CURRENT ALLERGY & CLINICAL IMMUNOLOGY

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GUEST EDITORIAL

FESTSCHRIFT FOR PAUL POTTER

Festschrift: A volume of articles and essays contributed by many authors in honour of a colleague, usually published on the occasion of retirement. Dictionary.com

It would be difficult to think of anyone in the field of Allergology in South Africa more deserving of a Festschrift in his honour than Paul Potter. It is also fitting that this Festschrift is published in *Current Allergy and Clinical Immunology*, because Paul is the Founding Editor of this journal.

In June 2016 Paul retired from his position as Head of the Allergology Department and the first full Professor in Allergology in the Department of Medicine at Groote Schuur Hospital and the University of Cape Town (UCT) after 42 years of service. At the time of his retirement, he was also the Director of the Allergy Diagnostic and Clinical Research Unit (ADCRU) at the UCT Lung Institute.

Paul graduated from UCT in 1974 after an illustrious undergraduate career in which he received many honours and awards. His post-graduate career continued in the same brilliant manner. Specialising in Paediatrics at the Red Cross War Memorial Children's Hospital, he was awarded the Fellowship of the Colleges of Medicines of South Africa in Paediatrics in 1982. He won the Robert McDonald silver medal for the best candidate in the FCP Paediatrics examination.

He developed an interest in allergy while he was a paediatrics registrar and, in 1982, was appointed as the first full-time paediatric allergy registrar in the Allergy Department of the Red Cross War Memorial Children's Hospital, under Eugene Weinberg. In the same year, he also obtained a BSc (Hons) degree in Immunology with distinction. In 1986 he was awarded a visiting Fellowship at the National Institutes of Health in Bethesda, Maryland in the United States, where he spent 18 months working with Dr Craig Venter and Dr Michael Kalliner. Here he began work on his MD thesis project in molecular biology entitled 'In-vitro-studies of factors which influence the ligand binding, function, immunogenicity and genetic regulation of the Beta-2 adrenergic receptor in asthma'.

On his return to South Africa, he directed his research efforts at developing the field of Allergology, focusing in particular on allergen characterisation. This began by defining the common regional allergens and the clinical sensitisation of patients with allergic diseases and it led to a number of 'firsts' in this area.

In 1984 he, together with Dr Frank Spracklen, reported the

first case of AIDS in South Africa. The first case of latex allergy was reported in 1997. Reports of hypersensitivity to kikuyu and buffalo grass, polymorphisms of the Beta-2 receptor, and effective prophylaxis with penicillin for patients with recurrent meningococcal meningitis due to C6 Complement deficiency – now a standard treatment – followed. He was a pioneer in the research and treatment of patients with Hereditary Angioedema (HAE) in this country. He was the first to use C1 esterase-inhibitor concentrate for the acute and prophylactic management of patients with HAE.

Paul supervised the first molecular characterisations of allergy to abalone, having identified the first clinical cases. He then went on to supervise the laboratory characterisation of other novel allergens in the indigenous allergen research unit (with Bartha Fenemore, Ruth Prescott and Andreas Lopata). This work included identifying allergens in imbuia wood, the Rhodesian flame lily, verbena, cobra venom and the African penguin. More recently, he collaborated with Dr Shiang-Ju Kung on unique African allergens from Botswana. Using samples obtained from Dr Kung, he identified novel indigenous allergens, including mopane worms, marula nut and other food allergens from that region. He was the first to establish Western blotting for allergen identification in South Africa and established the first assays for autoantibodies to the IgE receptor. He conducted the first double-blind placebo-controlled food-allergy challenges (DBPCFC) in patients with suspected seafood allergy. He also carried out the first house-dust mite (HDM) antigen assays for studies in the Western Cape and on the Highveld.

He supervised more than 30 post-graduate projects and

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References: 1: Picado C. Rupatadine: pharmacological profile and its use in the treatment of allergic disorders. Expert Opinion in Pharmacotherapy 2006;7(14):1989-2001.) **2.** Steubner P, Horak F, Zieglmayer R, Arnaiz E, Leuratti C, Perez I, Izquierdo I. Effects of rupatadine vs placebo on allergen-induced symptoms in patients exposed to aeroallergens in the Vienna Challenge Chamber. *Annals of Allergy, Asthma & Immunology* 2006;96:37.44. **3.** Gimenez-Arnau A, Donado E, Arnaiz E, Perez I, Izqierdo I. Fast onset of action of rupatadine in the reduction of pruritis in patients suffering from chronic urticaria: a pooled analysis. *Allergy* 2007;62 (suppl 83):306

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theses in allergy and immunology at the University of Cape Town. He has received numerous research awards. These include the SA Medical Research Council Post-Doctoral Scholarship, SA Druggists Research Award, basic research awards while in the MRC Cell Biology Unit from 1988 to 1993 and many research awards for clinical trials in asthma, allergic rhinitis, atopic eczema, chronic spontaneous urticaria, HAE, immunotherapy (especially SLIT), and also for cytokine studies.

Paul served on the World Allergy Organisation (WAO) Board of Directors from 2008 to 2011. He also served on the WAO Speciality and Training Council from 2004 to 2011 and was the first author of the inaugural *Global Guidelines on Training Requirements for Medical Students in Allergy* published in 2009. In addition, he served on the WAO Councils on Education, Communication, and Allergen Immunotherapy and on the Editorial Board of the WAO Journal. He was a member of the Executive Committee of the ARIA Guidelines from 2004 to 2013.

Locally, Paul has served on several South African advisory boards in the fields of rhinitis, asthma, eczema and on the South African Working Groups in these fields. He has studied and published extensively on latex allergy in South Africa, developing local guidelines for containing the latex allergy epidemic in this country while at the same time authoring guidelines on asthma, rhinitis, eczema and immunotherapy. He has also contributed several chapters to the WAO *White Book on Allergy*. He has published more than 370 articles: 130 of them in international peer-reviewed journals, including six citation classics, 60 articles in South African peer-reviewed journals, 130 articles in local CME journals and 34 chapters in books.

Paul was a founding member and the first Honorary Secretary of the Allergy Society of South Africa (ALLSA) and its second Chairman. He served as Treasurer of the society and head of the Research Awards Committee for many years. He was the Convenor of the first South African Allergy Congress in Cape Town in 1989. He also was the first Editor and founder of the *ALLSA Handbook of Allergy* and served as Editor for the 1994 and 2001 editions and as Co-editor for the 2010 edition.

In addition, he founded the ADCRU at the UCT Lung Institute in 1999. This rapidly developed into a nationally and internationally recognised centre of excellence for the diagnosis and management of complex allergic conditions for patients from all over southern Africa and the Indian Ocean islands. He was a co-founder, with Prof Monika Esser, of the South African Primary Immunodeficiency Register. He worked tirelessly for the recognition of Allergology as a sub-speciality in South Africa, his efforts finally being rewarded in 2012. He was most deservedly grandfathered as a sub-specialist in Allergology in 2013.

His academic activities in the UCT Faculty of Health Science and at Groote Schuur and the Red Cross War Memorial Children's hospitals included teaching generations of medical students and supervising BSc Honours in Immunology, Master's and PhD students. Many Diploma in Allergy students spent time in his unit, as did dental students, dieticians in training, registrars and allergy fellows, including international student electives in allergy.

Paul has given more than 200 invited lectures locally and more than 80 at international conferences and meetings. He serves on the editorial boards of six journals and is a referee for several international allergy journals. He has also served on special committees of the American Academy of Allergy Asthma and Clinical Immunology (AAAAI) and the American College of Allergy, Asthma and Immunology (ACAAI); he is an International Fellow of both of these academies.

It should be clear from the above that Paul's contribution to allergy and clinical immunology has been tremendous and is unlikely to be readily surpassed. Has there ever been a specialist in this area in South Africa with his wide range of interests combined with his unique skills, knowledge and ability? Paul is also endowed with tremendous enthusiasm for life in general and appears to have limitless interests in his surroundings wherever he may be in the world at the time. He enjoys playing the violin and loves scuba diving. He is blessed to have Anne as his wonderful wife and most supportive life partner and has a loving, accomplished and talented family.

This Festschrift issue includes five special articles selected by his colleagues. They all illustrate in a small way Paul's unique, pioneering and expansive range of interests in allergy research in South Africa – and no less so his significant, even monumental, contribution to and influence in the field. Each of these articles begins with a short introductory paragraph which details the circumstances that surrounded each particular research project at the time.

Besides these Festschrift articles, there are also two research articles in this issue. The first is 'House-dust mites' impact disease expression in children with allergic rhinitis: A retrospective study' by Refiloe et al. The second is a welcome article from Nigeria, 'Allergenicity of Dominant Aeropollen in Nigeria: Part 1' by Adeniyi et al. Our regular highly educational and very popular articles are also to be found in this issue: Ethics, ABC of Allergies, Allergies in the Workplace, Case report, PID article and PID123.

We hope that you will enjoy this special issue devoted to a wonderful and unique colleague whom we are proud to honour in this manner.

Eugene Weinberg and Di Hawarden
Editors

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Reference: 1. Simons FER, Ardusso LRF, Biló MB, El-Gamal YM, Ledford DK, Ring J, *et al.* World Allergy Organization Guidelines for the Assessment and Management of Anaphylaxis. *WAOJ.* 2011;**4**:13-37.

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Festschrift Article

HYDRATION IN SEVERE ACUTE ASTHMA

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A TRIBUTE TO PAUL POTTER

Paul Potter was appointed as the first paediatric allergy registrar in my Department of Paediatric Allergy at the Red Cross War Memorial Children's Hospital in 1982. He immediately showed a great interest in all aspects of paediatric allergy and this interest was accompanied by an enormous capacity for hard work and boundless enthusiasm. In this same year he also managed to find time to obtain a BSc (Hons) degree in Immunology (with distinction). Paul conducted a most important study on the fluid requirements of children presenting with severe acute asthma. At the time IV fluid supplementation was considered to be an important part of the management of children with severe acute asthma, but there was considerable debate about the correct volume of fluid to be given. One approach based on adult studies had shown considerable dehydration in these patients and recommended that double the maintenance volume of fluid was to be given. The liberal correction of fluid deficits was also thought to help to mobilise any mucus plugs blocking the airways of these patients. A second approach assumed that dehydration was not an important feature and that the over-correction of fluid deficits in children with severe acute asthma was likely to be harmful. Paul's study showed that mild dehydration was common in children with severe acute asthma and that fluid given at a rate of 50 mL/kg/24 hours was safe and appropriate.

This study significantly changed our approach to the use of IV fluids in treating children presenting with severe acute asthma. His article, published in a prestigious international paediatric journal, had a considerable impact on the management of fluid requirements of children with acute severe asthma in many countries. Even more amazing is that this important article was to be the first of more than 370 articles that Paul would go on to write and publish. Of these, 130 articles appeared in international peer- reviewed journals, including six citation classics.

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Archives of Disease in Childhood 1991;66:216–219

ABSTRACT

Twenty children were studied during severe attacks of acute asthma to determine how dehydrated they were on admission to hospital. Mean body weight on admission was 97.8% of their reference stable weight seven to ten days after the attack and in only three children was it less than 95% of the stable weight. Bedside assessment of dehydration was unreliable. The mean packed cell volume was significantly higher on admission than 7–10 days later (0.44 compared with 0.42, difference 0.02 SE 0.01). Serum sodium and potassium concentrations and osmolality on admission were within normal ranges. The degree of dehydration correlated best with a fall in blood pH. There was no association between the degree of dehydration and the recovery of the peak expiratory flow rate during the first 24 hours or thereafter. We conclude that mild dehydration is common in severe acute childhood asthma. Fluid given at a rate of 50 mL/kg/24 hours was safe and appropriate for these children.

INTRODUCTION

Treatment with intravenous fluid replacement is considered to be an important part of the management of severe acute asthma,[1] but there is no consensus about the volume of fluid that should be given during the acute phase. There are two contrasting approaches; the first, based on the results of adult studies, is that patients with severe asthma become appreciably dehydrated and that the degree of dehydration is directly related to the

severity and duration of the attack.[2–4] The liberal correction of fluid deficits is thought to mobilise mucus plugs and to decrease narrowing of the airways.[5–6] The second approach assumes that appreciable dehydration is not a feature of asthma and that the indiscriminate correction of fluid deficits in patients having severe attacks may be harmful. This approach is based largely on the finding of raised concentrations of antidiuretic hormone in patients with acute asthma.[7–8] Giving unnecessary additional fluids to such a patient could lead to fluid retention, pulmonary oedema and a deterioration in the patient's condition. This is particularly so if the increase in antidiuretic hormone represents an appropriate response to mild dehydration. The value of the measurement of acute weight changes as an index of dehydration in children has recently been stressed by Mackenzie et al.[9]

We therefore conducted a prospective study to assess the incidence and degree of dehydration in children with severe acute asthma who required admission to hospital, and to assess the effect of their degree of hydration on their rate of recovery while on a relatively restricted fluid intake.

PATIENTS AND METHODS

PATIENTS

Children aged between five and 12 years who attended the asthma clinic regularly and who knew how to use the Mini-Wright peak-flow meter were admitted to the study if they required admission to hospital for a severe attack of acute asthma. The criterion for admission was that the peak expiratory flow rate (PEFR) remained at less than half the predicted level after two doses of nebulised salbutamol 1 mL 0.5% solution with 1 mL 0.9% saline (Ventolin).[10]

TREATMENT PROTOCOL

During the first 24 hours after admission, each child received 50 mL/kg 5% dextrose/water intravenously. Oral fluids were withheld during this period, but thereafter intravenous fluids were discontinued and free oral fluids were allowed. Hydrocortisone 2 mg/kg was given intravenously every six hours for 24 hours, with prednisone 2 mg/kg/daily thereafter, reducing to their previous dose over five days. Theophylline 5 mg/kg was given orally every six hours and inhalations of nebulised 0.5% salbutamol were given every two to four hours as required. Respiratory function was monitored by daily readings of the PEFR.

ASSESSMENT OF HYDRATION

On admission the duration of the attack was recorded, as was whether the child had been vomiting before admission, and whether they were receiving maintenance treatment with steroids or theophylline.

A clinical estimation of the state of hydration was made at the bedside, taking into account skin turgor, mucosal moistness and eye signs. A sphygmomanometer with appropriate cuff was used to measure the difference between the inspiratory and the expiratory diastolic blood pressure.

On admission, each child was carefully weighed wearing underclothes only and was weighed again 24 hours, 48 hours and seven to ten days later. Weights were recorded by two independent observers to the nearest 10 g and the mean weight recorded. The scales were standardised at the start of the study and the same scales were used throughout the period.

Weight at the seven- to ten-day visit was assumed to represent the child's stable reference weight, and weights on admission, and 24 hours and 48 hours later, were expressed as a percentage of this.

Serum sodium and potassium concentrations (ion elective electrode, Astra 8 Beckman), serum osmolality (Micro-osmette Precision System), and packed cell volume (Coulter, Model 5+2) were measured at the same time that the children were weighed. Urinary osmolality and ketones (Ames, Ketodiastix) were tested only on the first specimen of urine passed after admission.

On admission PEFR was measured with a Mini-Wright peak-flow meter after the initial inhalation of salbutamol and again whenever the child was weighed. The maximal value of three efforts was recorded.

DATA ANALYSIS

Statistical analysis was carried out by the Biostatics Unit of the South African Medical Research Council, Parowvallei.

Univariate analysis of variance (Anova) was carried out using the following variables: weight, serum sodium and potassium concentrations, osmolality, packed cell volume, and percentage of predicated peak flow. This was done to identify those variables that changed significantly over time.

Sequential values of weight and hydration indices for each patient were compared using the paired two-tailed Student's *t* test, each patient acting as their own control. Spearman correlation coefficients were calculated to work out the relationship between individual variables and the degree of dehydration. Regression analysis was also carried out on all subsets to find out which variable correlated best with the degree of dehydration.

Informed parental consent was obtained for each child studied and the protocol for the study was approved by the ethics and research committee of the Faculty of Medicine, University of Cape Town.

RESULTS

CLINICAL EVALUATION OF THE PATIENTS

Twenty children with severe acute asthma (12 girls and

eight boys, mean (SD) 9.15 (2.57), range 5–12 years) were studied. The median duration of the attack from the onset of symptoms to the time of admission was 16 hours (mean (SE) 17.7, (2.09), range 5–48). In eight children the attack had lasted 12 hours or less, in ten from 13–24 hours, and only two patients had been ill for more than 24 hours. Of the 20 children, 18 were on maintenance treatment with oral theophylline and 11 with oral steroids. Eleven children had vomited at least once.

Thirteen children were thought to be clinically about 5% dehydrated, four were thought to have borderline dehydration (2.5%), and three were considered to be normally hydrated.

DEHYDRATION AND CLINICAL AND LABORATORY MEASUREMENTS

The mean admission weight was 97.9% of stable weight ($p < 0.05$) (mean percentage dehydration = 2.11). Using a repeated measures analysis of variance to detect an overall time effect on the variables shown in the table, significant overall changes in the serial measurements were found for weight ($p = 0.002$), and percentage predicted peak flow ($p = 0.001$).

Analysis of the significant day-to-day differences in the variables by Student's t test (see Table I) confirmed that a marginal increase in packed cell volume was present on admission. Despite unrestricted fluids, a small but significant fall from the 24-hour weight was recorded at 48 hours. Three children were more than 5% dehydrated by weight on admission (−5.4%, −5.7% and −6.2% of stable weight). There was no correlation between the calculated percentage dehydration and the clinical assessment (Spearman rank correlation coefficient = −0.007, $p = 0.98$). The degree of dehydration on admission correlated weakly with the duration of the attack. Only one of the two children whose attack exceeded 24 hours' duration was dehydrated (percentage dehydration 3.3). The children who had vomited had a mean percentage dehydration of 2.2, and those with no history of vomiting had a mean percentage dehydration of 2.0 ($p > 0.8$). The only baseline measurement that came anywhere near correlating with the degree of dehydration (using Spearman rank correlation coefficients) was a fall in arterial pH ($r = 0.413$, $p = 0.07$). All subsets regression analysis of the packed cell volume, oxygen and carbon dioxide tensions, bicarbonate, pH, the difference between inspiratory and expiratory diastolic pressure, and percentage of predicted PEFR, confirmed that the best predictor of dehydration was a fall in pH, but the level of correlation was not sufficient for practical use ($r^2 = 0.165$, $p = 0.10$).

CLINICAL COURSE

All 20 children had uneventful hospital stays and were discharged to their homes several days before their seven- to ten-day follow-up visit. Comparison of the rate of recovery in lung function (as shown by PEFR) between the children who were below the median value of percentage dehydration (−2.3%) with those who were above the median value (by analysis of variance) showed no difference between the two groups in the rate of recovery ($p = 0.38$).

No complications of asthma were encountered, and all patients were clinically well, with normal PEFR at the seven- to ten-day follow-up study.

DISCUSSION

Few controlled studies have investigated the incidence and treatment of dehydration in severe acute asthma.[10–12] None of these studies have assessed the degree of dehydration normally encountered in children with severe acute asthma, or the effect of a relatively restricted fluid regimen on the rate of recovery from the attack.

In this prospective study, by serially measuring clinical and laboratory indices of hydration normally available to the clinician, we found that a mild degree of dehydration was common among children presenting with severe acute asthma. Within the range of dehydration encountered, the degree of dehydration on admission bore no relation to the rate of recovery when children were treated with a relatively restricted fluid intake. Our results therefore support the argument that additional fluids above baseline requirements are unnecessary in severe acute asthmatic attacks.

Three lines of evidence support a conclusion that the children whom we studied had only mild dehydration. The first, obtained by comparison of the admission weight with the seven- to ten-day reference weight (baseline), showed that the mean (SD) percentage dehydration was 2.1 (2.3), range 1.6 to 6.2, median 2.3. Secondly, normal serum electrolyte concentrations and osmolality were recorded on admission and throughout the seven- to ten-day observation period, and the mean packed cell volume on admission was mildly but appreciably raised. Thirdly, there was no significant weight gain once the children were allowed unrestricted fluids orally after the initial 24-hour period, during which time fluids were restricted to 50 mL/kg/24 hours. This supports our conclusion that none of them had unrecognised significant dehydration on admission.

In our interpretation of the serial weight changes we have assumed that the weight of a child seven days after a severe acute attack who has recovered completely represents a stable baseline reference weight. We have also assumed that rapid fluctuations in weight reflect fluid balance. These assumptions are reasonable, given the fact that it is practically impossible to obtain a child's reliable weight as a reference scale immediately before the onset of the acute attack of asthma. It has previously been shown that in the

TABLE I: SEQUENTIAL MEASUREMENTS OF INDICES OF HYDRATION EXPRESSED AS MEAN (RANGE)

Time from admission	Weight (kg)	Percentage of stable weight	Packed cell volume	Serum potassium (mmol/L)	Percentage of predicted peak flow	Serum sodium (mmol/L)	Osmolality mmol/kg
On admission	27.9 (15.3–48.6)	97.9 (93.9–101.2)	0.44 (0.35–0.63)	3.9 (2.9–5.1)	30.9 (30–49)	137.9 (131–145)	248.9 (274–292)
At 24 hours	28.0 (15.0–48.5)	98.6 (20.0–103.0)	0.41 (0.34–0.49)	3.7 (3.0–5.0)	75.3 (41–114)	137.5 (132–147)	283.9 (275–297)
At 48 hours	27.6 (15.5–47.5)	97.3 (93.2–101.2)	0.42 (0.36–0.47)	4.0 (3.4–4.8)	103.6 (80–139)	137.8 (80–139)	286.1 (277–299)
At seven to ten days	28.5 (16.2–49.6)	100 (Reference weight)	0.42 (0.36–0.47)	4.0 (3.4–4.8)	103.6 (80–139)	137.8 (133–143)	286.2 (279–306)
p value Anova	0.0001	Not applicable	0.0784	0.1021	0.0001	0.5700	0.4227
SE of differences	0.16	Not applicable	1.16	0.12	5.15	0.73	1.59
Significant changes	0 *v* 7–10	0 *v* 7–10	0 *v* 7–10	24 *v* 7–10	0 *v* 24	None	None
$p < 0.05$ (Student's *t* test)	48 *v* 7–10	48 *v* 7–10			24 *v* 7–10		

short term respiratory tract illnesses (e.g. pneumonia and otitis media) cause no discernible retardation in the growth rate of most well-nourished children.[13]

The possibility that treatment with steroids could have influenced the seven- to ten-day baseline assessments must be considered. Short-term weight increases associated with treatment with steroids are usually attributable to mineralocorticoid effects, and some children do gain weight quite quickly on high doses of steroids. We do not consider that in our patients steroids caused any appreciable increases in weight, because in the first instance we noted a decrease in mean weight ($p < 0.05$) at 48 hours (see Table I), the point at which the patients had received their highest doses of their tailing courses of prednisone. Secondly, serial monitoring of the serum sodium and potassium concentrations and osmolality (see Table I) provided no evidence in support of mineralocorticoid activity at any of the assessments. Thirdly, we discontinued the short course of prednisone on day 5, at least 48 hours before the seven- to ten-day assessments of weight. In view of the short reported serum half-life of prednisone (3.6 hours), any pharmacological effects of prednisone at the seven- to ten-day assessments were likely to be minimal.[14]

If, however, we accept that treatment with steroids may have increased our patients' weight, we have over-assessed the degree of dehydration, and the mean percentage dehydration on admission is even less than the 2.1 that we found. This would strengthen our conclusion that dehydration in severe acute asthma is usually mild. To our knowledge, there are no published data on the effects of tailing-off doses of prednisone on weight in the presence of mild dehydration. Our data provide reasonable evidence that any weight gain caused by the steroids in acute childhood asthma is likely to be minimal.

Factors that are likely to influence the degree of dehydration encountered during an acute asthmatic attack include the duration of the attack, the ambient temperature, the maintenance drug treatment (e.g. steroids and theophylline), the presence of fever, the oral fluid intake before and during the attack, and vomiting. Several of these factors may occur to varying degrees in a given patient and some factors may compensate for others. We found that children with a history of vomiting were not significantly more dehydrated than those who had not vomited. The clinician over-estimated the degree of dehydration in 13 of 20 cases: mouth breathing by a distressed child causes drying of the buccal mucosa and can falsely suggest dehydration to the attending clinician.

In this study, the degree of dehydration correlated weakly with the duration of the attack. This finding suggests that most children with acute asthma maintain hydration where possible by continuing to take oral fluids. The severity of the attack as measured by the difference between inspiratory and expiratory diastolic pressure, and oxygen and carbon dioxide tensions, did not correlate with the degree of dehydration. A mild compensated metabolic acidosis (as shown by a fall in pH) did, however, correlate more strongly with a state of mild dehydration than did changes in any of the other variables measured by multiple regression analysis.

To assess the effect of the degree of dehydration on the rate of recovery of the PEFR, the recovery of children who were more dehydrated was compared to that of the children who were less dehydrated. A greater degree of dehydration on admission did not impair the rate of recovery, and because all the children received the same fluid regimen, it suggests that additional fluids are not required to assist airway recovery. Supporting this observation, Nadel emphasised that there have been no studies to support the assumption that fluids will reduce the viscosity of tenacious secretions or influence the mechanisms that could be expected to

assist their removal from the airways.[15]

In animal experiments, Marchette et al found that in allergic sheep with antigen-induced bronchial obstruction, tracheal mucus velocity (as an index of lower-airway mucociliary clearance) was impaired by 5% dextrose infusions of 25–35 m/kg, although no impairment was observed in normal sheep.[16] This raises further questions concerning the possible deleterious effects of too much fluid intake on children with severe acute asthma.

Baker et al studied the weights of asthmatic patients and warned about the dangers of over-hydration and the possibility of water intoxication.[7] In their study of seven patients (age range 7–52 years), concentrations of antidiuretic hormone were raised in all patients and serum osmolality was decreased. Dawson et al measured the concentrations of antidiuretic hormone in the plasma of ten asthmatic children and found that the concentrations were appreciably increased. If concentrations of antidiuretic hormone are inappropriately raised, abundant oral and parenteral fluids may aggravate mucosal oedema of the airways.[10] This will cause further airway narrowing, perhaps premature airway closure, and worsening of the asthmatic attack. On the other hand, Bahna and Kaushik could find no evidence of the syndrome of inappropriately raised antidiuretic hormone secretion in 26 children with acute severe asthma.[12] Singleton et al have, none the less, shown that children with severe acute asthma have impaired water excretion after water loading and are at risk of hyponatraemia if given hypotonic fluids for a prolonged period.[11] We believe that the most likely explanation for the raised concentrations of antidiuretic hormone that have been reported in severe acute asthma is the presence of mild dehydration.

Straub et al reported haemoconcentration in nine adult patients with asthma using erythrocytes labelled with ^{51}Cr and a slight increase in packed cell volume in 13 of 20 patients with severe attacks, but this did not correlate with their assessments of dehydration using serial measurements of weight. Our study also showed a slight, but significant, increase in the packed cell volumes of children presenting with severe acute asthma.

Taken collectively, these studies support our conclusion that additional fluids should be given with caution to a child with severe acute asthma. As concentrations of antidiuretic hormone are increased in acute childhood asthma, we believe that the most likely explanation is that this is an appropriate response to mild dehydration rather than a syndrome of inappropriate section of antidiuretic hormone. Until there is evidence that the liberal intake of fluids has a beneficial effect on the course of the disease, or that withholding fluids to achieve a state of mild dehydration is beneficial, it seems prudent to aim for a state of normal fluid balance in children with severe acute asthma. When fluids cannot be taken orally, calculation of the maintenance volume of low-solute intravenous fluid should be based on about twice the estimated insensible loss – that is, roughly 50 mL/kg/24 hours. This is a safe and appropriate volume, not accompanied by significant electrolyte changes or fluid retention, and is consistent with rapid recovery in the PEFR.

ACKNOWLEDGEMENTS

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Festschrift Article

COCKROACH ALLERGY IN DURBAN

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A TRIBUTE TO PAUL POTTER

I was a senior registrar at the Division of Allergy of the Red Cross War memorial Children's Hospital in Cape Town in 1990. We were very keen on using monoclonal antibody techniques to measure indoor allergen levels. This was the first study using this technology in South Africa. We were involved in a study on control of house-dust mites (HDM) using acaricides and other HDM-reduction methods. My supervisors were Prof Eugene Weinberg and Prof Paul Potter.

There were many papers published around that time on cockroach allergy and inner-city asthma. Cockroaches thrive in hot humid environments such as Durban. Prof Potter told a story about when he was an intern at Addington Hospital in Durban. He made tea every morning at the doctor's residence in the hospital using an old kettle. He filled water through the spout and continued with this practice for a long time until, one day to his horror he discovered a large number of dead cockroaches lying at the bottom of the kettle.

When I returned to Durban we decided to study the prevalence of cockroach allergy in Durban. We embarked on this study using monoclonal antibody technology to quantitate and characterise indoor cockroach allergen levels in patients who were allergic to cockroaches.

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INTRODUCTION

Cockroaches have become increasingly recognised as important components of HDM allergens causing allergic sensitisation. Cockroach allergy was reported to cause allergic reactions by Bernton and Brown in 1964 in a study that demonstrated that 40% of their asthmatic patients reacted on skin-prick testing (SPT) to cockroach extract.[1] Many subsequent studies have identified the cockroach as an important indoor allergen, responsible for causing asthma in urban inner-city dwellers.[2,3,4]

In a study by Kang in Chicago, the United States, on a group of 592 asthmatics, it was demonstrated that more than 60% were allergic to cockroaches. Bronchial-provocation testing in patients with cockroach allergen demonstrated reactively in 87% of patients. When compared with asthmatics in general, they found that cockroach-sensitive asthmatics had a more severe type of asthma, required higher corticosteroid usage and that more than 90% of these patients had multiple hospital

admissions for acute asthmatic attacks.[2] Kang et al have demonstrated the aetiologic role of cockroaches in asthma in many other studies.[2,3] Pollart and Gelber, in two separate studies in Charlottesville, Virginia, Altanta, studied the epidemiological trends in these patients. They showed that both sensitisation and exposure to cockroaches are major risk factors for hospital admission with asthma, especially in patients living in poor social circumstances.[4,5]

Cockroaches have also been reported to cause asthma in other parts of the world, for example Central America, Asia and South Africa.[6,8,9] In a study performed in Durban in the 1970s Fraser demonstrated that 30% of asthmatics

presenting at Addington Hospital reacted to cockroach allergen on SPT.[6]

COCKROACH ALLERGENS

There are many different species of cockroach but only three have been implicated in the pathogenesis of asthma. These are the American (*Periplaneta Americana*), German (*Blatella germanica*) and Oriental (*Blatella orientalis*) cockroaches. The important cockroach allergens have been identified using immunochemical techniques. They are designated as Bla g I, Bla g II and Per a I.[10] Bla g I is a *B germinica* allergen and 30–40% of cockroach-allergic patients have serum IgE to Bla g I. Bla g II is a specific blatella allergen and 60–80% of cockroach-allergic patients have specific IgE to Bla g II. Per a I cross-reacts with Bla g I. Its quantification using monoclonal antibiodies may indicate total allergen load in our area since it is a common allergen shared by important cockroaches associated with asthma.[11,12]

AIMS OF THE STUDY

Durban is a coastal city with a warm humid climate conducive to mite and cockroach infestation. It is well known that Durban has a heavy cockroach infestation. The only published data to date on cockroach allergy in Durban was a study conducted by Fraser in 1979. We therefore studied the prevalence of cockroach allergy and environmental contamination in a prospective trial in Durban. Our aims were to:

- assess the level of cockroach sensitisation in Durban asthmatics;
- determine the presence of cockroach allergen in the homes of cockroach-sensitive asthmatics using immunochemical methods;
- assess which assay is most suitable for local environmental surveys.

METHODS

SPTs were performed on 70 asthmatic children selected randomly from our practice. The skin-test extract used was the Dome Hollister Stier extract. The mixed cockroach extract consisted of extracts from *B germanica*, *B orientalis* and *P Americana*. SPTs were performed in a standardised manner with the following extracts: mite, grass, pollen, zea mays, cat, dog, Bermuda grass pollen, moulds, tree pollen, weed pollen and mixed cockroach extract. A wheal diameter of greater than 5 mm was regarded as positive.

Dust was collected from the homes of mite- and cockroach-sensitive asthmatics using a vacuum cleaner over a 1 m^2 area in the patient's bedroom. The dust was collected from the bedroom floors of 22 patients' homes and dust samples were sent to the Allergology Unit, University of Cape Town Medical School for immunochemical assays. The allergens Bla g I, II and Per A I were measured using monoclonal antibodies. The assays were performed using the method described by Schou et al[12] and the Bla g I antibodies were purchased from Dr Martin Chapman (United States).

RESULTS

The SPTs were positive to the mixed cockroach allergen extract in 29 of the patients tested (see Table I). This constituted 41% of patients. Skin-test reactively to HDM was demonstrated in 95% of patients. Cockroach sensitivity is therefore the second most common reactive allergen in SPTs in the Durban area.

TABLE I: SPT ON ATOPIC ASTHMATIC CHILDREN IN DURBAN

Allergen	% Positive
HDM	95%
Cockroach	41%
Cat	22%
Bermuda grass	14%
Dog	7%
Moulds	4%

ALLERGEN ASSAY

Bla g I levels were undetectable in six (27%) homes and found to be moderately high in 15 (68%) homes. It was very high in one home. Bla g II levels were undetectable in the majority of homes studied, that is 18 (81%) and detectable in only four (18%) homes (see Table II). Per a I levels were detectable in all the homes studies (see Figure 1). There were six homes in which Bla g I levels were undetectable but had measurable levels of Per a I, whereas 16 homes had detectable levels of Per a I and Bla g I. Per a I levels (ng/mL) in Figure 1 are shown in ng/mL and can be converted to µg/g dust by multiplying by a factor of 0.38.

TABLE II: COCKROACH ALLERGEN LEVELS (N = 22)

Levels		Number	%
Bla I	<0.02 (undetectable)	6	27.3
	0.02–5	15	68.2
	>5	1	4.5
Bla II	<0.02	18	81.8
	0.02–5	4	18.2
	>5	0	—

Bla g I and Gla g II levels are expressed in µg/gram dust

DISCUSSION

Our study has shown that cockroach allergy is the second most common allergy in asthmatic children following HDM allergy in Durban.

There are significant levels of Per a I allergen in the homes of our cockroach-sensitive asthmatic patients. Our data shows that Per a I is detectable in all the homes that we studied. The low and undetectable lows of Bla g I suggests that *B germanica* is a less important allergen of indoor house dust. The very high levels of Per a I in some

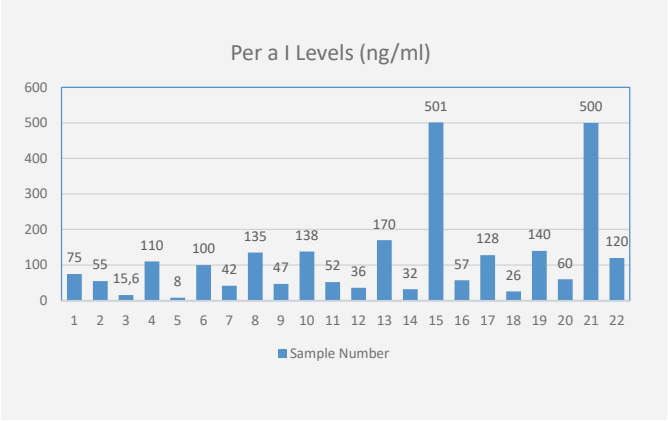

Figure 1: Per a I (ng/mL) can be converted to µg/g dust if multiplied by 0.38

homes suggests that other species of cockroaches such as *B orientalis* or other species may also be contributing to the total allergen load. Measuring Per a I level is a useful measurement since it quantifies the total allergen load and is not a species-specific allergen.

Considering the studies of Kang et al[2] and our studies, it is apparent that exposure to cockroach allergens contributes to the pathogenesis of asthma in our patients. The observation that cockroach-sensitive asthmatics are usually steroid-dependant and experience severe symptoms must be kept in mind when treating cockroach-sensitive asthmatics.[2] These patients often require frequent hospitalisation for acute asthma. Therefore prophylactic asthma therapy and environmental control need to be aggressively instituted in these patients.

Our study also has other important therapeutic implications. When considering immunotherapy for patients with mite-sensitive allergic rhinitis (AR) it is important that cockroach allergy be excluded in the Durban area. Immunotherapy may fail if measures are not introduced to reduce exposure to cockroach allergen.

In practice therefore, it may not be appropriate to de-sensitise against HDM for nasal allergy if the patient has a concomitant cockroach allergy. Avoidance measures that are used for HDM reduction also help with reducing cockroach exposure. However, the use of fumigation techniques and insect killer sprays must be combined with HDM avoidance protocols in patients with cockroach allergy.

One major drawback of our study was that we measured cockroach-allergen levels in the patients' bedrooms. Levels have been found to be highest in kitchens in other studies. This needs to be taken into account in future studies.

Our results also differ from the work of Pollart et al[4] in that cockroach infestation and sensitivity in their study affected mainly poor communities. In our study, cockroach allergy was shown to affect homes across the spectrum.

We therefore suggest that doctors practising in areas with a heave cockroach infestation look carefully for cockroach allergy in their asthmatic patients since this has important therapeutic implications We also suggest that skin-test kits in the Durban area should have the cockroach mix extract as part of the standard kit. If patients are unsuitable for skin testing, RAST (Pharmacia) can be performed as is available from commercial laboratories.

In conclusion, cockroach allergy is a problem in the Durban area and more detailed studies need to be performed to quantify the extent and significance of the problem.

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SANOFI

Festschrift Article

CRUSTACEAN ALLERGY IN SOUTH AFRICA

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A TRIBUTE TO PAUL POTTER

I have known Prof Paul Potter since 1993, when I joined the National Health Laboratory Services (NHLS) at Groote Schuur Hospital, and I was one of his first doctoral students. Paul was not only my supervisor, but inspired me to investigate new and indigenous allergens causing clinical symptoms in South African allergic subjects. His way of dealing with patients in South Africa, with its unique biodiversity of populations, flora and fauna, was central to my entire career in the field of allergy research.

He introduced me to the importance of focusing on patient service and management and not exclusively on the molecular side of laboratory sciences. Under Paul's guidance, we conducted the first studies in Africa on allergic sensitisation to various indigenous seafood species, for which there were no diagnostics available at the time. We identified a novel allergen in abalone (perlemoen) – the first African allergen given a formal designation by the International Union of Immunological Societies (IUIS), which was subsequently published in 1997 in the Journal of Allergy and Clinical Immunology, the premier journal in the field. A new ImmunoCAP for abalone was subsequently developed (f346).

Our work on indigenous allergens did not stop with perlemoen. We investigated in detail the allergens causing asthma through inhaling dust when breeding locusts. Not only critters but, in addition, pigeons generate dust that causes allergic alveolitis. All these studies were published in several international journals as well as in the South African Current Allergy and Clinical Immunology Journal, and they provided valuable information for practising clinicians.

Through Paul's dedicated training we were able to establish a strong research team, at the University of Cape Town and then the IDM, supported by seven ALLSA grants and several NRF and MRC grants. This team focused on various aspects of seafood allergy and their research resulted in eight doctoral and master's students' successfully completing their degrees in the field of allergy, including murine models on mechanisms of seafood allergy and sensitisation to fish through workplace exposure.

Whereas Paul started the African endeavour into seafood allergies with 100 patients from the Western Cape in 1996, 21 years later we continue this legacy of characterising indigenous allergens now Down Under, in Australia. With funding from Australia, the United States and Europe, our team has identified more than 20 novel seafood allergens and is now translating previous and current findings into the development of the first immunotherapeutic for fish and shellfish allergy.

I would like to highlight our milestone publication on the immunological cross-reactivity of South African patients with crustacean allergy to a range of related indigenous crustacean species. This study was one of the very first international studies to focus on other crustaceans species besides shrimps or prawns. The diagnostic workup of the subjects in this study was challenging as no specific diagnostics where available at the time. Furthermore, this study also demonstrated distinct different immune responses to specific allergens in individual subjects with crustacean allergy, in particular those to our South African crayfish.

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INTRODUCTION

Invertebrate species known to elicit adverse food reactions belong mainly to two phyla – arthropods and molluscs. These constitute the artificial grouping known as 'shellfish'.[1] The group of crustaceans (see Table I) contains more than 35 000 species (including barnacles) and the order Decapoda, including practically all edible species, contains approximately 8 000 species.

The local 'crayfish' in the Western Cape of South Africa is in fact a 'rock lobster' and it belongs to a different sub-order than the 'true' crayfish, which is a freshwater species not found in Africa (see Table II). This can result in missing the diagnosis of a crustacean allergy if the wrong species in the RAST is tested.

The sub-orders include shrimp, crab, lobster and crayfish, all of them closely related to one another. Consequently, similar antigenic structures occur and human IgE antibodies against antigens in one crustacean species may cross-react with those from other species.

The aim of this study was to analyse the immune response of subjects with food sensitivity to South African crustacean species. These subjects are part of a previous study published by our research group on seafood allergy.[2]

Sera from 80 subjects with perceived seafood allergy were obtained for RAST and Western-blot studies, of which 40 reported sensitivity to crustacean. All the subjects replied to a self-administered questionnaire concerning history and symptoms.

SPECIES

The most frequently reported sensitivity to crustacean species was to prawn (47%), followed by rock lobster (44%), shrimp (13%) and crab (12%) (see Figure 1).

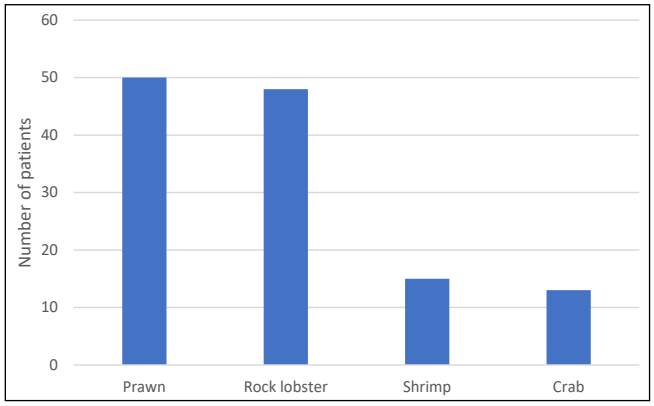

Figure 1: Frequency of crustacean species implicated in allergic reactions reported in the questionnaire by subjects with perceived seafood allergy

SYMPTOMS

The symptoms experienced by the subjects included: cutaneous, gastro-intestinal, respiratory and miscellaneous (see Table II).

Itching and swelling of the throat, lips and/or tongue were the most common symptoms; nausea and vomiting (78%) and, surprisingly, respiratory problems were also very common (61%).

The vast majority of IgE-mediated allergic reactions to crustaceans result from ingestion. However, we found that approximately one-third of the study group experienced clinical symptoms after handling seafood. The allergens from crustaceans can enter the atmosphere in steam aerosols and this explains the respiratory symptoms after 'smelling' cooked or grilled rock lobster, for example.[3] The inhalation of these aerosols could be a means of inadvertent exposure to crustacean allergens, producing reactions in sensitive patients;they could also be responsible for the fact that hypersensitive patients rarely lose their clinical reactivity. Aerosolised allergens are of particular importance in the occupational environment.[4,5]

IgE RESPONSE

In order to study the immune response of 40 subjects

TABLE I: CLASSIFICATION OF CRUSTACEANS, THEIR COMMON AND SCIENTIFIC NAMES AND AVAILABLE CAP-RAST

Class	Sub-orders	Common names	Some local species (scientific names)	Common southern African names	Available commercial CAP-RASTs
Crustacea	NATANIA	True schrimp; prawn	Tiger prawn: Penaus monodon	Tiersteurgarnaal	Shrimp: Pandalus borealis, f24
	REPTANTIA	Spiny lobster	Rock lobster - West Coast: Jasus Lalandli Rock lobster - East Coast: Panulirus homarus	Crayfish/kreef	
		Langoustine (Rock lobster)	Rock lobster - West Coast: Palinurus gilchristii Langoustine: Nephrops andamanicus		Langoustine/Spiny lobster: Palinurus sp, Rf304
	ASTACURA	Lobster; crayfish (freshwater crayfish)	No local species		Lobster: Homarus gammrus, f80 Crayfish: Astacus astacus, Rf320
	BRACHYURA	Shore, Stone and Fiddler crab	Cape stone crab: Neolithodes capensis Red crab: Chaceon sp.	Spinnekopkrap	Crab: Cancer pagurus, f23

TABLE II: FREQUENCY OF REPORTED SYMPTOMS IN 40 SUBJECTS WITH PERCEIVED CRUSTACEAN SENSITIVITY

SYMPTOMS	PERCENTAGE (%)
Oropharyngeal itching/swelling	100
Nausea/vomiting/abdominal pain	78
Asthma/wheezing	61
Anxiety	48
Urticaria/eczema	44
Flushing	22
Diarrhoea	17
Dizziness	13
Headache	9

with crustacean sensitivity their specific IgE reactivity was analysed in more detail.

The IgE response of 24 crustacean-sensitive subjects to langoustine, crayfish, lobster, shrimp and crab is illustrated in Figure 2A with means of 13.6, 6.4, 6.0, 5.0 and 5.0 ku/L respectively. The mean value for the langoustine RAST was far higher than the means for the other four crustacean species. Co-reactivity to all five crustacean species analysed was found in six subjects (see Figure 2B). However, the immune responses observed to the different crustacean species are not very different from one another.

ALLERGENS

Allergens from shrimp have been reported to be heat stable and to have molecular weights between 8.2 and 29 kDa.[6] However, few studies have focused on different lobster and crab species. More knowledge about the molecular characteristics of the allergens is needed to make a more specific diagnosis or prediction of cross-allergenicity among crustacean species. We therefore analysed six South African crustacean species by SDS-gel electrophoresis and Western-blotting to identify species-specific allergens.

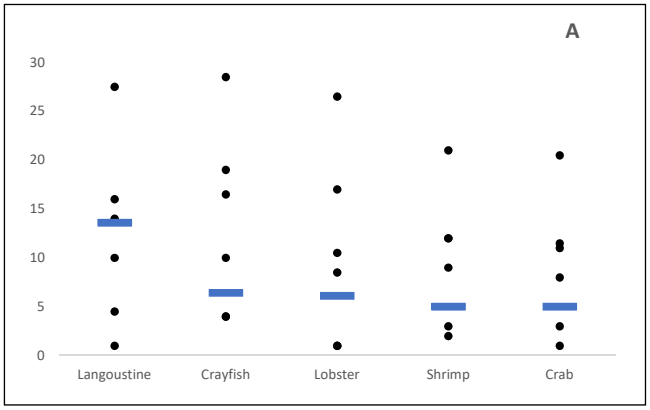

Figure 2A: Distribution of specific IgE response to different crustacean species in 24 subjects with seafood allergy and their mean values for each species: 13.6, 6.4, 6.1, 5.0 and 5.0 ku/L respectively. The following CAP-RASTs were used: langoustine (rf304), crayfish (rf320), lobster (f80), shrimp (f24), crab (f23)

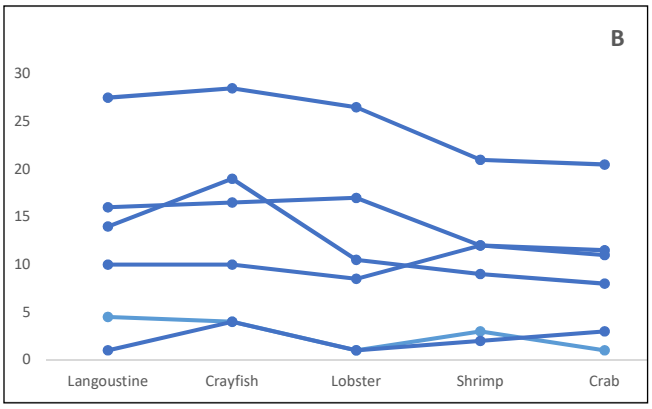

Figure 2B: Comparison of concurrent IgE response of six subjects for five different crustacean species

Immunoblots of several crustacean extracts with sera of two sensitised subjects (see Figure 3) demonstrated for subject A allergens in the molecular range of approximately 22 kDA for three of the six species investigated. In addition, all six crustacean species showed binding at apporximately 70 kDa for subjects A and B. Both subjects also demonstrated binding to allergens between 36 and 40 kDa in all analysed species. However, the immune response of subject A showed distinctly different allergens in rock lobster from the east coast compared to the other,

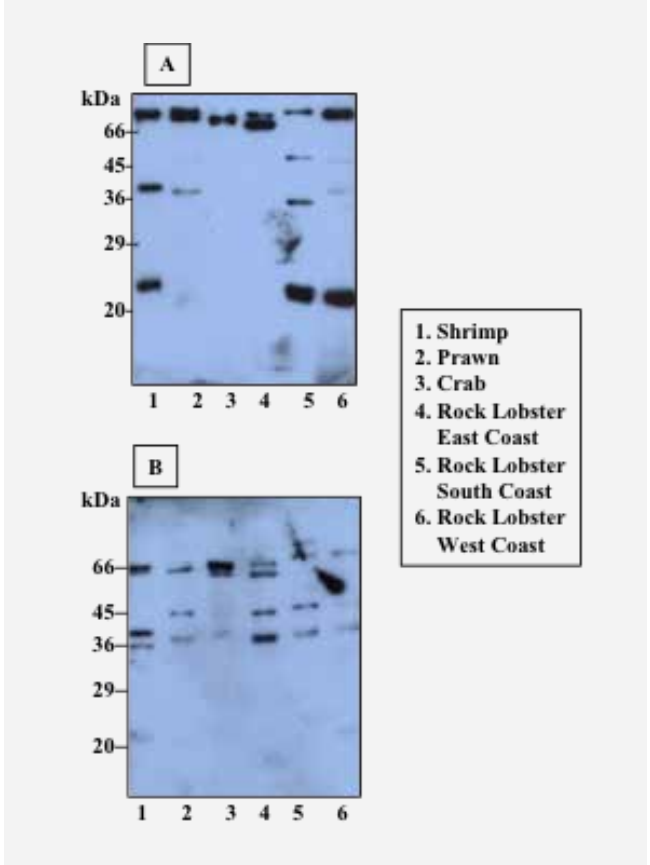

Figure 3: Western blot of IgE reactivity of two sensitised subjects (A and B) to South African crustacean species. The species' names are labelled from 1 to 6 and the molecular weights are indicated on the left side of kilodalton (kDa)

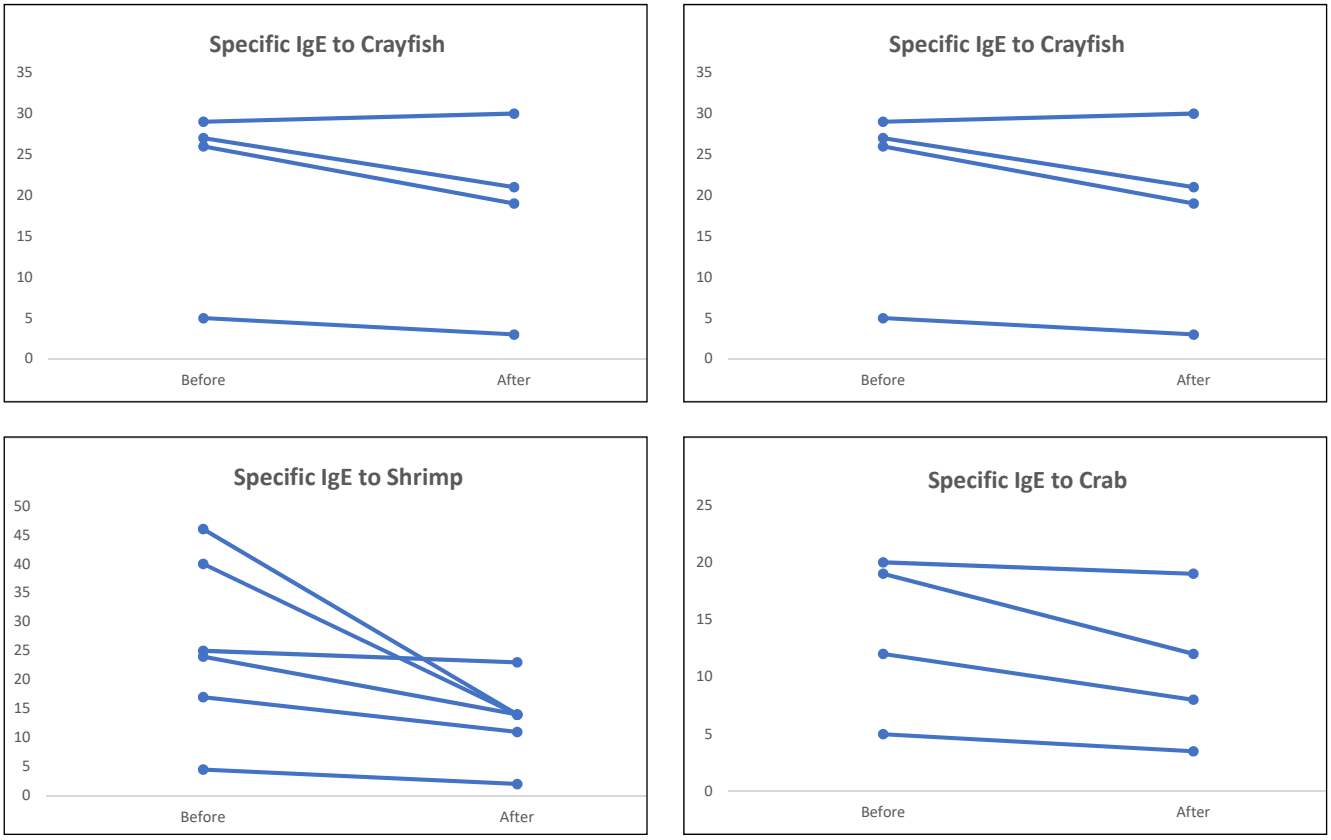

Figure 4: Specific IgE reactivity of six crustacean-sensitive subjects before consultation in our clinic and after 3–4 years, as measured by CAP-RAST in ku/L. All subjects, except one, demonstrated a decrease in IgE reactivity

very closely related rock lobsters from the south and west coasts.

Western-blot analysis of cooked crustacean species indicated decreased allergenicity. Nevertheless, some of the allergens are remarkably resistant to heat treatment. Other research groups have demonstrated that crustacean allergens are very stable; tropomyosin, a muscle protein of approximately 38 kDa, has been implicated in several other studies to be the major allergen of different crustacean species.[7]

However, the immunoblots produced in our study demonstrated higher molecular weight allergens in most of the species analysed. These results clearly indicate that specific allergens can vary among species and among individual subjects. Therefore, a detailed analysis of crustacean-sensitive individuals is important.

PERSISTENCE OF THE IMMUNE RESPONSE TO SEAFOOD

Six subjects, with concurrent allergy to crustaceans, were followed up 3–4 years after their first consultation in our clinic and the immune response to different crustacean species was compared by RAST reactivity (see Figure 4). In five subjects, the concentration of specific IgE antibodies to tested seafood species decreased by approximately 50%. The one subject who did not demonstrate a decreased immune response over three years admitted to occasionally ingesting seafood, in particular rock lobster and prawns, and taking oral antihistamines before meals to control symptoms.

CONCLUSIONS

Crustaceans are among the most frequently mentioned seafoods causing adverse reactions in food-sensitive individuals. Sensitivity should be regarded as lifelong.

The CAP-RAST results indicate a stronger immuno-response to the langoustine RAST with decreasing reactivity to crayfish, lobster, shrimp and crab. Because of the close phylogenetic relationship of our local rock lobster to langoustine, we recommend the use of langoustine CAP-RAST for identifying specific rock lobster IgE. We noted that crayfish is a freshwater crustacean species and lobster is a European crustacean species.

We observed that the IgE reactivity on Western-blots of the different local species is similar but not identical. Binding occurred to a 38 kDa allergen, which is probably the well-described tropomyosin also found in shrimps and prawns. However, additional allergens in the higher molecular weight ranges were also identified which have not been described previously in the literature.

Although we have encountered patients with sensitivity to

one crustacean but not to another, it is advisable to be very careful about avoiding all crustaceans if sensitivity to one is shown, unless double-blind challenges can be conducted to demonstrate that another crustacean is in fact safe to eat.

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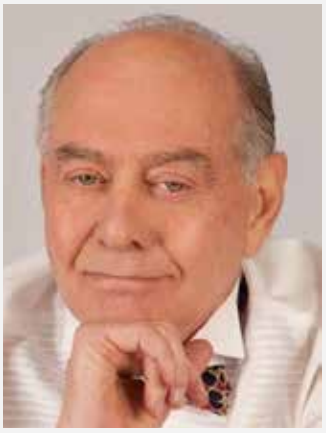
A SAD FAREWELL TO DR JOHAN JANEKE



Dr Johan Benjamin Janeke, an esteemed ENT surgeon died in September, 2017. He was a loyal member of ALLSA since it's founding in 1988.

After obtaining his MB ChB degree at the University of Pretoria Medical School in 1963, he went on to complete his PhD thesis in Otolaryngology at the University of Amsterdam in 1969. He continued his training in Otolaryngology at Baylor College of Medicine in Houston, Texas. Here he received the prestigious Ben Schuster Award for his outstanding research in nasal reconstruction.He returned to South Africa in 1975 and entered private practice as an ENT surgeon in Johannesburg.

Dr Janeke had the good fortune to meet many famous people during his career. Included among these were Elvis Presley and Bob Hope. He was a demonstrator in Anatomy under Prof Phillip Tobias at the University of the Witwatersrand. Most precious and memorable to him was a meeting with Nelson Mandela.

Dr Janeke was passionate about his profession and he had the ability to connect with his patients and their families in a special way. Although he was learned and gifted with great wisdom, he was a humble man who made it his mission to treat all of his patients with the greatest respect and compassion. He gave all of his patients the same level of care, dignity and respect no matter what what their station in life. In recognition of his unique ability, skills and dedication to his calling, he was awarded the prestigious Christiaan Barnard Memorial Award for his contribution to medicine and to the people of South Africa.

ENT Out of Africa, Dr Janeke's book

Alongside his private practice career, Dr Janeke was a passionate scholar, academic and author. He wrote and published several books in the field of Otolaryngology including 'ENT Out Of Africa' This book was translated into French and used for medical training in the Francophone countries of Africa. The English version was used as a textbook at the University of Witwatersrand Medical School and at several other medical schools in South Africa. He published articles in several peer reviewed journals in his field and was on the editorial Board of several journals in this country, including Modern Medicine

He was always innovative and sought new low cost ways to help the hearing impaired. He was the first ENT surgeon in South Africa to perform a cochlear implant. He developed the ABC implant as an alternative to the costly cochlear implant. When no longer able to practice,Dr Janeke turned his hand to painting. He soon developed his own unique style which he used to capture his fascination with nature, particularly trees.

Dr Janeke succumbed to pneumonia after a valiant 40 day battle. He was a committed Christian throughout his life. These values were constantly demonstrated in his personal life and in his practice. He leaves his wife Margaret, six children and six grandchildren.

Festschrift Article

LOW PREVALENCE OF LATEX SENSITIVITY IN SOUTH AFRICAN SPINA BIFIDA CHILDREN IN CAPE TOWN

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TRIBUTE TO PAUL POTTER

I began working with Paul at Groote Schuur Hospital on the 1st of February 2000. At that time, we were part of the Division of Laboratory Medicine. This was before the days of the NHLS. Our clinical responsibilities included a weekly allergy clinic at Red Cross Children's Hospital, run by Professor Eugene Weinberg. The big latex epidemic was taking hold and Paul was instrumental in getting powdered latex out of the hospital environment. We ran a latex clinic at GSH once a week, and diagnosed many new staff members weekly. In addition, it was at this time that Paul started up his unit at The Lung Institute.

We had, however crossed paths in the 80's, when I was still a medical technologist, and ran the routine allergy diagnostic laboratory at Medical School, under Professor Eugene Dowdle. The specific IgE's were conducted using a radioimmune assay. Paul was working on his MD thesis on beta receptors. We chopped up many a foetal lung to find him enough raw material. Perhaps the macabre nature of this work played a role in my decision to leave the laboratory and go back to UCT to study medicine. It was no surprise therefore that when he phoned me to offer me a job in his department in 1999 I easily relinquished the idea of Obstetrics and Gynaecology.

This article was published following the work done at Red Cross Hospital and GSH, mostly by a visiting dermatologist, Dr Asmah Johar from Malaysia. We collaborated with the Rubber Research Institute of Malaysia in Kuala Lumpur as well as the National University of Singapore. - Di Hawarden

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ABSTRACT

Studies from Europe and the United States report that spina bifida children have a high prevalence of latex allergy. This study investigated the prevalence of latex allergy in a cohort of 24 spina bifida children at the Red

Cross War Memorial Children's Hospital in Cape Town, South Africa. The children were investigated using a detailed questionnaire, skin-prick tests (ALK-Abello), ImmunoCap RASTs, Western blotting and ELISA, using the purified latex proteins Hev b1 and Hev b3 and whole latex preparation. A low overall prevalence of latex sensitisation of 16.7% was found in the children. Children who were sensitive reacted to water-insoluble Hev b1 and Hev b3 proteins. The low prevalence of latex sensitisation in the South African children may not be explained by stringent latex avoidance. The children were from a low socio-economic social status and 'hygiene' and other factors should be considered.

Key words: spina bifida; children; latex allergy; Hev b1; Hev b3

INTRODUCTION

Latex allergy affects approximately 0.1% of the general population.[1] Healthcare workers are frequently exposed to latex, especially in the form of surgical gloves. Therefore the prevalence among them is expected to be higher. Reports among healthcare workers from Europe and the United States give a prevalence of between 3% and 11% for latex allergy.[1] A survey among 2 316 staff members of Groote Schuur Hospital, South Africa showed a prevalence of clinical allergy in 9.2%.[2] Nevertheless, a high incidence of latex allergy is not necessarily an inevitable consequence of constant and repeated contact with latex. In contrast to what is observed in the West, prevalences of latex allergy in South East Asia are relatively low even among groups that are occupationally exposed to latex. Two Malaysian surveys among rubber tappers and glove factory workers gave prevalence figures of only 3% and 2%, respectively,[3,4] whereas only 1.3% of rubber tappers and 1.7% of latex glove factory workers in Thailand were allergic to latex.[5]

Spina bifida patients are frequently exposed to latex as they are in contact with latex products very early in life (e.g. gloves, catheters) and undergo multiple surgical procedures from an early age. A high prevalence of latex allergy of 29–64% has been found in spina bifida studies in the United States and Europe.[6–11] The prevalence reported in one European country, Italy, was substantially lower at 15%,[12] whereas a study from Venezuela put the figure at only 4%.[13] No reports are yet available in any Asian countries or South Africa. It has been also observed[14,15] that children with spina bifida in United State and Europe react to the water-insoluble Hev b1 (14.6 kDA) and Hev b3 (22 kDA) proteins to which healthcare workers are less commonly sensitised.

The aim of this study was to determine whether the high prevalence of latex allergy (particularly sensitisation to the rubber-particle proteins, Hev b1 and Hev b3) among spina bifida patients in the West was also evident in children with spina bifida living in Africa. To this end, we studied sensitivity to latex using a panel of latex skin-prick tests (SPT), ImmunoCap (Pharmacia) levels to latex (k82), ELISA and Western blot techniques using whole latex extracts and purified Hev b1 and Heb b3 proteins.

METHODS

Twenty-four patients between the ages of six months and 18 years who attended the spina bifida clinic at the Red Cross War Memorial Children's Hospital, South Africa, were studied.

Ten samples from adult patients with allergy to latex and ten samples from non-allergic children were used as positive and negative controls.

A questionnaire was used to obtain details of medical and surgical history, latex exposure symptoms and cross-reaction to fruits. Each patient underwent titrated SPT with the commercial latex preparations from ALK-Abello, and with purified Hev b1 and Hev b3 extracts at different dilutions of 1/1 000, 1/100, 1/10 and an undiluted sample obtained from the Malaysian Rubber Research Institute of Malaysia.

A positive reaction was a wheal of 3 mm or more greater than the saline control, and a wheal of 2 mm was regarded as borderline. Patient sera were tested using the Pharmacia ImmunoCap RAST (k82) test. A value of > 0.35 kU/L was regarded as positive.

PREPARATION OF HEVEA BRASILIENSIS LATEX, LATEX FRACTIONS AND THE PURIFIED LATEX ALLERGENS HEV B1 AND HEV B3

Fresh natural rubber latex from *Hevea brasiliensis* trees (clone RRIM 600) was collected into chilled containers at the Rubber Research Institute of Malaysia Experiment Station, Sungei Buloh. After centrifugation at 43 000 g for one hour at 4–7°C on a Sorvall RC 5C high-speed centrifuge, three main fractions were recovered: the rubber cream, the C-serum and the bottom fraction. Latex B-serum was prepared based on the method of Hsia.[16] The latex bottom fraction from centrifuged latex was washed by re-suspension in 0.4 m mannitol and recovered by centrifugation. The washed bottom fraction was then subjected to repeated freezing and thawing to rupture the lutoids that were its main constituents. The lutoidic fluid, the B-serum, was recovered as the supernatant after re-centrifugation. The rubber cream from centrifuged latex was sampled at the intersection between Moir's Zone 1 and Zone 2[17] so as to obtain rubber particles belonging to both the large and the small size classes.[14] The rubber particles were washed by re-suspension in 30% sucrose and proteins on the rubber-particle surfaces were then solubilised and extracted using a detergent mixture comprising 0.1% Triton-X 100 and 1% sodium dodecylsulphate (SDS). The two main proteins obtained were Hev b1 and Hev b3. These two proteins were separated and purified by

preparative electrophoresis on 15% SDS-polyacrylamide gel using the Prep Cell (BioRad, Hercules, CA) according to the manufacturer's instructions.

ANALYSES OF ALLERGEN–IGE BINDING BY WESTERN IMMUNOBLOT

The rubber-particle proteins extracted with SDS-Triton X-100 were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% gels according to the method of Laemmli[18] and transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% non-fat milk in phosphate-buffered saline (PBS-milk), pH 7.4, for 30 min and then incubated overnight with patient serum (diluted 1:5.25 in PBS-milk and 0.05% sodium azide) as the primary antibody. After three cycles of washing with PBS-milk, the nitrocellulose membrane was incubated for one hour with the secondary antibody, anti-human IgE conjugated to alkaline phosphatase. After a further three cycles of washing with PBS-milk, the nitrocellulose membrane was incubated for 10 min in Tris-buffered saline (TBS) before being immersed in 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) substrate to generate the coloured alkaline phosphatase reaction product. The results were recorded semi-quantitatively based on the intensity of the protein bands on the immunoblot. The results were reported in a semi-quantitative form (0, +, ++, +++).

ANALYSES OF ALLERGEN-IgE BINDING BY ELISA

The binding of proteins to IgE from latex-allergic and control patients was quantitated by ELISA. The proteins used in the assays were purified Hev b1, purified Hev b3 and a mixture of latex B-serum and C-serum (2:5 v/v) representing whole latex. ELISA plates were coated with the test proteins diluted in PBS, pH 7.4 and left overnight. The next day, the plates were washed three times in PBS containing 0.05% Tween-20 (PBS-T) and then they were blocked with 0.5% BSA in PBS-T for one hour before washing three times again with PBS-T. Patient serum diluted to 16% with PBS-T was used as the primary antibody source, with incubation carried out for three hours. After it was washed three times with PBS-T, biotinylated anti-IgE was added as the secondary antibody and it was incubated for one hour. The plates were washed once with PBS-T and then twice with Tris-buffered saline containing 0.05% Tween 20, pH 7.5 (TBS-T). Following this, 100 µL of streptavidin-conjugated alkaline phosphatase in TBS, pH 8.0, containing 0.2 mm magnesium chloride was added. The plates were incubated at room temperature in the dark for 30 minutes. They were then washed twice with TBS-T and once with TBS, pH 9.5 containing 50 mm magnesium chloride. Colour development was initiated by adding *p*-nitrophenyl phosphate in 10% diethanolamine buffer and the absorbance read at 405 nm using an ELISA plate reader. Blank values for each individual patient were subtracted from the ELISA plate-absorbance readings.

STATISTICAL ANALYSES OF SEROLOGIC TESTS

As ELISA absorbance readings do not normally vary linearly with IgE binding, a non-parametric analysis, the Kruskal-Wallis test was adopted. ELISA readings were taken from the different experimental treatments.[18] Comparisons of latex allergy prevalences between different studies were done using 2 × 2 contingency tables followed by Fisher's exact probability test.[19]

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics and Research Committee of the University of Cape Town.

RESULTS

PATIENT CHARACTERISTICS

Clinical characteristics and results of the serological evaluations of the latex-sensitive South African children are shown in Table I. The 24 children consisted of ten males (41.7%) and 14 females (58.3%). Their age ranged between 14 months and 15 years. The mean age was 8.34 years. All were diagnosed at birth except for one patient with lipomyelomeningocoele who was diagnosed at the age of two years. One patient had transverse myelitis at the age of three years and one patient had possible sinus pilonidal with a hypoplastic right thumb.

There was a personal history of atopy in six patients (16.7%); four (16.7%) had allergic rhinitis (AR), two had asthma (8.3%) and one had atopic dermatitis (4.2%). Ten patients had a family history of atopy: four (16.7%) had relatives with AR, nine (37.5%) had relatives with asthma, two (8.3%) had relatives with allergic conjunctivitis and two (8.3%) had relatives with atopic dermatitis. None of these patients had previously been diagnosed with latex allergy.

Of these patients, only one presented with symptoms of AR such as itchiness of nose and sneezing. However, these symptoms were aggravated neither at home nor in the hospital by any known latex exposure. All the patients were exposed to latex at home in the form of rubber toys, balloons, erasers, rubber lining splints, disposable nappies or rubber wheelchair cushions. None of the patients were having problems eating avocado, banana or kiwi, though a few had never been exposed to any of these fruits.

All the patients had undergone operations and one-third of them had had up to three operations. Those patients who had undergone more than three operations were those with complications of their ventriculo-peritoneal shunt. The common procedures done during operations were closure of myelomeningocoele, insertion of a ventriculo-peritoneal shunt and correction of ankle deformity, mainly for clubbed feet. All the patients had been exposed to latex gloves during operations, but only one had a latex-indwelling catheter.

TABLE I: CHARACTERISTICS OF THE SOUTH AFRICAN PATIENTS WITH POSITIVE OR BORDERLINE LATEX ALLERGY TESTS

							SKIN PRICK DIAMETER (MM)					ELISA‡			WESTERN BLOT	
Patients	Sex	Age (Yr)	Personal history of atopy	Relatives with atopy	Disturbing nasal symptoms	No of operations	ALK 100Hep*	Hev b1*	Hev b3*	Overall skin-prick reaction to latex†	IgE-Cap RAST (kUA/l)	Whole latex	Hev b1	Hev b3	Hev b1	Hev b3
1	M	10	No	Yes	No	3	0	2	3	Pos	<0.35	0.009	0.009	-0.02	0	0
2	M	6	No	Yes	No	4	4	3	0	Pos	37.9	1.258	1.377	0.335	+++	0
3	M	2	No	Yes	No	3	0	1	1	BL	<0.35	0.009	0.012	-0.024	0	0
4	M	3	Yes	Yes	Yes	2	3	0	2	Pos	<0.35	0.101	-0.009	-0.03	0	0
5	F	6	Yes	No	No	3	0	2	0	BL	<0.35	0.101	-0.017	-0.042	0	0
6	F	11	Yes	Yes	No	11	4	3	4	Pos	>100	>3.4	>3.4	>3.4	+++	+++
7	F	3	Yes	Yes	No	8	0	0	0	Neg	1.5	0.027	0.027	-0.018	+	0

** Results shown for reaction to highest concentrations tested.*
† Overall evaluation based on reactions to multiple dilutions of latex, purified Hev b1 and Hev b3. Pos, Positive; Neg, Negative; BL, Boderline
‡ Readings above 0.030 (which is equal to 3× the standard deviation of readings from non-latex-allergic pediatric controls) are regarded as positive.

SKIN-PRICK TEST AND SEROLOGY RESULTS

SKIN-PRICK TEST

The SPT was positive in four patients (Patients 1, 2, 4 and 6) (n ¼ 2 416.6%) and two patients had borderline results (see Table I). Three out of four patients reacted to the commercially available Alk 100 Hep latex allergen. The SPT with purified rubber-protein particle, Hev b1 and Hev b3 was positive in three patients (Patients 1 and 3), one of them not reacting to Alk 100 Hep. Two patients had borderline reactions to Hev b1 and Hev b3, of which one was only to Hev b1. The maximum wheal size diameter was only 4 mm.

For the patients who were aged seven years and older (median age ¼ 10), only one patient out of 12 tested positive.

IGE-CAP RAST TO LATEX AND ELISA TO PURIFIED RUBBER-PROTEIN PARTICLE

IgE-Cap RAST was positive in three patients (Patients 2, 6 and 7) with one patient having a level greater than 100 kUA/L. This patient had undergone 11 operations because of the complications with her ventriculo-peritoneal shunt. One of these patients (Patient 7) was negative to SPT with a low IgE level (1.5 kU/L) despite having had eight operations. ELISA testing was also positive for these three patients.

The paediatric controls were negative for both tests.

The latex-positive children were positive to the whole latex extract in the ELISA. One patient had a slightly raised reading of 0.101 and was also positive to SPT, despite being negative IgE-Cap RAST. Latex-positive adult controls had readings that were significantly above the whole latex ELISA pediatric controls (see Table I).

Western blots were positive to Hev b1 in three of the children and only one was positive to Hev b3. None of the ten adult-control latex-positive patients were positive to Hev b1 or Hev b3 in the Western blots.

DISCUSSION

Spina bifida patients[20,21] and adults undergoing multiple operations[22] are at risk of developing latex allergy. These patients may not be symptomatic even though they are sensitised to latex allergens. Testing for latex sensitisation is important for those requiring more than two operations in order to avoid clinical reactions. Two patients with positive serologic tests had a history of AR. They were, however, not symptomatic in the hospital environment. Latex gloves have not been used in the spina bifida clinic for the past six years and this probably this could explain why these patients were not symptomatic. The third positive patient, Patient 6, who had undergone 11 operations, is much older (11 years old) and had high levels in all the serologic tests. This patient would have been significantly exposed to latex gloves in the first few years of life.

It is possible that the low prevalence of latex sensitisation in the small cohort we have studied in spina bifida patients from the Cape Town area of South Africa might have been because the younger patients were exposed to much less latex during the past six years, during which time latex-avoidance measures have become more stringent. A comparison was made between the 12 patients in the sample who were seven years or older and who therefore had been exposed to latex with those who were younger than seven. The results (see Table II) showed that the prevalence of latex sensitisation was not significantly

TABLE II: LATEX ALLERGY PREVALENCE AMONG SPINA BIFIDA PATIENTS AS DETERMINED BY SPT

				Comparison with latex allergy prevalence in the present study* (p-value)	
Study	Country	Sample size	Latex allergy prevalence (%)	All South African patients	South African patients aged seven and above†
Present study (all patients)	South Africa	24	16.7	–	–
Present study (patients aged seven and above†)	South Africa	12	16.7	–	–
Yassin et al (1992)[6]	United States	76	64.5	< 0.0001***	0.0032**
Kelly et al (1993)[7]	United States	86	48.8	0.0050**	0.0600 NS
Niggemann et al (1996)[9]	Germany	81	44.4	0.0165*	0.1135 NS
Moneret-Vautrin et al (1993)[8]	France	25	32.0	0.3209 NS	0.4447 NS
Nieto et al (1996)[10]	Spain	100	39.0	0.3055 NS	0.5054 NS
Bernadini et al (1998)[11]	Italy	59	15.3	1.0000 NS	1.000 NS
Capriles-Hulett et al (1995)[13]	Venezuela	93	4.3	0.0545 NS	0.1388 NS

** Probability observed difference is because of chance occurrence, as determined by the Fisher's exact probability test. NS = not statistically significant.*
† Young patients who received treatment in a mainly latex-free environment excluded.

higher in the older group of patients who had had greater exposure to latex in our hospital before more stringent latex-avoidance measures were adopted.

We have also recently studied a smaller group of seven Singaporean children (data not shown) with spina bifida and found the prevalence of latex allergy to be 42%. A large study would be necessary to determine the true prevalence of latex allergy in this Asian region.

We have confirmed that the rubber-elongation factor (Hev b1) and a small rubber-particle protein (Hev b3) play an important role in the development of latex allergy in spina bifida patients using ELISA, Western blot and SPT. The negative results on ELISA and Western blot among the 'control' confirmed latex-sensitive adult healthcare workers confirm that they are not commonly sensitised to Hev b1 and Hev b3.[10] This observation is consistent with previous reports of European and American patients.

The major finding in this study is that the prevalence of sensitisation to latex and to the rubber-particle proteins among spina bifida patients in South Africa (16.7%) is significantly lower than that observed in Europe and the United States,[6–10,12] and it is similar to the prevalences reported from Italy[11] and Venezuela.[13]

In the study from Venezuela[13] it was suggested that the low prevalence was because of the strict avoidance of latex exposure in that group of children and a low frequency of surgical procedures.

The low prevalence of latex sensitisation at the Red Cross War Memorial Children's Hospital is not necessarily the result of a policy to avoid using latex gloves in the spina bifida clinic in the past six years, as the prevalence in the study group is the same as in the children who were exposed to latex. Airborne latex allergens may be a route of sensitisation, especially in those with AR or asthma. It is likely, though, that repeated and prolonged mucosal exposure is the major route of sensitisation. However, other factors such as population prevalences of allergy, 'hygiene' factors and 'Western lifestyle' may also influence the prevalence of latex sensitisation in spina bifida patients. All of the South African children in this study were of mixed ethnicity and from a poor community of low socio-economic status. None of the children in the cohort were from black African ethnic groups (i.e. Xhosa) in the region. We have recently found the prevalence of atopic sensitisation to aeroallergens to be 42.3% in urbanised Xhosa children and 45% in other urbanised children in the Cape Town area.[23]

High prevalences of latex allergy in spina bifida children have been reported from more affluent countries and only in the northern hemisphere.

The detection of latex allergy is important in spina bifida patients because they may not be clinically symptomatic, but may still be at risk of severe clinical reactions if they are inadvertently exposed to them. Most hospitals in developing countries are not latex-free.

Although the prevalence of latex sensitisation in the South African spina bifida children is low, it is probable that in countries where latex avoidance has more recently been strictly implemented that the high prevalence of latex allergy in spina bifida levels previously reported in the

United States[6,7] and Europe[8,9] should have decreased. Studies of the current prevalence among infants born in the past four years in these regions would be of great interest.

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Festschrift Article

REGIONAL-SPECIFIC POLLEN AND FUNGAL-SPORE ALLERGENS IN SOUTH AFRICA

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A TRIBUTE TO PAUL POTTER

Paul Potter shared work space with me when he took up his position as the first registrar appointed to the Allergy Department at the Red Cross War Memorial Children's Hospital. He was immediately intrigued by pollen sampling and centred his project around the daily pollen count and the application of the aeroallergens to the diagnosis and treatment of the allergy clinic patients. His interest in aerobiology did not wane when he moved to Groote Schuur where he played a major role in the publication of aerobiology findings. Paul included me in the design and preparation of a Cape Town Allergy Congress workshop and he arranged for congress delegates who wanted to learn how to identify aeroallergens to spend time in my laboratory. He coerced me into co-editing a textbook: Pollen and Mould Allergens in South Africa. When I left the Red Cross Hospital he arranged funding so that pollen sampling could continue under the auspices of his Allergy Diagnostic and Clinical Services Unit at the University of Cape Town Lung Institute. A long pollen data set had accumulated from the ground-breaking work of Eugene Weinberg, followed by later pollen monitoring, so with Paul's support and encouragement I am documenting a 30-year history of the Aerobiology of the Western Cape. He has invested countless hours in discussions and debates concerning this project. His energy and enthusiasm have been inspiring and have motivated me to turn a job into a lifelong career.

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ABSTRACT:

The selection of skin-prick and ImmunoCAP radio-allergosorbent test (RAST) testing panels for tree, grass, weed and fungal-spore aeroallergens is an easier task when pollen and fungal-spore levels for the particular geographic area are accessible to the allergologist. In South Africa, long-term pollen and fungal-spore level information exists for the Western Cape, where several areas in the greater Cape Town Metropole have been monitored. In Gauteng, sporadic pollen-sampling studies have been carried out in the vicinity of Johannesburg and Pretoria. In KwaZulu-Natal, short-term studies have sampled airspora in Durban and Richards Bay, and a three-year pollen and fungal-spore assessment has been conducted in Secunda, Mpumalanga. These findings are examined in relation to the choice of allergen-testing panels. There are vast unsampled areas of South Africa – containing major cities – where the major pollen allergens and their levels in the atmosphere are not known and, in addition, there is great variation in climate across these areas. Climate dictates the vegetation biome as well as the flowering times of allergenic plants. Guidelines for the selection of allergen-testing panels in clinical settings, where no pollen monitoring exists, are discussed.

HISTORICAL BACKGROUND

David Ordman, who pioneered the study of environmental aeroallergens in South Africa from 1945 to 1972, outlined many of the environmental problems relating to aeroallergens in different regions of southern Africa (South Africa, Zimbabwe and Namibia) providing a solid research base.[1–6] The discovery of the house-dust mite (HDM) provided evidence for his observation that house dust at the coast was more allergenic than dust from houses inland, as HDM prefer humid conditions.[5] Ordman's examination of the incidence of allergic disease in population samples in urban vs rural environments in the same ethnic group were illuminating.[5] Evidence-based studies in the second half of the 20th century confirmed his theories.[7,8] His examination of the allergenicity and flowering times of grasses, such as *Hyperrhenia hirta*, which he noted as 'the dominant grass found east of the Drakensberg escarpment' or *Themeda triandra,* found west of the Drakensberg mountains,[2] were expanded by Potter and Prescott.[9] They demonstrated IgE activity reactivity in >90% of patients tested for the

African grass *Pennisetum clandestinum* (kikuyu), which was introduced from East Africa. Their work was expanded to study the indigenous grass, *Stenotaphrum secundatum* (buffalo grass), which grows in many coastal areas on the east coast of South Africa, and *Eragrostis curvula,* a pasture grass that is widespread in the country. Ordman identified allergenic trees such as *Cupressus* (cypress) and *Schinus molle* (Brazilian pepper tree), and his understanding of the influence of climate on respiratory disease established the groundwork for aerobiology and the identification of specific pollen and fungal-spore allergens in South Africa.

WESTERN CAPE POLLEN-SAMPLING STUDIES

Emeritus Professor Eugene Weinberg, who was head of the Allergy Clinic at the Red Cross War Memorial Children's Hospital for three decades, reintroduced pollen sampling to the Western Cape in 1973. He established a pollen and fungal-spore monitoring programme at the School of Child and Adolescent Health, Department of Paediatrics, University of Cape Town. This monitoring system was set up to identify the allergenic pollen and fungal spores in the ambient air, and to measure the levels and assess the seasonality of the aeroallergens triggering symptoms in his allergic patients. The range of pollens and fungal spores that the unit has been able to identify is shown in Table I. On his retirement, the pollen-sampling programme was supported by Professor Paul Potter, head of the Allergy Diagnostic and Clinical Research Unit at the University of Cape Town Lung Institute, using a volumetric spore trap. This spore trap was initially sited at the Land Survey Building in Mowbray and later at the South African Astronomical Observatory, in the suburb which bears its name.

THE RONDEBOSCH SAMPLING SITE (1973–2007)

This aeroallergen-monitoring programme was set up at the Red Cross War Memorial Children's Hospital in Rondebosch primarily for the benefit of the patients attending the Allergy Clinic at the hospital. The findings were, however, also shared with the other academic hospitals in the Western Cape.

RONDEBOSCH POLLEN LEVELS

Grass was found to be the major aeroallergen in this area, which had a short tree season (August–October). The major tree-pollen loads were found to be *Platanus* (plane) and *Quercus* (oak) with low levels of *Ulmus* (elm), *Myrtus* (eucalyptus), *Olea* (olive), *Cupressus* (cypress), *Pinus* (pine) and Casuarina pine. There were occasional low counts for *Acacia, Betula* (birch), *Populus* (poplar) and *Salix* (willow). Weed-pollen levels were rarely significant and included: *Plantago* (English plantain), *Myrica* (sweet gale), *Chenopod* (goosefoot), *Urtica* (nettle) and *Taraxacum* (dandelion). Very low levels were recorded for fern spores (*Polypodium*), *Rumex* (Polygonaceae) and *Oenothera* (evening primrose). The typical Cape *fynbos* was represented by low background counts of Erica (heather), *Asteraceae* (daisy family) and occasional *Proteaceae* pollen grains.

TABLE I: POLLEN GRAINS & FUNGAL SPORES IDENTIFIED BY LIGHT MICROSCOPY

POLLEN GRAINS		FUNGAL SPORES
Cyperaceae	Ericaceae	*Alternaria*
Poaceae	Euphorbiaceae	*Ascospores*
Restionaceae	Iridaceae	*Aspergillus/Penicillium*
Typhus	Juncaceae	*Basidiospores*
Acacia	Liliaceae	*Bispora*
Betula	Loranthaceae	*Chaetomium*
Cupressaceae	Malvaceae	*Cladosporium*
Hippocastinaceae	Myricaceae	*Curvularia*
Moraceae	Oenotheraceae	*Epicoccum*
Myrtaceae	Oxalidaceae	*Nigrospora*
Oleaceae	*Plantago*	*Fusarium*
Pinaceae	Polygonaceae	*Helminthosporium*
Podocarpaceae	Polypodiaceae	*Leptosphaeria*
Platanus	Proteaceae	*Periconia*
Populus	Ranunculaceae	*Pithomyces*
Prosopis	Rosaceae	*Pleospora*
Rhus	*Rumex*	*Puccinia*
Quercus	Rutaceae	Rusts
Salicaceae	Solanaceae	Smuts
Ulmus	*Taraxacum*	*Spegazzinia*
Casuarina	Thymelaceae	*Stemphyllium*
Amaryllidaceae	Tiliaceae	*Tetraploa*
Asteraceae	Umbilliferae	*Torula*
Boraginaceae	Urticaceae	Mildew
Bruniaceae	*Parietaria*	
Caryophyllaceae		
Chenopodiaceae		

RONDEBOSCH FUNGAL-SPORE LEVELS

Fungal-spore counts were variable and weather-dependent but significant levels were consistently recorded for *Alternaria, Cladosporium* and *Epicoccum.* Ascospore levels peaked following rain and Basidiospore levels rose in autumn months. The highest fungal-spore levels were recorded during the spring and autumn seasons.[10]

THE MOWBRAY SAMPLING SITE (2008–2009)

Similar findings to those from the Red Cross War Memorial Children's Hospital were recorded for this nearby site, the Land Survey Building (see Figure 1), which was deliberately chosen for continuity of the airspora levels.

MOWBRAY POLLEN LEVELS

In addition to the pollen allergens listed above, significant

Figure 1: The Burkard spore trap at the Land Survey Building site in Mowbray

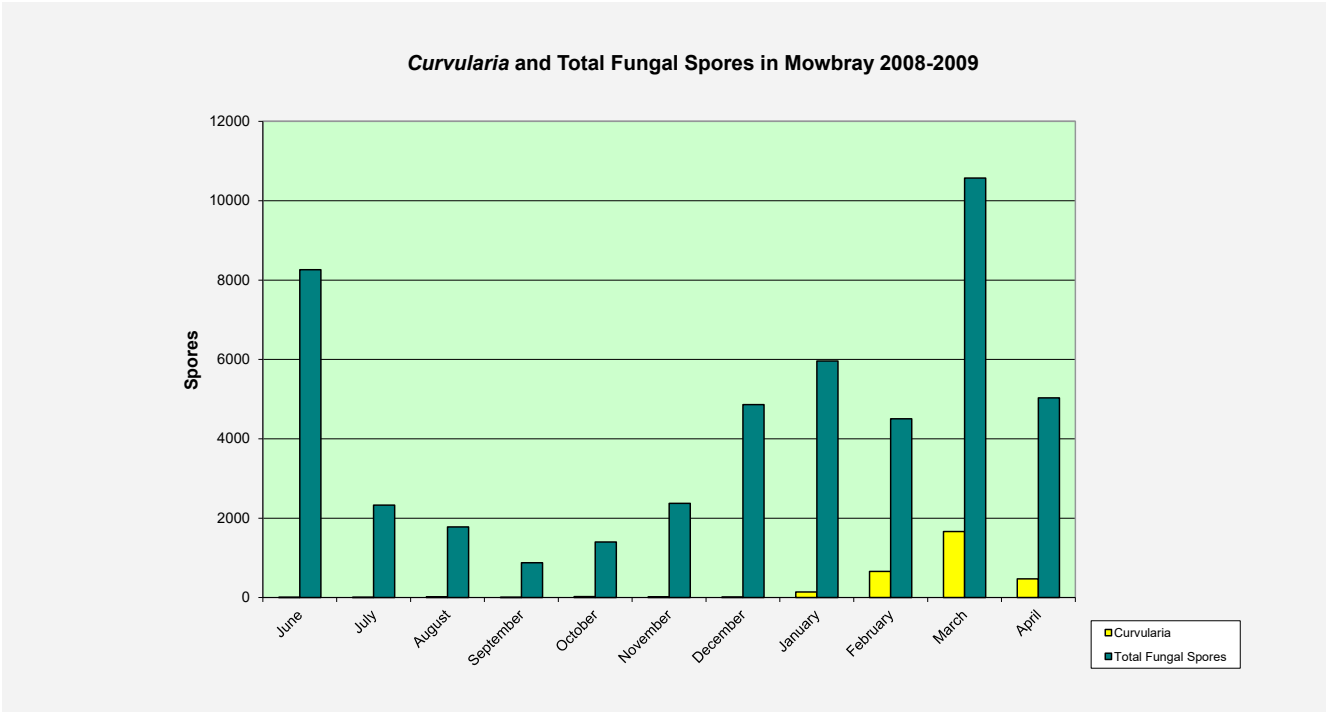

Figure 2: Culcularia and total fungal spores for Mowbray (2008–2009)

levels of *Rhus* (kareeboom) pollen were recorded. The grass-pollen load appeared to be slighter than previously measured levels at the Rondebosch site, but this may have been attributable to meteorological conditions and as the spore traps were not run concurrently, grass levels at the two sampling sites could not be compared.

MOWBRAY FUNGAL-SPORE LEVELS

The most important difference between this site and the other sites was the *Curvularia* peak each autumn when significant levels were seen for this taxon (see Figure 2).[11]

THE OBSERVATORY SAMPLING SITE (2010 TO DATE)

OBSERVATORY POLLEN LEVELS

The most important difference regarding the pollen trapped at this site was that there was a longer tree-pollen season with a larger tree-pollen load. Significant levels for *Cupressus* (cypress), *Quercus* (oak), *Rhus* (kareeboom) and *Platanus* (plane) tree pollen were recorded from early August until late October.

OBSERVATORY FUNGAL-SPORE LEVELS

The Observatory fungal-spore levels were unremarkable and showed the usual increases for *Alternaria, Cladosporium* and *Epicoccum* that are usually seen during spring and autumn months (D Berman – unpublished data).

THE TABLEVIEW SAMPLING SITE (1985 TO DATE)

The City of Cape Town added a pollen and fungal-spore monitoring system to their Air Quality Monitoring Project in 1985 and this has been continued intermittently.

TABLEVIEW POLLEN LEVELS

In keeping with the climate of this West Coast sampling site, grass was the dominant pollen while the tree season was scant and fleeting (September–October). For the sampling years 1995–2003 the grass season began two weeks earlier at this site than at the inland sites. Weed pollen was not significant but higher scores for weeds were obtained from this coastal site when compared with the weed-pollen levels at inland sampling sites.

TABLEVIEW FUNGAL-SPORE LEVELS

The fungal-spore levels were moderate overall at this sampling site, with sporadic autumn and spring peaks for *Alternaria* and *Cladosporium* (D Berman – unpublished data).

POLLEN MONITORING IN GAUTENG

ATMOSPHERIC POLLEN LEVELS

Ordman's aerobiological studies in Gauteng were extended by Cadman, who noted that the climate zone of this city is grassland, bordering savanna. Pretoria North is situated in the savannah belt. Despite the strong presence of northern hemisphere trees, which have been introduced and naturalised, the tree-pollen season is short (October–November).[12] Trees that make up the spring tree-pollen peak in this region are the following taxa:

- *Fraxinus (ash);*
- *Pinus (pine);*
- *Populus (poplar);*

- *Platanus (plane);*
- *Alnus (elder);*
- *Celtis (stinkwood).*

It is not surprising that the dominant pollen allergen in the sampled areas of Johannesburg and Pretoria is grass. The season runs from October to March, peaking later than the Cape grass season, as would be expected in this summer rainfall area. Similarly to Cape Town, weed pollen was not significant although weed-pollen allergens such as *Platanus* (English plantain) and *Tagetes minuta* (khaki weed) were present in the atmosphere.[12] Cosmos pollen would be identified as *Asteraceae*, a constant background presence in pollen assemblages and pollen-monitoring studies.

POLLEN RECORDS FROM KWAZULU-NATAL

Sampling sites have been set up in and around Durban and Richard's Bay in this subtropical region. These east coast towns enjoy milder temperatures, with abundant rainfall and increased relative humidity (RH) levels, and consequently higher perennial fungal-spore levels were recorded. Grass was a dominant aeroallergen and the dominant tree pollen was found to be *Morus*. The dominant weed pollen was *Cannabis*. The levels of this taxon exceeded the grass-pollen levels.[13]

THE MPUMALANGA POLLEN RAIN

A pollen-sampling study was carried out in the vicinity of Secunda in this lowveld region from 2006 to 2009 in order to assess the pollen and fungal-spore content of the atmosphere. This area of Mpumalanga is characterised by flat grasslands which include *Themeda triandra* (thatching grass).[2]

GRASS POLLEN

In this summer rainfall region, grass-pollen levels peaked in January and significant levels for grasses were recorded.

TREE POLLEN

Low tree-pollen counts were obtained from this area which is characterised by scrub vegetation. Indigenous tree pollen is infrequently allergenic as trees are invariably insect- or bird-pollinated and the pollen is not windborne or produced in large amounts. The tree-pollen load was dominated by alien trees such as plane, oak and eucalyptus.

WEED POLLEN

Weed-pollen levels were low, but small peaks for *Asteraceae* were seen during the autumn months of March and April, probably contributed by cosmos, which flowers in abundance in that region. The area around Secunda is often referred to as 'cosmos country', because of the thickly massed fields of these plants (see Figure 3) that are found in the vicinity. Cosmos belong to the *Asteraceae* family, introduced to South Africa from Mexico and naturalised.

Figure 3: Cosmos

FUNGAL SPORES

The major aeroallergens for this study were fungal spores, particularly *Epicoccum, Alternaria* and *Cladosporium.* This lowveld area experiences high RH, which frequently exceeds 90%. When this high RH coincides with temperatures that are >20°C, ideal conditions for the proliferation of fungal spores occur. The highest fungal-spore counts were recorded in February when counts >30 000/m^3 for *Cladosporium* were recorded (see Figure 4). This is in keeping with findings from other countries, where *Cladosporium* is often the dominant fungal spore.

GUIDELINES FOR THE SELECTION OF AEROALLERGEN-TESTING PANELS

AEROALLERGEN TESTING IN A NEW CLINICAL SETTING

It is difficult to select allergens for testing in a new environment. Ideally, skin-testing profiles are selected, based on pollen-monitoring records from the region. These pollen counts indicate the important pollen and fungal-spore levels in the atmosphere as well as the months of the year when the aeroallergens peak. It is sensible to choose testing panels for aeroallergens based on the experience of allergologists in other regions of southern Africa. A thorough knowledge of the seasonality of the major trees, grasses and weeds of the new study area is helpful. In cities, the allergenic pollen load has invariably been composed of alien pollen from northern hemisphere trees, which have been introduced and naturalised, particularly *Platanus* (plane) and *Quercus* (oak), but also *Olea* (olive). *Olea europeae* and *Olea capensis* are found in the Western Cape and the pollen may be an important allergen in areas where olives are cultivated, such as in Paarl in the Western Cape.

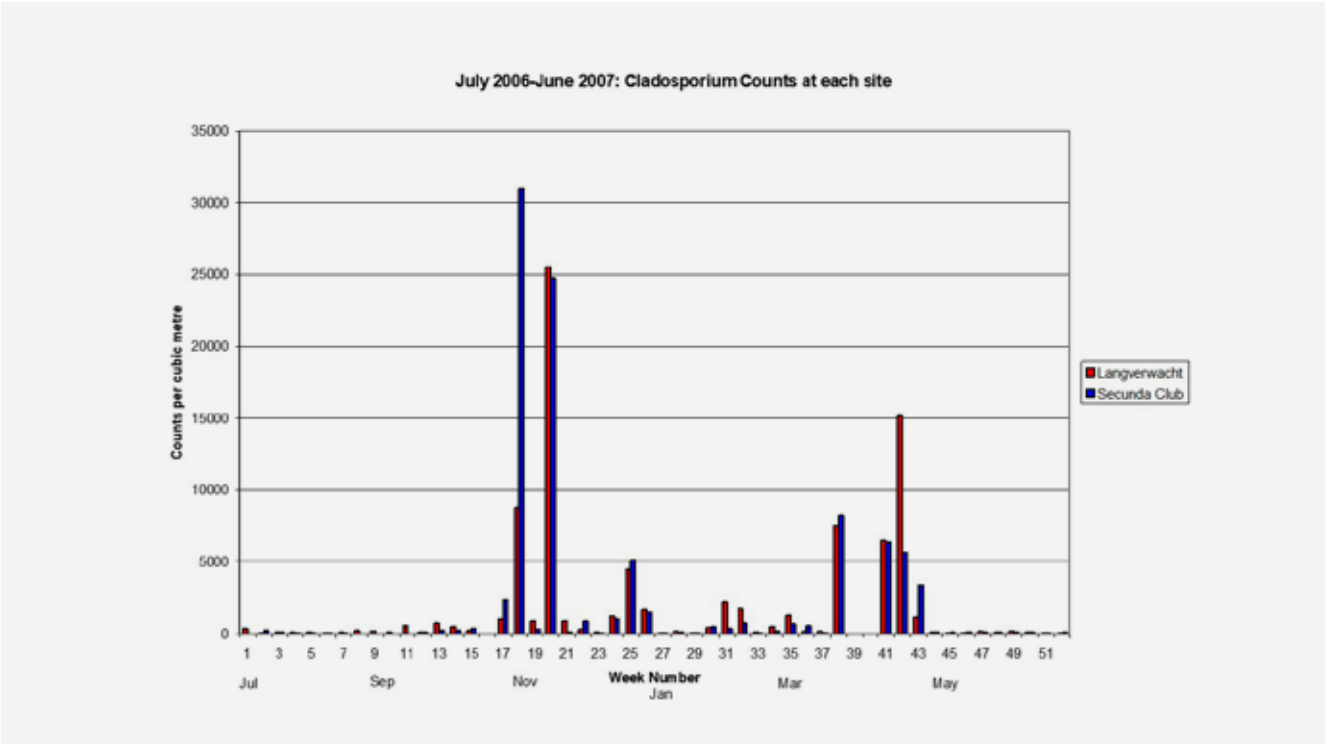

Figure 4: Annual Cladosporium levels (weeks) from Mpumalanga sampling sites

SUMMARY OF SUGGESTED GUIDELINES FOR THE CHOICE OF POLLEN/FUNGAL SPORE-TESTING PANELS IN A NEW ALLERGY CLINIC

1. If no pollen monitoring data exist for a new clinic, select pollen and fungal-spore allergens that have been found to be important allergens in other regions of southern Africa, that is, with a similar biome to the new clinic.
2. Audit the frequency of aeroallergen-specific sensitivity from positive skin-prick or ImmunoCAP RAST testing in the new clinic. This may prove useful when diagnosing patients with symptoms of seasonal allergic rhinitis (AR).
3. Set up contact with staff members of the Botany Department of the nearest university, technikon, botanical garden, parks and forest department or garden plant nursery. This will be invaluable when identifying allergenic plants, or plants that may cause contact dermatitis in your area. Pollen-flowering calendars are a very useful guide and are usually available from local botanists or botanical garden bookstores.
4. If there is a municipal air pollution department, a mutually beneficial relationship may develop between the staff of the allergy clinic and the air pollution personnel, in assessing triggers for seasonal allergy.
5. Take note of the local vegetation and record the flowering times of trees, weeds and grasses in the new area.
6. Note the weather patterns in your region. Weather parameters that influence pollen counts include the rainy season, humidity levels, temperature fluctuations and wind speed and direction.
7. If large, important sporting events especially running and cycling marathons, are held in your region, it is important to be informed about the seasonal allergens that may trigger symptoms of allergy in these elite athletes, so that they may be able to take appropriate and permitted medications.
8. Consider adding *Zea mays* to the testing panel in farming areas where maize is grown. It is interesting that sugarcane pollen does not appear to be allergenic.

POLLEN CALENDAR

Figure 5 summarises in calendar form the pollen frequencies from the sampling sites.

DISCUSSION

In Europe, pollen calendars are constructed for the benefit of travellers who suffer symptoms of seasonal AR. It has not yet been possible to design a comprehensive pollen calendar for South Africa because of the dearth of sampling sites in this country; however, pollen calendars of allergenic plants should be constructed when aerobiological studies are unavailable for a particular region.

Pollen sampling site	Pollen species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Western Cape	Tree												
	Grass												
	Weed												
Gauteng	Tree												
	Grass												
	Weed												
KwaZulu-Natal	Tree												
	Grass												
	Weed												
Mpumalanga	Tree												
	Grass												
	Weed												

Figure 5: Calendar of pollen from South African sampling sites

DECLARATION OF CONFLICT OF INTEREST

The author declares no conflict of interest.

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Allergies in the workplace

SKIN EXPOSURE, SYMPTOMS AND ASTHMA IN OCCUPATIONAL SETTINGS – IS THERE A LINK?

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ABSTRACT

Occupational contact with allergens through the dermatological and respiratory route can lead to various health effects, including allergic contact dermatitis, urticaria, rhinitis and asthma, which may result in a substantial disease burden in occupational settings. Inhalation is an obvious route for sensitisation and its pathophysiological mechanisms are relatively better defined. Recent studies have suggested that skin exposure may also play an important role in the sensitisation and development of respiratory outcomes in exposed workers. The review found that the evidence for such an association was limited and the immune mechanisms were not well understood. A better understanding of this association is required, more so for agents with a high molecular weight (protein), so as to identify important risk factors that may contribute to existing preventive efforts in reducing the incidence of allergy and asthma in occupational settings.

INTRODUCTION

Diverse immune responses have been associated with various environmental allergen exposures to the skin and these have resulted in the activation of the adaptive immune response.[1] In barrier-disrupted skin, type-2 lymphoid cells, mast cells and basophils are known to trigger pathogenic Th2 responses in murine models.[2] However, even when the skin barrier is intact; it still has properties that may allow, to some extent, permeability to some allergens. The 'brick-and-mortar' model – a schematic explanation of the permeability of the stratum corneum, in which corneocytes are the bricks and lipids are the mortar – presents the stratum corneum as a metabolically active structure with adaptive functions. This could be behind the mechanism in which some allergens could be absorbed by a healthy skin.[3]

Some epidemiological studies and animal experiments have demonstrated positive associations between skin and respiratory symptoms and positive exposure–response relationships, whereas others have not been able to replicate this.[4-9]

One of the main issues highlighted by these observations has been the challenges in investigating skin exposure.[10] Methodologies for assessing skin exposure are not as well advanced as for inhalational assessment and exposure data are limited.[11,12] In addition, airborne exposure to skin is variable and sporadic and there is uncertainty regarding the extent of skin uptake.[10] Furthermore, most workers are exposed to mixtures of occupational agents, making it difficult to identify the specific exposure responsible for the health outcome of interest. For example, exposure to beryllium and isocyanates typically occurs in mixed exposure settings and these agents may be present in various physical or chemical forms, namely, metal particles, oxides, salts and alloys with copper and other metals.[10]

The aim of this literature review is to assess the nature and extent of the associations between skin and respiratory symptoms following skin exposure to sensitisers with a high or a low molecular weight. The review includes all the relevant English publications that were retrieved from PubMed/Medline and Google Scholar. The key search terms that were employed in this review were terms used in other reviews; they included 'skin', 'asthma' or 'dermal', 'high molecular weight' or 'low molecular weight' and 'sensitisation' or 'dose-response' or 'link'. Other terms subsequently added included: 'allergen', 'allergy', 'atopy', 'occupational', 'work-related', 'respiratory', 'dermatological' and 'dermatitis'. Furthermore, some articles were retrieved using the functions 'similar articles search' in PubMed and 'related articles' in Google Scholar as well relevant articles that were cited in the references of these articles.

Figure 1: Bakery work handling various food proteins (high molecular weight agents)

EPIDEMIOLOGICAL STUDIES

CO-EXISTING OR SEQUENTIAL DEVELOPMENT OF DERMAL AND RESPIRATORY OUTCOMES

Workers in various occupations report both skin and respiratory symptoms. Table I illustrates case study reports in which both dermal and respiratory outcomes (diagnosed using patch tests and inhalation challenge tests respectively) were observed in occupational settings with diverse exposures. These allergens can cause both occupational asthma and allergic contact dermatitis.[13] To ascertain which health outcome precedes the other can be a challenge because of the nature of the study design used and the lack of a comparison group in such studies.

Arrandale[4] conducted an analysis of four cross-sectional pooled studies from a soda ash plant, a softwood planing mill, embalming and cabinet-making in which workers were exposed to ammonia, softwood dust, formaldehyde and/or glutaraldehyde and hardwood dust, respectively. Altogether, both skin and respiratory symptoms were reported by 11% (26/236). The highest prevalence of both dermal and respiratory symptoms was seen among embalmers, with no statistical significance.[13] Although this study does not show temporality, it reinforces evidence of the co-occurrence of both respiratory and dermal symptoms in occupational settings.[14] Similarly, previous studies among sewage-treatment and isocyanate workers reported a concomitant occurrence of dermal and respiratory symptoms in exposed workers.[15,16] However, no correlation was found between occupational hand dermatitis and flexural dermatitis, respiratory atopy (allergic rhinitis (AR) and bronchial asthma), serum IgE ± 100 IU/mL or metal sensitisation in the study of bakers by Bauer et al.[14]

Aside from the need to identify temporality, the co-existence of dermal and respiratory symptoms does not necessarily indicate that the same exposure is the trigger for both outcomes, unless the exposure assessment specifically characterised the nature and route of the exposures in affected workers.[13]

ASSOCIATION BETWEEN SKIN EXPOSURE, ALLERGY-SPECIFIC SENSITISATION AND SUBSEQUENT DEVELOPMENT OF RESPIRATORY DISEASE

Table II summarises the few key epidemiological studies that have investigated the role of skin exposure and symptoms and the subsequent development of respiratory symptoms, including asthma, in these workers.

Brisman et al[17,18] have alluded to the association between bakers' asthma and eczema, without indicating whether sensitisation to wheat flour was due to inhalation or due to contact with allergens through the skin (or both).

In a study of bakers exposed to wheat and autobody-shop workers (ABSWs) exposed to isocyanates, Arrandale et al[5] demonstrated an association between skin and respiratory symptoms. Work-related itchy skin was associated with wheeze, asthma-like symptoms and work-related asthma symptoms in ABSWs but not in bakers. The authors did, however, indicate that the autobody-shop workers had adhered to much stricter use of personal protective equipment, while such use of personal protective equipment had not been observed by the bakers.[5] A previous study by Arrandale et al[4] also found that eczema increases the odds

TABLE I: CO-OCCURRING OCCUPATIONAL ASTHMA (OA) AND OCCUPATIONAL CONTACT DERMATITIS (OCD) – DIAGNOSED USING SPECIFIC INHALATION CHALLENGE AND PATCH TESTING – CASE REPORTS REPORTED

EXPOSURE	OCCUPATION	AUTHOR, YEAR
2-hydroxyethyl methacrylate (HEMA)	Beautician	Moulin et al, 2009
Diglycidyl Ether of Bisphenol A (DGEBA)	Resin applier	Moulin et al, 2009
Diphenylmethane-4,4'-diisocyanate (MDI)	Manufacturing (automotive industry)	Valks et al, 2003
Potassium dichromate	Cement floorer	De Raeve et al, 1998
Aziridine hardener	Painter and varnisher	Kanerva et al, 1995
Onion	Homemaker	Valdivieso et al, 1994
Nickel	Manual grinding of metal castings	Estlander et al, 1993
Spiramycin	Poultry breeder	Paggiaro et al, 1979

Reproduced from Arrandale 2012[13]

TABLE II: STUDIES OF WORKING POPULATIONS INVESTIGATING THE ASSOCIATION BETWEEN SKIN EXPOSURE/SYMPTOMS AND RESPIRATORY SYMPTOMS/ASTHMA

AUTHORS, YEAR	ARRANDALE ET AL, 2013[5]	ARRANDALE ET AL, 2012[4]	LYNDE ET AL, 2009[19]	PETSONK ET AL, 20002[4]
Aim of study	To investigate associations between skin and respiratory symptoms in bakery and ABSW	To identify predictors of reporting concurrent skin and respiratory symptoms in a clinical population	To compare the prevalence of occupational cutaneous symptoms among professional indoor cleaners to other building workers (OBW) and their association with exposures and respiratory symptoms	To evaluate the respiratory health of workers exposed to methylene diphenyl diisocyanate (MDI)
Study design	Cross-sectional	Cross-sectional	Cross-sectional	Cohort
Population	723 bakers 473 ABSW	204 patients from the occupational health clinic	549 professional cleaners 593 OBW	214 wood product plant employees (144 completed initial, follow-up and occupational questionnaires)
Agent	Isocyanate and wheat	Multiple agents (animal dander, cement, isocyanates, pesticides, wet work, dust, fumes, paint)	Cleaning agents	MDI
Assessment tool(s)	Questionnaire, IgE for common inhalants, specific challenge tests and personal airborne exposure measurements	Questionnaire	Questionnaire	Questionnaire, serial peak flow, spirometry, methacholine challenge, specific IgE
Skin symptoms prevalence (%)	Work-related itchy skin: ABSW – 9%; Bakers – 17%	Possible work-related skin disease: 82%	Current rash: cleaners - 10%, OBW 6% Rash in the past 12 months: cleaners – 19%, OBW 16% (96)	Not reported
Respiratory symptom prevalence (%)	Work-related asthma symptoms (WRAS): ABSW – 4%; Bakers – 2%	Possible work-related respiratory disease: 18%	In males with rash: • physician-diagnosed asthma – 19% • new onset asthma 14% • ≥3 respiratory symptoms: 77% • ≥2 WRAS: 63% • ≥3 WRAS: 40%	Among the 178 (who participated in at least one follow-up survey): • initial asthma-like symptoms – 11% • follow-up asthma-like symptoms – 20% • new-onset asthma-like symptoms – 12%
Association between skin exposure/ symptoms and respiratory symptoms/ asthma	Association between work-related itchy skin and respiratory symptoms in: ABSW: • wheeze OR = 2.50 (95% CI 1.7–3.6) • asthma-like symptoms OR = 2.12 (95% CI 1.5–3.0) • WR asthma symptoms OR = 3.61 (95% CI 1.4–9.4) • Bakers: • wheeze OR = 1.60 (95% CI 1.1–2.3) • asthma-like symptoms OR = 1.54 (95% CI 1.2–2.0) • WR asthma symptoms OR = 2.15 (95% CI 0.7–6.3) Exposure-response relationship in ABSW: • itchy/dry skin PR = 1.55, 95 % CI: 1.2–2.0 • work-related itchy skin PR = 1.97 (95 % CI 1.2–3.3)	Eczema increases odds of concurrent work-related skin and respiratory symptoms: OR = 3.68 (95% CI: 1.7–7.8)	Rash in the past 12 months associated with: • physician-diagnosed asthma OR = 2.3 (95% CI: 1.1–4.5) • new-onset asthma OR = 3.0 (95% CI: 1.2–7.6) • ≥3 respiratory symptoms OR = 2.6 (95% CI: 1.6–4.3) • ≥2 WRAS OR = 3.2 (95% CI: 1.9–5.3) • ≥3 WRAS OR = 4.0 (95% CI: 0 2.2–7.2)	52% of workers who reported MDI skin stains reported asthma-like symptoms
Study limitations	• Poor correlation between airborne and skin exposure for particulates • Lack of information on other, potentially causal, exposures in the workplace • Potential role of Type IV allergy or irritant mechanisms in symptom development not modelled • Healthy worker effect owing to fewer symptomatic subjects at higher exposure levels	• Cross-sectional study – temporality cannot be ascertained • Not generalisable to all workers because of the selective clinical population	• Low response rate • Outcomes (rashes) were self-reported and not clinically confirmed	• Loss to follow-up • Lack of environmental exposure measurements

of concurrent work-related skin and respiratory symptoms. It needs to be borne in mind, though, that this does not necessarily indicate that skin exposure is always predictive of respiratory outcomes.

Lynde et al[19] investigated a group of predominantly male (84%) professional cleaners, 9.3% of them having a rash currently and 18.6% reporting having had a rash in the past 12 months. The study found that cleaners with a current rash and those reporting a rash in the past 12 months were at significantly increased risk of reporting work-related respiratory symptoms compared to those without a rash. This suggested an association between the presence of work-related asthma symptoms and dermatitis among professional cleaners.[19] However, atopy was considered as a possible underlying mechanism for this association, and a lack of personal protective equipment (PPE) use could have also been another factor influencing this association.[19]

Isocyanates, used in spray-painting and polyurethane foam products, have received considerable attention in relation to skin exposure and the resulting effects of asthma.[5,7,10,20,21] A key link for suggesting an association between allergic sensitisation via transcutaneous route for isocyanates and asthma has been the improvement in occupational health standards in workplaces due to the use of respirators to prevent isocyanate inhalation. However, the incidence of isocyanate-induced asthma remains persistently high, particularly in settings where skin contact with isocyanates has not been well controlled.[20] A laboratory study by Karol et al[22] demonstrated that isocyanate exposure to the skin, particularly TDI, could induce pulmonary hypersensitivity in guinea pigs. Subsequent studies have shown that similar patterns of sensitisation exist in humans.[1,5,7,23]

PATHOPHYSIOLOGICAL CORRELATES

Several studies in children have suggested that skin exposure and sensitisation precede the response in the airways. Dohi et al[25] compared eight atopic dermatitis (AD) patients without a previous report of asthma symptoms and eight mite-allergic asthmatic patients. These two groups were subjected to bronchial inhalation challenges (non-specific with methacholine and specific with house-dust mite (HDM)). Results demonstrated that both IgE and anti-mite IgE antibody were higher in AD' patients than mite-allergic asthmatic patients. The response for methacholine in AD' patients was from normal to asthmatic range. In a study by Lack et al,[26] peanut allergy did not show dependent association with intake of soymilk and soy formula. Peanut allergy was, however, associated with skin rash over the joints and flexures, crusted skin rash, and intake of soy milk and soy formula. In addition, the use of peanut-containing oils on the skin was associated with peanut allergy, suggesting that skin exposure could lead to generalised sensitisation. In more severe form, local skin contact with certain foods, medications, latex, metals and occupational allergens may result in generalised urticaria or systemic symptoms (angioedema, wheezing).[27]

It has also been suggested that beryllium sensitisation in those suffering from chronic beryllium lung disease (CBD) may also have percutaneous origins. Tinkle et al[28] observed that decreasing inhalational exposure had no effect on the incidence of the disease, suggesting that inhalation was not the only cause for the disease. The investigators suggested that following skin exposure to beryllium, the inhalational exposure to beryllium necessary to elicit an immune response and the formation of a CBD-related granuloma was significantly reduced. In further support of this hypothesis, the topical application of beryllium ointment to C3H/HeJ Heston mice showed an increase in beryllium sensitivity in the mice as determined through beryllium lymphocyte proliferation tests of lymph node and peripheral blood.[28] Furthermore, it has been shown that the stratum corneum (SC), the external layer of the skin, which although mechanically strong and resilient to stress and physical strain, may be penetrated by fine and ultra-fine beryllium particles.[28]

The most refined models demonstrating the link between allergic sensitisation and asthma from skin exposure have been from murine experimental studies. Spergel et al[29] applied the high-molecular weight molecule ovalbumin (OVA) via occlusive patch tests to tape-stripped mice skin. This experiment showed an OVA epicutaneous sensitisation and local allergic dermatitis. Later, when the sensitised mice were exposed to intravenous methacholine, the response was eosinophilia in the bronchoalveolar lavage fluid and airway hyper-responsiveness.[29]

In murine models, it has also been demonstrated that the dermal infiltration of eosinophils in response to dust-mite allergens in skin that has been sensitised by barrier disruption is greater than sensitisation through intact skin.[29–32] This suggests that there is a higher induction of Th2-dominant immunological responses to environmental allergens through skin that has the skin barrier disrupted, as is experienced in patients with AD,[33] and particularly following rubbing or scratching of skin, compared to intact skin. There is common agreement about Th2 response that allergen exposure requires both the involvement of barrier disruption and an adaptive immune recognition in order to induce an allergic response.[1,34]

PREDISPOSING FACTORS FOR ALLERGIC RESPIRATORY SYMPTOMS FOLLOWING SKIN EXPOSURE

Several predisposing risk factors could result in sensitisation and to the subsequent development of allergic respiratory symptoms following skin exposure to occupational and environmental allergens.

HOST RISK FACTORS

A. Pre-existing skin diseases

Atopic dermatitis (AD)

The integrity of the skin barrier is intrinsic to an individual's ability to respond to dermal allergen exposure.[35] In AD both cellular immune abnormalities and skin-barrier defects are known to be behind its pathophysiological mechanism.[36] Various non-invasive biomarkers of epidermal permeability are used to identify increased risk for allergic sensitisation through the skin. Trans-epidermal water loss (TEWL) has been directly, but not exclusively, associated with AD and the associated risk of respiratory allergy.[37] Kelleher et al[38] found that newborns with raised TEWL were at increased risk of developing AD at one year of age, and also at higher risk of IgE-associated food allergies, despite the absence of early-onset AD. Loo et al[39] also demonstrated that children with eczema before 18 months exposed to inhalant allergen (HDM) were more likely to present with positive skin-prick tests (SPT) at 18 months.

Irritant dermatitis

A damaged skin barrier, as is present in irritant dermatitis as a result of either physical, chemical or biological processes, is a potential route of entry for allergens.[13] Nielsen[40] demonstrated that skin treated with higher concentrations of sodium laurel sulfate (SLS), a known skin irritant, had greater overall penetration and a greater rate of penetration of chemical substances (pesticides with a wide range of solubility) compared to undamaged skin.

Ichthyosis vulgaris

Bremmer et al[35] demonstrated in a study of 491 patients with AD and ichthyosis vulgaris (IV) that those with higher severity of IV were more likely to report asthma, even after adjusting for AD severity, age, sex and season of the occurrence of symptoms. Bremmer suggested that the presence of severe IV could be used as a marker for patients who have a greater likelihood of developing allergic respiratory disease.

Trauma

Skin trauma is another risk factor for facilitating the entry of contact allergens into the body. This may increase a person's risk of sensitisation, as has been observed, for instance, in seafood-processing workers and animal-handling workers (rats), resulting in the development of allergic respiratory symptoms.[7,10,41]

B. Filaggrin gene mutations

One of the first-line factors associated with skin-barrier deficiencies is Filaggrin (FLG) defects,[42] which typically occur as a result of inherent FLG gene mutations.[43] FLG is an important protein responsible for the strength and integrity of the SC, regulating the permeability of the skin to water and antigens, involved with the packing of keratin filaments in the epidermis, and maintaining the skin's normal acid pH.[1] Studies in the general population evaluating allergen exposure via skin, sensitisation and respiratory outcomes have been reported.[43–45] Brough et al[46] found a positive association between FLG defects and peanut allergy. Filaggrin defects have also been identified in AD patients, and there appears to be a correlation between the extent of FLG defects, the extent of allergen exposure through the skin and the severity of AD.[46] Loss-of-function mutations in the gene encoding FLG are present in up to 50% of patients with moderate-to-severe AD (in European and Asian populations) and have been shown to increase the risk of inhalant allergic sensitisation, allergic rhinitis (AR), asthma and peanut allergy. In African American populations this defect is rare.[46,47]

ENVIRONMENTAL RISK FACTORS

Various high molecular weight (generally proteins) and low molecular weight (generally chemicals) allergens are capable of stimulating the induction of IgE antibodies and resulting in sensitisation and the development of allergic respiratory symptoms.[7] The extent to which they are able to do so will depend on the agent's physical properties, exposure intensity and duration.

A. Agent

Physical properties

Theoretically, liquid and solid allergens will facilitate skin contact and entry in contrast to aerosolised substances. Petsonk et al[24] found that after two years of working in a wood-manufacturing plant, subjects who worked with liquid methylene diphenyl diisocyanate (MDI) had a relatively higher risk of reporting asthma-like symptoms than those who did not work with liquid MDI. In addition, those who reported observing MDI skin stains at least once (suggesting skin exposure) also had a higher risk of reporting asthma-like symptoms compared to those who had never observed such stains. However, the prevalence of asthma-like symptoms was more prevalent among workers who reported brief removal of respiratory PPE while performing their routine tasks, which included liquid MDI handling. The investigators concluded that although inhalation was an important route of exposure for the

Figure 2: Spray-painting work with isocyanates (low molecular weight chemicals)

development of the respiratory symptoms, skin exposure may have also played a role.[24]

Chemical properties (molecular structure)

Transcutaneous chemical penetration also depends on the chemical characteristics of agents. Haptens must be structurally able to bind with a protein as a prerequisite for being recognised by the immune system. While lipophilic properties facilitate skin permeability for allergens,[40] hydrophilic properties are also needed to allow for movement of the chemical from the SC into the dermis. Therefore an efficient allergen should be amphiphilic (both lipophilic and hydrophilic).[48] Low molecular weight particles and molecules, such as nickel and poison ivy (urushiol), are known contact allergens that cause skin sensitisation by their action of binding to host proteins in the skin.[7] As a result of their chemical reactivity with host proteins, these particles cause otherwise innocuous compounds to be converted into hapten–protein complexes that stimulate the adaptive immune response.[1] High molecular weight protein allergens such as peanut, dust mite or water-soluble cat allergens, which range in size from 5 000 Da to 100 000 Da, are generally unable to pass through the skin's cutaneous permeability barrier owing to their size.[49] In such instances, it is generally understood that sensitivity to such high molecular weight compounds is possible only when the integrity of the skin is to some extent compromised.[1]

B. Exposure intensity and duration (dose–response relationships)

Apart from all other conditions that interfere with the skin integrity, the concentration of the agent on the skin surface and the duration of this contact may influence the body's response to an allergen. Some studies involving animal models have investigated trimellitic anhydride (TMA), phthalic anhydride (PA) and MDI and found that increasing the sensitising intradermal doses did not increase a respiratory response. However, Arakawa et al[50] found a dose-dependent response in guinea pigs for Dermatophagoides farinae (DF mite) and a dose–response relationship with increasing doses of intradermally administered TMA eliciting asthma-like symptoms.

Devos et al[51] investigated the asthmagenic capacity of methylisothiazolinone (MI) – a known cause of allergic contact dermatitis commonly used in cosmetics, household products and in different industries – in animal models. Fifteen days after the dermal administration of MI, the subjects did not show a significant respiratory response (either airway hyperreactivity or inflammation). The study concluded that whereas MI was a dermal sensitiser and irritant, it was unable to elicit an asthma-like response.[51]

Paulunh et al[9] also investigated the dose–response relationship of MDI exposure in animal models and the lung response after primary skin exposure. Surface area or dose-to-body weight did not show specific associations. On the contrary, the magnitude of the respiratory response was dependent on the frequency of inhalation challenge doses administered instead of the previous cutaneous doses.[9]

CONCLUSION

Skin is an important route of exposure to allergens leading to sensitisation and the potential to cause allergic skin symptoms that could co-exist or precede respiratory symptoms and other systemic health outcomes. However, much still needs to be investigated to enable us to understand better the modes of entry and sensitisation in occupational settings. Particularly among workers exposed to high molecular weight protein allergens, there is a need to ascertain the pathophysiological mechanisms involved in skin exposure that result in respiratory disease and other systemic effects. By addressing these issues, better interventions aimed at preventing occupational exposure to these allergens can be made possible.

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DECLARATION OF CONFLICT OF INTEREST

The author declares no conflict of interest.

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Research Article

HOUSE-DUST MITES IMPACT DISEASE EXPRESSION IN CHILDREN WITH CHRONIC RHINITIS: A RETROSPECTIVE STUDY

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ABSTRACT

Background: Asthma is the most common chronic childhood disease; it affects 10–20% of South African children, with allergic rhinitis (AR) being co-morbid in up to 80%. Data are lacking on the role of allergens in low- to middle-income countries, particularly in Durban, which is located in a sub-tropical region.

Objectives: First, to determine the common allergens in children with asthma and/or AR. Secondly, to determine the prevalence of atopy and to compare disease severity in those with and without atopy.

Methods: A retrospective chart review of asthmatic and AR children over a one-year period. The GINA 2015 and ARIA guidelines were used for grading the disease severity. Laboratory tests included Phadiatop®; FX5; RAST® for individual allergens. The Fisher's exact test and the Chi-squared test were used to test for associations between disease severity and atopy. The Kruskal-Wallis rank test was used to compare allergen load and disease severity. Ethical approval was granted.

Results: One hundred children of mean age 5.5 ± 1.5 years with 60% males were enrolled. Of these, 66% were atopic, with common aeroallergens being *D pteronyssinus* (57%) and *D farina* (56%) There was an association between severity of AR and presence of *D pteronyssinus* and *D farinae* sensitisation ($p = 0.012$ and $p = 0.013$), respectively. The persistence of AR was associated with the presence of *D pteronyssinus* and *D farinae* sensitisation ($p = 0.007$ and $p = 0.009$), respectively. There was no association between severity of asthma or the persistence of asthma and the presence of aeroallergens, all with $p > 0.05$. (There was no correlation between severity or persistence of asthma based on aeroallergen sensitisation.)

Conclusion: House-dust mites (HDM) are the commonest aeroallergens and conferred more severe and persistent AR in the sub-tropical region of Durban.

INTRODUCTION

The prevalence of allergic diseases is rising worldwide, and certainly in South Africa.[1] Asthma and AR are hypersensitivity disorders of the immune system that occur through allergic inflammation induced by an allergen-specific immunoglobulin E (IgE) mediated response.[2] AR occurs frequently in children with a prevalence of AR of 38.5% and asthma has a prevalence of 15% among South African children.[3]

Asthma and AR are closely linked, and physicians are well aware of this link in the form of the 'united-airway' theory.[4] It has been well described that co-management of these two conditions will reduce disease severity and improve quality of life (QoL).[4] Both these conditions form part of the united airway and management needs to be directed at both disease entities if outcomes are to improve.[4] Aeroallergens have been implicated in the pathophysiology of both these disease processes.[4] Those commonly implicated include HDM, grass, mould and cockroach, with a controversial minor contributor of food allergens with egg white and peanuts. Previous studies in South Africa have shown that the Oriental cockroach is the second predominant aeroallergen in KwaZulu-Natal, with sensitisation rates of 41%.[5] The prevalence of cockroach sensitisation in atopic patients in various parts of southern Africa is similar to that

TABLE I: DEMOGRAPHIC DATA, ATOPIC STATUS AND SENSITISATION OF CHILDREN WITH ASTHMA AND AR (N = 100)

Variable	Mean	SD
Gender (M/F)*	60/40	
Ethnicity Indian (N) African (N) Mixed race (N) White (N)	 73 20 4 3	
Age (months) 0–23 months (N/%) 24–60 months (N/%) >60 months (N/%)	95 5 (5) 38 (38) 57 (57)	72; 206
Height (z-scores)	–0.19	−4.53; 2.13
Weight (z-scores)	–0.08	−3.32; 5.45
BMI (z-scores)	0.07	−3.53; 6.04
Atopy (N/%)	67 (67)	
Poly-sensitisation (N/%)	63 (63)	
AR (N/%)	84 (84)	34; 175
Asthma (N/%)	78 (78)	22; 222

in Europe, the United States and Asia. In a Botswana study by Kung et al in Gaborone, cockroach sensitisation was noted in only 10% of their cohort.[6] This is less than the prevalence of 30–70% worldwide and the reported 40% (Western Cape), 38% (KwaZulu-Natal), 32% (Free State) and 32% (Gauteng) in the provinces of South Africa.[6]

A previous study comparing patients with sensitisation to HDM found higher levels of *Blomia tropicalis* sensitisation in KwaZulu-Natal, when compared to Johannesburg.[7,8,9] *Blomia tropicalis* sensitisation was significantly higher in northern KwaZulu-Natal, with 52% of individuals as opposed to only 2.9% for the Highveld.[4]

There are no recent data on the predominant allergens in KwaZulu-Natal; therefore we sought to describe the prevalence of allergen sensitisation in asthmatic and/or AR children in this region, with a secondary objective being to assess for the impact of allergen sensitisation on disease expression.

METHODS

SETTING

The study was a retrospective chart review conducted in a paediatric outpatient department's allergy clinic at the RK Khan Regional Hospital in Durban, KwaZulu-Natal, South Africa. KwaZulu-Natal is a sub-tropical region with a warm climate that registers temperatures ranging from 16ºC to 25ºC in winter and 23ºC to 33ºC in summer, which distinguishes it from most parts of South Africa. Durban is a coastal city at sea level with year-round humidity levels of between 71% and 83%. Children in this clinic come from both urban and peri-urban settings.

PARTICIPANTS

Participants included children who were all under the age of 14 years who were seen for follow-up between June 2014 and June 2015 with a diagnosis of asthma and/or AR. Global initiative for Asthma 2015 (GINA)[11] and AR and its Impact on Asthma 2014 (ARIA)[17] guidelines were used for grading disease severity. Asthmatic subjects were categorised into having no asthma, mild asthma, moderate asthma and severe asthma to assess the severity of the disease as per GINA. The severity of AR was classified according to ARIA 2014 guidelines into mild intermittent disease, moderate–severe intermittent, mild persistent and moderate–severe persistent disease.

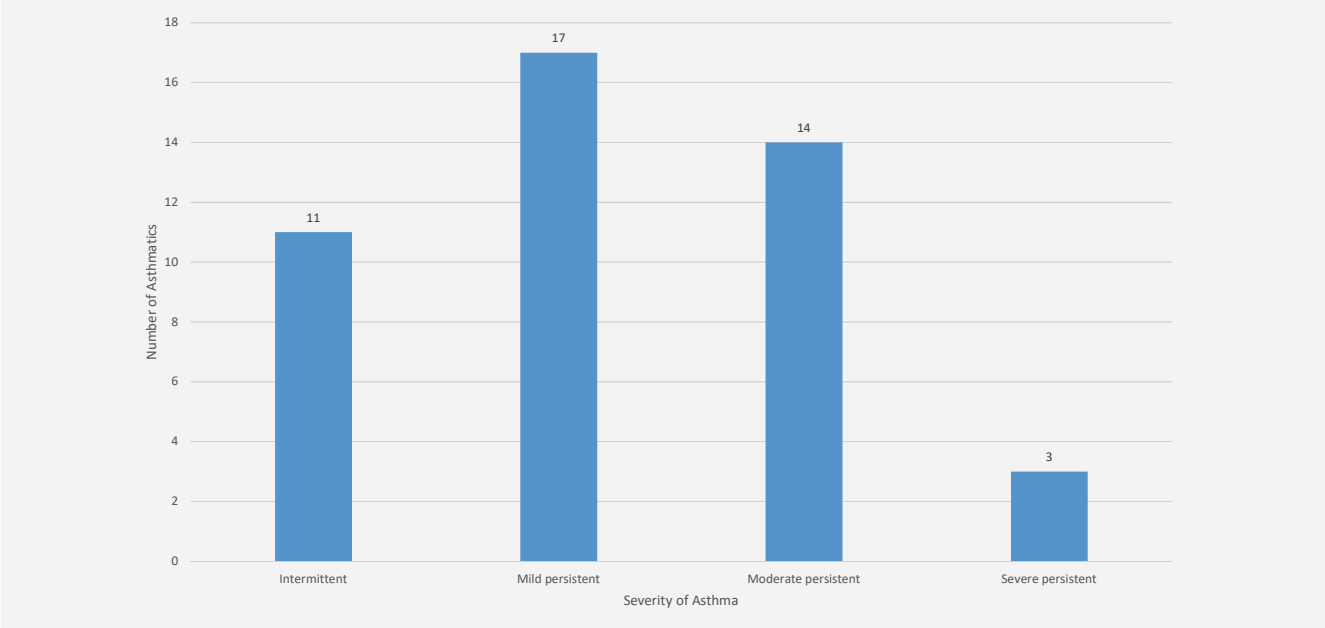

Figure 1: The severity grading for asthmatic children as per GINA (N=45)

TABLE II: CLASSIFICATION OF DISEASE SEVERITY GRADING FOR AR (N = 53)	
Grading of AR	Number of subjects (%)
Mild intermittent disease	23 (43.4)
Mild persistent	26 (49.1)
Moderate–severe persistent	7 (7.5)

INVESTIGATIONS

Laboratory results were obtained from electronic system of the National Health Laboratory Service (NHLS). Tests analysed included Total IgE; Phadiatop®; FX5; RAST® for individual allergens. The universal allergy panel included both the food panel (FX5) and the inhalants panel (Phadiotop). Instruments and reagents are sourced from Thermofisher, and the method used for assessment is fluoro-enzyme immunoassay. The interpretation of results for ImmunoCap allergen mixes is qualitative values and 0.35 Ku/L is used as a cut-off. The reagents used are specific IgE conjugate (A1HFH), development solution (BW1P8), stop solution (BNDJN) and control-specific IgE (CZ4A9-1.9–3.7 Ku/L). The reagents are manufactured by Phadia AB Rapsgatan, Uppsala, Sweden.

Data were analysed using the STATA statistical package (StataCorp, Version 13). Proportions were depicted as frequencies for individual aeroallergens. The Fisher's exact test and Chi-squared test were used to determine the association between aeroallergen sensitisation and disease severity. The Kruskal-Wallis equality of populations rank test was used to compare disease severity according to the different aeroallergens tested. A p-value of <0.05 was considered significant. Full ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (IRB number: 317/15).

RESULTS

A total of 200 charts were screened, with a 100 fulfilling inclusion in the final analysis. Of the 100 excluded patients, the reasons for exclusion were: incorrect patient numbers, illegible notes, lost result sheets or duplicate blank files. There were 60% males with a mean age of 5.5 ± 1.5 years (see Table I) – 73% were children of Asian (Indian) descent. Children ranged from 0 months to 13 years, with the majority of patients in the 5–13-year age group; 5% and 38% were in the 0–2-year and 2–5-year age groups, respectively. Of the study population, the majority of subjects 73% had both asthma and AR. Only 14% had AR as a single condition and 13% asthma alone. Two-thirds (66%) were atopic, with either a food or an aeroallergen positive result. Most of the children had mild persistent asthma and mild persistent AR (see Table II and Figure 1).

Aeroallergen sensitisation was found in 60% of the study population, with the commonest aeroallergens identified being HDM: *D pteronyssinus* (56%); *D farinae* (57%), followed by cockroach mix (16%) – Figure 2. Sensitisation to food allergens was found in 26% of patients, with peanut and wheat being the most common at 17% and 10%, respectively – Figure 3. Among those identified were egg white (10%), peanuts (17%) and wheat (9%). Other aeroallergens to which patients were sensitised included dog (6%), cat (5%), Timothy grass (7%), mugwort (5%) and latex (3%).

With regard to allergens and disease severity, there was an association between the severity of AR and the presence of *D pteronyssinus* and *D farina* (p = 0.012 and p = 0.013), respectively (see Table III). The presence of persistent AR was associated with the presence of *D*

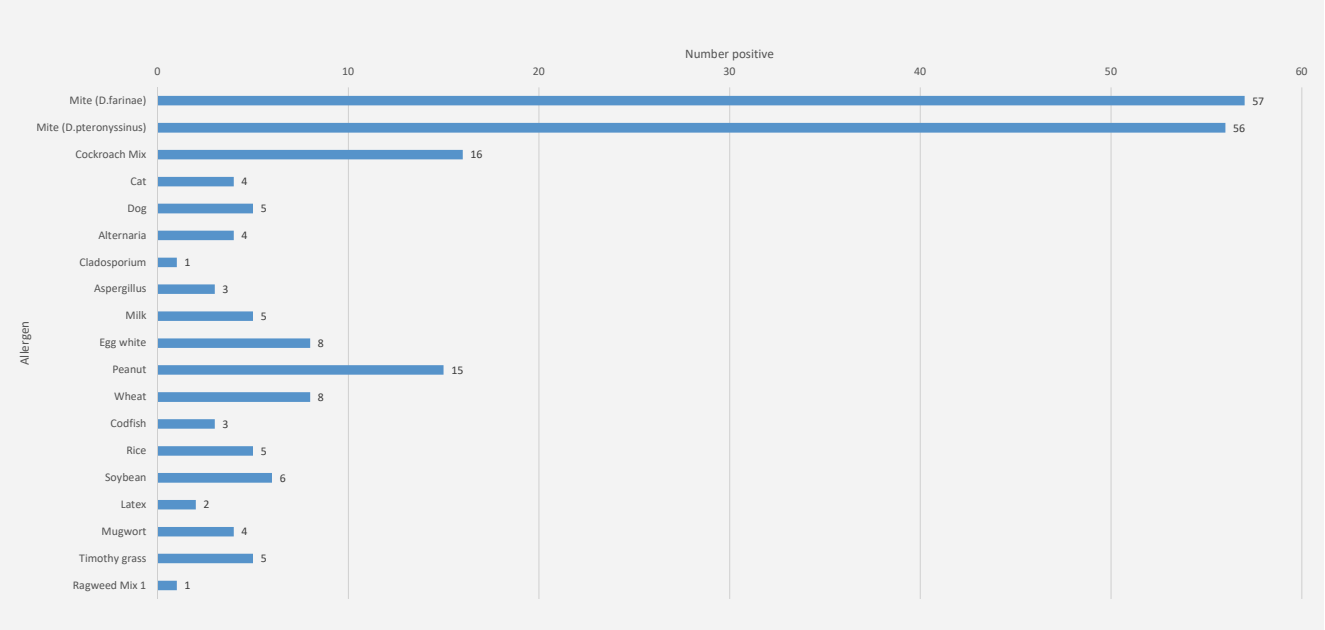

Figure 2: The predominant aeroallergens in children with asthma and allergic rhinitis. Number of children indicated at end of the column for each individual aeroallergen.

TABLE III: PRESENCE OF HDM SENSITISATION IN ASTHMA AND AR		
Asthma	*D pteronyssinus* sensitisation (n)	*D farinae* sensitisation (n)
No asthma	9	10
Mild asthma	28	27
Moderate asthma	16	15
Severe asthma	4	3
AR	*D pteronyssinus* sensitisation (n)	*D farinae* sensitisation (n)
No sensitisation	2	2
Mild intermittent	38	36
Mild persistent	9	9
Moderate-severe persistent	8	8

pteronyssinus and *D farina* sensitisation (p = 0.007 and p = 0.009), respectively. Persistent AR was not significantly associated with cockroach mix (p = 0.38). There was no association between severity and persistence of asthma with the presence of aeroallergen sensitisation: 31% of patients were monosensitised to HDM only, 38% polysensitised with other allergens out of a total of 59% sensitised to HDMs. Total IgE was not assessed owing to its limited value as a screening tool in allergy.

DISCUSSION

In the current study, we found high levels of sensitisation to common HDM in the study population, with fewer subjects sensitised to cockroach. Atopy was found in two-thirds of all children with asthma and/or AR, with three-quarters having both asthma and AR. Sensitisation to foods was present in 26% of the patients. The majority of children had mild disease, the majority of them being of Indian descent. Of significance was that the presence of HDM sensitisation was associated with more severe AR when compared to other aeroallergens, but this association was not found to be present in asthmatics.

AR and asthma frequently coexist: asthma occurs in 15–38% of patients with AR, whereas nasal symptoms are present in approximately 75% of patients with asthma.[11] In a Tucson study of 747 children followed up from birth to age six years, it was found that by the age of six years 42% had AR and that the onset of rhinitis in the first year of life was a risk factor for asthma by age six years.[12] A Canadian study demonstrated that 40% of AR patients have asthma, and as many as 94% of allergic asthma patients have AR.[13,14] The current study found this association in 73% of participants. Asthma was present in 83.9% (73/87) of patients with rhinitis, whereas rhinitis was present in 84.5% (73/86) of patients with asthma.

Mites make up a large part of house-dust allergens.[15] The majority of asthmatics and patients suffering from persistent AR are sensitised to mites.[16] Airborne exposure occurs with the active disturbance of contaminated fabrics and settles rapidly after disturbance.[16] It has been shown that 100 mites per gram of house dust (or 2 µg of allergens per gram of dust) is sufficient to sensitise an infant.16 With approximately 500 mites per gram of house dust (or 10 µg of allergens Der p1 the major allergen of *D pteronyssinus*), the sensitised patient shows a relative risk of approximately five times for the development of asthma at a later date.[16] The higher the number of mites, the earlier the first episode of wheezing.[16] The prevalence of mites has been found to be higher in humid regions (20–35%) compared to relatively dry regions (15%).[16] The humidity levels in Durban may explain the relatively high sensitisation of mites in this region of the country. The current study did not explore the age of onset of symptoms but the majority of children were over the age of five years.

Different mite species are found in different geographical areas. For instance, in the United States *D farinae* prevails, whereas in Western Europe the most common species is *D pteronyssinus*.[17] The dominant HDM species found, *D farinae*, would be expected in a drier climate such as the Highveld of South Africa.[18] HDM allergens were found in 100% of households in the Edenvale area of Gauteng, reaching levels deemed to be unsafe in 20% of households.[19] Relative humidity (RH) affects HDM reproduction and survival.[18] Critical equilibrium humidity (CEH) for *D pteronyssinus* is 73% at 25°C, below which dehydration occurs.[18] At 40–50% RH, mites survive for only 8–11 days, which is too short for active proliferation.[18] At very high RH, >85%, mites also struggle to survive because moulds proliferate in these conditions and produce toxins that may be detrimental to the mites.[20] Mites probably prefer a fluctuating environment, with periods of high RH to maintain water balance and low RH to minimise mould growth. In the current study, *D pteronyssinus* and *D farinae* sensitisation were found in high levels.[21] This is in keeping with a study by Speiksma et al, which found that more humid areas of the world carry higher concentrations of these mites in households, whereas mites and their allergens are reduced in colder, more arid regions.[22] HDMs are by far the most common aeroallergen implicated in allergic individuals in Asian countries:[23] the highest rates of HDM sensitisation were reported in Singapore (70 to >90%), followed by Taiwan (85–90%) and South India (89.7%).[23] The lowest sensitisation rates were described in North India (7.8%), Vietnam (9–23%) and the Philippines (33–47%).[23] Atopic Asians were mostly sensitised to the *Dermatophagoides* HDM species, with a slightly lower percentage sensitised to *Blomia tropicalis*.[24] However, exceptions were seen in Vietnam and Singapore in 1999, where *B tropicalis* was instead the predominant mite in sensitised atopic individuals.[23] Around the world, *B tropicalis* is abundant in the tropical and sub-tropical regions where there is high humidity and warm temperatures.[4] A study in Mexico City, which does not have a temperate climate, showed *B tropicalis* allergy to be highly prevalent in patients with

AR and asthma.[24] A previous local study by Jeevarathrum et al demonstrated that allergy to *Dermatophagoides spp* occurs both on the subtropical northern KwaZulu-Natal coast of South Africa and in the temperate Highveld region, with no statistical difference between the two regions.[4] However, the proportion of *B tropicalis* sensitisation was significantly higher in northern KwaZulu-Natal, with 52% of individuals as opposed to only 2.9% for the Highveld.[4] The group advocated routine testing for *B tropicalis* in warm, tropical environments.[4] Our study did not assess *B tropicalis* as it is not currently offered in the public sector as routine testing.

It has been known since at least the 1970s that atopy manifests far less in rural than in urban areas of poorer countries.[25,26,27] Worldwide, atopy is present in up to 40% of the population.[28] The interactions between urbanisation, migration and the changes induced by not only direct immunological exposure to allergen, but the context of exposure, have been studied in an excellent unique study by Steinman et al.[29] This is the only study in the region ever to have compared allergenicity and bronchial hyperreactivity (BHR) using histamine challenges in 2 157 children, 10–14 years old, living in different areas, from the Libode district in rural Eastern Cape (former Transkei), Marconi Beam (an informal settlement area in the Western Cape) and Kirstenhof, an established city suburb in Cape Town.[29] There are numerous important findings from this study: 17% of rural and 34.4% of recently urbanised black children now show increased BHR (by histamine challenge) compared with 0.03% and 3.1% respectively shown 30 years ago.[29] This compares with the current prevalence of increased bronchial reactivity of 33% in white urban children.[29] The acquisition of sensitisation to one or more aeroallergens as indicated by CAP RAST tests was present in 36% of the children with normal BHR and in 62% of the children with increased BHR.[29] Overall atopic sensitisation to one or more aeroallergens by skin-prick test (SPT) was found in 42% of the recently urbanised Xhosa children and 45% of urbanised white children.[29] In a cohort of 455 asthmatic black African children at Baragwanath Hospital, Soweto, South Africa, 70% of the patients had positive SPTs to allergens,[30] compared with 45% in a Pretoria.[31] The current study found the presence of atopy in 67% of the study population. Levin et al reported atopic sensitisation in 34% of urban black African children in Cape Town with asthma and AR and of these 26% were sensitised to HDM and 17% to German cockroach.[32] In Cameroon, Mbatchou et al found 42% sensitisation to aeroallergens, with *D pteronyssimus* (24.2%), *D farinae* (22.8%), *B tropicalis* (23.3%) and *Blatella germanica* (15.2%) being the most common allergens found in their cross-sectional study of 600 particpants.[33] With the study site being located in a coastal region at sea level, higher rates of sensitisation to HDM (42%) were demonstrated by Potter et al in a coastal study in Cape Town.[7,34] Previous residence in a coastal region was also found in a study in the inland province of Free State, South Africa, by Seedat et al to be a risk factor for HDM sensitisation.[35] A Turkish study showed that children with asthma who live at high altitudes are characterised by higher pollen but lower mite sensitisation rates than those living at sea level.[36] Different climatic conditions and altitudes may affect aeroallergen sensitisation in children with asthma.[36] The higher levels of sensitisation in our population may be explained by a combination high humidity and low altitude that favours more atopy. Whether ethnicity has an impact on sensitisation particularly to mites requires further exploration.

In West Africa (Nigeria and Ghana), a high prevalence of cockroach sensitisation was seen in asthmatic children and adolescents, and cat dander was also a more significant allergen than in Asia.[23] Atopic patients in Europe and the United States were also more highly sensitised to animal dander (cat and dog) and pollen than Asians.[23] Middle Eastern atopic subjects had the highest overall sensitisation rates to all aeroallergens – dust mites, cockroach, animal dander, pollen and mould.[23] A local Durban study conducted by Manjra et al showed that in SPTs 41% of asthmatic children in Durban were sensitised to cockroaches.[37] This was the second most common allergen in 16% of the current study population, but at lower levels than previously described. In a study in Germany, Lehmann et al found that *Alternaria* sensitisation was common and that it increased with age.[38] However, their study showed no significant correlation between asthma severity, hospitalisation rates and sensitisation profiles.[38] In west Sweden sensitisation to both cat and dog allergens increased the prevalence of asthma combined with rhinitis, and the number of sensitised components increased asthma severity.[39] We also found no correlation between these aeroallergens and disease severity.

A Korean study by Kyo Ha et al demonstrated that poly-sensitisation was weakly related to multiple morbidity.[40] However, the number of allergens to which a child is sensitised is related to the severity of IgE-mediated symptoms.[40] Most of the children in the current study were poly-sensitised but this did not correlate with disease severity. Food sensitisation was found in 26% of subjects in the current study. This is higher than the reported rates from a large epidemiological study with more than 38 000 children in the United States that showed a prevalence of 8% in children.[41]

The strength of the present study is that this is the first time in recent years that an attempt has been made to identify the prevalence of allergens in children in a peri-urban setting in KwaZulu-Natal. The study is, however, limited by its retrospective nature with a small sample size. Other limitations are the fact that data could not be obtained from the records for half of the patients who should have been included in the study and the limited number of allergens

for which sensitisation could be tested. The results are also reflective of black African and Indian children in a peri-urban setting in KwaZulu-Natal. There may be a bias of children with more severe disease presenting for follow-up and therefore skewing the data to reflect more severe disease in those sensitised to HDM.

RECOMMENDATIONS

We recommend that children with asthma and AR in KwaZulu-Natal should be tested for HDM sensitisation as this form of sensitisation was associated with moderate–severe persistent AR. Measures such as removing carpets for regular cleaning, and proper aeration and ventilation, may help to decrease the allergy burden and morbidity associated with AR. Adequate coverage of drains, pest control and barrier methods to decrease cockroach species are possible ways of minimising exposure.

CONCLUSION

HDM are the commonest aeroallergens and conferred more severe and persistent AR in children in KwaZulu-Natal.

This article has been peer reviewed.

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Research Article

ALLERGENICITY OF DOMINANT AEROPOLLEN IN NIGERIA: PART I

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ABSTRACT

Several pollen grains have been studied in detail for purification and characterisation of allergenic components in the advanced countries; however, many are yet to be studied in the tropics, including Nigeria. To close this gap, four pollen grains (Poaceae – *Cynodon dactylon, Panicum maximum;* Cyperaceae – *Cyperus rotundus* and *Mariscus alternifolius*) found dominant in the air from previous aeropalynological studies in Nigeria were selected. The pollen grains were harvested from fresh anthers and their proteins were extracted, quantified, separated and subjected to Western-blot analysis. The allergenic proteins were also identified. While *C. dactylon* had the highest protein content (17.09 mg/mL), *M. alternifolius* had the lowest (11.19 mg/mL). Western-blot analysis showed that individuals were most susceptible to the 35 kDa protein of *C. dactylon* (76%). Furthermore, only *C. dactylon* proteins of 14.5 kDa and 35 kDa were identified with their exact matches in the ProFound database (Cyn d 12 and Cyn d 1 respectively), whereas the peptide sequences of eight protein bands were newly added to the database. Of these, the Profilin protein group (14 kDa) is common to all studied pollen grains – an indication of veritable immunotherapeutic potential. This study is the first in Nigeria to record allergenic proteins in these pollen grains and to create a foundation for the development of immunotherapy drugs for allergy treatment in the country.

Keywords: allergenic proteins; immunotherapy; Nigeria; pollen grains; Western blot

INTRODUCTION

It is often difficult to believe that the seasonal catarrh or wheezing cough or 'apollo' (conjunctivitis) is merely an abnormal condition of our immune system. Allergic individuals will produce immunoglobulin E (IgE) upon first contact with an antigen, termed an 'allergen'. Upon re-exposure, IgE binds to the IgE-specific receptors (Fc epsilon receptors) of mucosal and cutaneous mast cells and circulating basophils.[1] This reaction occurs within minutes, and upon re-exposure to the allergen, there is a rapid uptake of calcium ions into the mast cells. This results in degranulation and the release of pro-inflammatory mediators such as histamine, leukotrienes, prostaglandins and tryptase.[1] These, in turn, result in the symptoms of immediate allergic reactions. Allergenic conditions are characterised by intense sneezing (rhinitis), watery eyes, nasal obstruction, itchy eyes and nose, wheezing, coughing and asthma.[1] Other organs reacting to allergenic attacks – according to Kay[1] – are skin (urticaria), gastro-intestinal tract, central or peripheral nervous system and cardio-vascular system. Allergens are antigens that stimulate allergic reaction.[2] Mites, fungi and endothelial tissues/dander from pets are common indoor allergens, whereas for outdoor allergens, pollen and fungal spores dominate.[3] According to Ziello et al,[4] more than 250 000 well-described pollen-producing plants exist but fewer than 100 represent potent sources of allergens. Pollen allergens belong to various protein families that have been identified in diverse plant species mainly in the temperate regions.[5,6,7,8,9]

Pollen causing allergy are quite variable in different ecozones, which makes it very important to identify allergy-causing species from every region and to prepare extracts from them for diagnosis and immunotherapy for the benefit of allergy sufferers. Although allergenic protein

characterisation and identification is rare in Nigeria, aerobiological studies have revealed some dominant pollen grains in the air which could be allergenic due to their abundance and the plant families they belong to. Examples of these dominant pollen grains are grasses and sedges.[10,11,12,13] This dominance is due to the cosmopolitan nature of this group of plants in Nigeria: they are found in gardens, fields, drainages, river banks, cleared lands, etc.

Common grass species in Nigeria include: *Cynodon* spp, *Panicum* spp, *Axonopus compressus, Pennisetum* spp, *Paspalum* spp, *Andropogon* spp, *Sorghum* spp, *Triticum* spp, *Zea mays, Oryza* spp and *Stylosanthes* spp.[14]

Common sedges in Nigeria are: *Cyperus* spp, *Carex* spp, *Kyllinga* spp, *Marisucs* spp and *Pycreus* spp.[14]

Furthermore, the pollen grains of grasses are all similar – spherical and monoporate.[15] It is often difficult for palynologists to differentiate pollen of grass species. Similarly, the pollen grains of sedges which are often in tetrads or monads split from tetrads are very alike.[15] Palynologists therefore group these identical pollen grains under their respective families – Poaceae for grasses and Cyperaceae for sedges. However, in allergenicity studies, a vegetation reconnaissance of the sampled area is conducted to determine the dominant species within the region. The pollen of such dominant species can then be collected for analysis. Since the majority of the aerobiological work in Nigeria has been conducted in the southern part of the country,[10,11,12,13] the authors selected grasses (*Cynodon dactylon* (L) Pers and *Panicum maximum* Jacq) and sedges (*Cyperus rotundus* (L), *Mariscus alternifolius* Vahl) common to southern Nigeria.[14]

As explained above, several pollen grains have been studied in detail for purification and characterisation of allergenic components in the advanced countries; none are yet to be studied in Nigeria. This has led to misdiagnosis of allergy and self-medication of many allergy sufferers in the country. In view of this problem, the characterisation and identification of the allergenic proteins in dominant pollen grains in Nigeria are necessary for diagnosing and treating allergies.

MATERIALS AND METHODS

POLLEN HARVESTING

Anthers of *C. dactylon, P. maximum, C. rotundus* and *M. alternfolius* were collected and dried at room temperature and then converted to powder form by being crushed with a glass mortar and pestle. The powder was filtered through 200, 300 and 400 µm mesh sizes to collect fresh pollen and sieve out anther and other floral components.

PROTEIN EXTRACTION

Pollen grains were incubated and rotated overnight in phosphate buffer saline (PBS) to extract water-soluble proteins and in detergents (SDS 4%) to extract non-water-soluble proteins. After a centrifugation at 20 000 rpm at 4°C for 15 minutes, supernatants were collected and stored at −20°C until used. The protein concentration was measured using the classical Bradford method.[16] Here, protein standard, bovine serum albumin (BSA) was diluted with 0.15 M NaCl to final concentrations of 0.00 (blank = NaCl only), 250, 500, 750 and 1 500 µg/mL. Serial dilutions of each pollen sample was also prepared. One hundred microlitres of each sample were added to 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 pollen samples was computed from the extinction coefficient.

PATIENTS' SERA

Blood samples were collected from 50 allergy patients with a history of allergies and asthma from Gbagada General Hospital, Gbagada, Lagos State after an approval from the Ethics Committee of the hospital. The patient's symptoms and biological data were documented (gender, age group and allergic conditions). The criteria for including allergy patients comprised:

- a history of allergies;
- the timing and seasonality of allergies;
- age between 18 and 65;
- the allergic condition is not life-threatening;
- the patient is Nigerian by nationality and resides in Nigeria.

Patients were excluded if they exhibited one or more of the following exclusion criteria:

- no records of allergy history;
- allergies' seasonality or timing unknown;
- do not fall between ages of 18 and 65;
- the allergic condition is life-threatening;
- the patient is not Nigerian or is Nigerian but not resident in Nigeria.

For each analysis, the sera from five healthy individuals were used as controls. The healthy patients were selected from volunteers who donated blood to the hospital. Blood collection was done by a medical expert using syringes and anticoagulant tubes. The tubes were inverted eight times immediately after collection 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 serum was placed in a properly labelled polypropylene vial and stored at 4°C in sodium azide (0.02%) (Ramavovololona et al 2013)[23] until used.

GEL ELECTROPHORESIS

Proteins from pollen extracts were separated according to their relative mass (Mr) in SDS-PAGE. SDS-Polyacrylamide gel electrophoresis was performed in 12% gel according to the method of Laemmli[17] with modifications from Mondal et al.[18] 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 three minutes and loaded into the well of the gel; electrophoresis was then performed using a Laemmli buffer system (0.05 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.4) at room temperature and 70 V for two-and-a-half hours. 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 rrythrocytes (MW 29 kDa) obtained from the Sigma Company, United States. After electrophoresis, 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.

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 (25 ºC), blocked twice with 0.3% TBST for 30 minutes, and washed again. They were then incubated overnight in a 1:10 dilution of sera from patients (n = 50) and controls (n = 5). The 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 three hours. Antibody binding was detected by staining with the chromogenic substrate nitro-blue tetrazolium, 5-bromo-4-chloro-3'-indolyphosphate.[19]

IDENTIFICATION OF ALLERGENS

The spots of interest were analysed using the peptide-mass fingerprinting method.[20] Protein spots were excised from a Coomassie blue-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 a-cyanohydroxy-cinamic acid and submitted to laser shot in a mass spectrometer (Applied Biosystem Voyager DE-STR instrument, Texas, United States). The instrument was calibrated using two trypsin autodigestion peaks (m/z 842.5099 and m/z 2211.1045) to draw a calibration curve. The tolerance was adjusted from 4 to 500 ppm. Peptide listings were then submitted to databases using a ProFound proteomic tool (ExPaSy Proteomics Server).

RESULTS

PROTEIN CONTENT

Cynodon dactylon had the highest protein content (17.09 mg/mL), whereas *M. alternifolius* had the least (11.19 mg/mL) (see Table I).

TABLE I: PROTEIN CONTENTS OF POLLEN

POLLEN	PLANT FAMILY	COMMON NAME	PROTEIN CONTENT (mg/mL)
C. dactylon	Poaceae	Bermuda grass	17.09
C. rotundus	Cyperaceae	Tiger nut sedge	14.25
M. alternifolius	Cyperaceae	Umbrella sedge	11.19
P. maximum	Poaceae	Guinea grass	12.47

GEL ELECTROPHORESIS

The protein profile of *C. dactylon* pollen showed five major bands in the molecular weight range of 14.5–55 kDa. *M. alternifolius* pollen showed two protein bands at 14 kDa and 40 kDa. The protein profile of *C. rotundus* pollen showed five major bands with a molecular weight range of 14–58 kDa. *Panicum maximum* pollen showed five protein bands with a molecular weight range of 14–58 kDa (see Figure 1).

WESTERN-BLOT

Three dominant protein fractions of 55 kDa, 35 kDa and 14.5 kDa in *C. dactylon* pollen extract were observed to indicate a 50% (25 individuals), 76% (38 individuals)

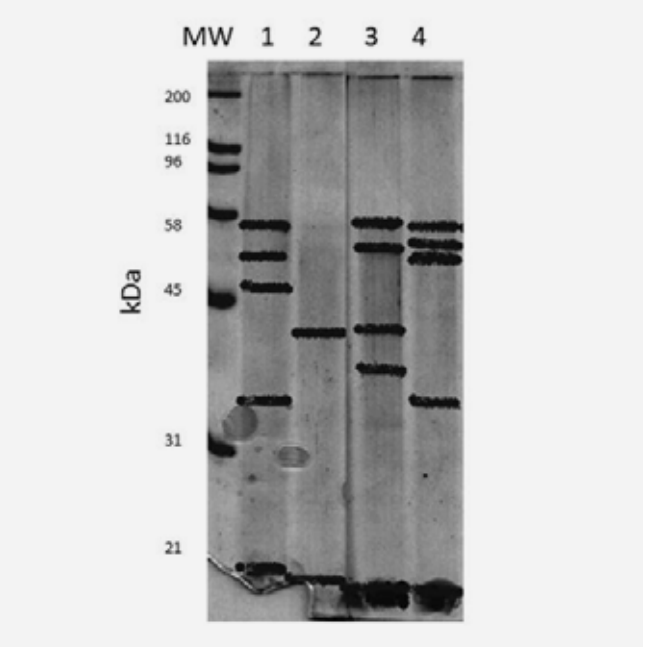

Figure 1: Protein profiling of different pollen types in 12% denaturing gel following SDS PAGE; 1 – C. dactylon, 2 – M. alternifolius, 3 – C. rotundus, 4 – P. maximum

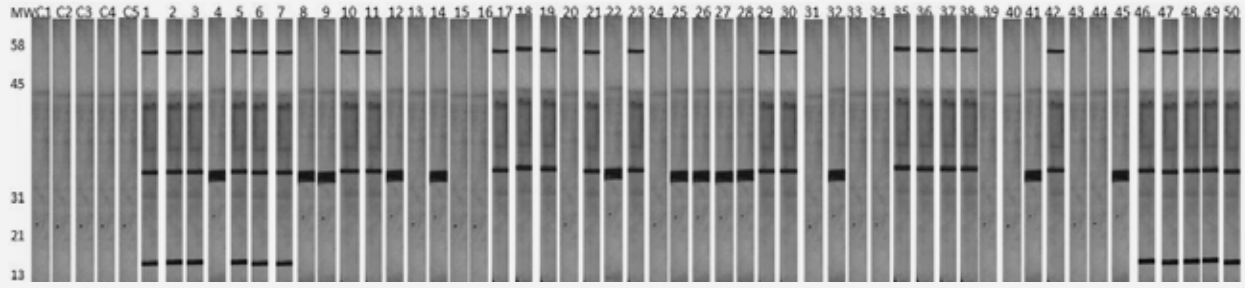

Figure 2a: Immunoblotting of C. dactylon pollen extracts. IgE-reactive protein bands are darker. MW = Molecular weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals

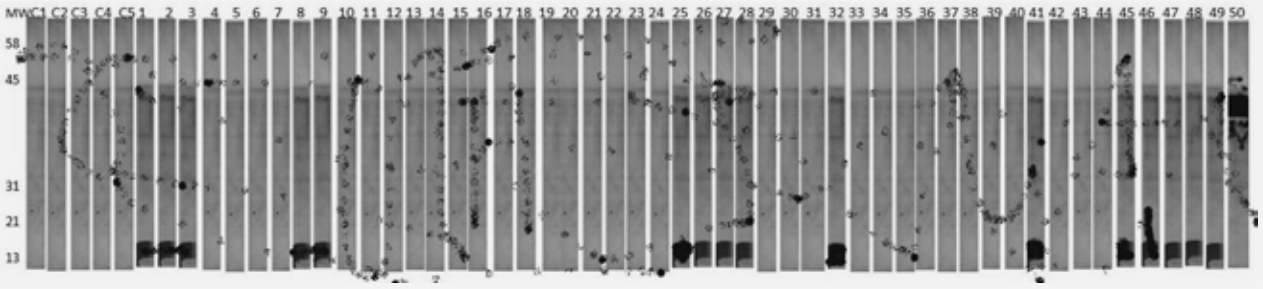

Figure 2b: Immunoblotting of M. alternifolius pollen extracts. IgE-reactive protein bands are darker. MW = Molecular weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals

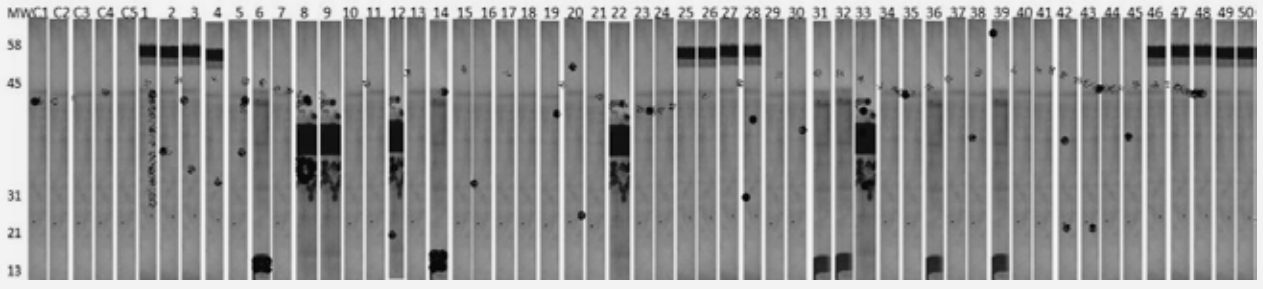

Figure 2c: Immunoblotting of C. rotundus pollen extracts. IgE-reactive protein bands are darker. MW = Molecular weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals

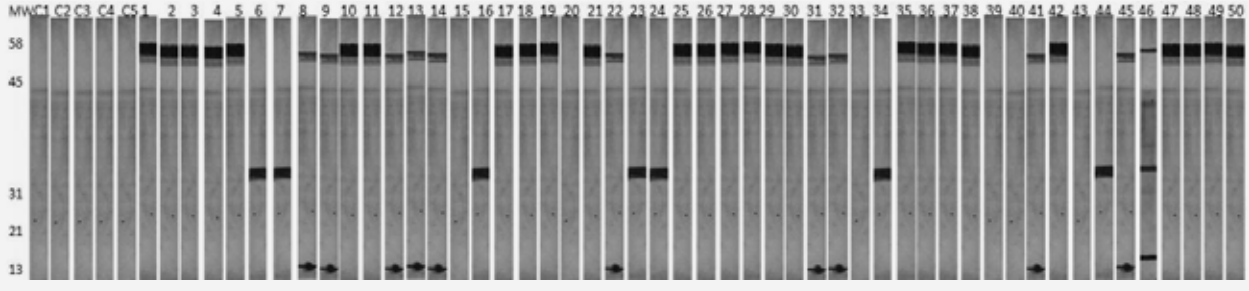

Figure 2d: Immunoblotting of P. maximum pollen extracts. IgE-reactive protein bands are darker. MW = Molecular weight. C1–5 = Controls, non-hypersensitive individuals. Lanes 1–50 = Sera of hypersensitive individuals

TABLE II: ALLERGEN IDENTIFICATION

POLLEN	PROTEINS			
	PROTEIN (DA)	NAME	HOMOLOGUE ALLERGEN	PLANT ORIGIN
C. dactylon	55 000	Polygalacturonase	Phl p 13	*P. pratense*
	35 000	Beta-expansin	Cyn d 1	*C. dactylon*
	14 500	Profilin	Cyn d 12	*C. dactylon*
M. alternifolius	14 000	Profilin	Phl p 12	*P. pratense*
C. rotundus	55 000	Polygalacturonase	Phl p 13	*P. pratense*
	37 000	Pectin methylesterase	Ole e 11	*O. europaea*
	14 000	Profilin	Phl p 12	*P. pratense*
P. maximum	55 000	Polygalacturonase	Phl p 13	*P. pratense*
	35 000	Beta-expansin	Zea m 1	*Z. mays*
	14 000	Profilin	Zea m 12	*Z. mays*

and 22% (11 individuals) IgE-binding capability. In *M. alternifolius,* 14 kDa protein indicated a 32% (16 individuals) while 40 kDa indicated a 1% (two individuals) IgE-binding capability. Three dominant protein fractions of 55 kDa, 37 kDa and 14 kDa in *C. rotundus* pollen extract were observed to indicate 26% (13 individuals), 10% (five individuals) and 12% (six individuals) IgE-binding capabilities respectively. Three dominant protein fractions of 55 kDa, 35 kDa and 14 kDa in *P. maximum* pollen extract were observed to indicate a 74% (37 individuals), 16% (eight individuals) and 22% (11 individuals) IgE-binding capability respectively (see Figure 2)

IDENTIFICATION OF ALLERGENS

All the protein bands, except the 40 kDa protein band of *M. alternifoliu*s, were linked to homologues. Only *C. dactylon* proteins of 14.5 kDa (profilin) and 35 kDa (beta expansin) were identified with their exact matches in the database (Cyn d 12 and Cyn d 1 respectively). Therefore, the newly sequenced eight allergenic proteins were deposited in the ProFound database with unique UniProt accession numbers. Profilin (14 kDa) was found in all the pollen sampled. However, the profilin of *C. rotundus* and *M. alternifolius* (both in the family Cyperaceae) were linked to allergen Phl p 12 of *Phleum pratense* while *P. maximum* profilin was related to Zea m 12 of *Zea mays*, also a grass. The 55 kDa protein bands of *C. dactylon, P. maximum* and *C. rotundus* were all polygalacturonases linked to allergen Phl p 13 of *Phleum pratense*. Also, *C. dactylon* and *P. maximum* had 35 kDa protein, which are beta-expansins and mainly related to Cyn d 1 and Zea m 1 respectively. The 37 kDa pectin methylesterase of *C. rotundus* was related to Ole e 11 of the tree *Olea europaea* (see Table II).

DISCUSSION

Pollen grains contain proteins that can be allergenic. A particular pollen may contain several types of protein, all or some of which may be allergenic. Also, the susceptibility of allergy sufferers to each protein allergen is different. This supports the reason for diagnosis of each hypersensitive individual to ascertain the set of allergenic proteins they are susceptible to. Stanley and Linskens[21] reported that the protein level in pollen generally ranges between 5.9 and 28.3 mg/mL of pollen residue. Similarly, in this work, the protein levels of the pollen grains studied were within the range 11.19 mg/mL to 17.09 mg/mL which is comparable to Stanley and Linskens finding.

This work is the first to document the protein profile of *C. rotundus* and *M. alternifolius.* Protein profiles of *C. dactylon* and *P. maximum* have been reported by other authors. The protein profile of *C. dactylon* pollen showed five major bands in the molecular weight range of 14.5–55 kDa, which was a similar range reported by Prescott and Potter[22] in South Africa and more recently by Ramavovololona et al[23] in Madagascar. *Panicum maximum* pollen also showed five protein bands with a molecular weight range of 14–58 kDa and this was a similar range reported by Ramavovololona et al[23] in Madagascar.

Qualitative observation of different immunoblots using a set of sera is very informative when no IgE tests for pollen taxa are commercially available, as is the case for three of the four plants in this study, the exception being *C. dactylon*. In this report, 38 individuals of 50 (76%) were observed to show IgE-positivity with *C. dactylon*. Ramavovololona et al[23] reported a similarly high percentage for *C. dactylon*, with 27 of 39 (69%) individuals showing IgE-positivity. These results suggest that seven to eight out of ten hypersensitive individuals are susceptible to *C. dactylon* pollen. Stemming from the fact that *C. dactylon* is a grass and a stubborn weed in the tropical and temperate regions, this high hypersensitivity warrants the control of the plant in gardens, parks, farms and drainages. In *P. maximum*'s IgE results, 44 individuals of 50 (88%) were observed to show IgE-positivity to the pollen protein. However, Ramavovololona et al[23] reported a very low percentage for *P. maximum*, with 13 of 64 (20%) individuals showing IgE-positivity. These results suggest that *P. maximum* pollen hypersensitivity may be attributed to the season of pollen collection, the type of pollen protein and the individuals in the region. Nonetheless, the ubiquity of *P. maximum* in

Nigeria also warrants the plant being controlled in gardens, parks, farms and drainages.

The work of Ramavovololona et al[23] did not highlight the specific protein bands in *C. dactylon* and *P. maximum* that were responsible for the sensitivity. Here, 35 kDa protein of *C. dactylon* and 55 kDa protein of *P. maximum* were the most allergenic of the two grasses. This indicates the diverse sensitivity of the two grass taxa which originate from different sub-families.

The 35 kDa protein of *C. dactylon* has been identified and registered in the database as a beta-expansin and named Cyn d 1. However, the 55 kDa of *P. maximum* is a polygalacturonase still being linked to Phl p 13 of *P pratense.* This work is the first to map the highly allergenic protein in *P. maximum* and register it in the database. Similarly to the Poaceae, the Cyperaceae plants *C. rotundus* and *M. alternifolius* are also stubborn weeds. Both pollen taxa exhibited low–mild allergenicity. However, the allergenic 14 kDa in both pollen taxa was similar to the Phl p 12 of *P pratense,* a Poaceae member. The similarity of a protein in the Poaceae to that of the Cyperaceae reaffirms several publications on the divergent evolution of both groups of plant from a common ancestral line.

The results of this investigation have identified allergenic proteins in the studied pollen which are often dominant in the air in Nigeria. The allergenic proteins can be used to develop immunotherapy drugs to aid allergy diagnosis and treatment in Nigeria. Of particular importance is the broad-spectrum protein (Profilin) present in all the studied pollen grains and, therefore, it has high immunotherapeutic potential.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

This article has been peer reviewed.

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Ethics Article

MICROETHICS: EVERYDAY ETHICS IN THE CLINICAL ENVIRONMENT

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TRIBUTE TO PAUL C POTTER

This Festschrift is a fitting tribute to one of the foremost scientists in the field of allergology in South Africa, and it is a privilege for me to write this short piece in honour of Paul Potter.

The legacy that Paul Potter has left us after his retirement as the first full professor of allergology at the University of Cape Town and in South Africa is a large body of knowledge about allergy and allergens in South Africa and Africa. He established a unit that put South African allergology on the international map, together with a laboratory that had the capability of characterising these allergens. After completing his paediatric training Paul studied immunology, and his subsequent research led to the characterisation of unique indigenous allergens, such as kikuyu and Eragrostis grasses, abalone and spitting cobra venom. He also was one of the foremost researchers on latex allergy in South Africa, and created awareness in our healthcare facilities about the dangers of latex.

Paul recognised the importance of defining the requirements of allergology training at under- and postgraduate level. He was the lead member of the World Allergy Organisation working group on education and advocated for the adaptation of the international guidelines to the South African situation. He was also one of the main advocates for the Diploma in Allergology and later for the subspecialty of allergology. Apart from his focused allergy research, Paul participated in numerous clinical pharmaceutical trials. This led to an interest in ethical issues in research and, in particular, ethical issues relating to paediatric asthma trials.

The greatest legacy any academic can leave is to promote and facilitate the careers of other researchers and academics. Paul, together with our other great allergologists Eugene Weinberg and the late Cassim Motala, certainly did that, and his publications bear testimony to this as well. He was a founding member of ALLSA and the founding editor of Current Allergy and Clinical Immunology. I credit Paul with encouraging me to study paediatrics, and for being one of the three major influences in my allergy career.

I wish Paul a long, happy and healthy retirement together with Anne. Thank you for what you have done for allergy and for the Allergy Society of South Africa, and for your influence on my own career.

ABSTRACT

Medical ethics education and discourse usually address the big moral dilemmas such as the extremes of life, analysed according to the established moral theories and ethical principles. Routine everyday clinical encounters provide many ethical dilemmas; this is termed 'microethics', or 'the view from inside'. Three microethics themes have been identified: respecting patient values and preferences; self-awareness, self-disclosure, and the management of physicians' values and biases; and the ethics of managing medical information.

INTRODUCTION

When we teach students medical ethics, it is often the big, headline-grabbing ethical issues that merit the most discussion and time. The same applies to public discourse: euthanasia; termination of pregnancy; newborn infants at the edge of viability; access to expensive therapy for children with rare, life-threatening medical conditions, and withdrawal of life-supportive treatment make the radio and print media regularly. Talk to any health professional in practice, however, and it is often the routine, everyday clinical encounters that cause the most ethical angst.

I have observed the best and the worst in medical and nursing professionals while supporting a friend of mine through serious illness. Poor communication with patients, caregivers and families makes it extremely difficult to navigate challenging circumstances. An acquaintance of mine told me that the way the surgeon communicated the proposed mastectomy to his wife who had been newly diagnosed with breast cancer was: "You have breast cancer. Don't worry, we will just chop it off!' Neither she as a person nor what she would be going through emotionally after this life-altering and life-threatening diagnosis was acknowledged. Contrast this with the two doctors who explained everything about my friend's illness to us in great detail, drawing diagrams, describing the options, and allowing us to ask questions and make as informed a decision as possible under the circumstances.

Patient-centred care requires shared decision-making. This entails the clinician presenting the patient with extensive information together with the management options, and the patient making an informed decision based on their values and preferences.[1,2] Epstein and Peters point out that this is relatively easy in uncomplicated situations, but 'in novel, unanticipated, and emotionally charged situations, preferences may not be elicited as much as they are constructed – shaped by how information is presented and by the opinions of family, friends and the media'.[2]

Rebecca Dresser is a bioethicist who was diagnosed with cancer in 2006.[3] She writes:

> 'This began a crash course in real-world medical ethics … The experience gave me a new understanding of what my profession is about … Experience as a cancer patient or family caregiver extends and deepens one's thinking about serious illness and bioethics.'

Some of the issues she addresses are how bewildering treatment decisions can be and how the cancer diagnosis exposes the limits of patient autonomy for the patient and family. What is striking, however, is her commentary on 'the ethical significance of what some might regard as trivial elements of patient care':[3]

> 'As Art Frank observed during one of our meetings, doctors and nurses make "constant small ethical decisions [in their] everyday clinical work", like whether to make eye contact with a patient or take seriously a patient's complaints about treatment side-effects. Their choices have a major impact on patients and caregivers. Concepts like beneficence and respect for persons are as relevant to these interactions as they are to conventional ethics concerns like decision-making about life-sustaining interventions.'[3]

Dresser alludes to the non-availability of clinical services outside normal office hours that forces cancer patients to go to an emergency centre rather than see their own doctors when they experience a complication of their disease or treatment. She describes this as 'the mundane ways in which clinical practice sacrifices generous care to organizational necessity'.

Regarding decision-making, Dresser points out that sometimes patients want to be fully informed and involved in management and treatment decisions, but at other times they want the health professional to make the decision for them. Sometimes they want an honest assessment of their situation, but at other times they prefer the optimistic version. Offering this truly individualised care for their patients requires health professionals to observe the highest ethical standards in their clinical practice.[3]

MICROETHICS: THE ETHICS OF EVERYDAY CLINICAL PRACTICE

Truog[4] terms this ethics of everyday clinical practice 'microethics' or 'the view from the inside', as opposed to macroethics, the more traditional form of ethics, informed by principles and moral theories, which he calls 'the view from the outside'.[4] 'The view from the inside, the microethical view, is unique to each situation, arises spontaneously at a particular moment in time, and is created in the relational space between the participants. It is inextricably connected to the verbal and nonverbal ways in which we communicate, and it is directly applicable to the front lines of practice.'[4]

Truog and his team from the Institute for Professionalism and Ethical Practice (IPEP) (www.ipepweb.org) at the Boston Children's Hospital have identified three themes in microethics arising from their work on decision-making in the clinical encounter:

- The ethics of respecting – and constructing – patient values and preferences.
- The ethics of self-awareness, self-disclosure, and the management of physicians' values and biases.
- The ethics of managing medical information.[4]

AUTONOMY AND PATIENT PREFERENCES

One of the principles of biomedical ethics is respect for autonomy. The term 'autonomy' was originally used to refer to the self-rule of independent city-states in ancient Greece, but is now applied to the individual. In this sense it 'encompasses self-rule that is free from both controlling interference by others and limitations that prevent meaningful choice, such as inadequate understanding'.[5] Truog writes:

> 'The traditional autonomy model of decision-making in medicine can be simplistically described as a type of recipe: clinicians contribute the "facts" (the projected risks and benefits of the available medical options), while patients provide the "values" (based on their

> own preferences and ethical commitments). When these are combined, medical decisions emerge.'[4]

As both Truog and Schneider point out, this model is far too simplistic: patients usually struggle to define their preferences clearly, particularly when confronted by difficult choices and life-threatening illness.[4,6] Dresser writes:

> 'At times, work in [bioethics] conveys the impression that seriously ill patients and their families would be in good shape if clinicians would only do things like give patients the proper information, respect patients' choices, and follow advance directives. We can assure that this is not true.
>
> 'Applying our individual preferences to treatment decision-making wasn't always simple. Few of us had confronted medical choices like this before. As John Robertson observed, "most of us are first-time players with no training". We weren't always sure which option was most consistent with our overarching values and goals.'[3]

Dresser asserts that, if experienced bioethicists are unable to make informed medical decisions about their cancer treatment, how can we expect it of patients without this background and training?[3] Epstein and Peters opine that patients' choices are likely to be fraught with indecision in 'unfamiliar, high-stakes, and uncertain situations, with potential outcomes that have not been considered or cannot be imagined'.[2] They use the term 'choiceless choices' to describe the difficulty of making decisions regarding bone-marrow transplantation as the patient would be unable to imagine what the treatment involves, despite their being well informed.[2]

Anaphylaxis is one area in allergy practice in which the risk of a severe reaction is often underpinned by a great deal of uncertainty. Severe peanut allergy creates significant anxiety for patients, families and clinicians. Last week I counselled a mother whose young daughter had developed facial swelling and urticaria after eating peanut butter and who tested positive to being allergic to many members of the legume family, with extremely high antibody levels to peanut and rAra h2. Despite the antibody levels, I believed her risk of developing a severe anaphylactic reaction to be very low. However, should I prescribe injectable adrenaline? Whose choice is it: mine or the mother's? And should the adrenaline be in the form of an autoinjector at high cost where the risk of a severe reaction is very small? How do we cope with this uncertainty in allergy practice? All we can do is to communicate the relevant information to the patient and parents/caregivers, explaining the risks and benefits, together with a recommended management plan, and the final plan should be decided upon through shared decision-making. This sounds wonderful in theory; in practice, though, who should make the final decision, and how do we arrive at a cogent decision?[7,8]

CLINICIANS' VALUES AND BIASES

Clinicians carry an enormous responsibility in helping patients and their families to make healthcare-related decisions. They should also be aware of how their own biases influence the recommendations to their patients. Taking peanut allergy as an example, if I had previously treated a child with a similar problem who died after an anaphylactic reaction to peanuts, my advice would be unequivocally in favour of recommending an autoinjector. Some of the ways in which we influence our patients' decisions are by outlining consequences in a positive (survival) or negative (death) way, or by presenting them with our preferred option first.[2] In the intensive care unit, discussion about the withdrawal of life-supportive treatment is often framed in a 'suffering' paradigm, whereas a recommendation to continue treatment would not even address this issue.[9]

Traditional medical ethics holds that clinicians' counselling should be value neutral and that clinicians' personal values should not influence their counselling.[4] Is this possible or even desirable? Would it not be preferable if clinicians acknowledged their experience and how that influences their preferences and, therefore, their counselling? The balance probably lies somewhere between the two extremes, and is situation dependent. As Truog says, 'Knowing when and how to reveal one's personal experiences and views is part of the art of medicine.'[10]

MANAGING MEDICAL INFORMATION

What information, and how much of it, should the clinician impart to the patient? When is it acceptable to withhold information from a patient? Epstein et al suggest that information should be provided according to the individual patient's requirements and situation.[11] When deciding on how much and what information to share, in making informed choices clinicians should not only take into account the benefit of the information, but also consider the emotional and psychological consequences of imparting that information. So, for example, when discussing the risk of peanut anaphylaxis, should the patient and family be informed that the risk includes death? And what of the extensive lists of allergy and asthma medication side-effects: would these possibly hinder adherence to the treatment?

Clinicians need to acknowledge their biases when making a 'clinical judgement' as to what information to impart to or withhold from the patient. Time (and therefore financial) pressures may influence the amount of information provided. Epstein et al suggest that doctors withhold information if they judge that it would cause anxiety to the patient without providing any benefit. On the other hand, the clinician should disclose information if the patient requests it and if not disclosing the information would

cause the clinician undue worry.[11]

COMMUNICATION, CONTEXT, CULTURE AND EVERYDAY CLINICAL ETHICS

Communication is an essential element of microethics. In everyday clinical practice doctor–patient encounters are often characterised by communication challenges, especially in a multicultural and multilingual country such as South Africa.[12] Social context influences patients' experience of disease to a great extent, and cultural and language barriers frequently hamper their access to quality medical care.[13] For example, in some cultures 'illness' refers to the subjective experiences of patients and how they interpret those experiences in terms of their belief system, whereas 'disease' is a malfunction of the body that can be recognised by the clinician.[14] A medical doctor 'cures' a disease by treating the underlying pathology, but the patient also needs to be 'healed'. Some patients may consult medical doctors as well as traditional healers to achieve both cure and healing. Clinicians should try to understand their patients' belief systems and address ideas about causation and traditional medicine when communicating with them.

Penn et al investigated the factors influencing patients' adherence to antiretroviral therapy, and found that there is an intricate relationship between illness, communication and cultural, socio-economic, contextual and systemic issues.[15] Their research revealed the limitations of a purely biomedical approach to addressing adherence: communication and context are both important. The authors suggest that the term 'concordance' is preferable to both 'compliance' and 'adherence' as it 'focuses on the process of interactions about medicines and the negotiation of when and how medications should be taken'. They propose that concordance should 'include negotiation of world views, an appreciation of patients' lifeworlds and sensitivity to contextual factors, all of which may influence medication taking behaviours'.[15]

CONCLUSION

The psychological and social context of sickness is extremely important, yet it is frequently absent from bioethics.[3] People suffering from serious illness have to deal with issues such as dignity, mortality and grief, and these issues should be incorporated into everyday ethics. Recognising the microethics of daily encounters with patients will make clinicians more aware and enrich their clinical practice. The traditional moral theories and ethical principles will help to frame and resolve these ethical dilemmas. To quote Komesaroff:

'We must not allow ethical debate in medicine to be restricted to discussions about embryo experimentation or life support systems, to the neglect of the actual, vital decisions that characterize our everyday work. Let us instead return to the living, ethical core of medicine – both as it is and as it ought to be.'[16]

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

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THE MAST CELL AND IgE

Shaunagh Emanuel & Di Hawarden

Dr Do-a-lot asks her students to write short notes on the role that mast cells and immunoglobulin E (IgE) play in allergic inflammation.

Mast cells are found in human tissue and derive from the bone marrow. They develop from myeloid stem cells to become granulocytes, which are white blood cells that contain secretory granules, or 'storage sacs'.

Mast cells play an important role in the body's defence against parasites and in allergic reactions. They are strategically situated at the borders of the body, where they are well positioned to protect the tissue against threats from the external environment.

Mast cells are found in large numbers at the mucosal surfaces of the respiratory tract and gut, as well as in the skin and around blood vessels. They can be activated by allergens, pathogens toxins, drugs and physiological mediators to produce a variety of inflammatory mediators. Activation may either be IgE-mediated or independent of IgE.

Mast cells are coated with hundreds of thousands of IgE antibodies. IgE that is specific to antigens to which the host has become sensitised can be found attached to the surface of mast cells. The base of the Fc region of the antibody attaches to the surface of the mast cell via the high-affinity FcεR1 IgE receptor. The Fab (or antigen-binding segment) is therefore free to bind to antigen.

Mast cell activation may occur when multivalent antigen cross-links membrane-bound IgE antibodies, leading to a number of effects and events:

- The mast cell degranulates.
- This results in the release of the contents of its secretory granules into the surrounding tissues or the bloodstream. The secretory granules contain substances such as histamine, cytokines, leukotrienes, prostaglandins and other inflammatory mediators.
  - Histamine causes increased vascular permeability and smooth-muscle contraction.
  - Cytokines, including interleukins -4 and -13,

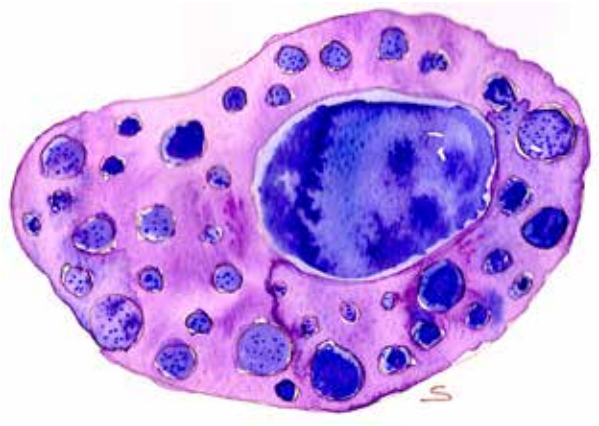

Figure 1: Toluidine Blue is an histological stain that is used by pathologists to highlight mast cell granules.In this sketch you can see a large nucleus and multiple granules in the cytoplasm of the mast cell.

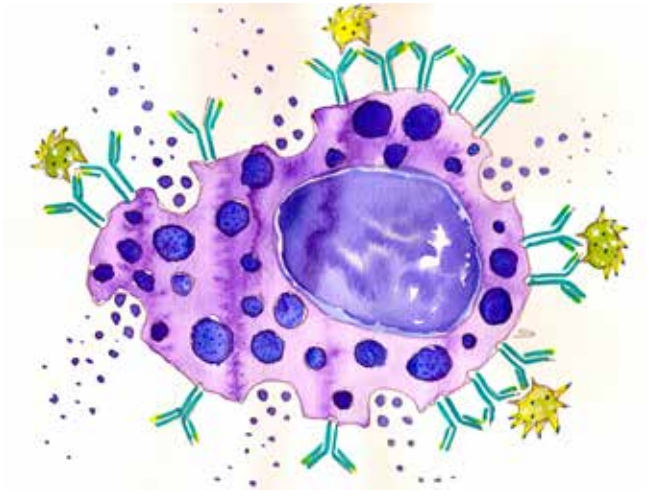

Figure 2: The mast cell degranulates as a result of stimulation by antigens that bind to and cross-link IgE attached to the surface of the mast cell.

promote Th2 differentiation and the production of IgE by plasma cells.
- Tumour necrosis factor-α (also a cytokine) promotes tissue inflammation.
- Lipid mediators such as leukotrienes and prostaglandins increase vascular permeability, cause smooth-muscle contraction, stimulate mucous secretion and chemically attract T-cells, eosinophils, mast cells and basophils.
- Mast cells also produce neutral proteases including tryptase and chymase that may affect the bronchial epithelium and contribute to airway remodelling.

- This activity results in inflammation, which typically causes tissues to become red, warm, swollen and painful, and to produce mucus.
- This may result in the clinical outcomes that we see in allergic conditions such as allergic rhino-conjunctivitis, asthma, urticaria, angioedema, food allergies or anaphylaxis.

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PID123

Primary Immunodeficiency

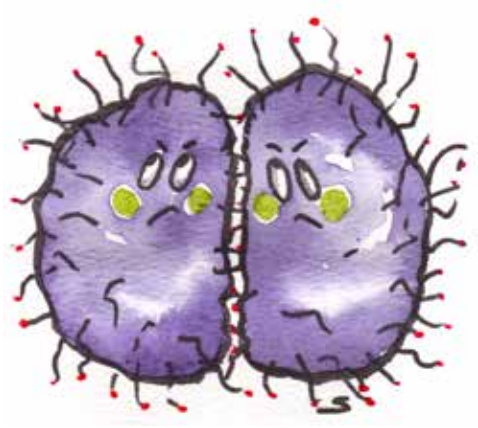
COMPLEMENT DEFICIENCY

The paediatrician, Dr Goodheart, is called to an urgent consultation in the Casualty section of her hospital. She has to review a four-year-old boy, Pieter, for possible intensive-care unit (ICU) admission. He was brought in by ambulance, referred by his local clinic for suspected meningococcal infection. Pieter had presented to the clinic with a one-day history of fever and confusion. A petechial rash was noted on the trunk and legs on the day of the clinic visit.

Dr Goodheart immediately assesses Pieter for signs of possible shock and initiates an intravenous line. She also notices rapidly spreading purpura on his back. The boy is isolated as he is potentially very infectious. She orders a sepsis screen, including a full and a differential blood count as well as a blood culture, with his initial laboratory tests. She does not perform a lumbar puncture because of the presence of petechiae over the trunk. She expects to find a positive blood culture for *Neisseria meningitides* (the positive culture is confirmed after 24 hrs) and starts appropriate intravenous antibiotics. Pieter is placed under close observation of his vital signs, as he may also be bleeding into the adrenal glands.

Of importance is the history of recurrence: At age three Pieter had also suffered from meningococcal bacterial meningitis. At age four he now presents with meningococcal septicaemia.

Dr Goodheart remembers the alerts for primary immunodeficiency that she has learnt as a student: severe, persistent, unusual or recurrent infections = SPUR! She suspects that Pieter's recurrent and severe infections might possibly be caused by a PID, specifically a complement deficiency.



'Mischievous' Meningococci (gram-negative Diplococci)

Regarding the family history, Pieter and his family live in an informal settlement close to the city. They stay in a two-roomed informal structure lacking indoor toilet facilities and running water. There is a younger brother and the family is generally healthy. Specifically, there is no relevant family history of recurrent infections or early unexplained infant death.

With the patient stabilised in the isolation room, Dr Goodheart calls the immunology specialist, Dr Bala, to discuss more targeted investigation. Dr Bala's laboratory requests a sample for total complement (on ice, as it is a functional assay of the intact complement cascade!) apart from the blood tests already sent. The next day Dr Bala telephones her colleague very excitedly because the complement activity level is indeed very low on repeat testing. She now suspects a late complement component deficiency and tests for the complement component C6, which is the commonest of these deficiencies. As it turns out, C6 is indeed absent from Pieter's blood.

While Pieter is still in hospital, Dr Bala recommends proceeding with **genetic testing**. She explains to Dr Goodheart that **late complement component deficiencies** are often inherited in an autosomal recessive fashion; therefore, the rest of the family must always also be tested for complement deficiency. Because a patient may not have been exposed

Pieter has recovered and wears his Medical Alert bracelet

to a 'PID signature bug', this immune defect may be missed in early life and the onset of a first infection may be delayed until adulthood. In certain regions, such as the Western Cape of South Africa, the frequency of a genetic cause for complement deficiency resulting in meningococcal disease is extremely high at 28% or 8% for black and mixed-race patients respectively![1] (Box 'Complement deficiencies').

The genetic screen tests for five common mutations in C6 and one in C5. Pieter tests homozygous positive for two of the mutations in C6, which confirms a **C6 deficiency**. The family is also investigated. The mother is heterozygous for C6 and hence not susceptible to meningococcal disease, the father is not available for testing. The good news is that the little brother Armin is not carrying the C6 gene defect.

Dr Bala explains that patients such as Pieter with C6 deficiency are at high risk for recurrent infections, specifically with meningococcus (meningitis or septicaemia) but also for extragenital gonococcal infection. This is an isolated genetic susceptibility, without susceptibility to other infectious organisms, and therefore it has a good prognosis otherwise.

Overcrowding in Pieter's home could have contributed to his recurrent meningococcal infections. Screening for carriers of meningococcus in the house is recommended in this case. Household members who were in close contact with Pieter also need short-term prophylactic treatment with antibiotics (Rifampin).

Dr Bala suggests the following for further patient management: empirically dosed systemic penicillin for the current infection; thereafter prophylactic penicillin must be taken (oral or intra-muscular) for life. Pieter should also receive pneumococcal, *Haemophilus influenzae b*, and meningococcal conjugate vaccines.

Pieter fortunately recovers without any problems and his skin lesions heal fully. He should never again suffer from further meningococcal infections if he stays compliant on lifelong penicillin. The local clinic sister receives a referral letter to make sure that his mother collects the medication regularly. He must also carry a Medical Alert bracelet. Pieter will attend the Immunology clinic for lifelong follow-up, too. If he stays well, this may be needed only every six months.

Box: Complement deficiencies[2]

Complement is an important part of the innate immune system. It comprises a series of proteins which activate each other in a cascade-like fashion. Although accounting for less than 2% of PID, defects are described for each of the individual components and clinical manifestation depend on the role of the affected component. One may distinguish:

1. Deficiencies in the classical pathway (e.g. C1 inhibitor defects – hereditary angioedema, C2 deficiency)
2. Deficiencies of the lectin pathway (e.g. MBL deficiency)
3. Deficiencies of the alternative pathway (e.g. Factor D deficiency; control/regulatory proteins: Factor H deficiency – for example Haemolytic Uraemic Syndrome)
4. Deficiencies of the terminal pathway i.e. **late complement component deficiencies** (e.g. C3, C5, **C6** [this case], C7, C8 and C9 deficiency).

Clinical indicators for possible complement deficiencies include recurrent bacterial infections, autoimmune disease, and angioedema.

Many complement deficiencies are inherited in an autosomal co-dominant fashion, which means that other family members may be affected. Diagnostic tests exist for individual complement components. Corrective therapeutic intervention (i.e. gene therapy) is still in its infancy and treatment currently includes life-long penicillin prophylaxis and vaccination. Genetic counselling of affected patients and screened family members is essential. Follow-up at a dedicated Immunology clinic is part of the management.

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A PRIMARY IMMUNODEFICIENCY DISEASES ODYSSEY – ANSWER FROM THE EXOME IN A CASE WITH IMMUNODEFICIENCY AND AUTOIMMUNITY

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ABSTRACT

Objectives: To illustrate the use of whole exome sequencing (WES) in the diagnosis of an unidentified primary immunodeficiency disease (PID) with a complex clinical phenotype of severe infections and autoimmunity.

Methods: Stored DNA from a 14-year-old deceased female of mixed racial ethnicity, treated for five years for an unidentified PID, was referred for WES to establish a post-mortem diagnosis. Previous laboratory investigations failed to confirm a PID diagnostic category. DNA extracted from whole blood was subjected to WES for the identification of potential disease-causing mutations. Variants of interest were confirmed by Sanger sequencing to ensure that they were not sequencing artefacts.

Results: A total of six PID candidate variants in five different genes were identified using WES and an in-house bioinformatics software package. A compound heterozygous variant was identified in the gene encoding the Lipopolysaccharide-responsive beige-like protein (LRBA). This gene has been associated with combined immunodeficiency (CID) previously classified as combined variable immunodeficiency disorder (CVID).

Conclusions: Next-generation sequencing (NGS) technologies such as WES are essential complementary investigations in the diagnosis of PIDs that manifest with a wide spectrum of phenotypes. Plausible candidate variants associated with a specific phenotype may be rapidly and cost-effectively identified by WES, and correct diagnosis can assist with targeted treatment. Candidate variants of unknown pathogenicity will, however, require detailed functional validation in the investigation of more complex and rare PIDs.

Key words: primary immunodeficiency diseases; common variable immunodeficiency disorders; combined immunodeficiency; whole exome sequencing; infections; autoimmunity.

SUMMARY

This article aims to illustrate the use of whole exome sequencing (WES) in the diagnosis of an unidentified primary immunodeficiency disease (PID) with a complex clinical phenotype of severe infections and autoimmunity. Stored DNA from a 14-year-old deceased female of mixed racial ethnicity, treated for five years for an unidentified PID, was referred for WES to establish a post-mortem diagnosis. Previous laboratory investigations had failed to confirm a PID diagnostic category. DNA extracted from whole blood was subjected to WES to identify potential disease-causing mutations. Variants of interest were confirmed by Sanger sequencing to exclude sequencing artefacts. A total of six PID candidate variants in five different genes were identified using WES and an in-house bioinformatics software package. A compound heterozygous variant was identified in Lipopolysaccharide-responsive beige-like anchor protein (*LRBA*) (*c 3296 C>G* and *c 3407 C>T*), a gene that has been associated with combined immunodeficiency (CID), previously classified as common variable immunodeficiency disorder (CVID). This diagnosis of our patient demonstrates the invaluable role of next-generation sequencing (NGS) technologies such as WES as essential complementary investigations in the diagnosis of complex PIDs that manifest with a wide spectrum of phenotypes. Plausible candidate variants associated with a specific phenotype may be rapidly and cost-effectively identified by WES, and correct diagnosis can assist with targeted treatment, which unfortunately was too late for our patient.

INTRODUCTION

Primary immunodeficiency diseases (PIDs), until recently considered rare diseases, are a group of inborn errors of immunity characterised by partial or complete loss of immune-system function.[1,2] Patients may present with recurring or atypical infections, allergy and/or malignancy and autoimmunity.[1,2] Incidence may be as frequent as 1:400 for the more common PID such as immunoglobulin A (IgA) deficiency.[3] The incidence and phenotypic spectrum of PIDs in Africa is dependent on genetic background, including consanguinity, but also on locally relevant factors such as prevalent infection or vaccination patterns.[4] This necessitates regionally appropriate warning signs and diagnostic guidelines with awareness for varied phenotype presentation. A timely and accurate diagnosis of PID is essential in the prevention of morbidity and mortality associated with these diseases.[5] However, a lack of recognition and restricted access to detailed immunological investigations commonly result in universal delay of PID diagnoses of many years.

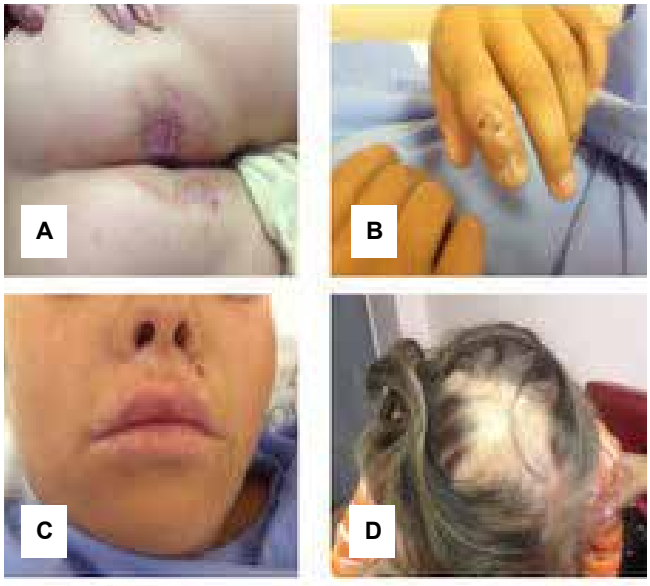

Figure 1: Clinical features of the index case. (A) Perianal non-infectious ulceration (B) Herpetic nailbed infection (C) Perioral scarring (D) Alopecia

NGS methods such as WES have proven effective in confirming suspected disease-causing mutations and for discovering mutations in novel PID-causing genes in patients with syndromes of unknown aetiology.[2,6–10] WES is the targeted sequencing of 1.2% of the human genome that is protein-coding and is reported to harbour an estimated 85% of all disease-causing variants.[11] There is significant potential for WES in PID patient care: it may provide a molecular diagnosis where a patient has remained unclassified, and in turn identify therapeutic alternatives such as haematopoietic stem-cell transplantation or justification of immune suppression in associated auto-immunity. It can also be relevant to screening of a patient's family members and offering genetic counselling.[2,6–9] Correctly selecting patients and their families for WES based on their clinical and immune phenotypes remains a crucial step towards increasing the chances of success.

Despite the technical and clinical developments attributable to NGS technologies such as WES, the identi fication of gene mutations associated with PIDs remains a major challenge.[12] These mutations may be of a disease-inherent nature or, conversely, of a technical nature. Even though WES is able to detect exonic variation only, its discovery rate for disease-causing mutations varies from 25% to 45%.[13,14] Patients who still remain undiagnosed may carry mutations in the 10% of exons that are not covered by WES or, alternatively, variants could be situated in intronic, regulatory or promotor regions of a patient's genome.[15] The latter types of variation could be detected using whole-genome sequencing; however, this method provides challenges of its own – including high costs.[16,17] Disease-associated challenges include the fact that mutations in different genes can result in very similar clinical phenotypes (locus heterogeneity), whereas mutations in different parts of the same gene can present with diverse phenotypes (allelic heterogeneity).[13,14] As the spectrum of distinct clinical entities and presenting phenotypes expands because of novel gene and variant identification, WES has become an effective strategy for the molecular diagnosis of patients with PIDs.[18]

In this study, we describe a fourteen-year-old patient who presented to Tygerberg Hospital with recurrent bacterial septicaemias, including Salmonella Group B and severe malnutrition. The initial laboratory investigations were unremarkable, except for IgA deficiency and a markedly elevated lymphocyte count. Social neglect was suspected at first presentation, as the patient recovered significantly on subsequent hospitalisation and with improved nutrition. The continued pattern of severe viral and bacterial infections, and the later onset of autoimmune manifestations and terminal disseminated tuberculosis (TB), confirmed a clinical pattern consistent with a diagnosis of combined immune deficit. Through the use of WES, a compound heterozygous mutation was identified was identified in the gene encoding the Lipopolysaccaride-responsive beige-like receptor anchor protein (*LRBA; c 3296 c>G* and *c 3407 C>T*) on a stored specimen of DNA. LRBA deficiency was previously included in both of the International Union of Immunological Societies (IUIS) combined as well as the Predominantly Antibody Deficiency Group, in the latter as a variant of CVID.[19] LRBA deficiency is now classified as affecting cellular and humoral immunity in the CID group.

METHODS

This study was approved by the Health Research Ethics Committee of Stellenbosch University (study no N13/05/075). Informed consent was granted by the parents and it included the genetic evaluation of the patient. The study adhered to the ethical guidelines as set out in the 'Declaration of Helsinki 2013'.[20]

The patient first presented for admission via the local day hospital at age nine years with a history of chronic gastroenteritis. She was marasmic, with a weight of 12.8 kg (44% of the expected weight for her age), with otitis media, severe *Herpes labialis* with secondary impetigo, extensive *Tinea corporis* and a herpetic lesion on her right index finger. A sacral pressure ulcer further raised the suspicion of parental neglect. Salmonella Group B was grown on the blood culture isolate. Her intellect appeared to be impaired and she presented with a very withdrawn nature. The child was treated with appropriate intravenous antibiotics and antifungals, and was referred to a chronic-care facility for nutritional rehabilitation and psychiatric evaluation. Baseline immune investigation was normal for full blood count (FBC), differential count, immunoglobulins (Ig's) and lymphocyte subsets, and the patient tested negative for human immunodeficiency virus (HIV) and TB. Previous medical and family histories were non-contributory. Despite good weight increase in the next months to 21.9 kg, prompt access to antimicrobials and intervention with intravenous immunoglobulin (IVIG) replacement, she developed further septicaemias and pneumonias, with resultant bronchiectasis. Further infections included viral encephalitis of unknown aetiology, extensive molluscum skin lesions and autoimmune manifestations such as vitiligo, alopecia, psoriatic skin lesions and non-infectious perianal ulcers (see Figure 1). Despite having access to clinical care interspersed with brief defaults from the outpatient clinic, the patient deteriorated, and the parents refused further hospitalisation until the terminal admission with disseminated TB.

DNA was purified from 5 mL of venous blood using the Nucleon BACC3 Kit (Amersham Biosciences, Buckinghamshire, United Kingdom). WES was performed on the Illumina HiSeq 2000™ (San Diego, California, United States). Variant prioritisation was performed using a custom-designed in-house method known as TAPER™.[21] Homozygous recessive and compound heterozygous variants were prioritised as potential candidate variants. In brief, TAPER™:

- submits VCF files to an online variant caller;
- removes all synonymous and non-frameshift variants;

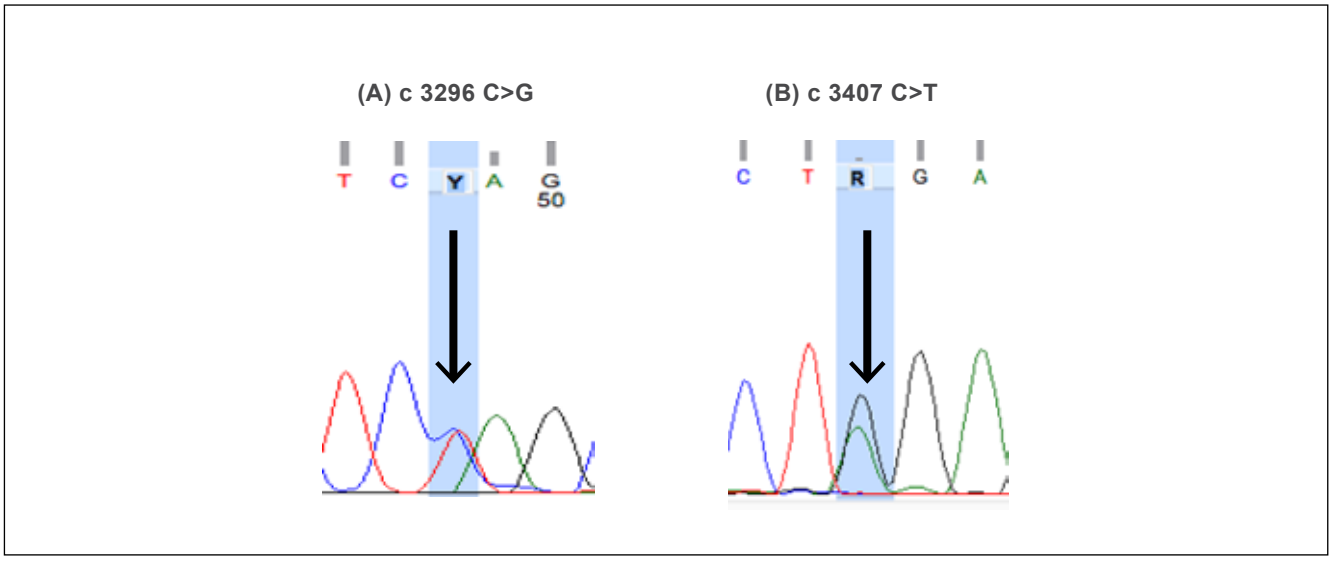

Figure 2: Sanger sequencing of the index case (the variant is indicated by black arrow). The index case is heterozygous for both variants identified in LRBA LRBA (c 3296 C>G (A) and c 3407 C>T (B))

- removes all variants with a frequency of greater than or equal to 1% if present in the 1000 Genomes Project[22] or Exome Aggregation Consortium (ExAC) databases;[23]
- removes all variants with negative Genomic Evolutionary Rate Profiling (GERP)+++ scores;
- removes all variants with Functional Analysis through Hidden Markov Models scores greater than or equal to 0.1, and, lastly,
- identifies associated disorders for prioritised genes.

A 367-base pair fragment containing both candidate variants was amplified from genomic DNA by polymerase chain reaction (PCR). The following forward and reverse primers, F: 5'CAACTGGCAAAGATTCAATGAC and R: 5'GCTGAAGACCCTGATGCTGT, were used to produce the fragment. Each amplicon was bi-directionally sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Applied Biosystems Inc., Foster City, California, United States), followed by electrophoresis on an ABI 3130XL Genetic Analyzer (Perkin-Elmer, Applied Biosystems Inc, Foster City, California, United States). All automated DNA sequencing reactions were performed at the Central Analytical Facility (CAF) at Stellenbosch University, Stellenbosch, South Africa. Sanger sequencing confirmed that the two variants in *LRBA* (*c 3296 C>G* and *c 3407 C>T*) were not sequencing artefacts (see Figure 2).

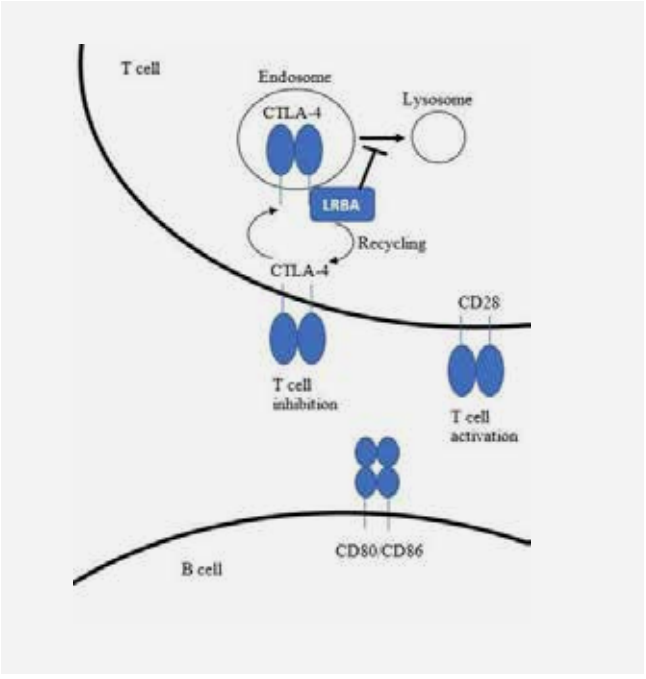

Figure 3: The interaction between CTLA-4 and LRBA is essential for immune system maintenance. LRBA is highly expressed on immune effector cells and is involved in trafficking of polarised vesicles, polarised responses of the immune effector cell, autophagy and cell survival regulation. CTLA-4 is an inhibitory TCR and negatively regulates immune responses. It contends with CD28 for binding to CD80/CD86 to prevent excessive T-cell activation and maintain immune tolerance. LRBA influences expression of CTLA-4 on cells and possibly rescues endosomal CTLA-4 from degradation through the lysosomal pathway. LRBA = Lipopolysaccharide-responsive beige-like anchor protein (LRBA); TCR = T-cell receptor; CTLA-4 = cytotoxic T lymphocyte-associated antigen 4; CD = Cluster of differentiation

RESULTS

Following filtering for rare or unreported variants, a total of six rare candidate variants were identified in five genes (see Table I). Following further analysis and database mining for gene-disease associations, we identified a compound heterozygous variant in *LRBA* (NM_006726) (*c 3296 C>G* and *c 3407 C>T*).

DISCUSSION

CVID is one of the most prevalent PIDs.[1,17] Its prevalence is estimated at between 1 : 10 000 and 1 : 50 000 in white patients and it has very rarely been described in Asian and African populations.[3,24] On the South African PID registry, 43 patients are registered with CVID diagnosis from a total of 335 PID entries.[25] The mean age of their diagnosis is 22.7 years and the median age is 19 years. Of the patients, 44% are males, whereas 56% are females. The oldest recorded age for CVID diagnosis is 59 years. The delay in diagnosis from onset of first symptoms was not traceable for all patients, but delays of up to two decades were reported anecdotally. The most prominent clinical presenting feature of CVID in patients listed on this registry is respiratory (91%), with gastrointestinal tract (GIT) associated symptoms in 7%. A total of 33 of 43 patients recorded are from two provinces only – Gauteng and the Western Cape. Forty-one of 43 patients are white and only two are of mixed racial ethnicity, reflecting a large gap in potential diagnoses in other provinces and ethnic groups. Although patients with CVID share numerous clinical and immunological features, the degree and the severity of the clinical phenotype may differ significantly between affected individuals.[1,5,26,24] The most consistent clinical feature is a marked increased susceptibility to infections. Patients may also develop significant complications as a result of disrupted immune homeostasis, such as autoimmunity.[1,5,26,24] Aside from impaired Ig production, abnormalities in almost all components of the immune system have been described.[24]

The CID group of PID, some of which may resemble CVID, includes the severe combined immunodeficiencies (SCID) which typically result in death from severe infections in the first year of life. For confirmed diagnosis of these overlapping PIDs, NGS techniques such as WES have enhanced the discovery of both autosomal-dominant and autosomal-recessive CVID-associated genes and variants, and assist with identifying the different entities of CID.[26]

By using WES, we identified a compound heterozygous variant in *LRBA* (*c 3296 C>G* and *c 3407 C>T*) in this patient. LRBA is a cytosolic protein that is localised in the endoplasmic reticulum, endocytosis vesicles, lysosomes and trans-Golgi apparatus.[27] LRBA is expressed by almost all cells, with significantly higher levels of expression in immune effector cells. LRBA functions in polarised

TABLE I. SHORTLIST OF SIX CANDIDATE VARIANTS IDENTIFIED AS PLAUSIBLE DISEASE-CAUSING VARIANTS

GENE	SNV	DBSNP	1000 GENOMES PROJECT FREQUENCY						EXAC GENOME BROWSER FREQUENCY							
			ALL	AFR	AMR	EAS	EUR	SAS	ALL	AFR	AMR	EAS	FIN	NFE	OTH	SAS
COL4A3	Q38E	rs201607115	0.0008	0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FBN1	E2796K	rs112310699	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LRBA	P1136L	rs113022115	0.0004	0.0015	0.00	0.00	0.00	0.00	7.423e-05	0.00007	0.00002	0.00	0.00	0.00	0.00	0.00
LRBA	A1090G	rs1782360	0.002	0.003	0.002	0.001	0.008	0.03	0.012	0.023	0.014	0.013	0.06	0.01	0.00	0.00
LRP1B	D3134G	rs115870540	0.0006	0.0008	0.00	0.00	0.002	0.00	0.0009	0.0006	0.0002	0.00	0.00	0.016	0.00	0.00
NFKB2	R853X	None	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SNV: single nucleotide variant; ExAC: exome aggregation consortium; COL4A3: collagen type IV alpha 3 chain; FBN1: fibrillin 1; LRBA: Lipopolysaccharide-responsive beige-like anchor protein; LRP1B: low-density lipoprotein receptor-related protein 1B; NFKB2: nuclear factor kappa B subunit 2; AFR: African; AMR: Ad Mixed American; EAS: East Asian; EUR: European; SAS: South Asian; FIN: Finnish; NFE: Non-Finnish European; OTH: Other; AA: African American; EA: European American

responses of immune effector cells, positive regulation of cell survival and autophagy.[26,27] The LRBA protein has recently been reported to play a major immuno-regulatory role in the expression, function and trafficking of cytotoxic T lymphocyte-associated protein 4 (CTLA-4).[28] CTLA-4 is a T-cell inhibitory receptor that is responsible for the down-regulation of immune responses.[28] CTLA-4 prevents excessive T-cell activation by competing with CD28 for binding to CD80/CD86, the classic co-stimulatory pathway which enables immune cell activation, thereby maintaining immune tolerance.[29,30] (See Figure 3.)

The clinical and immunological phenotype of LRBA-deficient patients is highly variable. Most patients that have been reported to be LRBA-deficient have been diagnosed with CVID, the common feature being early onset of severe autoimmunity, as seen in our patient with vitiligo, unexplained hair loss, perianal ulcers and psoriatic skin lesions. Targeted treatment options for the autoimmune manifestations include Abatacept, which contains the extracellular domain of CTLA-4, and therefore induces the activity normally ascribed to the native molecule.

The genetic basis of CVID is slowly being disentangled through the identification of novel disease-causing mutations predominantly identified using NGS technologies such as WES.[26] These mutations underline the broad and varied spectrum of phenotypes observed in CVID/CID patients, which partially explains the long delay in diagnosis seen in this PID category. Correct identification of the mutation may also result in reclassification of the patient's PID diagnostic category. Access to WES technology can potentially deliver a fast, potentially life-saving and therefore cost-effective diagnosis, where the clinical suspicion for CVID/CID or other PID categories is not readily confirmed by baseline immune investigations.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

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Case Report

SUCCESSFUL DESENSITISATION OF NON-IMMUNE TYPE SYMPTOMS SECONDARY TO SALICYLATE HYPERSENSITIVITY/INTOLERANCE

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ABSTRACT

Introduction: Salicylate allergy/intolerance is a well-described entity that may present with typical hypersensitivity symptoms such as rhinitis, wheezing and urticaria or non-immune-type features such as headache, migraine, fatigue and abdominal cramps. Whereas aspirin desensitisation has been associated with success in patients presenting with Samter's triad of recurrent nasal polyps, asthma and aspirin sensitivity, the procedure has not been described in patients presenting with atypical or non-hypersensitivity-type symptoms.

Case report: A 48-year-old female presented with a two-year history of chronic headaches, migraines, fatigue and occasional upper-body myoclonic jerk-like movements upon exposure to a host of sodium salicylate-containing foods and beverages. The Flow-Cellular Antigen Stimulation Test (CAST) (Bühlmann Laboratories AG, Schönenbuch, Switzerland) for sodium salicylate was positive (stimulation index-3.9 [normal <2], percentage basophils-6.4% [normal <5%]). Aspirin desensitisation was successfully conducted in the ICU over a two-day period. At one month follow-up, she reported complete tolerance of salicylate-containing foods as well as a much better quality of life (QoL).

Discussion: Salicylate hypersensitivity/intolerance presenting with atypical or non-hypersensitivity-type symptoms may be successfully treated with aspirin desensitisation, resulting in a significant improvement in QoL.

Key words: salicylate intolerance; salicylate sensitivity; salicylate allergy; salicylate hypersensitivity; sodium salicylate

CLINCIAL IMPLICATIONS

Aspirin desensitisation has been well described in Aspirin Exacerbated Respiratory Disease (AERD). We present the first case report of resolution of atypical and non-hypersensitivity-type symptoms after aspirin desensitisation in a salicylate-intolerant patient.

INTRODUCTION

Salicylates are natural or synthetic compounds that harbour anticancer, anti-aging and anti-oxidant properties as well as other beneficial health effects.[1–3] Natural salicylates are found mainly in plants and serve as a protection against environmental diseases and insects.[4] Varying concentrations of salicylates are present in various fruits, vegetables, teas, alcoholic beverages, herbs, spices, cosmetics, fragrances and medications.[5]

Symptoms of salicylate sensitivity/intolerance may include Gell and Coombs type-1 hypersensitivity related symptoms such as rhinitis, wheezing and urticaria as well as various non-specific and non-immune symptoms that include headaches, migraines, tinnitus, hyperacusis, hearing loss, palpitations, arrhythmias, irritable bowel symptoms (nausea, vomiting, reflux, bloating, flatulence, diarrhoea, constipation), joint pains, arthritis as well as possible behavioural and psychiatric disturbances (irritability, restlessness, inattention, Attention-Deficit Hyperactivity Disorder (ADHD), enuresis, depression, anxiety and panic attacks).[4] We present the first reported case of successful desensitisation in a patient that presented with non-specific and atypical symptoms of salicylate intolerance.

CASE REPORT

A 48-year-old female presented with a two-year history of

chronic headaches, migraines and fatigue upon exposure to a host of sodium salicylate containing foods and beverages. She had also reported six previous hospital admissions with myoclonic jerk-like movements of her upper body after either using various non-steroidal anti-inflammatory class drugs or consuming a high salicylate content meal. Her symptoms were not associated with rhinitis, nasal polyps, wheezing, urticarial rash, dizziness, hypotension, deranged consciousness or any other symptoms in keeping with a Gell and Coombs type-1 hypersensitivity reaction. Of significance, the Flow-Cellular Antigen Stimulation Test (CAST) (Bühlmann Laboratories AG, Schönenbuch, Switzerland) for sodium salicylate was reported positive (stimulation index -3.9 [normal <2], percentage basophils-6.4% [normal <5%]). Her symptoms had improved upon initiation of a low salicylate diet. Owing to her concern about the restrictiveness and unsustainability of this diet, she asked to undergo a trial for an aspirin desensitisation procedure. Desensitisation was carried out over two days as per the protocol described by the Scripps clinic.[6] As per this protocol, three doses of aspirin were administered on Day 1 (20 mg at 8 am, 40 mg at 11 am and 90 mg at 2 pm), three doses on Day 2 (150 mg at 8 am, 250 mg at 11 am and 325 mg at 2 pm) and a final dose (600 mg at 8 am) on Day 3. During the procedure, after ingestion of the 2nd (40 mg), 4th (150 mg) and 5th (250 mg) doses of aspirin, she again developed severe myoclonic jerk-like movements that lasted approximately 20 minutes each. She tolerated the final aspirin dose of 600 mg on Day 3 without any adverse effects, however. Prior to discharge from hospital with high-dose maintenance aspirin therapy (600 mg twice daily), she had also tolerated 1 g of turmeric powder (high salicylate content) without developing any of her previous symptoms. At one month follow-up, she reported complete tolerance of salicylate-containing foods and a significant subjective improvement in QoL with no associated headaches or myoclonic jerky movements.

DISCUSSION

Case presentations of salicylate intolerance-related non-immune-mediated symptoms are mostly self-described in web-based food and diet-intolerance forums.[4,7] Owing to the variation in presenting features and uncertain pathophysiology, the nomenclature is rather confusing. Terms such as 'salicylate allergy', 'salicylate sensitivity' and 'salicylate intolerance' have been used interchangeably in the literature. Although typical immediate hypersensitivity-type symptoms such as rhinitis, wheezing, urticaria and anaphylaxis have been well described on exposure to aspirin (acetyl salicylate acid) and related compounds, an IgE antigen-mediated mechanism has not been shown.[8,9] Non-hypersensitivity-type symptoms are believed to be non-immune-mediated.[10] Despite the positive CAST test result in our patient, which implies a non-IgE immune-mediated mechanism, her reported symptoms of headaches, migraine, fatigue and myoclonic jerky movements suggest a non-immune mechanism. It is possible that both immune and non-immune mechanisms were present in our patient. Hence, in our description we have included the terms 'hypersensitivity' and 'intolerance'.

Salicylate hypersensitivity/intolerance is mostly diagnosed in patients with Aspirin Exacerbated Respiratory Disease (AERD) who present with the triad of symptoms described by Samter in 1968.[8] Salicylate sensitivity is reportedly present in approximately 20% of adults with asthma.[11] However, the true incidence of salicylate intolerance is not known as the diagnosis of salicylate hypersensitivity/intolerance is seldom considered in patients presenting with headache, migraine, gastrointestinal tract symptoms and fatigue.[7] Dose-related myoclonic jerky or pseudo-seizure-like symptoms that were reported in our patient were rather atypical and have not been previously reported.

With regard to the desensitisation procedure, we followed the two-day aspirin desensitisation protocol with lifelong high-dose maintenance therapy (600 mg twice daily for 1 month, then 600 mg in the morning and 300 mg in the evening for the second month, then 300 mg twice daily thereafter).[6] Although the literature is clear with regard to the benefits of aspirin desensitisation in the setting of AERD, we have not come across reports of successful desensitisation following non-immune-type symptoms that manifested in our patient. Of added significance in this case is the reported resolution of symptoms and marked improvement in QoL.

The successful resolution of non-immune-type symptoms in our patient paves the way and opens a new avenue for potential therapy of patients manifesting troublesome symptoms from salicylate intolerance. A possible mechanism for success in our case may be attributed to an increase in the tolerance threshold. This is supported by the fact that food and other intolerances have been shown to be dose-dependent, as some individuals may safely tolerate lower concentrations of salicylate without manifesting clinical symptoms.[12]

Current uncertainties and areas of potential research relate to the following questions:

- Will salicylate desensitisation for non-immune-type symptoms be associated with the same success as that in AERD?
- Does high-dose maintenance aspirin therapy with aspirin have to be continued lifelong?
- If so, what is the optimal daily maintenance dose?
- Do the risks associated with daily aspirin (renal, GIT) outweigh the benefits of desensitisation and the resolution of troublesome symptoms?

In designing a prospective study, first the diagnosis of salicylate intolerance must be confirmed by improvement of symptoms while following a low salicylate diet, prior to a desensitisation procedure.

DECLARATION OF CONFLICT OF INTEREST

The authors hereby certify that this submission is not under consideration for publication elsewhere, and is free of conflict of interest.

FUNDING

None

ETHICS

Informed consent was obtained from the patient to make use of patient data and photographs/images for publication purposes.

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CPD QUESTIONNAIRE

Earn 3 CPD points after you have read the journal by completing the following questionnaire online on the ALLSA website at www.allergysa.org. To earn points you need to log in to the member section to access the questionnaire (contact ALLSA office if you do not know your user name and password). There is only one correct answer and you will have only two opportunities to submit the questionnaire, so please check answers carefully. A pass of 70% or higher is required to gain 3 points. This CPD activity is for ALLSA members only.

HYDRATION IN SEVERE ACUTE ASTHMA

1. *True or false:* Studies in adults with severe acute asthma showed that patients became appreciably dehydrated?
2. *True or false:* The measurement of acute weight loss is a good indicator of dehydration in children with severe acute asthma?
3. *True or false:* This study found that only mild dehydration occurred in children with severe acute asthma?
4. *Choose the correct answer:* The maintenance volume of low solute intravenous fluid required in children with severe acute asthma is:
   a. Double the normal maintenance volume per kilogram per 24 hours;
   b. 'Liberal' fluid replacement is beneficial in children;
   c. Fluid should be given at a rate of 50 ml/kg/24 hours;
   d. Fluid should be given at a rate of 75 ml/kg/24 hours;
   e. Fluid should be given at a rate of 100 ml/kg/24 hours.

COCKROACH ALLERGY IN DURBAN

5. *True or false*: Cockroach allergy is the third most common allergy in Durban.
6. *True or false:* Patients with cockroach allergy have a more severe form of asthma.
7. *True or false:* Cockroach allergy can only be diagnosed with an Immunocap test.
8. *Choose the correct answer:* The three species of cockroach found in Durban are:
   a. Blatella germanica;
   b. Perplanetta ameicana;
   c. Blatella orientalis;
   d. All of the above;
   e. None of the above.

CRUSTACEAN ALLERGY IN SOUTH AFRICA

9. *True or false*: The strongest IgE response of 24 crustacean sensitive patients was to Langoustine (ImmunoCAP).
10. *True or false*: All 24 crustacean sensitive patients were IgE reactive to all five crustacean species investigated.
11. *True or false*: All subjects experienced clinical symptoms through ingestion as well as non-ingestion related exposures to crustacean.
12. *True or false*: The major heat stable allergen in most crustaceans is parvalbumin.
13. *True or false*: IgE mediated sensitisation to the South African crayfish is best identified using the ImmunoCAP for Langoustine.
14. *Choose the correct answer:* What were the three most common clinical symptoms reported by the subjects with perceived seafood allergy?
   a. Oropharyngeal inchings/swelling; Asthma/wheezing; Urticaria/eczema
   b. Asthma/wheezing; Urticaria/eczema; Nausea/vomiting/abdominal pain
   c. Oropharyngeal inchings/swelling; Nausea/vomiting/abdominal pain; Asthma/wheezing
   d. Nausea/vomiting/abdominal pain; Asthma/wheezing; Urticaria/eczema

REGIONAL SPECIFIC POLLEN AND FUNGAL SPORE ALLERGENS IN SOUTH AFRICA

15. *True or false:* There is a long tree pollinating season in Table View.
16. *True or false:* Grass is the dominant aeroallergen in Rondebosch.
17. *True or false:* Fungal spore levels are low in the Lowveld.
18. *Choose the correct answer:* What is the dominant grass species east of the Drakensberg escarpment?
   a. Themeda triandra;
   b. Stenotaphrum secundatum;
   c. Cynodon dactylon;
   d. Hyperrhenia hirta.

ETHICS CPD QUESTIONS: MICROETHICS: EVERYDAY ETHICS IN THE CLINICAL ENVIRONMENT

19. *True or false:* Ethical issues in everyday clinical practice are referred to as microethics.
20. *True or false:* Respect for autonomy implies that patients should make decisions about their treatment once they have been fully informed of their choices by their doctors.
21. *True or false:* In the case of life-threatening illness, patients frequently want the doctor to make treatment decision for them.
22. *True or false:* Using the term "suffering" when counselling the family of a patient regarding withdrawal of life-supportive therapy ensures that the counselling remains value neutral.
23. *True or false:* In some cultures the terms "illness" and "disease" have completely different meanings.

ALLSA 2017 CONGRESS REPORT

The 26th Annual Congress of the Allergy Society of South Africa (ALLSA) took place at the Boardwalk Hotel in Port Elizabeth in September 2017.

This year the congress was held in parallel with the Primary Immunodeficiency Working Group (PIDDSA), which added depth to our understanding of the immune system and the manifestations associated with its malfunction. It was broadening too, to have contributions from the Allergy Dieticians' Interest Group (ADIG), as so much of allergy practice depends on informed dietary management.

Leigh Du Plessis and Yvonne Dias Fernandes of Londocor once again outdid themselves in their quest to organise an outstanding congress. The range of their detailed work, which lends itself to the potential dropping of multiple balls, is impressive. From finance to finger-food, and all the balls in between, they didn't drop a single one. We deeply appreciate their commitment without which our congress would not have got off the ground.

The staff at the Boardwalk hotel made our stay and our congress a pleasure, and the technical support was first class.

Our ALLSA secretary, Ruwayda Adams, and her consistent assistant, Jean April, did a sterling job in preparing for the congress, and tirelessly manned the stand, providing the executive committee with all the support that they needed to host the meeting.

The congress organisers put together an interesting and varied 'Inside Out' program centering on the theme of the microbiome. We extend our gratitude to them for the many hours that they contributed to making the congress a logistic and academic success.

The ALLSA invited international faculty included Professor John Warner, professor of paediatrics at Imperial College, London, and Dr Keser Pillai, specialist in internal medicine and respiratory medicine from Mauritius. We applaud them both for their valuable contributions to the program, which were greatly appreciated. Prof Aisha El-Marsafy, from Egypt, shared her knowledge on immunodeficiencies as part of the PIDDSA program.

We extend our heartfelt thanks to the local faculty which included the following speakers in order of appearance: Dr Sarah Karabus, Dr Jeanette Holtzhausen, Dr Candice Royal, Dr Carol Hlela, Dr Shaunagh Emanuel, Prof Jonny Peter, Prof Andre van Niekerk, Prof Mike Levin, Prof Sharon Kling, Dr Di Hawarden, Prof Eugene Wynberg, Dr Tamara Kerbelker, Prof Refiloe Masekela, Prof Mohamed Jeebhay, Dr Amy Burdzik, Dr Shahieda Adams, Prof Stan Ress, Prof Robin Green, Kamalina Coopasamy, Dr Abdullah Laher, Dr Sylvia van den Berg, Prof Eftyxia Vardas, Dr Sipho Duncan Ntshalintshali, Dr Kaarina Meintjies, Prof Leon Dicks, Prof Guy Richards, Prof Charles Feldman, Dr Marilee Kriel, Dr Suvarna Buldeo, Dr Claudia Gray, Prof Elvis Irusen, Dr Marinda Macdonald, Mrs Lindsay Archibald-Durham, Mrs Sasha Watkins, Dr Karin Ludwig, Dr Shirani Naidoo and Mrs Idonette van Zyl. It takes many hours of preparation and administration to develop and create the gift of an academic contribution to a congress. Without the exceptional efforts of these selfless and hardworking individuals we would not be able to offer a congress at all.

Along with academic input, the success of a congress depends on economic input. The contributions that we received from trade companies and organisations made it possible for us to host a splendid meeting. We are deeply grateful for the loyal and committed support shown to ALLSA by the following companies (in alphabetical order): Actor Pharma, Adcock Ingram Healthcare, Ampath, Aspen Pharmacare, AstraZeneca, Bayer, Beiersdorf, Cipla Medpro, Dr Reddy's Laboratories, Ethiqal, Euroimmun South Africa Pty Ltd, Genop, Immunospec, iNova Pharmaceuticals, Lancet Laboratories, Pharmaco, Mylan, National Bioproducts Institute NPC, Nestle, Novartis, Nutricia, Octapharma, PharmaDynamics, Sanofi, Thermofisher Scientific, Woolworths and Zentiva.

The Thermofisher Best Journal Article Award was awarded to AE Laher, F Motara, M Moolla, DA White

Prof Heather Zar received the GSK-Aspen Research Award

A) Abdullah Latief received the Thermofisher Best Oral Presentation Award
B) Idonette van Zyl received the Cipla Medpro Research Award
C) Tshego Mabelane received the MSD Research Award
D) The Thermofisher Best Journal Article Award was awarded to Greg Lamb, André van Niekerk and Robin Green

The Cipla Medpro team shared the Best Stand Award with Mylan

Dance-off for the Best Stand trophy between Mylan and Cipla Medpro

Honarary membership was awarded to the three international speakers – Prof John Warner, Prof Keser Pillai, and Prof Aisha El-Marsafy

The Thermofisher Scientific Journal Photograph award shared between Caitlin Hawarden and Shaunagh Emanuel

The stands in the exhibition hall were beautifully built and I had the pleasure of visiting each of them where I was welcomed by knowledgeable, and often entertaining, company representatives. The prize for the best stand, as judged by the congress delegates, was shared by Cipla Medpro and Mylan. We only had one trophy to award, so they gamely participated in an entertaining 'dance-off' on stage at the gala dinner in order to claim it. Mylan had the moves! We have couriered a duplicate trophy to Cipla Medpro, and we look forward to seeing them displayed on the stands in 2018. In addition, we greatly appreciate the contribution made by Thermofisher for their sponsorship of the registration stand and the congress bags.

The Allergy Foundation of South Africa hosted an educational event for members of the public over the same weekend, which was well attended.

The Gatsby-themed gala dinner was a 'roaring' success. We extend special thanks to Cipla Medpro for kindly sponsoring this fabulous event. The entertainment and catering were superb, and delegates must be commended for taking such care with their wonderful outfits

It is a great privilege to be able to meet. We are extended both professionally and personally. Strangers become colleagues, and colleagues become friends. A network of collegial support is developed throughout the country and beyond our borders. The Allergy Society of South Africa will continue to work hard to make this extraordinary luxury possible, because it makes us better so that we do better.

Dr Shaunagh Emanuel
Honorary Secretary, ALLSA

On behalf of the ALLSA congress organising committee: Dr Debbie White, Dr Shaunagh Emanuel and Dr Di Hawarden.



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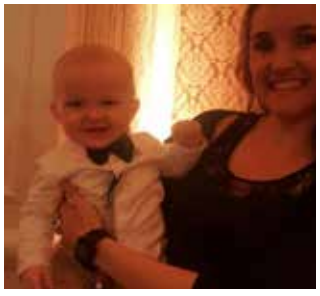








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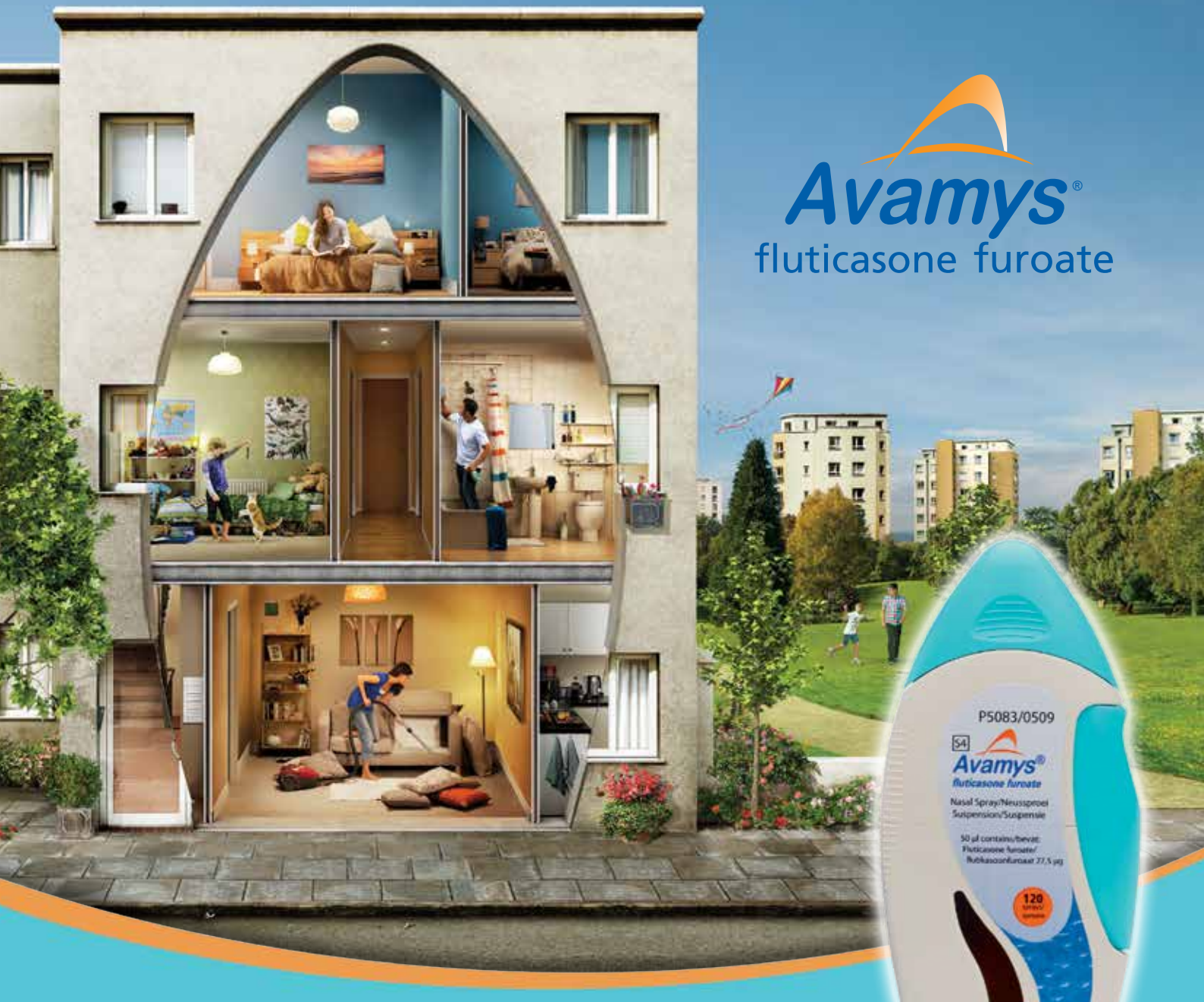

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