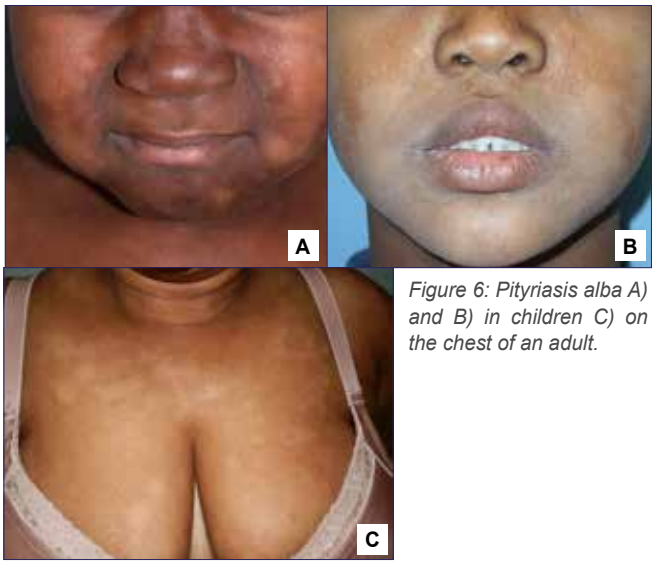


Figure 6: Pityriasis alba A) and B) in children C) on the chest of an adult.

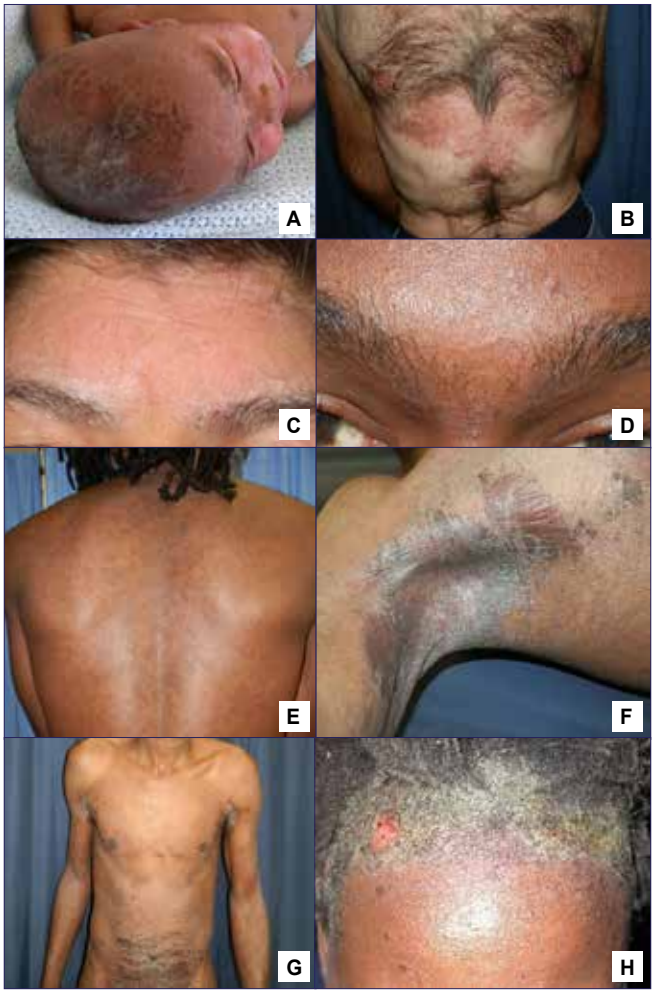


Figure 7: Seborrheic eczema; A) cradle cap in a baby, B) on the chest of an adult, d) on the scalp and eyebrows of two adults, D) & E) petaloid variant on the eyebrows and the back of an adult, F) & G) severe weepy variant in an HIV-infected adult, H) scalp involvement in HIV

of seborrheic eczema include sebopsoriasis, seborrheic dermatitis, dandruff and pityriasis capitis again reflecting a wide clinical spectrum and uncertain pathogenesis. The incidence of seborrheic eczema peaks in the first 3–6 months of life, puberty and after the age of 50. Androgens, *Malassezia furfur* (a fungus) infection, neurologic and psychiatric disease, and altered immunity (as seen in HIV and organ transplant) are thought to play a pathogenic role.[9] An autosomal recessive inherited seborrheic eczema has been identified in mice, although a similar pattern is yet to be identified in humans.[10] The morphology of seborrheic eczema ranges from that of acute eczema, psoriasis-like, petaloid (petal-like), greasy or dry flaky scale. In moist areas the lesions becomes wet and weepy. Erythema in pigmented skin is less visible and post-inflammatory hyperpigmentation is common (see Figure 7).[11] In HIV-infected patients the incidence can be as high as 80%, being reported as the second most frequent cutaneous finding is some series. In this setting, it is more severe, usually starting in the typical areas then becoming more widespread.[11] Treatment is directed at treating known causes and triggers, clearing signs and symptoms of the disease and maintaining remission with long-term therapy. Based on this, the most common treatments are topical antifungal and anti-inflammatory agents. Other therapeutic modalities used include coal tar, keratolytics such as salicylic acid and lithium gluconate/succinate.[12]

NUMMULAR ECZEMA

Also referred to discoid eczema, nummular eczema is a relapsing, itchy condition characterised by round, well demarcated 'coin-like' lesions. Two forms of the condition are recognised: exudative acute nummular eczema which presents with weepy blisters and plaques; whereas dry nummular eczema, the subacute or chronic variant is characterised by dry plaques that sometimes have an annular configuration (see Figure 8). In darker skin it is often associated hyperpigmentation. Nummular eczema is most prevalent on the lower legs, although arms and trunk can be affected. Although the exact pathogenesis is not

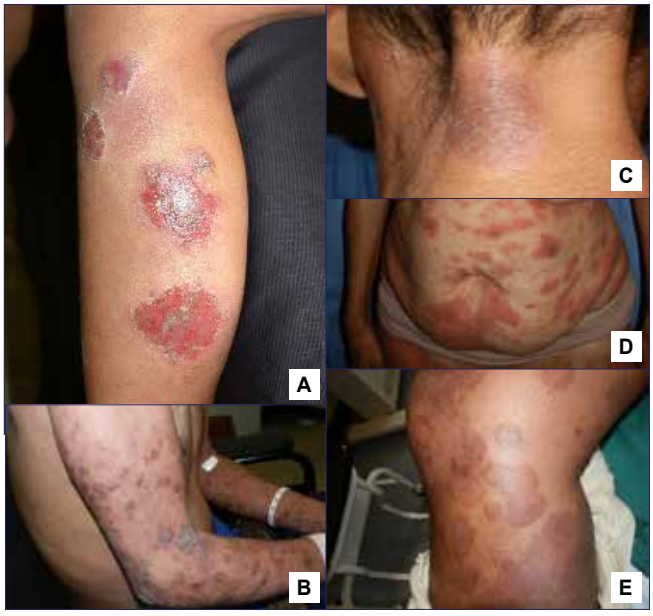


Figure 8: Nummular eczema variants. Note E) the annular variant that is often confused with tinea corporis.

clear, background of atopy, previous contact eczema and stasis eczema have a strong association with nummular eczema. Allergens, bacterial infection, drugs, xerosis and varicose veins are among known precipitants. Nummular eczema is fairly resistant to treatment but responds to emollients and potent topical steroids, sometimes under occlusion. In resistant cases, phototherapy and oral immunomodulators are used.

STASIS ECZEMA

This is also referred to as gravitational or venous eczema. It is characterised by oedema of the lower legs that is associated with itchy erythematous plaques. The plaques can be weepy, crusted or scaly. Over time, they become confluent, circumferential, hyperpigmented and may ulcerate (see Figure 9). Hyperpigmentation is a result of haemosiderin deposition from extravasated red blood cells. In advanced stages of stasis eczema, there is fibrosis of the lower legs resulting in narrowing and the 'inverted wine bottle' appearance and ulceration.[13] Stasis eczema can also be seen following breast surgery and lymph-node dissection.[14] Apart from topical corticosteroids and emollients, lifelong compression stockings are the mainstay of treating stasis eczema.[13]

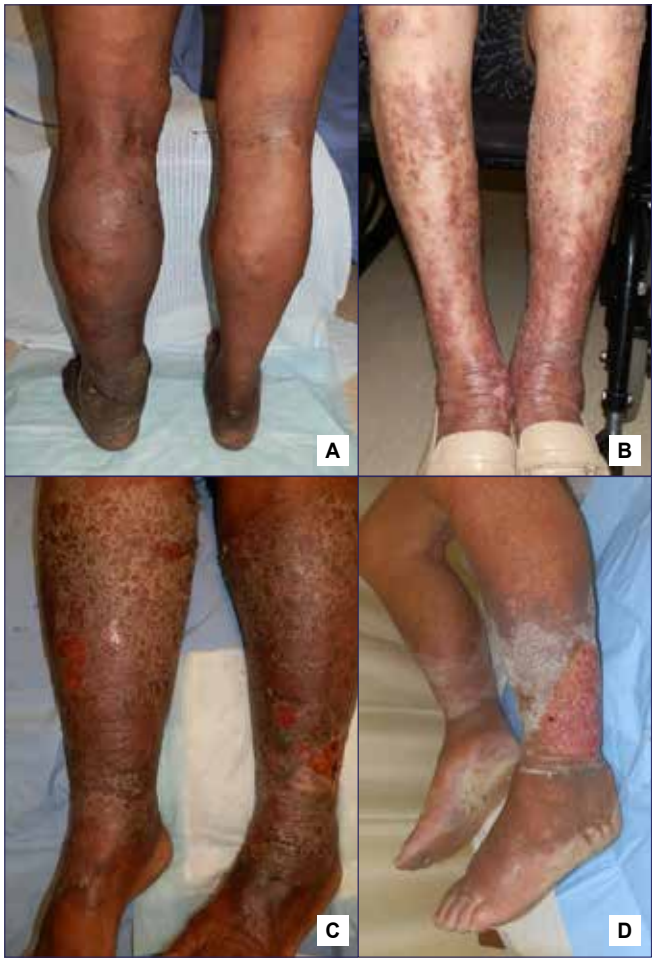


Figure 9: Stasis eczema. A) on the background of varicose veins. The left leg had a previous fracture, B) acute weepy variant, C) early ulceration, D) dry scale with ulceration.

ERYTHRODERMIC ECZEMA

Erythroderma is defined as an erythematous eruption affecting more than 90% of the body surface area as a result of any inflammatory condition. Erythroderma refers more to the extent of erythema rather than the cause. Chronic erythroderma results in excessive scale, referred to as exfoliative dermatitis (see Figure 10). Erythroderma should be differentiated from flushing, which has no associated scale. Causes of erythroderma include eczema, psoriasis, drug reactions, pityriasis rubra pilaris, cutaneous T-cell lymphoma, congenital ichthyosis disorders and internal malignancies. Complications of erythroderma include impaired temperature control, fluid loss, secondary infection and hypoalbuminaemia. Children are more susceptible to hypothermia. Management includes treating the underlying disorder, managing complications, emollients and topical steroids.[15]

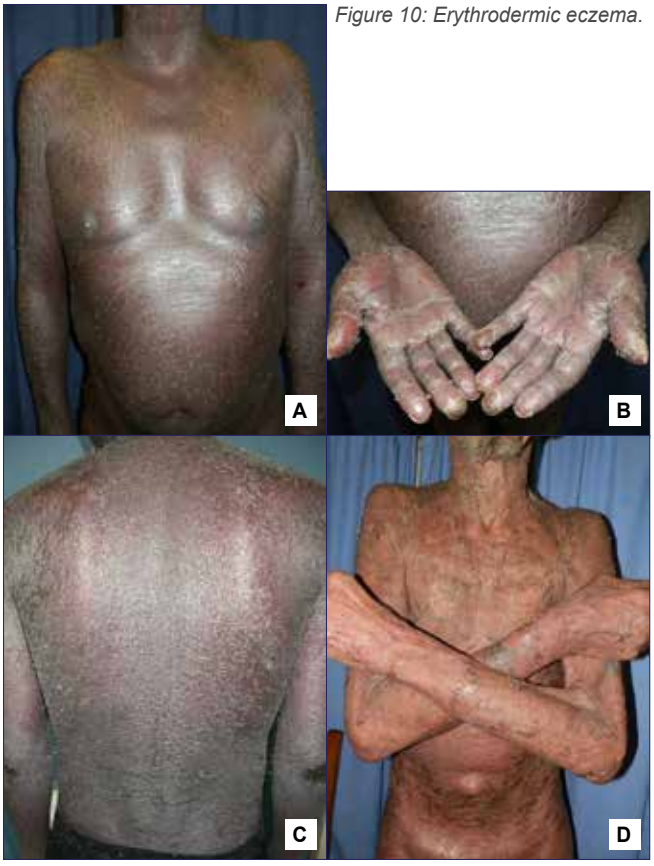


Figure 10: Erythrodermic eczema.

DYSHIDROTIC ECZEMA

Dyshidrotic eczema, also known as pompholyx, is a chronic relapsing disease characterised by itchy vesicles and blisters on the palms and soles. On resolution and, if untreated, it results in thick scale and deep painful fissuring of the hands and feet (see Figure 11). The disease does not affect sweat glands and the term 'dyshidrosis' is a misnomer. Dyshidrotic eczema has been associated with atopy, allergic contact eczema, irritants, hyperhidrosis and fungal infection.[16] Management is challenging and includes identification and avoidance of triggers.

Topical therapies include corticosteroids, calcineurin inhibitors, bexarotene and phototherapy. Systemic agents that have been tried in severe recalcitrant cases include corticosteroids, azathioprine, methotrexate, mycophenolate mofetil and retinoids. Botulinum toxin has been successfully used in a small series of patients.[16]

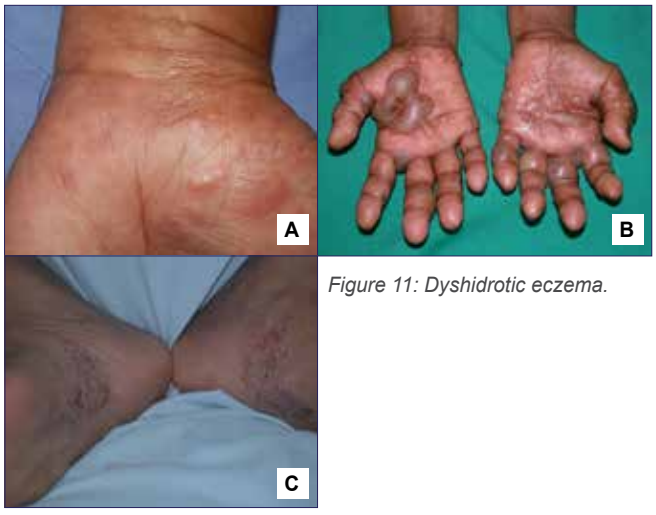


Figure 11: Dyshidrotic eczema.

NIPPLE ECZEMA

Nipple eczema can be a manifestation of atopic eczema or exist in isolation. The clinical presentation is variable and ranges from erythema, scale, blisters, scale crust, fissures, erosions and lichenified plaques (see Figure 12). Nipple eczema is usually bilateral but can be unilateral, particularly when it is not associated with atopy. Although most commonly seen in young women, nipple eczema has also been reported in males.[17] The pathogenesis of nipple eczema has not been clearly defined, but it has been shown to have a strong relationship with atopy and allergic-contact eczema.[17,18] Paget's disease is the major differential diagnosis of nipple eczema, although the latter has an insidious onset and is almost always unilateral.[19] In breastfeeding women, allergic or irritant contact eczema and superimposed candidiasis have to be considered in the differential diagnosis. Eczema on the nipple is treated like eczema in other parts of the body.

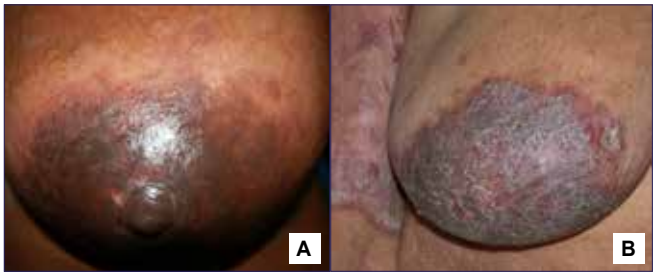


Figure 12: Nipple eczema.

NODULAR PRURIGO

Nodular prurigo is a manifestation of severe prolonged scratching due to multiple underlying conditions. Nodular prurigo has the strongest association with atopic eczema which is reported in 80% of cases. Other associated disorders include liver failure, chronic renal failure, nerve compression and HIV-associated papular urticaria. The lesions present as symmetrical firm nodules with a warty or eroded surface. In darker skin they are often hyperpigmented (see Figure 13). Nodular prurigo is resistant to treatment and standard treatment modalities include potent topical and intralesional corticosteroids, calcineurin inhibitors and phototherapy. Cryotherapy, capsaicin, systemic immunomodulators, gabapentin, thalidomide and tricyclic antidepressants have also been tried.[20]

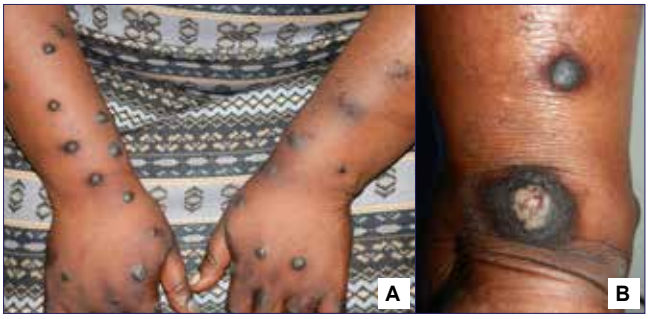


Figure 13: Nodular prurigo showing verrucous warty nodules in B.

LICHEN SIMPLEX CHRONICUS

Lichen simplex chronicus also referred to as lichenification, is a result of chronic scratching from any cause, eczema included. It characterised by exaggerated skin markings, leathery induration, pigmentary changes and scale (see Figure 3D and Figure 14). Broken hairs and scratch marks may be a feature. Back of the scalp and neck, wrists and forearms, lower legs and genitalia are the most commonly affected areas. Apart from identifying and eliminating the underlying cause, topical corticosteroids, emollients, topical calcineurin inhibitors, intra-lesional corticosteroids and phototherapy are the major treatment modalities of lichenification.[21]

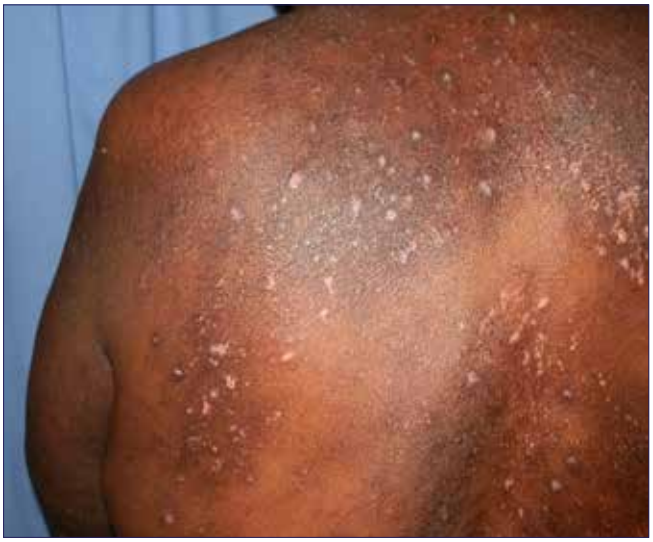


Figure 14: Lichen simplex chronicus.

ASTEATOTIC ECZEMA

Asteatotic eczema, also known as eczema craquele because of its 'cracked appearance', is a common disorder in the elderly characterised by a crazy paving appearance of the skin associated with pruritus. It is most common on the lower legs, but it may occur elsewhere including upper limbs and trunk (see Figure 15). The exact pathogenesis is not clear but it is thought to be a result of desiccation of the stratum corneum resulting in cracked skin. The cracks expose free nerve endings on the skin, triggering itch. Soaps, detergents, excessively hot baths and dry environments aggravate asteatotic eczema. The conditions can be associated with old age, malnutrition, hypothyroidism, inherited and acquired forms of ichthyosis, internal malignancies and drugs (e.g. retinoids). Emollients and mild topical corticosteroids are the mainstay of asteatotic eczema treatment. Avoidance of triggers and exacerbating factors improves treatment outcomes. Although infrequent, it is important to rule out underlying disease.[22,23]

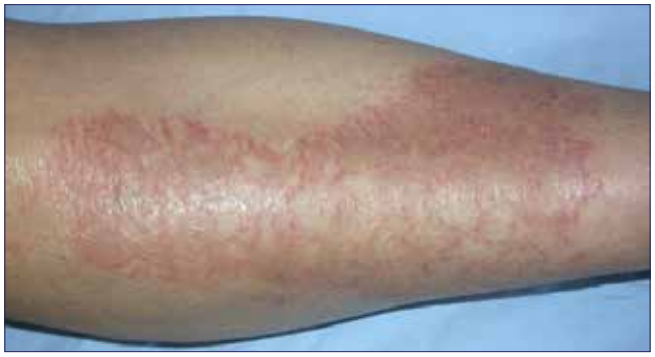


Figure 15: Asteatotic eczema.

CONTACT ECZEMA

Contact eczema refers to a group of disorders in which the skin reacts to a direct contact with the causative agent. Contact eczema is classified into allergic, irritant, photo and systemic forms based on the underlying mechanism and clinical appearance. There is often overlap between the different forms.

ALLERGIC-CONTACT ECZEMA

In allergic-contact eczema, the material coming into contact with the skin is an allergen that induces a delayed type hypersensitivity reaction only in susceptible people. Impaired barrier function of the skin as seen in ulcers and atopy predisposes to allergic-contact eczema. The reaction usually mirrors the area of contact although it may extend beyond the margins (see Figure 16). Although common in the general population, allergic-contact eczema tends to cluster in certain occupational groups such as hairdressers, cleaners and florists. Common causes include nickel, acrylates in glue, fragrances, rubber, hair dye, preservatives, topical antibiotics and topical steroids. A good history and pattern recognition are critical in the diagnosis of this and other forms of contact eczema. Patch tests are used to confirm the allergen.

IRRITANT-CONTACT ECZEMA

Irritant-contact eczema occurs when exposure to chemicals (body fluids, water, detergents) and physical agents (friction, heat, cold) damage the skin resulting in impaired barrier function and subsequent inflammatory response. The speed of onset and severity depend on the anatomical location, strength and quantity of irritant, as well as frequency and duration of exposure. Unlike in allergic-contact eczema, anyone with adequate exposure to the irritant can develop irritant-contact eczema. The rash is usually confined to the area of contact with the irritant (see Figure 17). Common examples of irritant-contact eczema include napkin eczema, drool eczema and chemical burns. There are no tests that can confirm irritant-contact eczema.

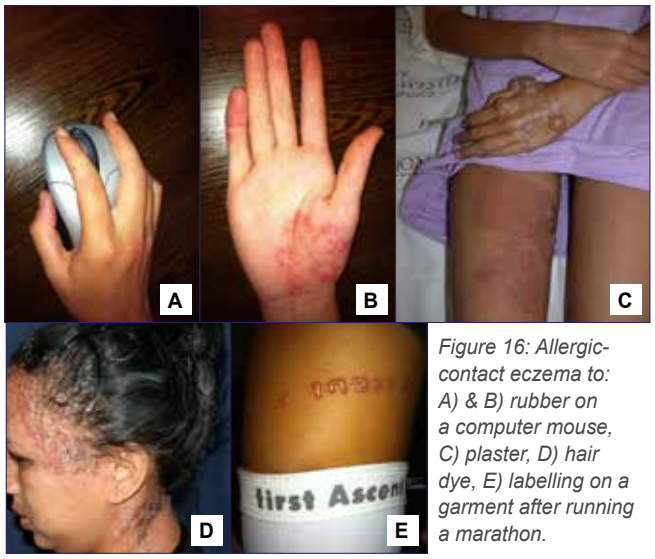


Figure 16: Allergic-contact eczema to: A) & B) rubber on a computer mouse, C) plaster, D) hair dye, E) labelling on a garment after running a marathon.

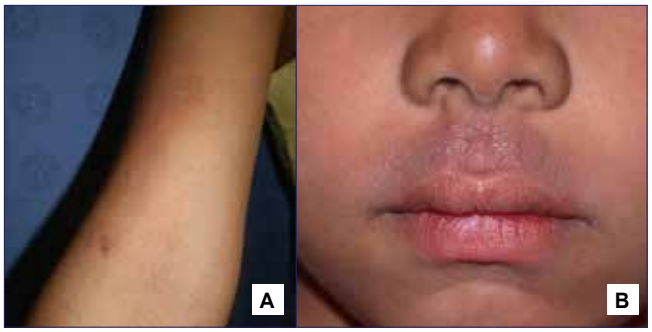


Figure 17: Irritant-contact eczema to A) a liquid B) chronic lip sucking.

PHOTOCONTACT ECZEMA

Photocontact eczema refers to an eczematous reaction that develops when a chemical applied to the skin interacts with ultraviolet (UV) radiation. The reaction can either be photoallergic or phototoxic. Photoallergic reaction is a delayed type hypersensitivity reaction to the UV-activated chemical, whereas phototoxicity results from direct tissue damage by the UV-activated chemical. Phototoxicity resembles severe sunburn, whereas photoallergy presents as an eczematous eruption in sun-exposed areas.[24]

SYSTEMIC-CONTACT ECZEMA

Systemic contact eczema refers to development an eczema when a person with existing allergic-contact eczema is exposed to the allergen via a systemic route (oral, inhalation, injection or

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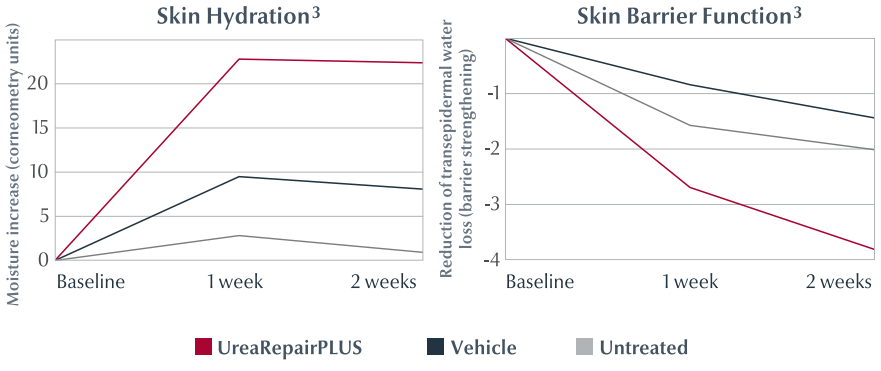

(1) 2-week randomised double-blind vehicle-controlled study, test products were applied twice daily on the inner forearm on 44 female patients (50 to 70 years) suffering from very dry skin, skin hydration was measured by corneometry at baseline and after one and two weeks. (2) Treatment of [very dry rough skin] with a topical formulation containing glyceryl glucoside, natural moisturising factors and ceramide; Weber et al., JCAD 2012. (3) A new skin care approach for [very dry rough skin]: topical treatments containing natural moisturising factors, glycerol glucoside, and ceramide 3.

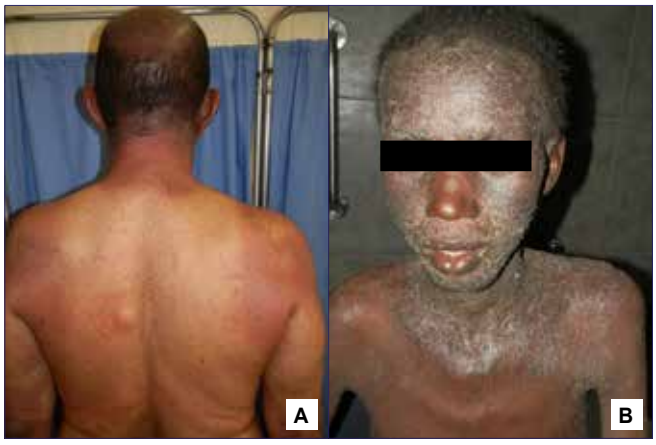

Figure 18: Photocontact eczema.

transmucosal). The most common feature is a flare-up at the site of previous contact or patch test, although the rash may become generalised. The spectrum of lesions is as wide as eczema itself but dyshidrotic eczema is particularly common. Another unique presentation of systemic contact eczema is baboon syndrome. In baboon syndrome, the erythematous rash is accentuated in the buttocks and upper inner thighs, hence the name. This should be differentiated from symmetrical drug-related intertriginous and flexural exanthema (SDRIFE), a form of cutaneous drug reaction with a similar clinical presentation.[25,26]

DISSEMINATED SECONDARY ECZEMA

Disseminated secondary eczema is also referred to as auto-eczematisation or ID reaction. It is a generalised acute eczema that follows another localised inflammatory skin disease. The lesions are intensely pruritic, symmetrical and usually affect the trunk and limbs. The morphology ranges from blisters, papules, nummular, follicular papules, morbilliform, targetoid and dyshidrotic. The preceding inflammatory diseases include other forms of eczema (stasis, nummular and acute contact), infections (fungal, bacterial and viral) and infestations (scabies and lice). The pathogenesis of disseminated secondary eczema is unknown. It is postulated to be an immune response to circulating antigens, skin components or messenger proteins. Treatment of the underlying condition is the mainstay of management. The secondary eczema should be managed based on the phenotype.[27]

CONCLUSION

Eczema is an umbrella term for multiple conditions with differing pathogenesis, clinical presentation and natural history. Clinicians need to be able to distinguish between the variants as this affects management and prognosis.

DECLARATION OF CONFLICT OF INTEREST
The author declares no conflict of interest.

This article has been peer reviewed.

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Review Article

AN OVERVIEW OF RECEPTORS AND GRANULAR CONTENTS OF MAST CELLS AND BASOPHILS AS THEY PERTAIN TO ALLERGY DIAGNOSIS

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ABSTRACT

Mast cells, related basophils and basic biology underpin many allergic diseases. The activation and subsequent degranulation of mast cells manifests with both the well-known clinical presentations of anaphylaxis, urticaria and angioedema and more chronic disorders such as mastocytosis or mast-cell activation syndrome. IgE-dependent and independent pathways can be activated by an increasingly diverse range of allergens, including foods, insect venom, and chemicals and drugs. Allergy diagnostics must exploit our understanding of this basic biology in order to provide clinicians with biomarkers for disease identification and in vitro systems to identify triggering allergens without the risk of harm to patients. This brief review outlines mast cell and basophil surface characteristics, receptors and activation markers, emphasising their potential for complex immune interactions beyond IgE. Important signalling pathways and key degranulation products used as biomarkers and exploited in current and research-laboratory allergy diagnostics are described.

INTRODUCTION

In 2017, we celebrated the discovery of IgE as the antibody of allergic disease; but without the effector mast cell and basophil its impact would be null. The presence of granulated cells in unstained connective tissues was first reported by Friedrich von Recklinghausen (1833–1910).[1] In 1878 German physician Paul Ehrlich (1854–1915) named these cells 'Mastzellen'. This means 'well-fed' cells, because the thinking was that they were derived from connective tissue cells that had ingested large amounts of nutrients. A year later, Ehrlich described the first basophil during studies on the staining properties of blood cells distinguishing neutrophilic, eosinophilic and basophilic cells – the last of these susceptible to staining by basic dyes.[2]

Mast cells and basophils are amazing, phylogenetically ancient immune cells, possibly with a shared common progenitor in urochordates;[3] in human beings they both originate in the bone marrow from the pluripotent common myeloid progenitor. They are the critical effector components of allergic inflammation – exaggerated type-2 immunity. Going head-to-head on basic statistics: mast cells are bigger than basophils, contain many more granules (1 000 vs 80 per cell), and are long-lived (up to months) tissue-resident cells that fully differentiate only within peripheral tissues.[4,5] Basophils have bigger granules (1.2 µm vs 0.2 µm), are the rarest blood granulocytes and have a short lifespan (< 3 days). Immature mast cells and basophils disperse to mucosal and epithelial tissues throughout the body, localising at the host-environment interface, for example the skin.[6] Mediators stored preformed in the cytoplasmic granules of mast cell and basophils are numerous, defining, and hint at their diverse functionality. They include: mast cell-specific proteases (tryptase, chymases and carboxypeptidase A3), non-mast cell proteases, lysosomal enzymes, biogenic amines (histamine), cytokines and proteoglycans.[6] In this review, we highlight the unique surface characteristics of mast cells and basophils as well as the key activation pathways and granule contents that we try to measure or replicate in vivo for allergy diagnosis.

BASIC MAST CELL AND BASOPHIL BIOLOGY

SURFACE MARKERS AND IMMUNE INTERACTIONS

Figure 1 shows a basophil and a mast cell highlighting cell-type defining surface markers in resting and activated states. Basophils and mast cells can be identified as they carry specific surface markers. Mast cells in a resting state carry a number of surface markers, including four chemokine receptors of the CXC and CC families; CD23 – the low-affinity IgE receptor – FcεRII – and the IL-33/IL-1 receptor-related protein ST2 and the stem-cell factor receptor KIT – both important for mast-cell survival

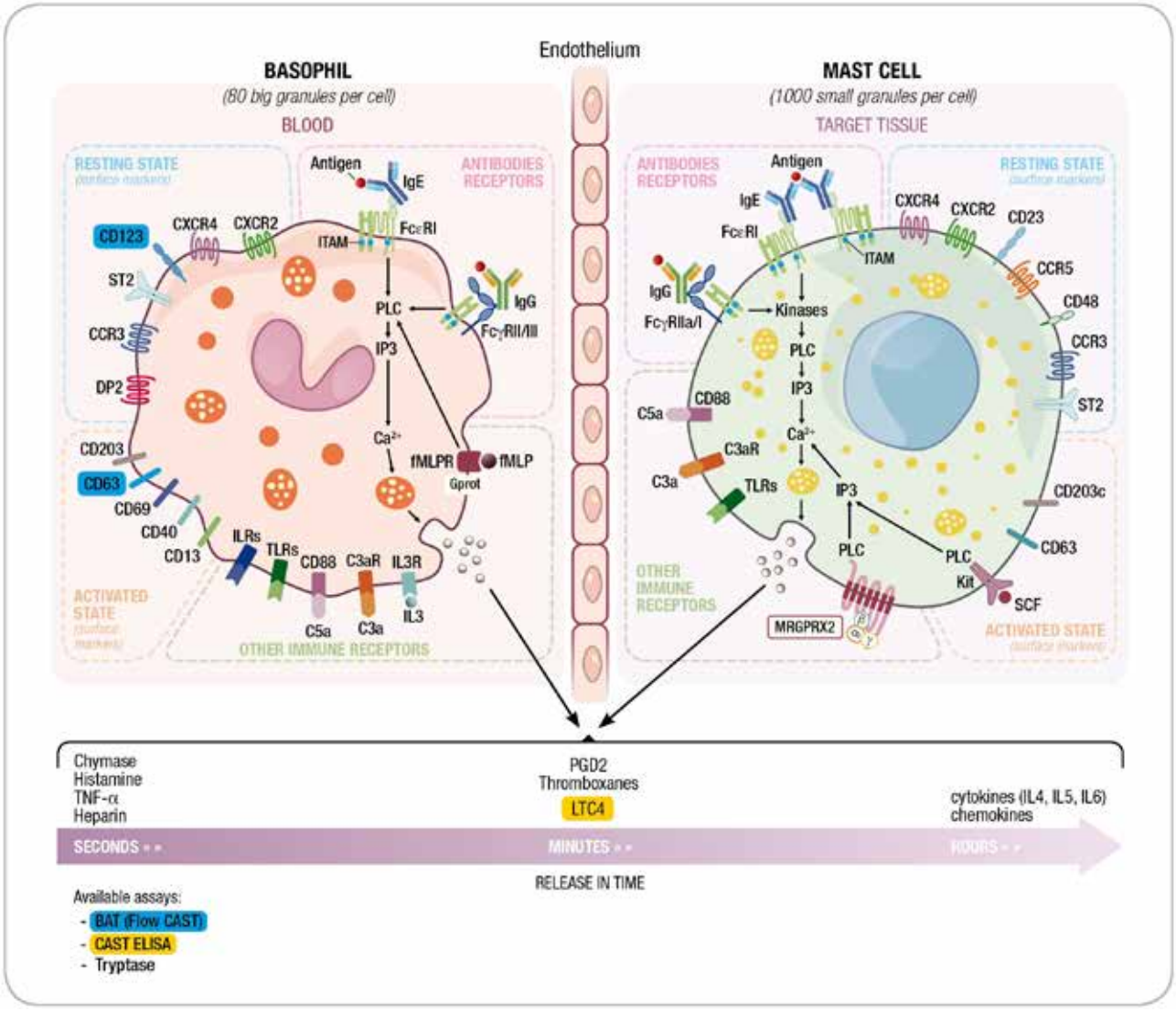

Figure 1: Important surface receptors of human mast cell and basophil in resting and activated states, with corresponding signalling pathways.

Cell surface receptors help to define different immune cells and are also useful in identifying when cells become activated. Binding of appropriate ligands activates cognate receptors with signal transduction. The figure provides a basic schematic of IgE-dependent and IgE-independent signalling cascades that result in the degranulation of cell mediators. Mast cells and basophils can also be characterised by granule contents, some of which are shared, for example, histamine and lipid-derived inflammatory mediators (leukotrienes). A number of these markers and granule contents are useful for the diagnosis of immediate hypersensitivity reactions, and the important markers and mediators have been highlighted in different colours: in blue the markers investigated used in Flow CAST and in yellow the ones in CAST ELISA.

Abbreviations: CXCR2: C-X-C motif chemokine receptor 2, CXCR4: C-X-C chemokine receptor type 4, CCR3: C-C chemokine receptor type 3, DP2: prostaglandin D_2 receptor 2, ILRs: interleukin receptors, IL3: interleukin 3, IL3R: IL3 receptor, TLRs: toll-like receptors, C3a: complement component 3a, C3aR: C3a receptor, C5a: complement component 5a, fMLP: N-Formylmethionyl-leucyl-phenylalanine, fMLPR: fMLP receptor, Gprot: G protein, IgE: immunoglobulin E, FcεRI: high affinity IgE receptor I, IgG: immunoglobulin G, FcɣR: low-affinity IgG receptors, ITAM: immunoreceptor tyrosine-based activation motif, PLC: phospholipase C, IP3: inositol trisphosphate, Ca^{2+}: calcium, SCF: stem cell factor, CD30L: CD30 ligand, CCR5: C-C chemokine receptor type 5, TNF-α: tumor necrosis factor *alpha, PGD2: prostaglandin D2, LTC4: leukotriene C4, BAT: basophil activation test, CAST: cellular antigen stimulation test, ELISA: enzyme-linked immunosorbent assay*

and proliferation. KIT (CD117) expression is independent of tissue site, maturation or activation status. In systemic mastocytosis, KIT is often expressed in mast cells in a mutated and constitutively activated form.[6] Following immune activation through either IgE- or non-IgE-mediated pathways, different markers can be detected – including a tetraspanin protein CD63, the ectocenzyme E-NPP3 (CD203c) and a member of the TNF superfamily, CD30L. They also express FcεRI and FcɣRIIa/I, the receptors that mediate IgE and IgG antibody binding respectively. CD63 and CD203c expressions are upregulated following aggregation of FcεRI, and they are overexpressed on neoplastic mast cells in systemic mastocytosis.[7]

Mast cells possess an array of immune receptors that allow complex immune interaction and direct recognition of invading pathogens. They recognise pathogen-associated molecular

patterns (PAMPs) of all classes of microbiotas and danger-associated molecular patterns (DAMPs) through surface toll-like receptors (TLRs), with protein expression of nine out of ten TLRs on various tissue mast cells.[8] TLR ligand stimulation leads to the release of cytokines (TNF-α, IL-5, IL-1ß, GM-CSF), leukotrienes (cysLT, LTB_4, LTC_4) and chemokines (CCL1).[8] In addition, TLR ligation can act to enhance the response of mast cells to antigen, sensitising the cells to stimulation through FcεRI, a factor that might underline the role of co-infection in allergic diseases.[9] Mast cells express receptors for the complement components C3a and C5a and binding can cause degranulation.[10] Interestingly, they induce degranulation mainly in MC_{TC} mast cells (mast cell granules that contain both tryptase and chymase) such as skin mast cells, but not, for instance, in lung mast cells, since MC_T mast cells (mast cell granules that contain tryptase only) are the predominant cell type present in the lung.

In chronic urticarial pathogenesis, the relevant autoantibodies belong to complement-fixing subtypes IgG1 and IgG3.[11] The vascular leakage of such autoantibodies by local events facilitates their binding to either FcεRI or IgE, the cross-linking of the receptor, and complement activation that generates C5a. C5a interacts with the receptor for complement anaphylatoxin (C5aR) localised on the surface of mast cell MC_{TC} and participates in mast-cell activation.[12]

Basophils possess an overlapping but distinct set of surface markers, with unique markers in the resting state that include CD123 – the IL-3 receptor, and the chemoattractant receptor homologue expressed on TH2 cells (CRTH2 or DP2); and, in the activated state, CD69 – a C-type lectin protein, the co-stimulatory molecule CD40 and the alanyl aminopeptidase (AAP) – CD13.

In resting basophils, CD63 is located primarily in secretory granule membranes and is translocated to the cell surface when basophils are activated to degranulate, for example after aggregation of FcεRI, where it can be detected by flow cytometry. The bimodal surface expression of CD63 is typically detected on allergen-activated basophils in whole blood, whereas CD123 expression is seen on nearly all basophils regardless of their purity. Basophils do not express the HLA-DR marker, a MHC class-II cell-surface receptor found only in antigen-presenting cells (dendritic cells and monocytes) and used as a negative marker of basophils. Therefore, CD63, CD123 and HLA-DR are complementary for assessing basophil activation (see below).

Basophils also express TLRs – TLR2 and TLR4 – and secrete cytokines such as IL-4 and IL-13 in response to peptidoglycan.[13,14] TLR ligands enhance the production of cytokines and mediators when basophils are stimulated through IgE-dependent or IgE-independent pathways.[14] They also express fMLPR, the receptor for the bacteria-derived peptide fMLP.

THE IgE-RECEPTOR AND IGE-DEPENDENT SIGNALLING

IgE and mast cells are synonymous with type-1 hypersensitivity, manifesting in various allergic diseases, including allergic asthma, rhinitis and certain food allergies. When an antigen comes into contact with IgE on the surface of the mast cell, it cross-links two or more FcεRI molecules, which leads to a cascade of phosphorylation that results in activated Syk kinase; these in turn result in autophosphorylates and phosphorylated phospholipase C. The activated PLC hydrolyses PI(4,5)P2 to form D-Acylglycerol and IP3.[22] IP3 activates the IP3-receptor on the endoplasmic reticulum, allowing an increase in intracellular calcium. These events lead to exocytosis and the subsequent release of granular mediators; the production of newly formed lipid-derived mediators; and the transcription of cytokine genes (see Figure 1).[5] This cascade is rapid, releasing mast-cell mediators, for example histamine and tryptase, which occurs within minutes of antigen binding and receptor cross-linking. These are defining features of immediate hypersensitivity reactions.

IgE-INDEPENDENT ACTIVATION AND RELEVANT RECEPTORS

There are many examples of immediate allergic reactions, with the clinical phenotype of mast cell degranulation but without significant tryptase release or the detection of allergen-specific IgE. These types of reaction have been given various terms, including ‘pseudoallergic’ or, when severe, ‘anaphylactoid’. Recent statistics show that pseudoallergic reactions may represent as many as two-thirds of all immediate hypersensitivity reactions. In this context, mast cell activation is completed in an IgE-independent manner through different surface receptors (see Figure 1). Our understanding of the specifics of these receptors has recently been improved with the identification of certain non-IgE-mediated drug reactions as being the result of binding to a human G-protein-coupled receptor (GPCR) MRGPRX2, the human orthologue of the mice mast-cell receptor mrgprB2. This receptor recognises cationic neuropeptides, drugs (e.g. Vancomycin), fluoroquinolones and opioids, and also insect venom peptides.[15] Typically, non-IgE reactions have a reduced cardiovascular symptomatology, but are otherwise not easily distinguished from an IgE-mediated allergy.

The MRGPRX2 receptor is not the only GPCR to be found on mast cells, and, given the diversity of this group of receptors, their importance in the pathogenesis of allergic diseases continues to grow. In fact, only recently, a missense substitution in the adhesion GPCR E2 (ADGRE2) on mast cells was identified as the pathology in autosomal-dominant vibratory urticaria.[15] Mast cell function can be altered by GPCRs by modifying FcεRI-mediated mast cell activation or by inducing direct mast cell mediators’ release. Other examples of such GPCR activity include those for: C3a and C5a; sphingosine-1-phosphate – an important lymph node retention molecule; and chemokines such as MIP-1α. GPCRs do not appear to require tyrosine kinases to initiate degranulation or cytokine generation, but they do regulate their function through Gα subunits and βγ subunits.[16,17] Therefore, the way in which GPCRs influence mast cell function depends directly upon the class of G protein engaged and the manner in which the individual subunits regulate effector proteins.

Although the initiating events used by the FcεRI and GPCRs may differ, there are common elements to these pathways at more receptor-distal stages. GPCRs activate PLCγ[18], which leads to the generation of IP3 and DAG, which in turn results in calcium mobilisation and PKC activation. GPCRs also activate the p110γ subunit isoform of PI3K;[19] this induces the formation of membrane-associated PIP3, which allows the membrane recruitment of PH domain-containing signalling molecules (see Figure 1).[20]

IgG-DEPENDENT ACTIVATION

Unsurprisingly, IgE is not the only immunoglobulin isotype that can activate mast cells and basophils. In fact, IgG autoantibodies against the FcεRI and a host of other skin antigens are currently hypothesised to be the main driver of chronic autoimmune urticaria.[21] IgG antibodies have been known to activate mast cells in vivo and in vitro, but were somehow overlooked when IgE antibodies were discovered and their potent biological properties were recognised in human beings. Human mast cells also express FcɣRs: FcɣRIIA[22,23] and in some cases FcɣRI (CD64) when induced by IFN-γ.[24] Mast cells express one ITAM-containing receptor for IgG (two in IFN-ɣ-treated cultured human mast cells) capable of inducing essentially the same secretory responses as high-affinity IgE receptors. In addition to chronic urticaria pathogenesis, a wide array of physiological and pathological roles, which do not involve IgE antibodies but IgG antibodies, are played by mast cells – as observed in murine models of arthritis[25] and encephalitis.[26] Mast cells play critical roles in IgG-dependent tissue-specific autoimmune diseases.

DIAGNOSTIC EXPLOITATION OF MAST CELL AND BASOPHIL BIOLOGY

The major roles of a clinical allergist include:

- confirming that a patient's symptoms can be attributable to allergic disease, and
- identifying the offending allergen(s) so that treatment can be applied.

The diagnostic measurement of mast cell mediators release, the activation of basophils by offending allergens, and the measurement of specific IgE are at present the mainstay of in vitro allergy testing (see Table I) and they relate directly to the basic biology discussed. In the next section we outline the methods and application of some of these methods, building from the basic biology.

MEASUREMENT OF MAST CELL DEGRANULATION

Mast cell degranulation occurs a few seconds after cross-linking and releases the myriad inflammatory mediators stored in granules. Different techniques and challenges present when measuring mediators.

B-TRYPTASE, HISTAMINE AND B-HEXOSAMINIDASE

Tryptases are serine proteases found in high concentration in mast cells; the majority is α- and β-tryptase, with β-tryptase the isoform that is stored in mature granules and released only on activation.[27] Demonstrating a rise and return to baseline of β-tryptase during the course of a reaction is very useful in confirming the likelihood of an immediate, usually IgE-mediated, hypersensitivity reaction. The available serum tryptase assay (ImmunoCap Tryptase, Phadia Laboratory Systems, Uppsala, Sweden) uses quantitative fluorescence to levels in blood or plasma.[28] Some precautions are worth noting for the results to be valid:

- specimens are stable at room temperature for up to two days, but should be stored at 2–8°C if the analysis is to be performed only five days following collection, and
- serial tryptase demonstrating a rise and return to baseline is more helpful than a single measurement in the course of a suspected anaphylaxis.

If anaphylaxis is suspected, a sample collection should take place between 15 minutes and three hours after the onset of allergic signs and symptoms. Elevated levels can be detected 3–6 hours after the reaction and normalise within 12–14 hours.

TABLE I: ALLERGY LABORATORY DIAGNOSTICS (ROUTINE AND RESEARCH)

TEST NAME	MEASURED PARAMETER	MAIN LAB TECHNIQUE	CELL OF INTEREST	IgE-DEPENDENT	IgE-INDEPENDENT
• **Routine clinic use in South Africa**					
Specific IgEs (including mixes)				x	
Basophil activation tests:					
▫ CAST Elisa	Sulfidoleukotriene release	Elisa	Basophil	x	x
▫ Flow CAST	CD63 upregulation	Flow cytometry	Basophil	x	x
B-tryptase release	B-tryptase	Fluorescent Enzyme Immunoassay	Mast cell	x	x
• **Available only in the research setting**					
Histamine release	Histamine	Fluorescent Enzyme Immunoassay	Mast cell/ basophil	x	x
β-hexosaminidase release	β-hexosaminidase	Fluorescent Enzyme Immunoassay	Mast cell	x	x
PGD2 generation	PGD2 release	Elisa	Mast cell	x	x
Cytokine release	Cytokine release		Mast cell	x	x
Autoantibody detection	IgG anti-FcεRI				

Abbreviations: IgE: immunoglobulin E, CAST: cellular antigen stimulation test, ELISA: enzyme-linked immunosorbent assay, PGD2: prostaglandin D2, IgG: immunoglobulin G, FcεRI: high-affinity IgE receptor I.

Serum or urinary histamine are another biomarker to measure to confirm anaphylaxis. However, in contrast to tryptase, histamine-release measurement is not widely available. The half-life of histamine is short and its peak serum concentration occurs five minutes after the onset of anaphylaxis, declining to baseline levels by 15–30 minutes.[27] However, histamine and its primary metabolite, N-methylhistamine, are excreted in the urine, and can be measured using an enzyme immunoassay. Tryptase and histamine levels may be persistently increased in people with mastocytosis.[29]

Finally, β-hexosaminidase can also be assessed. Because β-hexosaminidase release is slow and the process persists for longer than histamine release, β-hexosaminidase could be a better candidate for mast cell degranulation detection than histamine. Currently, the most widely used method for quantifying β-hexosaminidase is a colorimetric assay. B-hexosaminidase release can be assessed in patient plasma, serum and tissue homogenates.

CYCLO-OXYGENASE DERIVATIVES (LTC4, PGE2 AND PGD2)

Mediators other than tryptase, histamine and β-hexosaminidase can be considered as clinical markers of mast cell activation. These include leukotriene C4 (LTC4) and prostaglandins (PGD2, PDE2). To measure these products in blood or urine, commercially competitive ELISA kits are used.

In the routine clinical practice measurement of serum tryptase is the mainstay of adjunctive diagnostics to confirm mast cell degranulation in immediate hypersensitivity. However, tryptase measurement lacks sensitivity as the single measurement method of an increased release of mast cell mediators, especially for diagnostic confirmation of mast cell activation syndromes. In this context, measures of N-methylhistamine, β-hexosaminidase or PGD2 in urine have been studied and have been found to be useful.

MEASUREMENT OF BASOPHIL ACTIVATION THROUGH IgE-DEPENDENT AND IgE-INDEPENDENT PATHWAYS

In 2018, the 7th EuroBAT focus meeting will converge about the use of basophil activation testing (BAT) in the diagnosis and study of allergic diseases. The basic biology of basophils underpins these assays, and they have two readouts or methods (see Table I). BAT is also referred to as a Cellular Antigen Stimulation Test (CAST), as the cell being stimulated with this *in vitro* allergy-provocation test is the basophil.

In brief, after allergen stimulation, basophil activation can be detected either by the quantitative determination of leukotriene C4 (LTC4) – known in South Africa as CAST ELISA – or by determining the upregulation of CD63 expression on the surface of basophils by flow cytometry – known in South Africa as Flow CAST.[30]

The major advantage of BAT is the detection of IgE-dependent and IgE-independent allergen activation. Another major advantage of the BAT test is that it can be seen as an 'oral food challenge' in a test tube, where, instead of giving the food to a child by mouth, basophils involved in acute allergic reactions are exposed to a food extract in a test tube. BAT can be used to monitor the clinical response to immunomodulatory treatments for food allergy. The following complexity to these functional assays applies:

- for the CAST ELISA, patients should be free of antiallergenic drugs such as antihistamines, corticosteroids or chromoglycic acid[31] and for the Flow CAST, patients should be free of systemic steroid and cyclosporin 3–7-days before blood sample.[32,33]
- blood sampling from individuals undergoing in vivo skin or oral-provocation testing must be drawn before any exposure to the allergen(s), and
- cell stimulation needs to be performed within 24 hours after blood collection, the blood samples stored at 2–8°C.

The BAT test reflects a functional response as basophil activation. BAT is used regionally and not harmonised globally; however, we offer both methods of BAT in South Africa, with utility in clinical settings where in vivo testing is not available, for example, in food additives and certain drugs; where in vivo skin or intradermal testing is negative and non-IgE-mediated reactions are suspected; or as adjunctive testing where in vivo tests lack sensitivity or are unsafe.

CONCLUSION

Our understanding of the basic biology of mast cells and basophils continues to expand in the '-omics' era. This review has introduced clinicians into this exciting world. We hope to have improved both the understanding of the basic biology of these amazing cells and to have provided insights into their use for allergy diagnosis, in particular non-IgE-mediated reactions.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

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DIFFICULT ASTHMA

1. *True or false:* Current primary care diagnosis of asthma is usually accurate.
2. *True or false:* Most children with asthma do not benefit from raising the dose of inhaled corticosteroids above 200 mcg/day fluticasone equivalent.
3. *True or false:* Adherence can reliably be assessed by taking a history in the clinic.
4. *Choose the **INCORRECT** answer:* Treatment of which of the following may be beneficial in difficult asthma:
   a. Obesity with weight loss;
   b. Exercise induced laryngeal obstruction by a respiratory physiotherapist;
   c. Gastro-oesophageal reflux with a proton pump inhibitor;
   d. Allergic rhinitis with nasal steroids and oral antihistamines.
   e. All of the above.

UNDERSTANDING THE HETEROGENEITY OF ATOPIC DERMATITIS IN CHILDHOOD

5. *True or false:* Atopic dermatitis (AD) is a heterogeneous disease including several types of long-term courses.
6. *True or false:* The frequency of the presence of Filaggrin loss-of-functional mutations vary across ethnicity.
7. *True or false:* Latent class analysis (LCA) is one of data-driven approaches to subtyping of complex diseases based on temporal patterns of symptoms.
8. *True or false:* Characteristics of AD phenotypes based on LCA have been consistent across studies.

THE VALUE OF SINGLE AND COMBINATION SEROLOGY TESTS IN THE DIAGNOSIS OF PAEDIATRIC COELIAC DISEASE IN THE ERA OF LATEST ESPGHAN GUIDELINES

9. *True* or false: It is mandatory to do endoscopy and duodenal biopsy to confirm the diagnosis of coeliac disease in symptomatic children.
10. *True or false:* HLA coeliac genetics is positive in about 30% of the European population.
11. *True or false:* Delaying the introduction of gluten in the infant weaning process can prevent the onset of coeliac disease.
12. *Choose the correct answer:* Coeliac Disease is associated with:
    a. Down Syndrome;
    b. Hirschprung's Disease;
    c. Crohn's Disease;
    d. Prematurity;
    e. Vegetarians.

AN OVERVIEW OF RECEPTORS AND GRANULAR CONTENTS OF MAST CELLS AND BASOPHILS AS THEY PERTAIN TO ALLERGY DIAGNOSIS

13. *True or false:* Basophils and mast cells express common surface markers.
14. *True or false:* Basophils and mast cells can only be activated in a IgE-dependent manner.
15. *True or false:* Mast cell degranulation can be measured in the laboratory.
16. *Choose the correct answer:* What is the relevant technique used in the laboratory to determine basophil activation?
    a. Western Blot;
    b. Flow CAST;
    c. PCR;
    d. All of the above;
    e. None of the above.

AUTO-INFLAMMATION AS A CAUSE OF URTICARIA

17. *True or false:* Auto-inflammation describes dysregulation of the innate immune system and as such is characterised by spontaneous episodes of systemic or localised inflammation caused by cells of the myeloid lineage (e.g. macrophages and eosinophils) and without identifiable auto-antibodies or antigen specific T-lymphocytes.
18. *True or false:* Muckle-Wells syndrome is characterised by fever, urticarial rash and sensorineural deafness.
19. *True or false:* Key features of CAPS include arthritis, urticarial rashes, conjunctivitis, symptoms on exposure to heat and sensorineural deafness.
20. *Choose the correct answer:* Useful drugs in the treatment of auto-inflammation include:
    a. Anakinra;
    b. Canakinumab;
    c. Colchicine;
    d. Tocilizumab;
    e. All of the above.

ETHICS: DECIDING FOR CHILDREN AT THE END OF LIFE – WHOSE DECISION?

21. *True or false:* The British High Court ruled in favour of withdrawal of life-supportive therapy from Charlie Gard based on the best interests principle.
22. *True or false:* The best interests principle requires weighing of the benefits and burdens of a treatment.
23. *True or false:* The harm principle is a beneficence standard.
24. *True or false:* The Zone of Parental Discretion (ZPD) tool guides the application of the harm principle to disagreements between parents and doctors.
25. *True or false:* The ZPD imposes limits on parental authority in all cases of disagreement between parents and doctors.

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2. Adco-Desloratadine Syrup Sugar Free Approved Package Insert, January 2010

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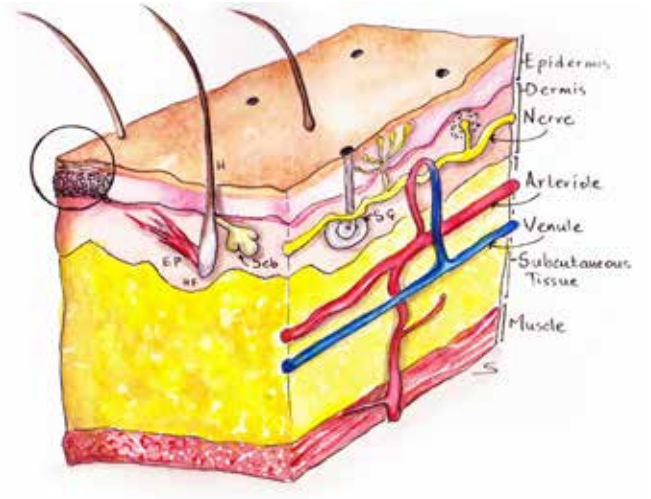
DERMATITIS

Shaunagh Emanuel and Di Hawarden

Dr Do-a-lot explains that **dermatitis** refers to inflammation of the skin.

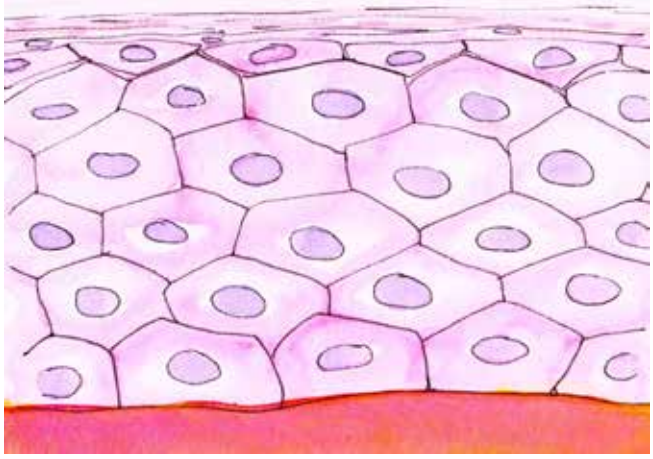
The definition is, however, used inconsistently. The term may be used to describe a broad range of inflammatory skin diseases. Some prefer to use the term 'eczema' instead of dermatitis. Many use the term eczema synonymously with 'atopic dermatitis', although others are more specific and use the term 'atopic eczema'. The dermatopathologists prefer the term 'spongiotic dermatitis'.



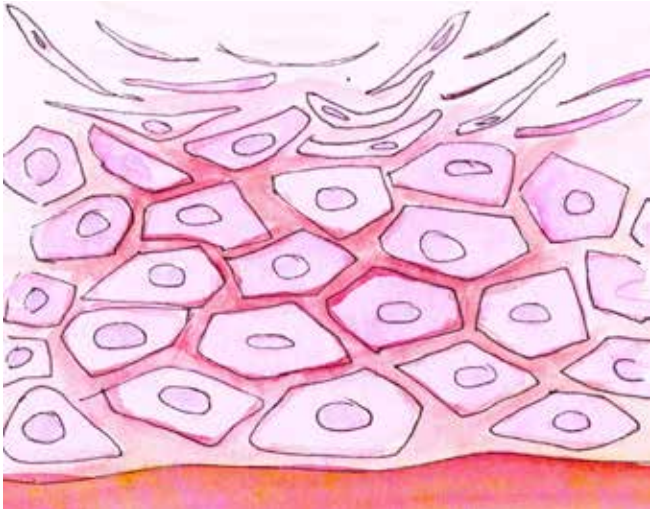
This is a simplified diagram of the layers that constitute the skin, showing the epidermis, dermis, subcutaneous tissue and muscle, with hairs (H), hair follicle (HF), sebaceous glands (Seb), erector pili muscle (EP), sweat glands (SG), arterioles, venules and nerves.

The two figures on the right show the area of epidermis within the black circle.

Dermatitis has multiple clinical patterns, characterised histologically by a spongiotic tissue-reaction pattern within the epidermis.



This diagram is a magnification of the circled area on the simplified diagram of the skin, showing a normal epidermis. Notice how the skin cells are tightly packed together forming layers that are impenetrable.



This diagram is a magnification of the circled area on the simplified diagram of the skin showing how the keratinocytes in the epidermis of a patient with dermatitis are haphazardly packed together resulting in large intercellular spaces. This makes the skin easily penetrable.

Spongiosis refers to intercellular accumulation of fluid resulting in swelling. Movement of skin cells (called keratinocytes) to the skin surface is accelerated, which results in parakeratosis, where nucleated skin cells reach the epidermis. The intercellular bridges widen, allowing fluid accumulation resulting in the formation of vesicles in the epidermis. The skin becomes oedematous and the perivascular space becomes infiltrated with lymphocytes, histiocytes and occasional eosinophils and neutrophils. Acanthosis occurs in chronic forms of dermatitis – with a thickening of the epidermis.

Common types of dermatitis include atopic dermatitis, contact dermatitis, stasis dermatitis and seborrhoeic dermatitis.

Atopic dermatitis is a common chronic inflammatory skin condition that may have an hereditary component. It usually begins in infancy, and is characterised by a relapsing red, itchy rash with a broad clinical spectrum. There is no objective laboratory or genetic test for the disease and the diagnosis is made clinically. Food allergies are more common in this population. Lichenification is a feature in atopic eczema where the skin has been chronically scratched or rubbed causing it to become leathery, thickened and shiny.

Contact dermatitis is a rash that occurs as a result of coming into contact with substances that may irritate the skin or cause an allergic reaction. Examples of such substances include soap, essential oils or poison ivy. The rash may burn, sting or itch, and vesicles may be present.

Stasis dermatitis, also called 'gravitational dermatitis', 'venous eczema' and 'venous stasis dermatitis', typically occurs in the lower legs as a result of circulatory insufficiency. The area becomes oedematous and the skin becomes discoloured, scaly and dry. Varicose veins are common and, in severe cases, ulceration may occur.

Seborrhoeic dermatitis results in scaly patches, erythematous areas of skin and stubborn dandruff. In infants it presents as cradle cap. It typically affects oily areas of the body such as the face, upper chest and back. This form of dermatitis tends to have a relapsing course.

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Ethics Article

DECIDING FOR CHILDREN AT THE END OF LIFE – WHOSE DECISION?

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ABSTRACT
The recent cases of Charlie Gard and Alfie Evans in the United Kingdom focused attention on decision-making for children at the end of life. Frameworks for decision-making include the best interests standard, the harm principle and the zone of parental discretion. Ultimately, the decision to withdraw life support was made by the court, but I contend that this is not ideal in the clinical context

INTRODUCTION

The outcome of two end-of-life paediatric cases decided by the British courts generated much discussion in the public and academic media over the past months. Charlie Gard and Alfie Evans both had life-supportive treatment withdrawn from them against their parents' wishes, after the doctors and hospitals applied to the court to do so. This poses the question as to who should make treatment decisions for young children, and what standards should be used when making such decisions. I outline the clinical histories of these two infants, discuss frameworks for decision-making for incapacitated infants, and then suggest an approach to making end-of-life decisions in similar cases.

CHARLIE GARD[1]

Charlie Gard was born at full term in August 2016 and at the age of two months at Great Ormond Street Hospital (GOSH) in London was diagnosed as having a rare severe mitochondrial disorder, infantile-onset encephalomyopathic mitochondrial DNA depletion syndrome (MDDS). The type of MDDS from which Charlie suffered causes rapid deterioration in all organ systems with death in infancy. Charlie required ventilation for respiratory failure and manifested involvement of his cardiac, hepatic and renal systems. His parents became aware of an experimental nucleoside treatment that had previously been used in another form of MDDS. The medical team at GOSH considered using this for Charlie, but his condition deteriorated in January 2017 and they believed that continued ventilation and the proposed nucleoside treatment were both futile. A doctor in the United States offered to provide treatment for Charlie and his parents started fundraising for the journey to that country. The hospital went to court to prevent Charlie's parents from flying him to the United States and, despite appeals to higher courts, the judges all ruled in favour of the GOSH doctors' plan to withdraw life support. Subsequent public intervention delayed the withdrawal of life support, but new imaging and expert opinion confirmed that nucleoside treatment would not benefit Charlie, and his parents withdrew their court application in July 2017. He died on 28 July 2017.

ALFIE EVANS[2,3]

Alfie Evans, born on 9 May 2016, was diagnosed with a severe neurodegenerative disorder at Alder Hey Children's Hospital in Liverpool at the age of 6 months, although the exact diagnosis was never established. He was ventilated for a prolonged period in the paediatric intensive-care unit without any improvement. The medical team sought to withdraw life-supportive treatment from Alfie as he had been in a semi-vegetative condition for more than a year and his prognosis was extremely poor. His parents disagreed with this and wanted to transfer him to the Bambino Gesu Hospital in Rome, Italy, for further treatment. The Alder Hey medical team contended that not only was continued treatment futile, but it was also 'unkind and inhumane', and obtained a court order authorising the withdrawal of ventilation, with the High Court ruling that continued ventilation was not in Alfie's best interests. Alfie's parents contested the ruling, saying that they should be allowed to make decisions for their son. Protracted legal battles ensued, but the courts upheld the original ruling. Life support was withdrawn on 23 April and Alfie died on 28 April 2018.

In both cases, the debate raged over Charlie's and Alfie's right to live, and whether doctors and the courts ought to have overridden the parental right to decide on their sons' treatment. Who should make decisions for children at the end of life, on what grounds should courses of treatment be decided, and how should disagreements between parents and the hospital team be resolved?

THE CONCEPT OF 'BEST INTERESTS' AS A STANDARD FOR END-OF-LIFE DECISION-MAKING

Young children are not autonomous, and thus the best-interests principle is the standard against which healthcare decisions are made for them in both ethics and the law.[4] This is in accordance

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with the ethical principle of beneficence. 'Best interests' requires a benefit–burden and quality of life (QoL) analysis, and may therefore be a difficult standard to apply.[5] Criticism of the best-interests standard holds that it is impractical and restricted, but it is important to realise that the application thereof should be reasonable rather than ideal, and that other interests may be affected by the decision.[5,6,7] Another criticism of the best-interests standard is that its interpretation depends on the values of the person applying the test.[7,8,9]

Can it be in a child's best interests to die? If the child is suffering and their life is unbearable then it can be argued that dying is in their best interests.[9] Can this criterion be applied to Charlie Gard and Alfie Evans? We could argue that both children were being ventilated in the intensive care unit, but their pain and discomfort would have been controlled with analgesia and sedation, and thus it may not have been in their best interests to die.[9]

THE 'HARM PRINCIPLE' AS A STANDARD FOR END-OF-LIFE DECISION-MAKING

Diekema proposed that a harm-based standard should be used in determining the appropriate threshold for intervention by a higher authority in paediatric decision-making.[10] He argued that the best-interests standard is a poor guide when considering various treatment alternatives for children.[10] The 'Harm Principle' originated in the powers granted to the state to protect the health and welfare of the public.[10] According to Diekema: 'Parents should be overruled if they are making decisions that significantly increase the risk of serious harm to the child compared with other available options.'[10,11] A parental decision that may pose harm to the child can therefore justify state intervention.[10,11]

Wilkinson[11] argues that the use of the harm principle can be justified by the following reasons:

1. *Medical or value uncertainty:* the evidence for a particular therapy may be lacking, or the parents' values may differ from that of the doctors.
2. *Parental autonomy and interests:* paediatric end-of-life decision-making will have a major impact on the parents and the rest of the family.
3. *Consistency:* we do not usually interfere in parents' decision-making in other areas of life, even if we do not agree with them. Similarly, therefore, we ought not to interfere with end-of-life decision making unless we believe it will result in significant harm to the child.[11]

Would the proposed continued ventilation and transfer of Charlie to the United States and Alfie to Italy, respectively, have caused significant harm to either of the two children? I believe not.

THE ZONE OF PARENTAL DISCRETION (ZPD)[12]

Gillam[12] proposed the zone of parental discretion (ZPD) as a tool to guide the practical approach to using the harm principle if there is disagreement between parents and doctors regarding a child's medical treatment. She uses the ZPD to 'refer to the ethically protected space where parents may legitimately make decisions for their children, even if the decisions are sub-optimal for those children (i.e. not absolutely the *best* for them).' In other words, "'good enough" parental decisions should be tolerated, until the point where they would cause harm to the child.'[12]

The ZPD approach is set out below (adapted from Gillam).[12]

STAGE I: HARM FROM PARENTS' DECISIONS

1. What are the parents' wishes or decision?
2. What would the effects be on the child if the decision were carried out?
3. Are these effects sufficiently bad as to cause probable *significant* harm to the child?

STAGE II: HARM FROM OVERRIDING THE PARENTS' DECISION

1. What would the effects on the child be of overriding the parents' decision?
2. If the effects were likely to be negative, would this constitute greater harm to the child than the harm likely from the parents' original decision?

Doctors have to assess the benefits and burdens of the parents' decisions on the child, and whether significant harm is likely to result. If the assessment is that the parents' decision will not significantly harm the child, the decision should be respected, even if it is sub-optimal for the child. If the parents' decision is likely to result in significant harm to the child, it should not be adhered to and an assessment made as to the harms resulting from overriding the parents. If overriding the parents would cause minimal harm to the child then it should be done; if significant harm is likely to result, then the parents' original decision should stand.[12]

Gillam explains that the ethical justification for the ZPD is the following:[12]

1. 'Parents have an ethical right to make medical decisions for their children, based on their own conception of the good life.
2. Parents are not morally obliged to maximise the well-being of their child.
3. The limit to parental authority lies at the point where significant harm is likely to be caused to the child. Parents' decisions should only be overridden if the child is likely to suffer significant harm from the decision.'

Using the ZPD, I would argue that the parents' decisions should have been respected for both Charlie and Alfie, as I do not believe that *significant* harm would have been caused to either of them by so doing.

FUTILITY AND RESOURCES

Limiting treatment at the end of life may also be based on the fact that scarce medical resources may be consumed with little hope of a positive outcome, or even if the outcome has a chance of success, it is not justifiable in the context of others' needs.[13] Savulescu argues:

> 'Treatment limitation decisions are best made, not in the alleged interests of patients, but on distributive justice

grounds. When parents request "futile" or very-low-chance-of-success treatments …, their requests can be legitimately denied on distributive justice grounds.'[14]

WHO SHOULD DECIDE FOR CHILDREN AT THE END OF LIFE?

Gillon argues that Charlie Gard's parents should have made the decision about his treatment, and not the doctors or the court.[15] He bases his argument on the following understanding:

> 'wherever possible, people's views about moral dilemmas should be morally and legally respected unless substantial harms or substantial injustices would result; and in the case of children who can't decide for themselves, the law should allow parents to decide their children's best interests unless, once again, substantial harms or substantial injustices would result.'[15]

He argues that, in this particular case,

> 'neither substantial harms nor substantial injustices would have resulted had Charlie Gard's parents been allowed to pursue their views of their son's best interests and, therefore, both morally and legally those views should have been respected.'[15]

I believe he would conclude similarly about Alfie Evans.

How should we resolve the ethical dilemma posed by cases such as Charlie and Alfie? I do not believe that the court is the ideal authority to do so, and I also agree with Gillon that the court should have upheld the GOSH doctors' rights not to continue futile treatment that they felt was against Charlie's best interests, while at the same time legally permitting his parents to seek the experimental treatment elsewhere that they desired for him.[15]

A Clinical Ethics Committee, where available, would be the ideal body to address and mediate such moral dilemmas, but it may require that ethics consultants from a different hospital be involved to minimise conflicts of interest.

As Wilkinson and Savulescu state,

> 'Paediatric medical care is, at its best, a partnership between professionals and families, working together to promote the wellbeing of children. These prolonged and painful disputes about treatment are devastating for families and traumatic for the medical and nursing staff. There are no winners, only losers.
>
> 'There is a pressing need now for professionals to come together with families to explore and implement new constructive solutions to avoid, mitigate, and resolve disagreements about treatment. That would be in the best interests of all children.'[16]

DECLARATION OF CONFLICT ON INTEREST

The author declares no conflict of interest.

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Research Article

OCULAR ALLERGY: AN UNDERESTIMATED DISORDER OF THE 21ST CENTURY

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ABSTRACT
Until recently, studies regarding allergic diseases were much more focused on asthma, rhinitis and other dermatological manifestations rather than ocular manifestations. It is now clear that the eye serves as one of the organs that exhibit early indications of an allergic condition but research is still rudimentary in terms of prevalence studies, environmental impact, genetics, pharmacological treatment implications and the economics of its control and treatment. This article highlights the apparent underestimation of ocular allergy and the prospect it holds in bringing perspective to both ocular and systemic diseases of clinical importance in order to inform action in the domain of public health.

Keywords: allergy; oxidative stress; conjunctivitis; hypersensitivity; pollen

INTRODUCTION

Ocular allergy (also called 'allergic eye disease') is not a single disease entity but assorted forms of hypersensitivity disorder that result from an abnormal immunological response of the eye to harmless antigens – commonly pollen, mites, dander and dust, among others. It is characterised by itching (pruritus), tearing (lacrimation), a burning sensation, a foreign-body sensation and ocular dryness.[1–3] It is usually known to sufferers as 'allergic conjunctivitis'; nevertheless, it presents in characteristic diverse forms with ocular itch as the hallmark symptom.[2,3] Allergies in general are deemed the third most-chronic disorder among vulnerable groups – essentially children – and is accountable for most paediatric eye consulting.[4,5] However, unlike other diseases of the eye, it has yet to receive the attention it deserves. Every aspect of ocular allergy – ranging from epidemiology and natural history, pathomechanism and its interrelationships, ocular allergy and the environment, genetics of ocular allergy, pharmacological treatment and its associated economics – has far-reaching consequences, which are most often neglected or underestimated. The primary purpose of this article is to bring to light the extent of the underestimation of this ocular disease burden and to emphasise the need for a concerted effort in addressing this ubiquitous ocular morbidity.

EPIDEMIOLOGY AND NATURAL HISTORY OF ALLERGY

Allergies in general are by far underreported because their incidence is dependent on geographical location, which affects the estimation of prevalence of allergic eye disease, among others.[6] It is obvious that several geographical locations have no data about ocular allergy. The global estimate of 15–20% prevalence of ocular allergy may not be sufficiently profitable for country- and region-specific policy intervention, because the field lacks information on key contributing factors such as age, environmental peculiarities, genetics and childhood exposures.[7] Allergens of different geographical locations have interesting particularities that provide clues to the improved understanding of the pathogenesis, diagnosis, and management of allergic diseases.[8] But little effort has been made, especially in low-income countries, to study ocular allergy as most are without the empirical data needed to inform policy direction.

Children are the most affected group of people: before age 15, males predominate in ocular allergic disease and females preponderate after that age. However, nearly all ocular–allergic patients have a family history of atopy.[9] Wherever there have been studies to estimate the prevalence of ocular allergy, it is found to far outweight the prevalences of the priority eye diseases of cataract and glaucoma. For example, across the globe over the past two decades there has been an 11.4% and a 20.2% decline in the number of people affected by blindness and moderate or severe visual impairment, respectively, due to cataracts.[10] The global prevalence of glaucoma for the population aged 40–80 years is 3.54%, with primary open-angle glaucoma (POAG) being high in Africa (4.20%) and primary angle-closure glaucoma (PACG) the most common among Asians (1.09%).[11] Studies rarely indicate that these priority eye diseases have attendant co-morbidities and, even if there are, the frequency and consistency cannot be compared to ocular allergy, which almost always presents with noisome co-morbidities.[6,12,13] Predominantly children have had to live with these co-morbidities such as asthma, allergic rhinitis (AR), and atopic dermatitis, with manifest symptoms of food and drug allergies.[13] In most

Close to allergens.
Far from allergies.

Optimal relief.[1]

- Multiple action provides rapid and sustained relief from signs and symptoms of ocular allergy[1]
- Neutral pH (approximately 7.0)[4]
- Prescribe for patients aged 3 years and older[3]

SCHEDULING STATUS: S2 **PATANOL®** Eye Drops, solution Reg. No. 33/15.4/0189. **Composition:** Contains 1.11 mg/ml olopatadine hydrochloride equivalent to 1 mg/ml olopatadine, preserved with benzalkonium chloride 0.01% (m/v). **Pharmacological Classification:** A 15.4 Ophthalmic preparations, other. **Indications:** PATANOL is indicated for the temporary prevention of itching of the eye due to allergic conjunctivitis. **Contraindications:** Hypersensitivity to PATANOL or any of the excipients. **Warnings:** Benzalkonium chloride, which is commonly used as a preservative in ophthalmic products, has been reported to cause punctate keratopathy and/or toxic ulcerative keratopathy. Close monitoring is required with frequent or prolonged use in dry eye patients, or in conditions where the cornea is compromised. PATANOL should not be administered while wearing contact lenses. Patients can insert contact lenses 10 minutes after instillation of PATANOL or at any time later. **Dosage and Directions for Use:** Instil one drop in the conjunctival sac of the affected eye(s) twice daily. To prevent contamination of the dropper tip and solution, care must be taken not to touch the eyelids, surrounding areas or other surfaces with the dropper tip of the bottle. Keep the bottle tightly closed when not in use. PATANOL may be used in paediatric patients (3 years or older) at the same posology as in adults. **Side Effects and Special Precautions: Side Effects:** ***Common:*** ocular discomfort (burning and stinging). ***Uncommon:*** ocular pruritis, ocular hyperaemia, ocular discharge, keratitis, lid oedema, dry eye, foreign body sensation, photophobia, headache, asthenia, dizziness, dry nose, taste perversion. ***Post marketing experience:*** blurred vision, dry mouth, rhinitis, erythema. **Interaction with other medicinal products:** In case of concomitant therapy with other topical ocular medicines, an interval of 5 minutes should be allowed between successive applications. No human clinical drug interaction studies were performed with PATANOL. Experimental studies have shown that olopatadine did not inhibit metabolic reactions, which involve cytochrome P-450 isozymes. No significant drug interaction was noted following systematic administration of olopatadine and the CYP3A4 inhibitor itraconazole in studies evaluating pro-arrhythmic potential. These results indicate that olopatadine is unlikely to result in metabolic interactions with other concomitantly administered active substances. **Pregnancy and Lactation:** ***Pregnancy:*** Safety and efficacy have not been established, the use of PATANOL in pregnancy is not recommended. ***Breastfeeding:*** PATANOL is not recommended for breastfeeding mothers. **Effects on ability to drive and use machines:** If blurred vision occurs at instillation, the patient must wait until the vision clears before driving or using machinery. **Packs:** 5 ml Opaque low-density poly-ethylene bottle with dispensing plug and polypropylene screw cap. **Note:** Before prescribing consult full prescribing information.

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instances, sufferers may have to receive treatment not only for their ocular allergy but for its co-morbidities. This disease menace is unlikely to reduce substantially in the face of climate change and incessant pollution around the globe[14] and the low priority accorded it, despite the fact that ocular symptoms may be the initial and the most outstanding signs of allergic diseases.[15]

Chronic forms of the disease result in more severe symptoms such as pain, blindness from corneal scarring, cataract or glaucoma, disfigured skin and eyelid changes.[16] Paying careful attention to allergic conjunctival disease (ACD) has the potential to reduce the component contribution of ocular allergy to these notorious priority eye diseases of global concern.

PATHO-MECHANISM OF OCULAR ALLERGY AND ITS INTERRELATIONSHIPS

It is important to note that ocular allergy presents in diverse forms: seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), giant papillary conjunctivitis (GPC) and contact dermatoconjunctivitis (CDC).[17] Pathophysiologically, allergic conjunctivitis is mediated by the Th2-immune response and the production of IgE.

The entire process involves an initial sensitisation phase. First, picograms of allergens such as pollens, dust mite, faecal particles, animal dander and other proteins reach the conjunctival mucosa. These particles are then processed by antigen-presenting cells (APCs) such as Langerhans and dendrites. Antigens break away by means of proteolysis and after that they attach to the antigen-recognition site of the major histocompatibility complex (MHC) class II molecules. The antigens are then presented to local Th0 lymphocytes that express antigen-specific receptor by means of antigen-presenting cells (APCs) for recognition. Several contacts and cytokine interactions between APCs and T-cells are needed to induce a Th2-type response. The cytokines freed by the type-2 helper T-lymphocytes mainly interleukin-3, IL-4, IL-5, IL-6, IL-13 and granulocyte-macrophage colony stimulate factor (GM-CSF) stimulate the synthesis of IgE by B-cells.

Phase two of the disease process in conjunctival allergy involves the activation of the native mast cells in the conjunctival mucosa, the sensitised host bearing specific IgE antibodies on the cell surface by means of high-affinity receptors. Contact with allergens in sensitised individuals causes cross-linking of IgE at the level of the mast-cell membrane; this leads to mast-cell degranulation, causing the release of histamine, tryptase, prostaglandins and leukotrienes. These mediators trigger clinical manifestations of the acute phase of the disease: itching, redness, and swelling. The degranulated cells also induce stimulation of vascular endothelial cells, and as a result chemokine and adhesion molecules are expressed. These molecules include 'regulated-upon-activation normal T-cell expressed and secreted (RANTES), monocytes chemotactic protein-1 (MCP-1), intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM), and p-Selectin and chemotactic factors (IL-8, eotaxin). These parameters set up the conscription phase of activated inflammatory cells in the conjunctiva. The late-phase reaction to allergen stimulation occurs hours after allergen exposure and is distinguished by the recurrence or persistence of symptoms owing to the permeation of eosinophils, neutrophils and T-lymphocytes into the mucosa. The late-phase response remains a key contributor to the disease process of severe forms of ocular-allergic inflammatory disorders.[18]

Current studies have defined such essential pathogenic mechanisms of ACD, extending our knowledge of the cellular and mediator mechanisms involved, and enabling pathophysiological correlates to be made between the allergic diseases.[16] Oxidative stress – a major pathogenic factor in allergic eye disease – has implications for the development of complications, co-morbidities and other systemic diseases of clinical importance.[19] This pathogenic factor involved in an underrated disease has several insights to offer in the study of ageing and other age-related diseases that plague the ageing population. It is not uncommon to attend to patients with chronic ocular allergy accompanied by other ocular surface disorders such as dry eye, pterygium, conjunctival chalasis, ocular surface epithelial damage and, rarely, keratoconus.[20,21] In most instances, ACD serves as the precursor of these complications via oxidative injury similar to that which drives the disease process of ocular allergy itself.[22]

It is evident that prioritising ocular allergy promises to provide not only a clue as to the therapeutic intervention for other ocular disorders of global priority but also an insight into the science of their pathogenesis. Oxidative stress has been implicated in the functional distortions of several structures at the centre of most blinding eye diseases. These structures include membranes, DNAs, proteins, and mitochondria.[23] Mitochondria, in particular, are predicted to be the key intracellular target for most drugs with antioxidant properties. It is clear that oxidative stress plays a role in the pathogenesis of POAG, a mechanistic pathway that is rarely targeted by conventional drugs. Studies have shown that oxidative DNA damage significantly increases in the trabecular meshwork (TM) of glaucomatous patients compared to non-glaucomatous patients. The oxidative stress occurs also in retinal neuronal cells and is complicit in the neuronal cell death that results in visual field defects in POAG.[24] This suggests that models of allergic eye disease could offer beneficial correlation for glaucoma because oxidative stress is a common driver in the pathogenesis of both diseases. Another, blinding, disease of the eye is age-related cataract; enhanced oxidative stress is considered an important pathophysiological process.[25] The oxidative stress sets the tone for other processes such as alteration of epithelial cell metabolism, impairment of calcium homeostasis, apoptosis, DNA damage, activation of m-calpain, and insolubilisation of proteolysed β- and α-crystallins.[26]

A good understanding of the oxidative process as seen in allergic eye disease has the advantage of providing therapeutic hints for eye disease in general. Some prevalent blinding eye diseases of developed countries, such as age-related macular degeneration (AMD), have been found to be associated with cataracts, especially the early incidence of AMD where there is

an increase in pigmentation and indistinct drusen formation.[27] This makes it obvious that the same or a similar etiopathology underpins these diseases: there is a correlation between the pathogenesis of ocular allergy and uveitis, diabetic retinopathy, Pseudoexfoliation syndrome and retinitis pigmentosa.[28,29]

A myriad systemic diseases share the same or similar etiopathogenesis as ocular allergy. This means that, in the face of ocular allergic episodes, these diseases may worsen or be a risk factor among susceptible individuals. Until recently, epidemiological studies had shown that allergic diseases were associated with an increased risk for Alzheimer's disease (AD). Nevertheless, a recent study that investigated the impact of allergic disease on AD using experimental models concluded that allergy may have harmful effects on the normal brain but favourable effects on AD. This beneficial effect is through the stimulation of immune responses induced by allergens.[30] In this study the authors recommended further studies aimed at clarifying the impact of allergy on AD pathogenesis and progression. It is clear that ocular allergy may be helpful in this regard as an initial manifestation of most allergic diseases.[15]

In another study, patients with asthma were found to have an increased risk of developing Parkinson's disease (PD).[31] Other studies have pointed out the necessity of assessing patients with PD for allergic conditions and allergy to common food as it has the prospect of improving their symptoms, treatment and quality of life (QoL).[32] Several unresolved controversies remain regarding allergies and PD for which ocular allergy could provide a lead in its resolution.

Catatonia has been hypothesised to be associated with allergic triggers. It is a well-known fact that histaminic receptors (H_3) result in the release of neurotransmitters such as dopamine, acetylcholine, gamma-aminobutyric acid, (GABA) and serotonin. This receptor has implications for the pathogenic correlation between allergy and pediatric psychiatric disorders which may include schizophrenia and mood disorders.[33] Although many consider it to be a trivial disease, AR, in addition to the nasal and ocular symptoms, is remarkably associated with impairments in information-processing and changes in attention-related cognitive processes.[34] The tendency for rhinoallergic conjunctivitis to interrupt sleep has long been established: it is associated with poor sleep quality, which can result in decreased learning ability, diminished efficiency at work or school, driving, and by and large affects QoL negatively.[35,36] The release of inflammatory mediators due to allergens activates the biosynthesis of inflammatory cells, which results in nasal congestion and ocular itch, causing disrupted sleep and subsequent daytime somnolence. The presence of IgG- and IgA-producing plasma cells and lymphocytes in the basal lamina associated with an accessible pathway to the lumen strongly suggests that the vagina may be able to mount a mucosal immune response similar to the lung, eye, nose and skin.[37] Some studies have reported allergy as a significant cause of vulvovaginitis.[38] Included among the allergens identified as triggers for allergic vulvovaginitis are:

- seminal fluid components;
- medications taken by the partner and present in his semen;
- spermicides;
- soaps;
- sanitary napkins;
- latex, and
- intestinal parasites.

Candida albicans may possibly provoke immediate hypersensitivity reactions. In addition, allergens such as pollen, dust or food particles can be accidentally introduced into the vagina by means common to the eye, nose, and skin.[39]

OCULAR ALLERGY, ITS COMPLICATIONS AND THE ENVIRONMENT

The environment plays a major role in the dynamics of ocular allergic diseases, their complications, and other disease correlates. With the advent of climate change and incessant global pollution, the interaction between the environment and the dynamics of allergic eye diseases has become more complex but they have yet to be fully examined. It has been observed that the risk of ocular allergy is increased by up to 20% in children who are exposed to environmental tobacco smoke. This is associated with the likelihood of unborn children developing strabismus increasing up to 6.55 times. Exposure to tobacco smoke is also associated with nuclear and posterior subcapsular cataract formation, and AMD.[40,41]

This highlights a fascinating interaction between triggers of ocular allergy and other ocular disorders worthy of attention. Smoke from either smoking tobacco or industrial fumes affects the ocular surface, which leads to symptoms such as itchiness, redness and irritation of eyes characteristic of allergic and dry-eye diseases. Smoke causes an alteration in the lipid layer of tear film, reduced tear secretion and decreased corneal and conjunctival sensitivity. Apart from triggering allergy, smoking increases the risk of developing chronic open-angle glaucoma.[42] Such an interrelationship that borders on allergens, allergic diseases and ocular diseases of peculiar characteristics among Africans is not clearly outlined in the literature. The level of humidity also affects the eyes: dry-eye symptoms worsen in dry and windy weather, but increased humidity is soothing for dry eyes. In well air-conditioned places such as department stores, malls and aircraft, low humidity has implications for ocular allergic complications, particularly dry eyes.

Furthermore, an allergic reaction in the form of dermato-conjunctivitis may occur as a result of a reaction to eye drops, cosmetic products, clothing, jewellery, plastics, animal or vegetable matter, or industrial waste and chemicals being instilled into the eyes.[43] Eye medications and preservatives commonly known to induce ocular allergic reaction include neomycin, gentamicin, idoxuridine, atropine, thimerosal and penicillin.[44] A hypersensitivity response may also occur in reaction to the toxic protein of bacterial disintegration, as in the case of chronic staphylococcal blepharoconjunctivitis, *Candida albicans*, *Coccidioides immitis*, chlamydia, parasites and lymphogranuloma venereum.[45]

Allergens are now everywhere and are posing a threat to susceptible eyes, throats and skin. Human activities and climate change are synergistically creating a brutal allergy season that is predicted to worsen with passing years. In particular, the impact of climate shift on temperatures and greenhouse gases has affected the timing of the onset of allergenic pollen production, which may influence pollen abundance and/or potency.[46]

GENETICS OF OCULAR ALLERGY

The interplay of genetics and the environment in the patho-mechanism, severity and natural history of allergic diseases has long been pinpointed.[47] However, most studies on the genetic basis of allergic disease have focused attention on other manifestations such as asthma, eczema and rhinitis. The patho-mechanistic and clinical relationship between ocular-allergic diseases and this cohort of allergic diseases has for a long time been extrapolated to enhance our understanding of the genetic predisposition to ocular allergy. Hereditary studies indicate that atopic conditions frequently occur within families and that children born to allergic parents have an increased risk of developing an atopic condition in their lifetime. For instance, of the various entities of allergic eye diseases, acute allergic conjunctivitis has a strong hereditary tendency;[48] and a 64% correlation was found between the incidence and family history of atopic keratoconjunctivitis, which is highly suggestive of a genetic association.[49]

But aspects of the genetics of ocular allergy indicate peculiarities independent of other allergic diseases. The inheritance of ocular–allergic disease does not obey the classical Mendelian order, indicating that the genetic underpinnings of ocular-allergic disease are complex and influenced by multiple factors.[50] Numerous studies have shown that allergen recognition and antibody response are affected by the HLA-Linked genes; however, studies that attempted to investigate a similar link between HLA-Linked genes and ocular-allergic disease have been conflicting.[51] Chromosomal studies through genetic mapping have indicated anomalies in the gene cluster encoding for cytokines, including IL-4, IL-13, IL-3, IL-5 on chromosome 5 and interferon-gamma, and these have implications for asthma and other atopic diseases. This has always been extrapolated to allergic eye diseases, but recent evidence suggests the involvement of chromosomes 5, 16 and 17 for allergic conjunctivitis, with additional weak linkage detected for chromosome 6 for specific allergens.[52] It can therefore be hypothesised that the genetic linkage for allergic conjunctivitis differs from that reported for asthma and other atopic disorders because organ-specific disease-susceptible genes, together with general atopy genes, may interact in the allergic response to specific mucosal tissues. The genetic correlation between ocular allergy, comorbidities and complications remains largely a grey area in need of further scientific investigation.

PHARMACOLOGICAL MANAGEMENT AND ITS RAMIFICATIONS

It is common for ocular allergy suffers to not seek immediate treatment for their ailments owing to the perception that their symptoms are not serious and will resolve by themselves.[53] This attitude often leads to complications and may, therefore, require treatment with one or more pharmaceutical agents. Steroidal preparations, antihistamines and mast-cell-stabilising agents have long been the mainstay treatment for allergies. Some of these, after long-term use, have been linked to grave adverse ocular and systemic consequences such as cataract formation and glaucoma.[54] The use of systemic antihistamine for the management of ocular allergy has been found to be a common practice among most practitioners due to the fact that most patients present with other manifestations such as AR.[55] The use of systemic antihistamines has also been associated with a high incidence of dry eye.[56-58]

Systemic antihistamines decrease mucous and aqueous production, which is crucial to the maintenance of the ideal pre-corneal tear film.[59] Vasoconstrictors should be prescribed with caution when this treatment is intended for patients with a history of hypertension, cardiovascular disease, arrhythmias, diabetes mellitus, hyperthyroidism or angle-closure glaucoma.[60] This is because these individuals have anti-cholinergic tendencies, which can result in mydriasis and therefore potential exposure to retinal photo-oxidative damage.[61] Lately, topical immunomodulators, including cyclosporines and tacrolimus, have been gaining recognition in the long-term treatment of severe allergic eye disease. However, owing to their lipophilic nature, they require an alcohol–oil base for dissolution, which causes ocular irritation such as burning, tearing, erythema, itching and headaches upon administration.[7]

Most of the readily available anti-allergic medications meant for allergic eye diseases are sold over the counter, despite their potential to contribute to the high prevalences of glaucoma, cataract and dry eye.[62,63] The component contribution of treating allergic eye disease pharmacologically to the prevalence of dry eye, cataract and glaucoma, especially in poor regions of the world, cannot the underestimated.

ECONOMICS OF OCULAR ALLERGY

The multifactorial dimensions of ocular allergy suggest an enormous financial implication not only to suffers but to healthcare planners, policy-makers and governments across the globe. The cost of treating AR is determined and pegged at a total direct medical cost of US$3.4 billion, with almost half of this cost attributable to prescription medications.[64] An attempt at estimating the cost of allergic eye disease highlighted only seasonal allergic conjunctivitis; the study alluded to an obvious potential for underestimation, since its emphasis was on patients who accessed private and not public healthcare services – the total mean per-sufferer cost of subscriber acquisition cost (SAC) was €348.50.[65] In another study in the United Kingdom, the total for both the public healthcare and private out-of-pocket costs of SAC was £64.61 for retirees and £123.69 for individuals in paid employment.[66] The estimated cost burden in treating ocular allergy in the United States alone is approximately US$5.9 billion.[67] The management of severe and chronic forms of

An **advancement in corticosteroid therapy** that treats the full spectrum of inflammatory mediators[1-3]

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Scheduling status: S4 **Proprietary name and dosage form: Alrex** Ophthalmic Suspension. **Composition:** Each 1 ml contains: Loteprednol etabonate 2,00 mg (0,2 % m/v) and Benzalkonium chloride (preservative) 0,01 % m/v. **Pharmacological classification:** A 15.2 Ophthalmic preparations with corticosteroids. **Registration number:** 38/15.2/0203.

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allergic eye diseases also poses a cost burden resulting from specialists' consultation to sufferers because patients may need care from allergists, dermatologists and paediatricians for the same condition and its comorbidities.[1]

The ideal means of ensuring the control and prevention of allergies other than the mere pharmacological treatment of allergic episodes in its various forms may even have far-reaching economic consequences. The multi-dimensional aspects of genetics, global pollution and climate change, if well evaluated, could give a clear perspective of the economics of ocular allergy. The study of ocular allergy and its co-morbidities has always been associated with its cohort of asthma, rhinitis, etc. Critical areas such as the additional costs of treating comorbidities with glaucoma, cataract, dry eye, keratoconus and pterygia are yet to be assessed.

CONCLUSION

It is clear that ocular allergy could be more than an isolated common disease entity. The relationship between allegic diseases and other medical conditions requires careful attention if we are fully to appreciate the consequences of these diseases. This may require the concerted effort of diagnosticians, researchers, governments, and healthcare planners and policy-makers to prioritise ocular allergy, among other diseases.

DECLARATION OF CONFLICT OF INTERESTS
The authors declare no conflicts of interest.

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Research Article

ALLERGENICITY OF DOMINANT AEROPOLLEN IN NIGERIA (PART II)

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ABSTRACT

Until the publication of the Part I of this research series, the purification and characterisation of allergenic components in pollen grains did not exist in Nigeria. To close this gap, Part II is presented here. Four pollen grains – *Alchornea cordifolia* (Schumach et Thonn) Mull Arg (Christmas bush), *Amaranthus hybridus* L (African spinach 'green'), *Casuarina equisetifolia* L (whistling pine) and *Terminalia catappa* L (Indian almond tree) – found dominant in the air from previous aeropalynological studies in Nigeria were selected. Pollen grains were collected from anthers (through crushing and sieving) and their proteins were extracted and quantified (using standard methods), separated (using SDS-PAGE) and subjected to an allergenicity test (using Western-blot). After this, the allergenic proteins were identified and their peptide sequences were deposited in the proteomics database. *A hybridus* had the highest protein content (19.15 mg/mL), whereas *C equisetifolia* had the lowest (7.48 mg/mL). However, hypersensitive individuals were most susceptible (84%) to the 58 kDa protein in *A cordifolia*. Each of the pollens studied had one dominant allergenic protein. These protein bands were newly registered in the database, since there were no previous entries. Profilin (14 kDa) was the group to which two of the protein bands belong (*T catappa* and *A hybridus*) – an indication of the broad-spectrum immunotherapeutic potential of Profilin. Part II is the first study in Nigeria to report allergenic proteins in these pollen grains, and it is hoped that the results will spur further research in allergenic proteins to aid allergy diagnosis and treatment in Nigeria.

Keywords: allergenicity test; immunotherapy; Nigeria; pollen grains; Profilin

INTRODUCTION

Allergies are a heavy socio-economic burden worldwide. Gilles and Traidl-Hoffmann[1] remarked that there is a deficit in public awareness, education and training on allergies; therefore efficient prevention strategies are urgently needed. Pollen grains are the major cause of allergies in urban areas.[2] Consequently, pollen grains that trigger allergies are often specific to the local region where the pollen-producing plant is dominant or exists. This makes it very important to identify allergy-causing pollen grains from each local region.[3] Furthermore, the allergenic proteins in these pollen grains can be extracted, identified, characterised and purified for immunotherapeutic purposes. The overall goal is often to develop pollen-specific allergy immunotherapy vaccines for allergy sufferers in the form of immunotherapeutic treatment.[4,5]

Research involving allergenic protein identification and characterisation was non-existent in Nigeria before Adeniyi et al[3] began research on the allergenicity of dominant aeropollen in the country. They investigated the dominant pollen grains of grasses (*Cynodon dactylon* – Bermuda grass and *Panicum maximum* – Guinea grass) and sedges (*Cyperus rotundus* – tiger-nut sedge and *Mariscus alternifolius* – umbrella sedge). In addition, their allergenic proteins were identified and documented appropriately. In continuation of the series of research to investigate the allergenicity and allergenic proteins of popular aeropollen in Nigeria, the authors selected four pollen grains. These prevalent pollen taxa are *Alchornea cordifolia* (Schumach et Thonn) Mull Arg (Christmas bush), *Amaranthus hybridus* L (African spinach 'green'), *Casuarina equisetifolia* L (whistling pine) and *Terminalia catappa* L (Indian almond tree) found to be dominant based on results of various aeropalynological studies across Nigeria. These include Agwu,[6] Agwu et al[7] and Njokuocha[8] in southeast Nigeria; Adeonipekun,[9] Adeniyi et al[2] and Adeonipekun et al[10] in southwest Nigeria; and Ezike et al[11] in north-central Nigeria.

MATERIALS AND METHODS

POLLEN HARVESTING

Anthers of *A cordifolia, A hybridus, C equisetifolia* and *T catappa* were collected, dried at room temperature and then converted to powder by being crushed with a glass mortar and pestle. Powder was filtered through 200, 300 and 400 µm mesh sizes to collect fresh pollen and sieve out anther and other floral components.[3]

PROTEIN EXTRACTION

Pollen grains were incubated and rotated overnight in phosphate buffer saline (PBS) and detergents (SDS 4%). After centrifugation at 20 000 g at 4°C for 15 minutes, supernatants were collected and stored at −20°C. Protein concentration was measured using the Bradford method.[12] Protein-standard bovine serum albumin (BSA), was diluted with 0.15 M NaCl to final concentrations of 0 (blank = NaCl only), 250, 500, 750 and 1 500 µg/mL. Serial dilutions of each unknown pollen sample were also prepared. One hundred microlitres of each sample were poured into a spectrophotometer tube and 5.0 mL of Coomassie Blue was added to each tube; the mixture was then vortexed. Spectrophotometric readings were taken at a wavelength of 595 nm, and blanked using the tube which contained no protein. The absorbance of the standards versus their concentration was plotted and the protein content of the unknown pollen samples was calculated from the extinction coefficient.[3]

PATIENTS' SERA

Approval from the Ethics Committee of Gbagada General Hospital, Gbagada, Lagos State, was obtained before the research commenced. Afterwards, a registered immunologist collected blood samples from 50 allergy patients with a history of allergies and asthma. Patients were selected based on the following inclusion criteria:

- history of allergies;
- timing and seasonality of allergies;
- age range between 18 and 65;
- allergic condition is not life threatening, and
- Lagos State residency.

For each analysis, the sera from five individuals with no history of allergy were used as controls. Blood was collected using syringes and anticoagulant tubes. The tubes were inverted eight times to distribute the additive evenly. Centrifugation was done immediately at 1 300–1 800 rpm for 10–15 minutes. The cell-free supernatant plasma (serum) was removed carefully using a syringe. The extracted sera were placed in polypropylene vials and sodium azide (0.02%) was added. The vials were stored at 4°C.[3]

GEL ELECTROPHORESIS

Proteins from pollen extracts were separated according to their relative mass (Mr) in SDS-PAGE using 12% gel.[13,14] Ten microlitres of the protein sample was heated with an equal amount of sample buffer (0.06 M is HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5% β-mercaptoethanol, 0.01% Bromophenol Blue at 100°C for 3 minutes. It was then loaded into the well of the gel and electrophoresis performed using the Laemmli buffer system (0.05 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.4) at room temperature at 70 V for 2 hours 30 minutes. The gel was calibrated with a marker mixture consisting of myosin from rabbit muscle (MW 205 kDa), β-galactosidase from *Escherichia coli* (MW 116 kDa), phosphorylase from rabbit muscle (MW 97.4 kDa), albumin from bovine (MW 66 kDa), albumin from egg (MW 45 kDa) and carbonic anhydrase from bovine erythrocytes (MW 29 kDa) obtained from Sigma Company, United States.[3] Afterwards, the gel was stained with 0.1% Coomassie Brilliant Blue R 250 mixture to take photographs before being blotted onto a cyanogen-bromide activated nitrocellulose (NCa) membrane for further Western blot.[3]

WESTERN BLOT

Protein bands obtained by SDS-PAGE were transferred by semi-dry blotting (45 minutes, 0.8 mA/cm^2) onto nitrocellulose membranes. The membranes were cut into strips, washed with 0.05% Tris-buffered saline-Tween buffer (TBST: 50 mM Tris, 150 mM NaCl pH 7.4, 0.05% Tween20) for five minutes at room temperature (25ºC), blocked twice with 0.3% TBST for 30 minutes, washed again, and incubated overnight in a 1 : 10 dilution of sera from patients (n = 50) and controls (n = 5).[3] Strips were subsequently washed four times with 0.05% TBST buffer and incubated with a 1 : 750 dilution of goat antihuman IgE-alkaline phosphatase (AP) for 3 hours. Antibody binding was detected by staining with the chromogenic substrate nitro-blue tetrazolium, 5-bromo-4-chloro-3'-indolyphosphate.[3,15]

IDENTIFICATION OF ALLERGENS

The spots of interest were analysed by the peptide mass fingerprinting method.[16] Protein spots were excised from the stained gel. Proteins were then reduced with di-thio-threitol, alkylated with iodoacetamide and in gel-digested with porcin trypsin. The resulting peptides, in solution, were added to the matrix (α-cyanohydroxy-cinamic acid) and submitted to laser shot in the mass spectrometer (Applied Biosystem Voyager DE-STR instrument, Texas, United States). Peptide listings were then submitted to databases using a ProFound proteomic tool (ExPaSy Proteomics Server).[3]

TABLE I: PROTEIN CONTENT OF POLLEN

POLLEN	PLANT FAMILY	COMMON NAME	PROTEIN CONTENT (MG/ML)
A cordifolia	Euphorbiaceae	Christmas bush	16.85
A hybridus	Amaranthaceae	African spinach 'green'	19.15
C equisetifolia	Casuarinaceae	Whistling pine	7.48
T catappa	Combretaceae	Indian almond tree	16.49

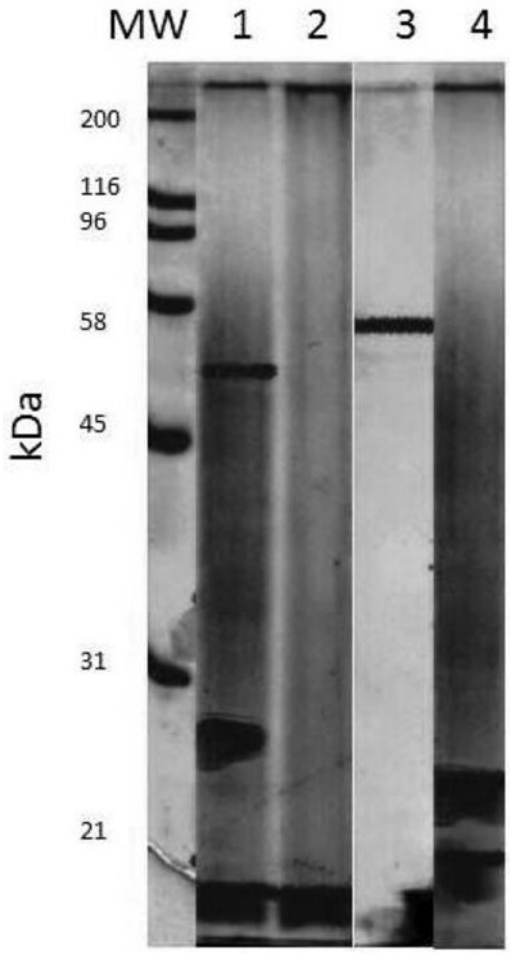

Figure 1: Protein profiling of different pollen types in 12% denaturing gel following SDS PAGE; 1 – T catappa, 2 – A hybridus, 3 – A cordifolia and 4 – C equisetifolia

RESULTS

PROTEIN CONTENT

A hybridus had the highest protein content (19.15 mg/mL), whereas *C equisetifolia* had the lowest (7.48 mg/mL) (see Table I).

GEL ELECTROPHORESIS

The protein profile of *A cordifolia* pollen showed one major band (see Figure 1, Lane 3) with a molecular weight of 58 kDa. *A hybridus* pollen showed one protein band at 14 kDa (see Figure 1, Lane 2). *C equisetifolia* pollen showed two protein bands at 17 kDa and 25 kDa (see Figure 1, Lane 4). The protein profile of *T catappa* pollen showed two major bands (see Figure 1, Lane 1) with molecular weights 14 kDa and 50 kDa.

WESTERN BLOT

A dominant protein fraction of 58 kDa in *A cordifolia* pollen extract was observed to indicate 84% (42 individuals) IgE binding activity (see Figure 2a). The dominant protein fraction of 14 kDa in *A hybridus* pollen extract was observed to indicate 24% (12 individuals) IgE binding activity (see Figure 2b). The dominant protein fraction of 17 kDa in *C equisetifolia* pollen extract was observed to indicate 54% (27 individuals) IgE binding activity, while the 25 kDa protein was not allergenic (see Figure 2c). The dominant protein fraction of 14 kDa in *T catappa* pollen extract was observed to indicate 40% (20 individuals) IgE binding activity, while the 50 kDa protein was not allergenic (see Figure 2d).

ALLERGEN IDENTIFICATION

All the allergenic protein bands were linked to homologues while no proteins had their exact matches in the database. Therefore, the newly sequenced four allergenic proteins were deposited

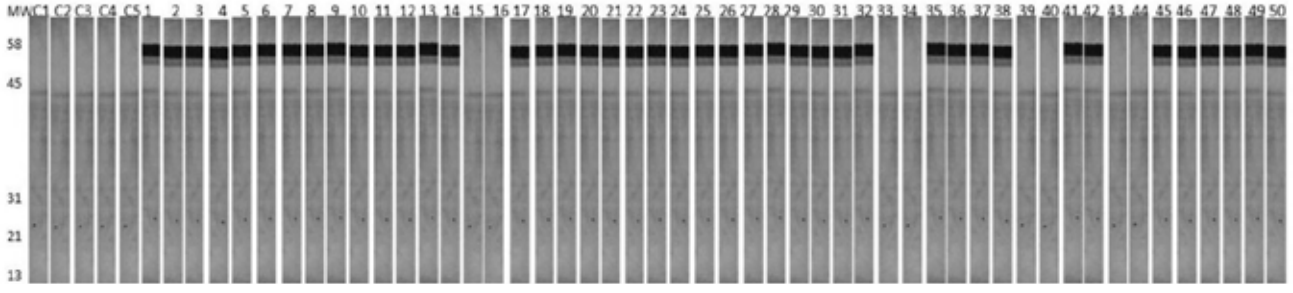

Figure 2a: Immunoblotting of A cordifolia pollen extracts. IgE-reactive protein bands are darker. MW = Molecular Weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals.

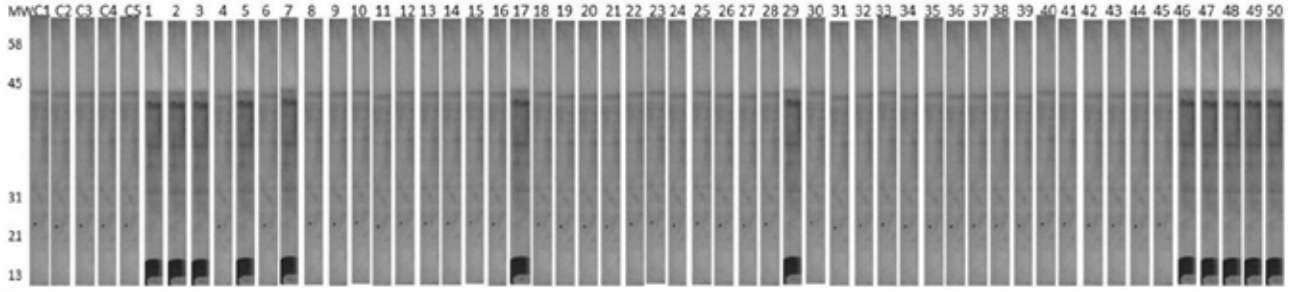

Figure 2b: Immunoblotting of A hybridus pollen extracts. IgE-reactive protein bands are darker. MW = Molecular Weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals.

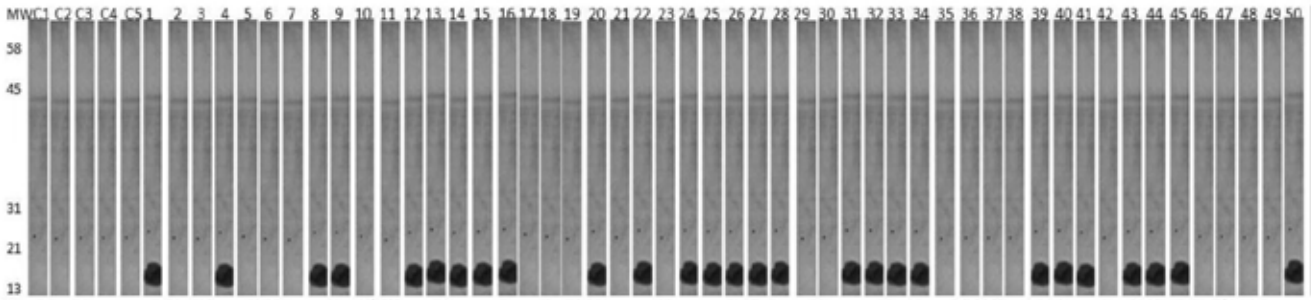

Figure 2c: Immunoblotting of C equisetifolia pollen extracts. IgE-reactive protein bands are darker. MW = Molecular Weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals.

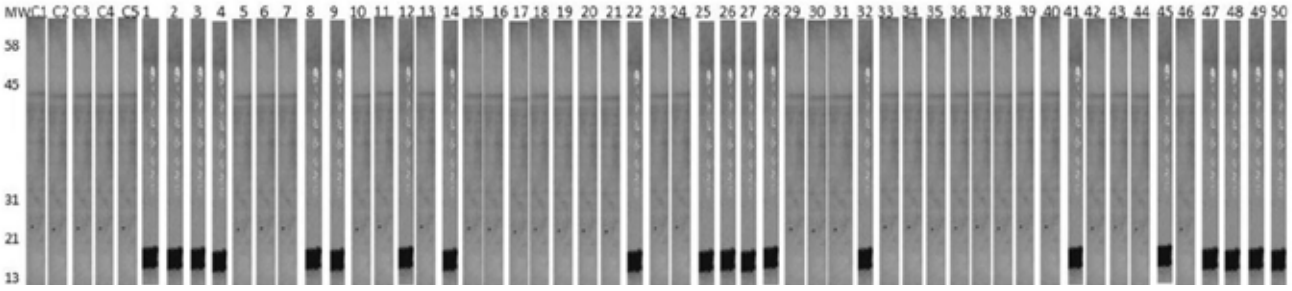

Figure 2d: Immunoblotting of T catappa pollen extracts. IgE-reactive protein bands are darker. MW = Molecular Weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals.

TABLE II: ALLERGEN IDENTIFICATION

POLLEN	PROTEINS			
	PROTEIN (DA)	NAME	HOMOLOGUE ALLERGEN	PLANT ORIGIN
A cordifolia	58 000	Lecithinase	Hev b 4	*Hevea brasiliensis*
A hybridus	14 000	Profilin	Ama r 2	*Amaranthus retroflexus*
C equisetifolia	17 000	PR-10	Bet v 1	*Betula verrucosa*
T catappa	14 000	Profilin	Ole e 2	*Olea europaea*

in the ProFound database with unique UniProt Accession numbers. Profilin (14 kDa) was the group to which most of the protein bands belong (2 – *T catappa* and *A hybridus*). However, the profilin of *T catappa* was related to allergen Ole e 2 of *Olea europaea,* whereas *A hybridus* profilin was related to Ama r 2 of *A retroflexus*. The 58 kDa lecithinase of *A cordifolia* was related to Hev b 4 of the tree *Hevea brasiliensis*, which is also in the Euphorbiaceae family. The 17 kDa PR-10 protein of *C equisetifolia* was related to Bet v 1 of *Betula verrucosa*, which is also a tree in the order Fagales (see Table II).

DISCUSSION

Allergy-causing pollen grains contain allergenic proteins. The susceptibility of allergy sufferers to each protein allergen is different and this necessitated this research series. Part I of the series[3] investigated the allergenic proteins in the aero-dominant pollen of grasses and sedges. The protein level of the dominant pollen grains studied range from low (7.48 mg/mL in *C equisetifolia*) to moderately high (19.15 mg/mL in *A hybridus*). This is within the pollen protein range reported by Stanley and Linskens[17] (5.9 and 28.3 mg/mL). For *C equisetifolia* pollen,a similar protein content (7.18 mg/mL) was reported by Prakashkumar et al[18] for pollen grains of the plant collected from India in 1998. This similarity and the ability of the plant to retain a particular protein content range despite weather, climatic and geographical changes, together with differences in the samples could be an adaptive potential. The plant should be studied for its resilience and possible adaptive properties to these changes, especially climate change.

Regardless of the protein contents, allergenicity studies revealed that *A hybridus* (with the highest protein content) had low reactivity (12 individuals out of 50), whereas *C equisetifolia* (with the lowest protein content) had a higher reactivity (27 individuals out of 50). Prakashkumar et al[18] and Adeniyi et al[3] recorded similar trends of results in which high pollen protein content did not necessarily indicate a high number of susceptible individuals. The allergenicity ranking of pollen grains is therefore dependent on the type of allergenic proteins in the pollen and the nature of the susceptibility of the individuals selected for the study. Although this is the first documentation of the *A hybridus* pollen protein profile, which showed one protein band at 14 kDa, species in the same genus have been documented previously. Singh and Dahiya[19] reported that the protein profile of *A spinosus* pollen had a range of 14–70 kDa. Also, Tehrani et al[20] reported that the protein profile of *A retroflexus* pollen had a range of

10–85 kDa. However, the differences in protein profiles could be attributed to factors such as weather, soil type, herbivory, and several environmental parameters to which the plants could be responding to.

In the case of *A hybridus* in this report, the allergenic 14 kDa protein had low hypersensitivity (12 of 50 individuals). The protein was identified as a Profilin with homologous similarity to Ama r 2 of *A retroflexus.* The low allergenicity of this profilin protein was also reported by Tehrani et al[20] (3 of 16 individuals) in *A retroflexus*. However, unlike *A hybridus* in this report, where only one allergenic protein was identified (14 kDa Profilin), Tehrani et al[20] reported six allergenic proteins in *A retroflexus,* of which the 45 kDa protein was the most allergenic. This protein was not identified in this work in *A hybridus*, suggesting either that the protein may be unique to *A retroflexus* or that several other environmental factors (as listed above) may be responsible for the protein content of the plant. Pollen grains from trees in the order Fagales are a major cause of pollinosis in the temperate regions of the northern hemisphere.[21] Similarly, the pollen grains of *Casuarina* (Family Casuarinaceae; Order Fagales) have been implicated in allergies; especially in the tropical regions of the northern hemisphere where the tree was introduced in the early 19th century. The major Fagales pollen allergen is the 17 kDa PR-10 protein, Bet v 1 of *Betula verrucosa*, which is highly allergenic[21]. In this work also, the 17 kDa *C equisetifolia* pollen protein with a medium allergenicity of 54% is homologuely related to Bet v 1. Therefore, the cultivation of this tree as an ornamental in southern Nigeria should be discouraged so as to reduce the volume of *C equisetifolia* pollen in the air and its risk to hypersensitive individuals. Studies should be done on better ornamental trees that can provide shade and which possess non-allergenic pollen as replacements for *C equisetifolia.* Furthermore, extensive studies need to be carried out on trees and other plants before their introduction into different climes.

Another tree pollen grain of high significance in the present work is *Terminalia catappa.* This is the first report to implicate this taxon of the family Combretaceae in allergy sensitivity. *T catappa* is a tree mainly grown for its fruit, for ornamental purposes and its shade properties in Lagos State. The only allergenic protein of 14 kDa identified in *T catappa* pollen is a profilin similar to Ole e 2 of *Olea europaea.* The protein had a mild sensitivity of 20%. Nonetheless, the authors suggest that there should be a reduction in the planting of the tree as a shade plant in public areas such as roadsides and academia so as to minimise the exposure of hypersensitive individuals to its pollen.

Alchornea cordifolia is in the family Euphorbiaceae, where two major plants have been implicated in allergies – the rubber tree (*Hevea brasiliensis*) latex and the castor bean (*Ricinus communis*) pollen. The results of Parui et al[22] on *R communis* pollen indicated that there were no similarities between the allergenic proteins identified and the 58 kDa allergenic protein of *A cordifolia* pollen identified in this work. However, the allergenic 55 kDa protein (Lecithinase – Hev b 4) identified by Palosuo et al[23] in *H brasiliensis* latex has similarities to the 58 kDa allergenic protein of *A cordifolia* pollen identified in this work. The 58 kDa *A cordifolia* pollen protein showed a high allergenicity of 84%. This poses great risks for hypersensitive individuals, especially those within close proximity to freshwater swamps, canals, wetlands, secondary forests and riverine forests[24] where the plant has been seen to be dominant. Also, its abundant production of pollen grains, which are widely dispersed and often remain in the atmosphere throughout the calendar year,[2,10] poses another major risk. Because of these properties, the authors suggest an integrated weed management approach for *A cordifolia*: the plants' populations should be reduced to the barest minimum in urban canals/swamps and such areas should be re-vegetated to allow the animal species to survive; *A cordifolia* should remain in urban wetlands (where they certainly perform ecological functions), but they can be pruned regularly to prevent flowering; cut/thinned *A cordifolia* plant parts should be disposed of in an environmentally friendly manner (for instance, sold to medicinal practitioners, used as compost) to prevent regrowth in another environment.

The results of this investigation have identified allergenic proteins in the pollens studied: that of *A cordifolia, A hybridus, C equisetifolia* and *T catappa*). These pollens are often dominant in the air in Nigeria. The allergenic proteins can be used to develop immunotherapy drugs to aid allergy treatment in Nigeria. Furthermore, the Profilin protein which was present in all pollen (*Cynodon dactylon, Cyperus rotundus, Mariscus alternifolius* and *Panicum maximum*) investigated in Part 1 of the series is also present in two of the studied pollen (*A hybridus* and *T catappa*). This further buttresses the need to research the immunotherapeutic potential of this broad-spectrum allergenic protein.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

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SHOE-ALLERGIC DERMATITIS AFFECTING A SANITATION WORKER

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ABSTRACT
Occupational skin disease is a common condition globally, affecting men and women of all ages. The majority of cases are due to irritant and allergic contact dermatitis (ACD) of the hands and feet. Sanitation workers are an occupational group commonly affected by shoe dermatitis. The prevalence of shoe-allergic dermatitis varies from region to region and is between 1.5% to 25% of those attending specialist contact dermatitis clinics, depending on the types of shoe allergen commonly found in a region. The prevalence also varies depending on the nature of the work performed, the type and style of the shoes worn, pre-existing medical conditions, social disparity, and climatic and geographical conditions. Sanitation workers are exposed to a variety of hazards, including sun exposure, infective organisms, sewage, dirt and sharp objects, which necessitate the consistent use of impervious safety shoes. The prolonged use of these safety shoes creates an occlusive, moist environment, with the retention of perspiration from heat, physical activity and hyperhidrosis contributing to skin irritation, maceration and barrier dysfunction, which then facilitates exposure to shoe allergens present in leather, rubber, dyed fabric, plastic and adhesives. This article highlights a case of a non-atopic sanitation worker with shoe-allergic dermatitis and a fungal infection, and emphasises how to approach the diagnosis and management of shoe dermatitis.

Keywords: allergic contact dermatitis; shoe-allergic dermatitis, allergens, occupational skin disease

INTRODUCTION

Occupational skin disease (OSD) is common globally and accounts for up to 34% of reported occupational diseases (ODs).[1,2] In South Africa, OSD outside the mining industry accounted for 14% reported ODs in 1999[3] and 6% between 2000–2005.[4]

Of all OSDs, the majority (90–95%) are due to irritant contact dermatitis and ACD.[2,5] Burdzik et al found that 81% of 31 patients attending the Occupational Dermatology Clinic at Groote Schuur Hospital (Cape Town) with OSD had either allergic (68%) or irritant (13%) contact dermatitis. The most common primary site of involvement was the hands and/or the forearms (76%) followed by the hands and the feet (8%).[6]

Foot dermatitis can be classified as exogenous if it is caused by an external agent such as an infection, contact allergens and/or irritants, or endogenous if it is caused by an underlying skin condition such as atopic eczema or psoriasis.[7]

The prevalence of OSD varies from region to region and is between 1.5% to 25% of those attending specialist contact-dermatitis clinics, depending on the types of shoe allergen commonly found in a region.[7–11] OSD affects both sexes and any age group. It is difficult to compare prevalence rates between countries owing to a number of factors:

- the wide variety of shoe fashion trends in different regions of the world;
- the closely guarded shoe-production techniques and raw materials of differing quality;
- the components of the socks and stockings worn;
- daily foot- and shoe-care techniques, such as washing and maintenance;
- the use of treatment, especially topical antifungal foot powders and antibiotic preparations, and
- the wide-ranging climatic and geographical conditions in which shoes are worn.[7–10]

These discrepancies furthermore pose a therapeutic challenge in the diagnosis and treatment of shoe dermatitis.[7,13] Freeman notes that diagnosis becomes even more challenging when the shoe dermatitis is superimposed on a pre-existing underlying skin disease.[14]

Shoe dermatitis can be of either irritant or allergic type. Shoe-allergic dermatitis is a form of delayed type-IV hypersensitivity reaction which arises after feet come into contact with potentially sensitising substances such as those used in the processing of leather, rubber, dyes and adhesives.[5,7] These substances can be found in shoe components comprising the uppers (the shoe straps, vamp and tongue), the cap (made of steel in safety shoes), the soles (inner and outer sole), the sock found within

Figure 1: Anatomy of a safety shoe, showing the inner mesh lining (Source: Google Images)

the shoe, the materials used to make the heel and, occasionally, metal buttons and ornaments, as illustrated in Figure 1.[5]

Despite accurate diagnosis and treatment, shoe dermatitis can be chronic and debilitating, resulting in difficulties with walking during a period of greatest physical activity in adolescence and adulthood.[14,15] Rose and Rees of the National Institute of Occupational Health (NIOH) in South Africa interviewed 93 of 128 patients, seen between October 2006 and March 2008 with occupational contact dermatitis, and found that it was associated with job insecurity, difficulty completing work, financial loss and a poor self-esteem compounded by stigmatisation from colleagues and the employer.[16]

The following case report highlights a case of a non-atopic sanitation worker with a shoe-allergic dermatitis superimposed on a fungal infection referred to the Occupational Dermatology Clinic at a tertiary Cape Town hospital because of poor response to topical anti-fungal treatment.

CASE REPORT

A 55-year-old, non-atopic, previously well gentleman presented with a three-month history of severe tinea pedis with associated itch, mild swelling and interdigital maceration, pain and difficulty in walking – which improved after a month on an antifungal ointment prescribed by his general practitioner. The symptoms recurred within a month of completing the topical treatment, with increased dryness and fissuring of the feet, itch, moderate scaling and hyperkeratosis of both feet but now also involving the hands and sparing the rest of his skin. He was referred to a dermatologist for an opinion. At the time he was seen, he had developed crusting, redness, erosions and weeping over the dorsum of both feet, suggesting a secondary bacterial infection. He was therefore admitted for systemic antibiotic treatment with ciprofloxacin and potassium permanganate soaks.

He is employed as a municipal sanitation worker and performs a wide variety of tasks, including street sweeping, drain cleaning, road tarring (with exposure to tar and cement hardener), paving, and spraying insecticides and pesticides. He wears protective clothing consisting of an overall, leather safety boots, gloves and cotton socks, which he washes and changes on alternate days. The gloves were of the rubber-dipped, cotton, wrist-length type usually issued for crayfish handlers. He asserts that the onset of his skin symptoms followed the receipt of a new pair of leather safety boots provided in the workplace. He has a ninepack per year smoking history and he does occasional maintenance work including appliance repairs and painting using acrylic paint.

A diagnosis was made of a secondarily infected, partially treated inflammatory fungal infection of the feet with an id reaction involving the hands. The closed environment of the safety boots, vigorous manual work under warm weather conditions, perspiration and constantly wet feet from the prolonged exposure to drain spillage encountered when unblocking and cleaning municipal sewers/drains provided the ideal environment for the development of irritant contact dermatitis and microbial growth. ACD to components of his shoes could not be excluded, especially as he reported developing the lesions after he had been provided with a new pair of safety boots in the workplace.

He was discharged after four days on fluconazole 200 mg weekly for a month and Augmentin® (Amoxicillin + Clavulanic acid) 1 g twice daily for a week, to continue potassium permanganate soaks for infection and exudate control and liquid paraffin soaks to soften and hydrate the hyperkeratosis. A request that he be accommodated away from duties that required the use of safety boots was made to his employer, while acute management and investigations continued.

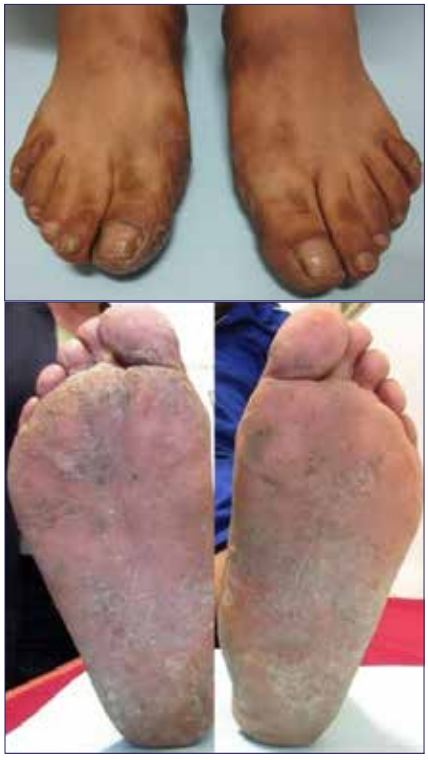

Figure 2: Patchy erythema, infiltration and scale crust on the dorsum of the feet, peri-ungual skin and the odd webspace. Individual dystrophic and discoloured toenails. Hyperkeratotic scaly plaques with fissures and the odd dry vesicle of both soles.

He was reviewed at the Occupational Dermatology Clinic, where a marked improvement was noted with no maceration between the toes. Ongoing patchy erythema, infiltration and scale crust was noted on the dorsum of the feet, peri-ungual skin and the odd webspace. Also noted were individual dystrophic and discoloured toenails and patchy residual dry vesicles, fissures and hyperkeratotic scaly plaques of both soles (see Figure 2).

A standard battery of 46 common commercial skin allergens was performed and graded according to the International Contact Dermatitis Research Group Guidelines[34] and left *in situ* for 48 hours. A weak (+1) reaction for neomycin, wool alcohol and lanolin was recorded 72 hours after application. These compounds are known shoe allergens (see Table I) but their relevance to this gentleman's presentation has not been conclusively demonstrated. To investigate further a diagnosis of an ACD to shoes, a specific patch test with samples from the safety boots and shoes he had used and those proposed for future use was performed. Pieces of leather, inner sole, breathable inner mesh and tongue, 1 cm^2 in size, were placed in individual Finn patch-test chambers and water-pre-moistened filter paper discs overlaid on each sample. The loaded chambers were applied to the back skin, after 48 hours of occlusion the patch tests were removed, and the reactions were then read 24 hours later (see Figure 3). Irritant reactions to several samples from the used safety boot and shoe were noted. A doubtful reaction (±) to the mesh from the used safety shoe and a weak (+1) reaction to the mesh from the Fuel safety boot proposed for future use were recorded. The findings from the specific patch test suggested a diagnosis of an occupational contact dermatitis with possible sensitisation to components of the safety boot mesh. The shoe allergy was superimposed on an irritant dermatitis and presumed fungal infection of the feet with an id reaction of the hands. No fungal cultures were done.

He was to continue with his prescribed treatment which, following confirmation of shoe dermatitis, included salicylic acid 10% as a keratolytic, Lassar's paste for sealing fissures and potent corticosteroid ointment for controlling the eczema. He was to keep his skin moisturised to limit the hyperkeratotic areas' fissuring. Socks should be cotton and changed daily in order to limit foot maceration and they should be washed with bland soap, preferably in hot water. He was advised that all safety boots proposed for use in the future should be evaluated for allergens with patch tests if necessary. In addition, the employer and the patient were provided with a resource on the products available to be used in instances of shoe-allergic contact dermatitis (see Table II).

His condition improved following workplace accommodation that reduced the time spent doing wet work and wearing the safety boots. However, he expressed a desire to return to his normal duties due to a lack of stimulation in his accommodated role as a gardener. Considering the occupational hazard of exposure to polluted water, impervious gloves and safety boots of the necessary length and material are essential personal protection equipment should he resume his work in the sanitation unit. Those he was using previously were inadequate as they allowed him to be contaminated by polluted waste. New Fuel safety boots for future use were tested in the special patch test; however, the mesh lining produced a positive reaction. It was impressed upon the employer and worker that permanent accommodation in areas that do not require the use of safety shoes should be considered.

EPIDEMIOLOGY OF SHOE DERMATITIS

Sanitation workers are responsible for maintaining cleanliness in many urban areas across the world.[17] In the developing world, however, this work is often done manually and exposes the workers to a variety of hazards, including sun exposure, infective organisms, sewage, sharp objects, and chemicals and substances released into the environment from industries (such as the construction, electroplating, tanning, dyeing and food- and drug-manufacturing industries) or households (cleaning agents, medications and gardening materials, including pesticides).[17] These exposures necessitate the consistent use of personal protective equipment such as impervious safety shoes, aprons, overalls, gloves and masks.[17–18]

A survey and general dermatologic evaluation of 87 street sanitation workers in India found the most common general skin problems included tinea versicolor (18%), dermatophytosis (16%) and contact dermatitis (10%). Some had more than one skin problem and there was inconsistent use of gloves, masks and footwear. No specific mention was made of foot or shoe dermatitis.[17] Yan et al found a statistically significant higher rate of occupational skin disease in the 273 sanitation workers in China compared to the 113 controls (administrative staff).[19] The most common skin problems related to overexposure to UV radiation and included 'prickly heat' (26% versus 8%) and dermatitis aestivale (15% vs 5%). In this case, no specific

TABLE I: PATIENT'S POSITIVE PATCH-TEST RESULTS

STANDARD BATTERY OF 46 ALLERGENS		
1+	Neomycin	Potent allergen; found in prescribed topical medication
1+	Wool alcohol and lanolin	Weak allergen; derived from sheep fleece used as textile finisher, shoe polish and on leather
PATCH TEST WITH OWN SHOE SAMPLES		
IR*	Shoe mesh (safety boot)	Possible exposure from dyes, biocides and fabric finishers used to produce mesh
IR*	Shoe mesh (safety shoe)	Possible exposure from dyes, biocides and fabric finishers used to produce mesh
Doubtful	Inner sole & leather (safety shoe)	Possible exposure from inner-sole adhesives, dyes, biocides and fabric finishers used to produce inner soles and leather chemicals
+1	Shoe mesh (Fuel safety boot)	Possible exposure from dyes, biocides and fabric finishers used to produce mesh

**Irritant reaction*

TABLE II: ALTERNATIVE PRODUCTS AVAILABLE FOR PATIENTS WITH SHOE-ALLERGIC CONTACT DERMATITIS*

MANUFACTURER AND CONTACT DETAILS	PRODUCT OFFERING
Askin Shoes www.askin.it; info@askin.it	Products excluding the following materials: Metal tanning agents: chromium salts, aluminium, zirconium, titanium, nickel, lead, copper, cadmium Acids: sulphuric, formic, oxalic, hydrochloric Biocides: trichlorophenol, pentachlorophenol, tributyltin oxide Formaldehyde resins Epoxy resins Amine catalysers Azoic catalysers Bactericides Isocyanides Carbamates
Ausangate Socks www.ausangatesocks.com/default.asp	Lanolin-free socks made of breathable alpaca fibre
Birkenstock Express www.birkenstockexpress.com	Cork and polyurethane foot-bed replacements
Birkenstock USA, LP info@birkenstockusa.com	Sandals and shoes do not use carba mix or allergens related to carba mix: zinc diethyldithiocarbamate diphenylguanidine zinc dibutyldithiocarbamate Products do not use: abitol, abietic acid, colophony, phthalate, p-tert-butylphenol formaldehyde resin (PTBP-FR) or thiuram mix Chrome VI is not used in the tanning process of leathers Soles do not contain mercaptobenzothiazole (MBT) Birkenstock buckles, rivets and staples are all made of steel and nickel free
The Cordwainer Shop info@cordwainershop.com	Glue-free linings for allergy-sensitive customers
Loveless Orthopedic Appliance contact@lovelessboots.com	Hypoallergenic liners for shoes and boots are offered
Microair Barrier Socks Alpretec www.alpretec.com/eng (for the list of distributors)	Barrier socks prevent skin from coming in contact with allergens
Multnomah Leather Shop www.multnomahleather.com/Allergy	Offers clogs made of chromate-free vegetable-tanned cowhide For those with rubber allergy; they use a wood midsole, isolating the wearer's feet from the cemented outsole
Po-Zu ww.po-zu.com; contact@po-zu.com	Use natural materials such as hemp, cork, coir and latex (from pure vegetable rubber), wool (100% unbleached and often undyed) and organically tanned leather Avoid glues
PW Minor and Son, Inc consumerhotline@pwminor.com	Offers hypoallergenic footwear, including chrome-free leather and the mnimal use of adhesives
Simple Way, The Leather Kit People www.simpleway.co.uk	Can make shoes to order without adhesives
Snipe http://www.snipe-shop.de/	Offers chrome-free tanned leather, natural cotton uppers, nickel-free metal, soles and laces made of natural materials
West Coast Shoe Company info@wescoboots.com	Offers chrome-free vegetable-tanned leather products

**Copied from Matthys et al[20]*

mention was made of foot or shoe dermatitis. The sanitation workers were supplied with gloves, broad-brimmed hats and long-sleeved clothes before the survey and it was found that younger workers used this protective equipment significantly more than older workers.[19]

Other occupations making use of heavy occlusive shoes, such as factory workers, security guards and military personnel, are prone to developing shoe-allergic contact dermatitis.[5,20]

Shoe dermatitis has been found to occur in all age groups; however, individuals between the second and fifth decade have been found to be most vulnerable, probably due to the increased frequency and duration of footwear use in this economically active group.[5,21] A prospective cross-sectional study at a dermatology outpatient clinic in India found that 81% (87/108) of patients with shoe dermatitis were between the ages of 20 and 50 years of age, which was in keeping with other studies from India.[7,22–24]

An increased prevalence of shoe-allergic dermatitis has been found in females compared to males (3 : 1) in Indonesian patients who were patch-tested. This is attributed to more frequent use of shoes of various types in females and the direct exposure of shoes to skin in warmer months when stockings are not worn.[13] The higher prevalence may also be because of better health-seeking behaviour in females compared to males and more regular exposure to water and household cleaning agents when doing household chores with bare feet in slippers.[13]

Shackelford et al reported a bimodal age distribution in American patients, noting that ACD of the foot was most common in those under 19 years of age and those between 41 and 60 years of age and in those who were atopic and male.[9]

AETIOLOGY AND RISK FACTORS

The major factors that contribute to the development of shoe-allergic contact dermatitis relate to the environment within shoes and the type and duration of exposure to shoe allergens.[15,20] The prolonged, occlusive, moist environment of shoes promotes skin contact with potentially immunogenic/allergenic substances/chemicals leached from the leather, rubber, fabric, plastic and adhesives used in their manufacture. The retention of perspiration from heat, physical activity and hyperhidrosis contributes to skin irritation and maceration, which then facilitates the skin penetration of these substances/chemicals.[8,15] Srinivas et al demonstrated that the chrome used for tanning leather is leached from the leather in the presence of perspiration.[25] It is postulated that it is probable that other chemicals from shoes may also be released by perspiration.[15] Other risk factors to consider are a history of eczema or skin disease, friction and medical conditions such as chronic venous insufficiency or those patients predisposing to hyperhidrosis.[8,20]

CONTACT ALLERGENS

Common shoe allergens are summarised in Table III. A North American retrospective, cross-sectional analysis of patch-test results documented between 2001 and 2004 aggregated patch-test data according to allergen groups. They found rubber additives to be the most common allergens (40.4%), followed by adhesives (32.5%) and leather components (20.1%).[5] The most common individual allergen was the adhesive, p-tertiary butylphenol formaldehyde resin (PTBFR), which accounted for 24.7% of positive patch-test results, followed by potassium dichromate (17.5%) used for tanning leather, and carba mix

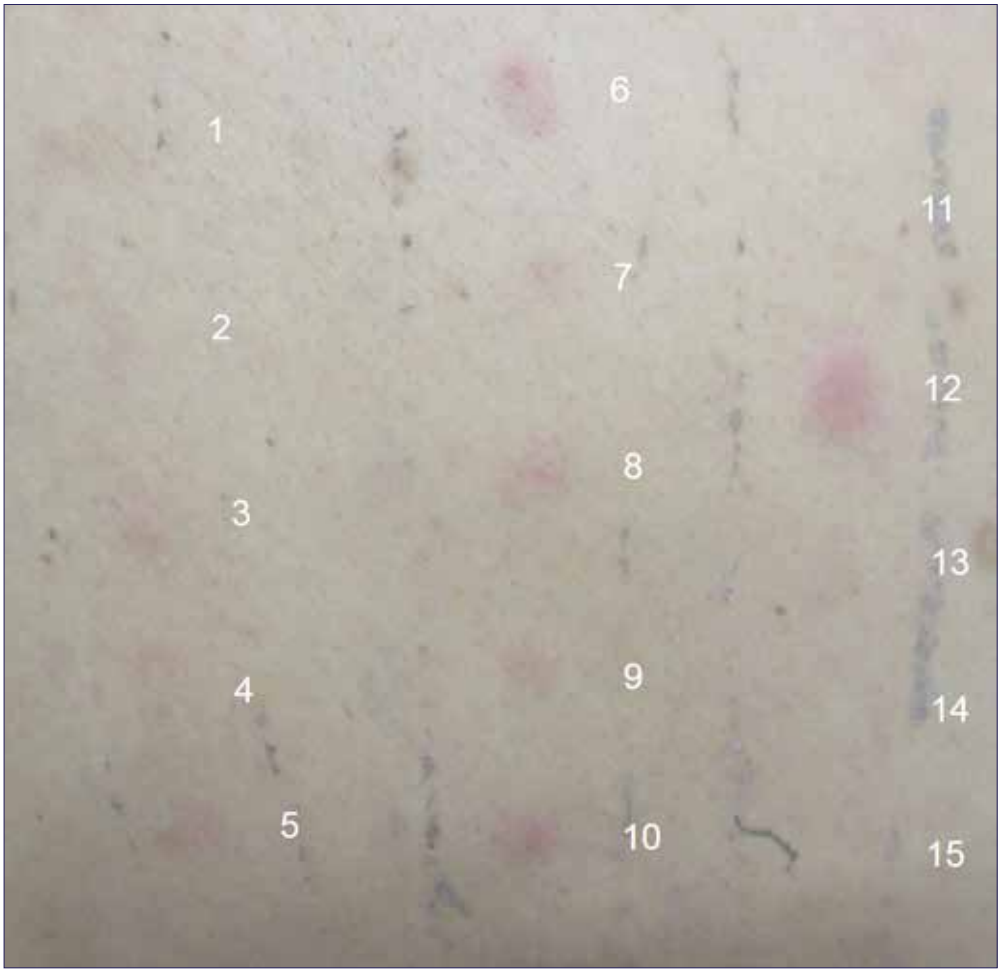

Figure 3: Patch-test results using pieces of safety shoes.
Safety boot: 1 polyurethane tongue; 2 boot leather; 3 blue inner sole; 4 tongue mesh; 5 yellow inner sole; 10 grey inner sole
*Safety shoe: **6 mesh** (±); 7 leather tongue; 8 white inner sole; 9 grey inner sole; 11 Empty*
*Fuel safety boot: **12 red mesh** (+); 13 leather tongue; 14 red inner sole; 15 black inner sole*

(11.7%), a fungicide and rubber additive found in rubber adhesives.[5]

Landeck et al found that among 2 671 foot-dermatitis patients, potassium dichromate, PTBFR, colophony, mercapto mix and mercaptobenzothiazole were significantly associated with foot dermatitis when compared to other body sites.[8] Nardelli et al, who patch-tested 1 168 Belgium patients with foot dermatitis, reported that of the 474 (5.5% of the total group) who had at least one positive reaction, potassium dichromate, cobalt chloride, p-phenylenediamine and rubber components were the most common shoe allergens.[10]

Landeck et al caution that significant variations in top-ranking shoe allergens should be expected as a consequence of distinct local shoe styles and customs, differing concentrations of chemicals and additives used in the production of shoes and polishes, including distinct environmental and weather patterns.[8] For example, in Africa, India and Europe, potassium dichromate predominates,[10,23,26–27] whereas rubber additives and adhesives appear to predominate in North America and Pakistan.[5,28] Chowduri and Ghosh argue that potassium dichromate allergy predominates in India due to the infrequent use of chrome-free leather because it is unavailable and expensive, whereas in Western countries rubber chemicals are more widely used and these countries are also moving towards using chrome-free leather.[23]

TABLE III: COMMON AGENTS CAUSING FOOT AND SHOE-CONTACT DERMATITIS [5,7,15,23]

AGENT	ALLERGENS
Leather chemicals	*Potassium dichromate *Formaldehyde *Glutaraldehyde
Rubber chemicals	*2-Mercaptobenzothiazole *Mercapto mix *Thiuram mix *Carba mix *Black rubber mix Dibutylthiourea Diphenylguanidine N-Cyclohexyl-2-benzothiazyl sulfenamide
Plastics	Hydroquinone monobenzylether Dioctyl phthalate
Dyes	*4-Phenyldiamine base 4-Aminobenzene
Adhesives	*Para-tertiary butylphenol *Epoxy resins *Colophony *Balsam of Peru Dodecyl mercaptan
Metals	*Nickel sulphate *Cobalt chloride
Textiles and fabrics	Socks and stockings
Topical medications	*Neomycin Monobenzylether of hydroquinone
Other accessories	Shoe polishes

** Shoe allergens in the standard commercial patch-test series*

CLINICAL APPROACH TO SHOE-ALLERGIC DERMATITIS AND THE EXCLUSION OF COMMON FOOT CONDITIONS

The diagnosis of shoe-allergic dermatitis is based on a history, the presence of suggestive skin lesions on examination and positive patch-test results, indicating sensitisation to one or more shoe allergens.

PATIENT HISTORY

A detailed history should be taken regarding the symptoms and duration of the dermatitis. Symptoms frequently include mild-to-severe itch together with redness and pain. As a delayed hypersensitivity reaction, shoe-allergic dermatitis can start within hours to as late as a week after exposure.[21] It is important to enquire about the type of shoes used (rubber, plastic, leather), the frequency and duration of use, and whether the onset of symptoms can be linked to a particular pair of shoes; this is because a temporal relationship would suggest shoe allergy and help to exclude other forms of dermatosis due to perhaps socks or topical medications.[7,14,21] Remission from or relief of symptoms upon removal of the shoes and exacerbation when shoes are used or during certain seasons where the weather is hot, humid or wet is also suggestive; however, this could also be due to an irritant contact dermatitis or tinea pedis.[21]

A personal and family background of atopy and other medical conditions such as hyperhidrosis and chronic venous insufficiency should be explored.[21] Atopic individuals have been found to have increased susceptibility to developing shoe-contact dermatitis while excessive perspiration in hyperhidrosis may increase the leaching out of shoe chemicals and subsequent sensitisation. Rasool and Sehgal found that in a cohort of 108 outpatients with shoe dermatitis, 31% had a personal and/or family history of atopy whereas the remaining 69% had no history of atopy.[7]

A social and occupational history on the type of work done and the use of occlusive protective footwear, exposure to water and chemicals at work and in the household and participation in hobbies such as fishing could provide a clue to the aetiology of the foot dermatitis. It may be helpful to enquire about toiletries used and the use of topical medication, as these have also been found to cause sensitisation (eg neomycin on patch test).[5,7]

PHYSICAL AND SKIN EXAMINATION

A meticulous physical examination will often reveal characteristic shoe-allergy morphology, namely, eczema, which includes erythema, vesico-papules and blisters of varying sizes, weeping, crusting and peeling at the site of contact. Left untreated, spread to adjacent areas, superadded infection, and features of chronicity including dry leathery skin with increased skin markings, hyperkeratosis, scale and altered pigmentation may develop. Shoe-allergic dermatitis is mostly bilateral and symmetrical, with a pattern that corresponds to the design of the footwear.[21] The dorsal aspect of the foot is the most commonly affected, usually starting with involvement of the big toe and extending to the rest of the foot.

An Indonesian prevalence study found the dorsum was affected in 47.6% of the patients.[13] A hallmark finding is the relative sparing of areas not in contact with the shoe, including the sparing of

the interdigital spaces and instep.[14,21,24] The involvement of other areas of the body should be examined, because ACD of the feet may also involve the hands, and *vice versa*.[29] Feet and hands may be exposed to the same allergy-causing agent such as leather chemicals in shoes and gloves or may be due to an infective process, as in the case presented where a fungal infection of the feet led to an id reaction (or autoeczematisation, a generalised acute cutaneous reaction to a variety of stimuli, including infectious and inflammatory skin) involving the hands.[14,29] A similar id reaction can occur with contact dermatitis affecting the feet. Shackelford and Belsito – in a five-year, retrospective, cross-sectional study – found that of the 70 patients with foot dermatitis at an American tertiary outpatient clinic, 29% had dermatitis confined to the foot whereas 71% had foot dermatitis plus the involvement of other areas (foot and hands at 40%).[9]

THE VALUE OF PATCH-TESTING

Patch-testing is ideal for testing for type-IV hypersensitivity reactions and is regarded worldwide as the gold standard for diagnosing and managing contact dermatitis. Freeman notes that the diagnosis of shoe allergy is often difficult to make without patch-testing, especially when superimposed endogenous foot dermatitis is present.[14] Disease-free skin should be used for patch-testing if the offending allergen is to be identified.[14]

Chemicals from a commercial battery of common allergens should be tested and expanded to include a shoe series. Thin samples (1–2 cm^2) taken from various parts of the patient's shoes are placed in patch-test chambers under 2 mm-thick filter paper discs moistened with saline. This has been found to reduce false negative results.[30] The filled chambers are applied to the skin and kept under occlusion for 48 hours. Reactions are read at 48, 72 and 96 hours after application. Less common is the extraction of chemicals from shoe materials using methods which include ultrasonic baths that enable the testing when a chemical is suspected but patch-testing is negative.[20] Common shoe allergens are listed in Table III.

DIFFERENTIAL DIAGNOSIS

Despite a thorough history and examination, shoe allergy can be difficult to diagnose clinically.[14] Consequently, other foot dermatoses should be considered, including fungal infections (tinea pedis), atopic dermatitis, psoriasis, dyshidrotic eczema, nummular eczema and juvenile plantar dermatitis.[9,31] Irritant dermatitis should also be considered, especially because of the occlusion, heat and friction that are associated with shoes. Shackelford and Belsito – in a five-year, retrospective, cross-sectional study – found that only 33% of their patients with foot dermatitis had ACD after patch-testing, whereas 43% had psoriasis.[9] Additional investigations, such as a skin scraping for fungal identification or skin biopsy, may be warranted.[21]

TREATMENT

Once it has been diagnosed, shoe-allergic dermatitis should be treated similarly to the treatment of contact dermatitis involving other parts of the body (see Table IV).[11,12]

Although these topical and systemic treatment options may be useful in the control of the eczema, the patient should be empowered to avoid using shoes, socks, toiletries or medicaments that contain the offending allergen, once identified. However, avoidance may be a challenge, particularly where no definitive cause has been identified.[11,12] Important adjunct control measures include the avoidance of products with unnecessary excipients; these include perfume, fragrances, preservatives and dyes.[21]

PREVENTION

The cardinal preventive measure for shoe-allergic dermatitis involves the use of products with little to no potential allergen exposure, such as chrome-free leather shoes, or the replacement of the normal leather shoes every few months in order to prevent chromium leaching out from the shoe onto the skin.[20] The patient should be provided with information on the various shoes available made with substitute material that do not cause allergic reactions. Matthys et al compiled a list of available products for shoe-allergic contact dermatitis that may be a useful resource for practitioners; this is summarised in Table II.[20] In the occupational

TABLE IV: TREATMENTS FOR VARIOUS FOOT DERMATOSES

PRESENTATION	TREATMENT
Acutely weeping lesions	The use of moist compresses, followed by air drying, has been found to be beneficial in reducing the drainage.[21,24]
Inflammation and itch	Can be reduced through the application of emollients and topical corticosteroids in an appropriate delivery vehicle[21] – the vehicle plays a vital role in the topical corticosteroid absorption and can improve its efficacy.[32]
Acute, wet dermatitis and hairy skin areas	Water-based lotions are ideal.
Moist or weeping lesions	Creams are appropriate.
Dry, scaly lesions that may also be thick and fissured	Ointments.
Hairy areas	Gels are most effective.[24]
Chronic lichenified dermatitis	Most effectively managed with regular steroid-containing ointments.[21,24,32]
Pruritis	Oral antihistamines may be effective.[24]
Secondary bacterial infection, the most common being β-haemolytic streptococci (group A, B, C, F and G), *Staphylococcus aureus*, *Streptococcus pneumoniae* and gram-negative bacilli, especially those of the *Pseudomonas* species[31]	Systematic antibiotics may be indicated.[31]

setting, personal protective equipment must be chosen in relation to the task and the potentional hazards. No single shoe meets all the requirements, and different shoes may be needed for different tasks. Damaged, ill-fitting or tight shoes and those that are re-dyed should be avoided. Excessive perspiration can be controlled through regularly (two to three times a day) changing cotton socks, taking breaks to air-dry the feet and using antihydrotics such as aluminium chloride hexahydrate to control foot perspiration.[21,24] A recent innovation in textile engineering has led to the development of a technological fabric, Microair®. Socks made from this three-layered fabric are designed as a barrier to prevent skin contact with shoe allergens and irritants but to provide good perspirability.[11] An improvement in quality of life (QoL) and a reduction in pain and itch in patients who tested these barrier socks was noted.[11]

CONCLUSION

Sanitation workers are exposed to a variety of hazards that require them to use personal protective clothing, including safety shoes, for prolonged periods. This case illustrates the role an occlusive shoe environment plays in promoting fungal growth and the leaching of shoe chemicals onto macerated skin, resulting in sensitisation. A definitive diagnosis of shoe-allergic dermatitis remains difficult in the absence of patch-testing, especially when there are other underlying skin diseases. The gentleman's initial suboptimal response to treatment was most likely due to undiagnosed shoe dermatitis being treated as a fungal infection only, without other causes such as shoe-allergic dermatitis, being considered. Other foot-skin diseases should also be considered, including fungal infections and psoriasis. The mainstay of management involves using products with little to no shoe-allergen exposure and addressing the risk factors specific to each patient.

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I wish to thank the patient for his consent to have his case discussed in this article. A special thank you must go to Dr Amy Burdzik of the Department of Occupational Medicine and Emeritus Professor Gail Todd of the Department of Dermatology, both at the University of Cape Town, for their guidance during the study of this case and in writing the article.

DECLARATION OF CONFLICT OF INTEREST

The author declares no conflict of interest.

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PID123

Primary Immunodeficiency

SPECIFIC ANTIBODY DEFICIENCY (SAD)

Liezel is a ten-year-old girl. She has been attending Dr Goodheart's paediatric clinic since she was five; first, with recurrent respiratory infections and later with recurrent bacterial meningitis.

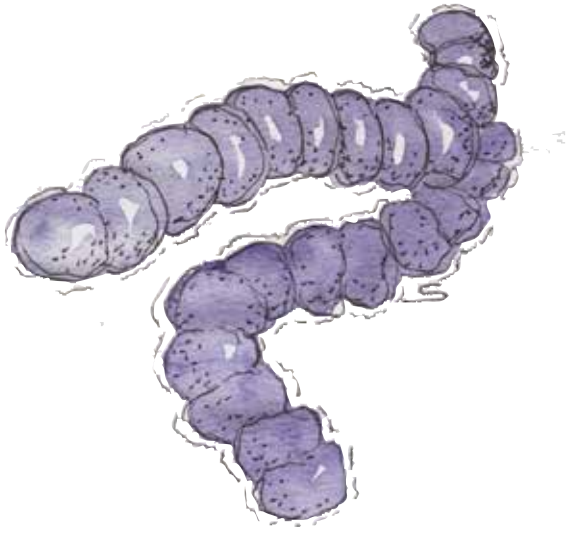
Liezel

Liezel is an only child born into a mixed-race family living in the Cape. She has no known family history of recurrent infections. She was born with congenital deafness caused by a deformed cochlea. Her birth weight was average (3.2 kg) and she was breastfed for three-and-a-half years. She was fully vaccinated (including unconjugated polyvalent Pneumococcal vaccine and *Haemophilus influenzae* Type B [HIB] conjugate vaccine). Her neurodevelopment was normal and there were no dysmorphic features observed at the time.

Liezel was initially referred at age five years with a history of recurrent lower respiratory tract infections, which started at age two years. She had nine hospital admissions for pneumonia which resulted in lung damage with bronchiectasis. Presenting at age five years she was also failing to thrive. Some baseline investigations had already been done: two Mantoux tests and several TB cultures were negative, and she also tested HIV-negative. Other blood and sputum cultures were also negative. A 'sweat test' was normal, excluding cystic fibrosis – a relevant exclusion especially with recurrent respiratory infections in childhood.

Dr Goodheart requested a number of blood counts and immunological tests, as she considered some form of immune defect. Full and differential blood counts showed that white cells, red blood cells and platelets were within reference range, however, neutrophils were elevated. A phagocyte burst test done on flow-cytometry was normal, excluding Chronic Granulomatous Disease (CGD). Serum IgG was elevated and IgA, IgM, IgE were within reference range. B-cells, T-cells (CD4, CD8) were elevated, which may indicate a compensatory/ secondary response. NK cell count and the CD4 : CD8 ratio were normal. Due to the recurrence of bacterial meningitis, Dr Goodheart requested complement tests, but C3 and C4 were within reference range. At this stage Dr Goodheart could exclude X-linked agammaglobilinaemia (XLA; with peripheral B lymphocytes normal in number and with normal immunoglobulins), autosomal recessive agammaglobulinaemia (for the same reason as XLA), Hyper IgM syndrome (HIGMS, characterised by hypogammaglobulinaemia and normal or elevated serum levels of IgM), Common Variable Immune Deficiency (CVID; characterised by low serum IgG and IgA levels) and CGD (the neutrophil burst test normal). Two important culprits for complement deficiency, C3 and C4 were also normal.

Liezel was started on penicillin prophylaxis. Despite these precautions, she had a further episode of pneumonia. A lung (lingula) biopsy showed peribronchial chronic inflammation. She also started having recurrent episodes of meningitis. Cerebrospinal fluid cultures were positive predominantly for *Streptococcus pneumoniae* ('pneumococcus'; 6 ×) but also for *Haemophilus influenzae* (1 ×) and *Streptococcus pyogenes* (1 ×), amazingly without neurological impairment! She was investigated for skull defects and a cochlear malformation was discovered (Mondini dysplasia). Despite surgical repair of this, however, she developed further episodes of pneumococcal meningitis.



Nasty *S pneumoniae*

Liezel is now ten years old. Dr Goodheart feels that she is missing the diagnosis of an underlying immune defect. She consults her friend Dr Bala, an immunology specialist.

Dr Bala agrees that this is a difficult case and that Dr Goodheart has excluded a number of important causes. She decides to investigate for deficient vaccine responses as most of the infections appear to be bacterial and frequently related to *S pneumoniae.*

It turns out that *H influenzae B* antibodies are not protective pre- and post-vaccination. *S pneumoniae* antibodies are borderline protective despite recurrent documented infections and the levels do not increase after vaccination. *Clostridium tetani* and *Corynebacterium diphtheriae* antibodies, on the other hand, are protective.

Dr Bala explains to Dr Goodheart that deficient antibody responses especially to polysaccharide antigens may persist after infancy and result in recurrent respiratory infections despite normal levels of immunoglobulins, as in Liezel's case. She arrives at a preliminary diagnosis of **specific antibody deficiency (SAD)** (see Box). Liezel is started on intravenous immunoglobulins (IVIG) infusions three-weekly to prevent further infections.

Given her susceptibility to severe infections and already established bronchiectasis, Dr Bala recommends that Dr Goodheart should continue IVIG and penicillin prophylaxis long-term despite the lack of a confirmed genetic diagnosis. Liezel is currently thriving and doing well.

Recently Liezel also developed a left-sided foot drop and atrophy of the calf with a tarsal coalition of the foot for which immobilisation was applied. Whether the congenital deafness and the abnormal bone structure of the foot are related to her immune defect remains to be explored in the future with the help of the geneticists.

Box: Specific antibody deficiency (SAD)[1]

SAD is a primary immunodeficiency disease (PID) characterised by:

- normal immunoglobulins, IgA, IgM, total IgG and IgG subclass levels;
- diminished antibody responses to polysaccharide antigens following vaccination; abnormal IgG antibody response to a pneumococcal vaccine;
- recurrent or severe bacterial infections (the sentinel pathogen is *S pneumoniae*);
- increased prevalence of atopy (allergies must be treated by standard interventions).

Practice points:

- Infections encountered in SAD mimic those in other PIDs; exclude Wiskott-Aldrich syndrome (WAS), Common Variable Immune Deficiency (CVID); T-cell deficits, etc.
- The diagnosis should not be conferred until after two years of age (by that time solid immune responses and robust responses to polysaccharide vaccines are typically reached in healthy children).
- Antibiotic prophylaxis is widely used to treat SAD and in selected cases IgG replacement therapy.

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Primary Immune Deficiency

AUTO-INFLAMMATION AS A CAUSE OF URTICARIA

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INTRODUCTION

Our understanding of auto-inflammation and the associated clinical syndromes is expanding rapidly – advances in genetic testing have revealed many monogenic auto-inflammatory diseases. While classic auto-inflammatory conditions such as Familial Mediterranean Fever have been recognised as distinct diseases for centuries, the relatively recent concept of auto-inflammation has led to better insight being gained into many distinct immune disorders and their classification. Auto-inflammatory conditions can present with a wide range of symptoms, which overlap with the presentation of allergic disease, and multiple organ systems can be involved. A basic understanding of auto-inflammation is therefore required if generalists, particularly allergologists, are to be prompted to a correct diagnosis, and triage to optimal treatment for these potentially devastating conditions.

INDEX CASE

A five-year-old Caucasian girl has presented to a multidisciplinary team of paediatric allergologists, immunologists and a paediatric rheumatologist with recurrent urticarial rashes since early infancy (see Figure 1). Her younger sister experienced similar, but less severe, symptoms. Her family had consulted numerous specialists over the preceding years, but extensive allergy testing and symptomatic treatment provided neither a clear diagnosis nor a sustained improvement. Her rashes appeared to be exacerbated by cold or pressure and appeared to worsen during times of infection. A delay in her speech development prompted a hearing test that demonstrated mild to moderate sensorineural deafness. She suffered recurrent upper respiratory tract infections and otitis media, necessitating myringotomies. On occasion, she had experienced difficulty walking, especially going down stairs in the morning. At age four, after complaining of eye pain, she was diagnosed with and treated for blepharitis by an ophthalmologist; however, resolution was slow, and the eye pain recurred intermittently. Her parents noted a deterioration in her mood and general condition, with urticaria, describing her as grumpy and lethargic, and with diffuse body pain. Additional clinical features were: allergic rhinitis (AR); chronic recurrent intermittent abdominal pain and diarrhoea. There was a strong family history of deafness and urticaria.

She was asymptomatic at the time of the visit, and her clinical examination was completely normal, apart from mild speech difficulties. However, the history and previous clinical findings indicated the likely diagnosis was an auto-inflammatory condition in the spectrum of Familial Cold Auto-inflammatory or Muckle Wells syndrome – one of the Cryopyrin Associated Periodic Syndromes (CAPS), where the pathology is driven by an overproduction of active Il-1β. The patient was referred for genetic testing because about 60 per cent of patients with CAPS have a specific mutation in the NLRP3 gene that codes for a protein in the inflammasome.[1] The results in her case were negative, but in view of her strong clinical picture, the team opted for a trial of therapy with Kineret (Anakinra) – a specific IL-1 inhibitor given subcutaneously once a day. It is dramatically

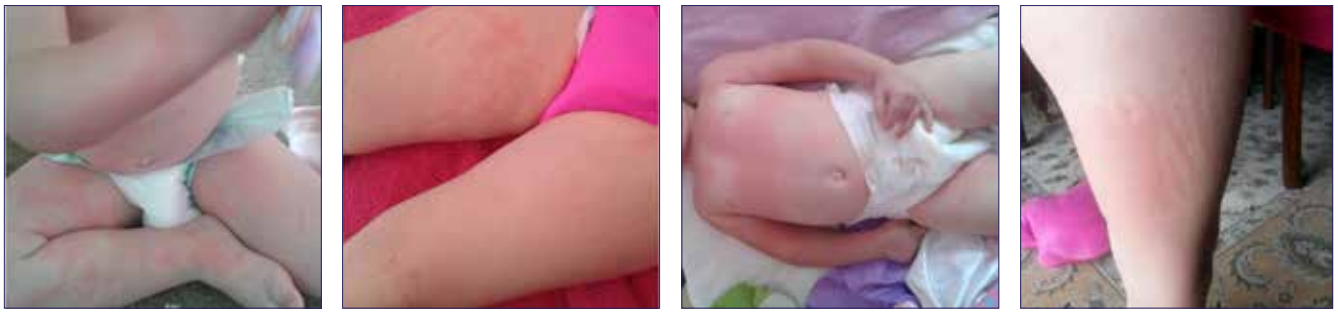

Figure 1: Diffuse urticarial rashes and dermatographism in the index case during an acute flare. (Pictures published with consent)

effective in CAPS and a response would support a diagnosis of Il-1β-mediated auto-inflammation. A dramatic improvement in her condition was noted with Anakinra, with no urticaria, and there was a sustained improvement in her wellbeing and systemic symptoms, including increased energy, appetite and mood. Her parents were ecstatic at the response, describing it as 'life changing'.

WHAT IS AUTO-INFLAMMATION?

The concept 'auto-inflammation' was first discussed in 1999, in the context of Familial Mediterranean Fever (FMF) and Tumour Necrosis Factor Receptor Associated Period Fever syndrome (TRAPS), after the discovery of the pathogenic mutations responsible for these inherited conditions.[2,3] Auto-inflammation describes dysregulation of the innate immune system and it is characterised by spontaneous episodes of systemic or localised inflammation caused by cells of the myeloid lineage (e.g. macrophages and neutrophils) and without identifiable auto-antibodies or antigen-specific T-lymphocytes.[4] In this sense, pure monogenic auto-inflammatory disease represents the opposite scale of the immune dysregulation spectrum to monogenic autoimmune diseases such as the Immunodysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) syndrome, where the gene defect involves failure of antigen-specific immune tolerance generated by regulatory T-cells (see Figure 2).[5] Nearly 20 years later, more than 20 monogenic auto-inflammatory conditions are described, and a larger number of polygenic auto-inflammatory conditions have been identified.[4] In fact, many conditions previously identified as autoimmune – such as systemic Juvenile Idiopathic Arthritis (sJIA) and Crohn's disease – are being conceptualised with an important auto-inflammatory component.[6]

THE INFLAMMASOMES

The inflammasomes (see Figure 3) are intra-cellular protein complexes that are formed in response to microbial and danger signals and mediate the release of the pro-inflammatory cytokine Interleukin 1β.[7] In the classic inflammasome-related conditions, known as the CAPS, for instance, mutations in the NOD-like receptor 3 gene (NLRP3) cause gain-in-function of the NLRP3 inflammasome and an overproduction of inflammatory cytokines Il-β. Excessive production of Il-1β leads to an exaggerated automated inflammatory response and triggers the production of downstream cytokines such as Il-6. This results in clinical manifestations that range from the mild Familial Cold Urticarial syndrome (FCAS) to the more severe Muckle Wells syndrome (MWS) characterised by fever, urticarial rash and sensorineural deafness. The most devastating CAPS is NOMID (Neonatal Onset Multisystem Inflammatory Disease), alternatively known as Chronic Infantile Neurologic, Cutaneous and Articular (CINCA) syndrome, which is characterised by destructive arthritis, deafness, progressive chronic meningitis and intellectual impairment (see Table I). Mevalonic aciduria/HIDS (Hyper-IgD syndrome) is a condition of excessive IL-1β signalling which is triggered by a metabolic disorder: a mutation in the mevalonate Kinase gene results in high levels of Mevalonic acid, a precursor in the cholesterol pathway, which in turn, via unclear mechanisms, results in excessive

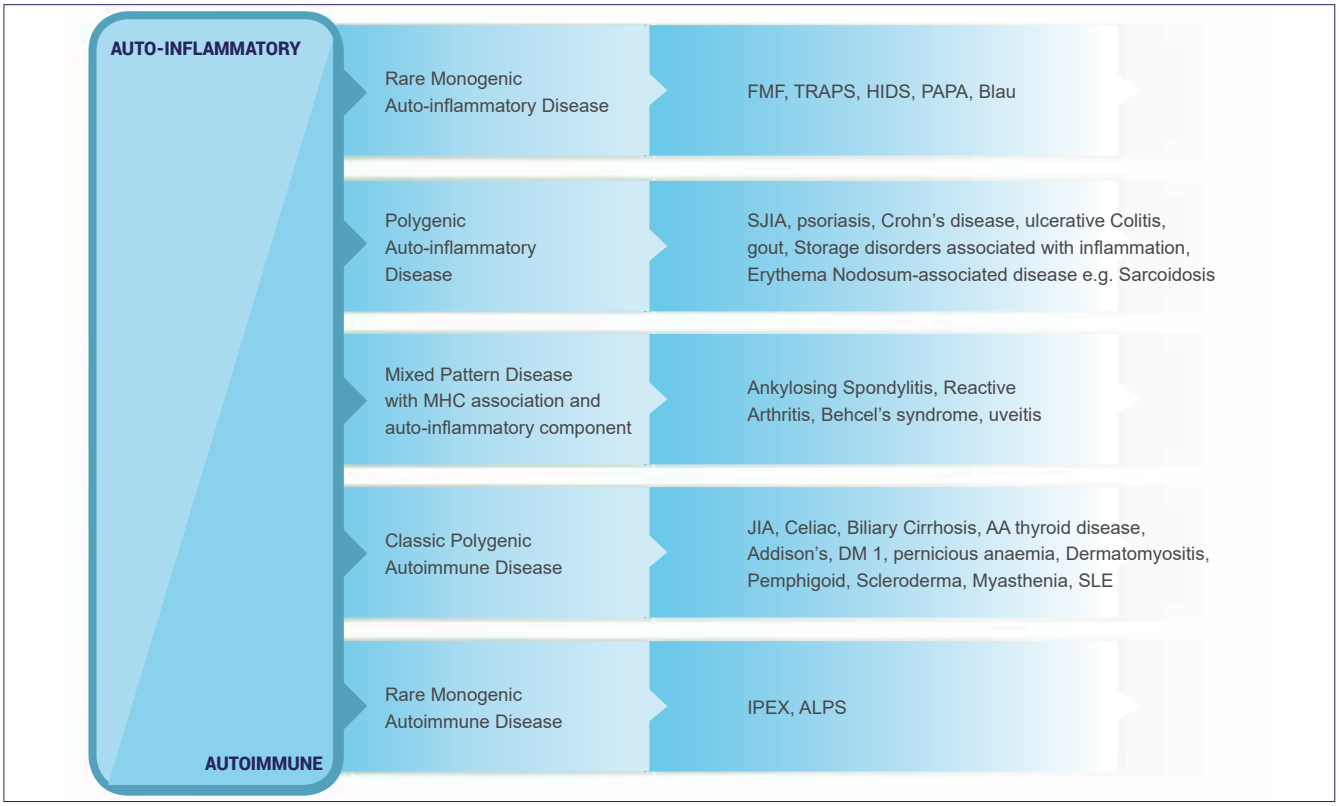

Figure 2: List of known monogenic auto-inflammatory diseases as well as a conceptualisation of the relative pathological involvement of non-specific innate immune dysfunction (auto-inflammation) and antibody or cell-mediated autoantigen-directed autoimmunity in rare and more common immunopathologies.

TABLE I: REPRESENTATIVE AUTO-INFLAMMATORY CONDITIONS SHOWING PATTERN OF INHERITANCE, EPISODE DURATION, CLINICAL FEATURES AND TREATMENT OPTIONS.

	FMF	HIDS	TRAPS	MW, FCAS, CINCA
Mode inheritance	AR	AR	AD	AD
Age of onset	< 20	Childhood	Variable	Infancy/neonatal
Length of episode	1–4 days	3–7 days	Several days	Variable
Abdominal pain	Very common	Very common	Common	Rare
Musculoskeletal	Monoarthritis	Arthralgias	Myalgias	Destructive arthritis
Chest pain	Pleuritis	Unusual	Yes	No
Rash	Rare	Very common, papular, maculopapular	Common, erysipelas-like	Urticaria, exanthema
Other	Pericarditis, scrotal attacks, splenomegaly	Headaches, cervical nodes, HSM	Orbital oedema	Cold sensitivity, dysmorphism, papilloedema
Amyloidosis	Yes	Yes	Yes	Yes
Treatment	Colchicine	None effective, ?TNF inhibitor	Steroids, TNF inhibitors	Steroids, IL 1 inhibitor

IL-1β signalling.[8] Similarly, mutations in the pyrin gene and the resultant dysfunction of the pyrin inflammasome, result in the clinical phenotype of Familial Mediterranean Fever.[3]

OTHER MECHANISMS OF AUTO-INFLAMMATION

While the most classic periodic fevers are associated with inflammasome dysfunction, other components of the innate system of downstream signalling from the inflammasome may also be involved in auto-inflammation (see Figure 4 and Table I). Abnormalities in NF-kB activation cause Crohn's disease and Blau syndrome;[6] Protein Folding Disorders are implicated in the pathogenesis of TRAPS[2] and spondyloarthropathies; complement disorders in Haemolytic Ureamic syndrome; Cytokine Signalling disorders in Cherubism; and Macrophage Activation disorders in Familial HLH.[8] Disorders of excessive type-1 interferon signalling have been associated with CANDLE syndrome[9] and Aicardi Goutières syndrome.[10] CANDLE syndrome is thought to be caused by proteasome dysfunction; proteasome degradation of ubiquinated inflammasomes is a key regulatory method of this innate signalling pathway.

These conditions fulfil a different clinical phenotype from the inflammasome-related conditions and a full description of these conditions is beyond the scope of this article. On the other hand, some conditions which have very clear features of auto-inflammation are at this stage not possible to classify according to disease mechanism. PFAPA (Periodic Fever Aphthous Stomatitis, Pharyngitis and Adenitis), for instance, is the most common auto-inflammatory condition in children; it presents in young children as clockwork-regular attacks of fever, pharyngitis, aphthous stomatitis and cervical adenitis. Attacks come regularly every three to six weeks and last 3–7 days, with completely asymptomatic intervening periods.[11]

HOW TO RECOGNISE AN AUTO-INFLAMMATORY SYNDROME: THE CLINICAL SPECTRUM

While the spectrum of auto-inflammatory diseases can be broad, certain combinations and patterns occur with increased frequency and are more likely to predict auto-inflammatory characteristics. Urticaria is a common feature of CAPS, NLRP12 FCAS, TRAPS, sJIA and HIDS.[12] Distinguishing between the

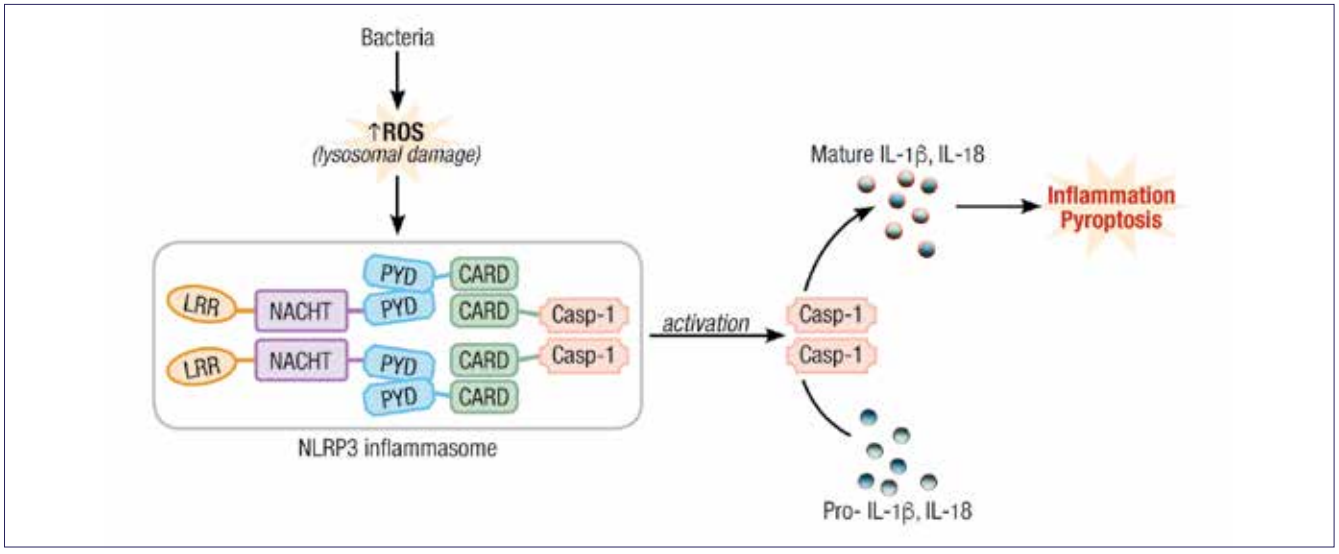

Figure 3: The inflammasomes

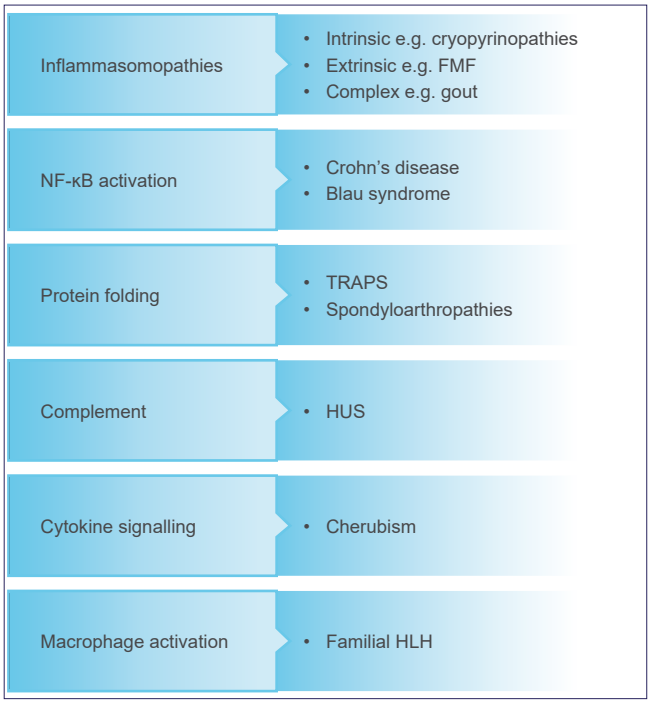

Figure 4: Classification of auto-inflammatory conditions by innate immune defects

different phenotypes of chronic urticaria and urticaria associated with auto-inflammation can be difficult, and is the key reason for allergologists to be aware of these systemic conditions. Chronic autoimmune and auto-inflammatory urticaria can both have physical inducibility, but chronic urticarias tend to be more intensely pruritic, respond better to antihistamines, and individual wheals tend to disappear within 24 hours. Auto-inflammatory urticaria tends to be associated with fever, arthralgia and longer duration, frequently with a family history.

In the case of the CAPS, urticarial rashes, arthritis, conjunctivitis and sensorineural deafness are key features.[12] Cold exposure is a well-known trigger and attacks are shortlived (1–2 days) in FCAS and MWS but continuous in NOMID.

TRAPS presents with longer periods of fever (up to six weeks) in addition to conjunctivitis, periorbital swelling, pleuritis, pericarditis, myalgia, arthralgia, abdominal pain and erythematous rash (often overlying areas of myalgia or arthritis). Familial Mediterranean Fever presents with shortlived attacks (1–3 days), with arthritis, peritonitis or pleuritis and erythematous rashes, especially over the lower limbs. Hyper IGD syndrome presents in infancy with recurrent attacks of rash (from petechial to urticarial), fever, cervical lymphadenopathy, joint pains, abdominal pain and vomiting or diarrhoea.

A number of auto-inflammatory conditions are known to cause sterile bone inflammation. Chronic Recurrent Multifocal Osteomyelitis (CRMO), PAPA (Pyogenic Arthritis with Pyoderma Gangrenosum and Acne), DIRA (Deficiency of IL1 Receptor Antagonist) and DITRA (Deficiency of Interleukin Thirty-six Receptor Antagonist) are well recognised as conditions presenting with fever, pustular skin lesions and sterile bone abscesses.[13]

Clinical combinations that warrant the consideration of auto-inflammatory conditions include those with recurrent discrete attacks of fever, often self-limiting, with skin rashes (from urticarial-like lesions to diffuse erythematous exanthems), arthritis or sterile osteomyelitis, serositis and abdominal pain (see Table II). Eye inflammation, deafness and neurological involvement are also associated with several auto-inflammatory conditions. The periodic nature of these conditions is highly suggestive, especially when symptoms cluster together. In some of these conditions periodic attacks are so regular that attacks can be accurately anticipated. Laboratory tests which demonstrate high CRP and inflammatory markers during the time of an attack, which improve spontaneously after the resolution of symptoms, can be a telling sign. However, in more severe conditions such as NOMID or conditions characterised by prolonged attacks, such as TRAPS, inflammatory markers may remain elevated. In milder variants, inflammatory markers may not be very helpful. Clinical classification criteria for many of these conditions have been proposed.[14]

TABLE II: WARNING SIGNS FOR AUTO-INFLAMMATION

WARNING SIGNS FOR AUTO-INFLAMMATION
Recurrent urticaria or erythematous rash plus any of the following:
Fever (self-limiting, with a predictable recurrence)
Arthritis
Sterile osteomyelitis
Serositis
Abdominal pain
Raised inflammatory markers during attacks
Additional features:
Sensorineuronal deafness
Family history

TREATMENT OF AUTO-INFLAMMATORY DISEASES

Given the differences in the mechanisms of inflammation, different auto-inflammatory conditions require different therapeutic approaches. In the case of inflammasomopathies, where IL-1β is overproduced, IL-1β-modulating or -blocking drugs can be instantly and dramatically effective. Three IL-1β-blocking drugs have been developed and approved for use. Unfortunately, these agents are not freely available in South Africa owing in part to a lack of demand, because these conditions are rare, and also because there is a scarcity of skills to diagnose and manage them. Cost is another limiting factor of importing these agents, which range from approximately R20 000 per month for the daily subcutaneous injection of Anakinra to approximately ten times that per month for Canakinumab, which is given monthly. IL-1 inhibitors are also useful in the management of Hyper-IgD syndrome and TRAPS.[15-17]

OTHER THERAPEUTIC AGENTS USED FOR AUTO-INFLAMMATORY CONDITIONS

COLCHICINE

This is Used frequently in FMF and can be dramatically effective. Colchicine acts by inhibiting neutrophil function and a good response is usually quite helpful in confirming the diagnosis of FMF. It has also been used successfully in other polygenic auto-inflammatory conditions such as gout and Behcet's disease.

TOCILIZUMAB

This IL-6-blocking biologic agent is useful in the management of systemic Juvenile Idiopathic Arthritis.

JANUS KINASE INHIBITORS

Agents in this class include Baricitinib, Tofacitinib and Ruloxitinib. While they have only recently been approved by the FDA for the management of rheumatoid arthritis, reports have emerged of efficacy in interferon alpha type-1-related diseases as well as in the proteasome dysfunction-related auto-inflammatory condition, CANDLE syndrome.

TNF INHIBITORS

TNF inhibitors such as Infliximab, Etanercept and Adalimumab have been used in the management of TRAPs as well as other auto-inflammatory conditions such as Blau syndrome, PAPA and CRMO.

CORTICOSTEROIDS

Used for decades in all forms of inflammatory disease, this agent is frequently used in auto-inflammatory diseases and offers a short-term option to treat acute attacks, especially when more specific agents are not available.

NON-STEROIDSAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

These are used as adjunctive and supportive agents, especially in sJIA and Hyper IGD syndrome; but they are effective only for symptom control.

OTHER MISCELLANEOUS THERAPIES

PFAPA tends to respond dramatically to tonsillectomy, although the reasons for this effect are not clear. Statins are sometimes used in MVK/HIDS because of the nature of the underlying metabolic disorder in cholesterol metabolism.

AMYLOIDOSIS

Apart from the obvious clinical impact of auto-inflammatory disease and the organ systems affected by inflammation, amyloidosis can complicate long-term uncontrolled inflammation. FMF and TRAPS are two conditions where this complication is prominent, and they can have dramatic effects on long-term health. Amyloidosis results from tissue deposition of Serum Amyloid A fractions produced in the liver as an acute-phase reactant. Renal deposition and subsequent kidney failure occurred in 60–80% of patients with untreated FMF after a median duration of 6,4 years (Gattorno and Martini).

CONCLUSION

Auto-inflammatory conditions are increasingly being recognised as multi-faceted diseases. Advances in genetic testing have led to the identification of more than 20 monogenic auto-inflammatory diseases with distinct phenotypes but many overlapping features. Recognising the patterns that suggest auto-inflammation will lead to improvements in diagnosis for patients and enable more appropriate therapeutic interventions. Given the range of specific and often complex and costly therapies, accurate diagnosis is imperative.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no potential conflict of interest related to this article.

This article has been peer reviewed.

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S4 RELVAR ELLIPTA 92/22 µg (48/21.5.1/0249) Powder for Inhalation. S4 RELVAR ELLIPTA 184/22 µg (48/21.5.1/0250) Powder for Inhalation. INGREDIENTS: Each pre-dispensed dose contains 100/25 µg (delivers 92/22 µg) or 200/25 ug (delivers 184/22 ug) of fluticasone furoate/vilanterol (as trifenatate). INDICATIONS: Asthma: the maintenance, preventive treatment of asthma. COPD: the maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and/or emphysema and to reduce exacerbations of COPD in patients with an exacerbation history. CONTRA-INDICATIONS: In patients with severe milk-protein allergy or who have demonstrated hypersensitivity to either fluticasone furoate, vilanterol or any of the excipients. WARNINGS AND SPECIAL PRECAUTIONS: Not to be used to treat acute asthma symptoms or an acute exacerbation in COPD. Increasing use of short-acting bronchodilators to relieve symptoms indicates deterioration of control and patients should be reviewed by a medical practitioner. Do not stop therapy without HCP supervision. Paradoxical bronchospasm may occur - treat immediately with a short-acting inhaled bronchodilator. Discontinue immediately and assess patient and alternative therapy instituted if necessary. Asthma-related adverse events and exacerbations may occur. Patients should be asked to seek medical advice if asthma symptoms remain uncontrolled or worsen after initiation. Use with caution in patients with cardiovascular disease, or heart rhythm abnormalities, hyperthyroidism or uncorrected hypokalaemia. Hypokalaemia may occur. High dosages may increase the risk of serious side effects, including cardiac dysrhythmias. This risk is further aggravated if administered concomitantly with other medicines that cause hypokalaemia and cardiac dysrhythmias, or in the presence of hypoxia and acidosis. The maximum dosage should not be exceeded. Beta-adrenergic blockers may cause bronchospasms and may weaken or antagonise the effect of beta2-adrenergic agonists - concurrent use of both non-selective and selective beta-blockers should be avoided unless there are compelling reasons for their use. Increases in blood glucose levels in diabetic patients have occurred. Systemic corticosteroid effects (e.g. HPA axis suppression, decrease in bone mineral density, growth retardation in children and adolescents, cataract and glaucoma) may occur, especially at high doses. Administer with caution in patients with pulmonary tuberculosis or with chronic or untreated infections. An increase in pneumonia has been obsaerved in patients with COPD. Risk factors for pneumonia in patients with COPD include current smokers, patients with a history of prior pneumonia, patients with a body mass index < 25 kg/m^2 and patients with a (forced expiratory volume) FEV1< 50 % predicted. 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Common: pneumonia, upper respiratory tract infection, bronchitis, influenza, candidiasis of mouth and throat, oropharyngeal pain, sinusitis, pharyngitis, abdominal pain, arthralgia, back pain, fractures, pyrexia. Uncommon: extrasystoles. MANAGEMENT OF OVERDOSAGE: No specific treatment for an overdose. If overdose occurs, the patient should be treated supportively with appropriate monitoring as necessary. Cardioselective beta-blockade should only be considered for profound vilanterol overdose effects that are clinically concerning and unresponsive to supportive measures. Cardioselective beta-blocking medicines should be used with caution in patients with a history of bronchospasm. Further management should be as clinically indicated or as recommended by the national poison centre, where available. HCR: GlaxoSmithKline South Africa (Pty) Ltd (Co. reg. no. 1948/030135/07), 39 Hawkins Avenue, Epping Industria 1, 7460. Trademarks are owned by or licensed to the GSK group of companies. 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