The Prevalence and Patterns of IgE-mediated Food Allergy and Sensitisation in South African Children with Atopic Dermatitis

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Abstract: The Prevalence and Patterns of IgE-mediated Food Allergy and Sensitisation in South African Children with Atopic Dermatitis

Background: The prevalence of food allergy in South Africa is unknown, but previously thought to be low, particularly in black South Africans. We hypothesised that food allergies would be low in Xhosa patients, even those at increased risk of food allergy such as children with atopic dermatitis (AD). This study aimed to determine the prevalence of, patterns and risk factors for, IgE-mediated food allergy in South African children with moderate to severe AD. It is the first food allergy prevalence study in South Africa to utilise controlled food challenges and component analysis, and is unique for its comparison of food allergy patterns between ethnic groups in the same geographical area.

Methodology: This was a prospective, observational study in a paediatric university hospital in Cape Town. Children with moderate to severe AD, aged 6 months to 10 years, were randomly recruited from the dermatology clinic. They were assessed for sensitisation and allergy by questionnaire, skin prick tests (SPT), Immuno Solid Phase Allergen Chip (ISAC) test and incremental food challenges. Sensitised patients were also tested for specific IgE by ImmunoCAP test.

Results: One hundred participants (59 black Africans and 41 of mixed race) were enrolled, median age 42 months. There were high overall rates of food sensitisation (66%) and food allergy (40%). Egg (25%) and peanut (24%) were the most common allergies. Black participants had comparable sensitisation (69% vs 61%) but lower allergy rates (34% vs 46%) than mixed race participants. This was especially evident for peanut allergy (15% vs 37%, p=0.01). Early onset AD (< 6 months), severe eczema, and young age < 2 years were significant risk factors for food allergy. The ISAC test was less sensitive than SPT and ImmunoCAP tests. Only 42% of cases of perceived food allergy were confirmed as true food allergy.

Component tests for peanut followed similar trends in both ethnic groups with *Ara h 2* being the most strongly associated with peanut allergy. However, the likelihood of peanut allergy with a positive *Ara h 2* was significantly lower in Xhosa than mixed race patients (53% v 93%, p=0.01). *Ara h 8* and *9* were associated with tolerance to peanut. The SPT to peanut achieved best Receiver Operating Characteristic (ROC) area under the curve indicating the best predictive value for peanut allergy in both ethnic groups. Internationally derived 95% positive predictive values (PPV) for SPT, peanut specific IgE and ImmunoCAP *Ara h 2* performed well in the mixed race group, but poorly in the Xhosa group. High degrees of asymptomatic sensitisation in Xhosa patients could not be explained by cross-

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reactive carbohydrate determinants or parasitaemia, or by a difference in peanut consumption patterns.

Egg allergy was common in both ethnic groups, and the best predictive value for egg allergy was achieved by SPT to fresh raw egg white. Internationally derived 95% PPV for egg allergy did not perform well in our population. Cow's milk allergy was uncommon in this study.

The prevalence of co-morbid allergic conditions, including aeroallergen sensitisation, asthma and allergic rhinitis, was high.

Conclusion: The prevalence of food allergy is high in South African children with moderate to severe AD, and comparable with food allergy rates in AD patients of similar severity in westernised countries. There are ethnic differences, with significantly lower peanut allergy rates in black Africans compared to mixed race patients.

Internationally derived 95% PPV for peanut, egg and cow's milk allergy did not produce good predictive values in our study population, particularly in Xhosa patients. SPT remains an excellent test in both ethnic groups for diagnosing peanut allergy, and SPT to raw egg white and fresh milk are the superior tests in egg and milk allergy diagnosis. *Ara h 2* is the most useful component in differentiating between peanut sensitisation and true allergy.

The reasons for ethnic differences in food allergy patterns are unclear, and may reflect environmental or genetic differences. A change in protective environmental factors may presage an increase in food allergy in South Africa.

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Contents Page:

Chapter	Page
1. Introduction	6
2. The Epidemiology of Food Allergy	10
3. The Relationship between Atopic Dermatitis and Food Allergy	27
4. Food Allergy Prevalence in South Africa	43
5. Study Aims, Objectives and Methodology	48
6. Overall Results for Food Sensitisation and Allergy, and Risk Factors for Food Allergy	58
7. Peanut Sensitisation, Allergy and Component Patterns in South African Children with Atopic Dermatitis	77
8. Egg Sensitisation, Allergy and Component Patterns in South African Children with Atopic Dermatitis	111
9. Sensitisation and Allergy Patterns to Cow's Milk, Soya, Wheat, Fish and Tree Nut	138
10. Allergic Co-Morbidity of Patients with Atopic Dermatitis and Food Allergy	153
11. Patterns of Introduction of Complementary Feeds and Solids	174
12. Conclusion: Clinical Application of Study Findings and the Increasing Burden of Food Allergy in South Africa	180

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Chapter 1:

Introduction

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

ISAC: Immuno Solid Phase Allergen Chip

sIgE: Specific IgE

PPV: Positive Predictive Value

ROC: Receiver Operating Characteristic

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There is evidence of a significant increase in the prevalence of food allergy, mostly studied in westernised countries, over the past 2-3 decades.[1-3] The rise in food allergies has followed a few decades after a documented increase in respiratory allergies, the latter having also been observed in South Africa.[4] Food allergy prevalence studies are methodologically difficult and time consuming. Standardised definitions of food allergy and standardised diagnostic work-ups are needed in order to be able to compare data between studies and regions.[5]

The prevalence of food allergy in South Africa is unknown. A handful of studies have looked at food sensitisation profiles and food allergy diagnosed according to self-report, but neither is a good representation of clinically relevant food allergy. The gold standard diagnostic test in equivocal cases of food allergy, or when there is a discrepancy between history and screening test results, is the controlled incremental food challenge.[6] Double blind placebo controlled food challenges are considered the gold standard, but require more time, staffing and preparation of foods. Incremental open food challenges are usually sufficient to confirm or refute food allergy in young children, who have few subjective symptoms.[7] Prior to this study, there have been no food allergy prevalence studies in South Africa utilising oral food challenges for the diagnosis of food allergy.

Atopic dermatitis is a significant risk factor for concomitant IgE-mediated food allergy. Children with moderate to severe eczema have a 5-8 fold higher prevalence of IgE-mediated food allergy than the general population. [8-10] The most common food allergies in children with atopic dermatitis are to hen's egg, cow's milk, peanut, tree nuts, soya, fish and wheat. However, the prevalence of sensitisation to foods (by skin prick test or specific IgE test) in children with atopic dermatitis far exceeds the

prevalence of true food allergy, thus there are many cases of "false positives" if sensitisation is taken as a measure of food allergy. Therefore, in the assessment of food allergy, a thorough history should always be combined with results of laboratory tests, and in equivocal cases an oral food challenge is necessary.

As food challenges are time and resource consuming, and carry the risk of the patient reacting, an attempt has been made at reducing the number of food challenges by the creation of "95%" or "100%" positive predictive values (PPVs) for skin prick tests or specific IgE, which differ for each individual food. [11,12] If the patient's result for that test falls above the 95% PPV, the chance of the patient reacting to that food during a challenge is deemed to be $\geq$ 95%. These internationally derived PPVs are used in clinical practice to diagnose patients with values $\geq$ 95% PPV as allergic without needing to perform a food challenge. However, 95% PPVs are population specific, and may be affected by selection criteria of that population, environmental, socio-economic and ethnic factors.

This study investigated sensitisation and allergy patterns to commonly allergenic foods in 100 children with atopic dermatitis, attending a dermatology clinic at a university hospital in Cape Town, South Africa (Red Cross Children's Hospital). These children were all of South African descent, either Xhosa (black African from the Western Cape) or mixed race (of mixed black African and Caucasian descent). These 2 ethnic groups make up by far the majority of children attending the Red Cross Children's Hospital hence could be recruited in meaningful numbers. One of the main drivers for undertaking our study was the widespread perception that food allergy is uncommon in South African children, particularly in children of black South African origin. We chose a population of children with moderate to severe atopic dermatitis to evaluate the prevalence of food allergy in a population at high risk for food allergy.

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The thesis is structured as follows:

Chapters 2 and 3 review the epidemiology of food allergy and the relationship between atopic dermatitis and food allergy. Studies on food allergy prevalence in South Africa are detailed in chapter 4. In chapter 5, the aims, objectives and methodology of our study are described in detail. Results of the study for sensitisation and food allergy patterns, as well as risk factors for food allergy, are described in chapter 6, including an analysis of ethnic differences. More detail on patterns for individual foods are provided in chapters 7 (peanut), 8 (egg) and 9 (other foods). Children with atopic dermatitis, as well as food allergy, are at increased risk for other atopic conditions: allergic co-morbidity is described in chapter 10, looking at the interaction between eczema, food allergy, aeroallergen sensitisation, asthma and allergic rhinitis. Chapter 11 focuses on food consumption

patterns, including the timing of introduction of commonly allergenic foods and more detail on peanut consumption patterns, to try and ascertain whether this may influence food allergy patterns. Finally, chapter 12 concludes the study findings and looks at some of the possible reasons why food allergy may be on the rise in a country such as South Africa.

This study has several unique features which have helped to create a vast database of information:

Firstly it is unique in its utilisation of controlled food challenges as part of a diagnostic algorithm for food allergy, giving accurate results for food allergy prevalence.

Secondly it is unique in the use of component derived diagnostics for food allergy testing. The study utilises the ImmunoCAP ISAC technology, a microchip technology allowing testing to 103 allergenic components simultaneously. At the commencement of the study, this technology was new to South Africa.

Patients who were sensitised by skin prick test or ISAC test also underwent standard ImmunoCAP testing for specific IgE's to whole extracts as well as components. Therefore in this study we are also able to compare the performance of 3 diagnostic tests in the prediction of food allergy: skin prick tests, ISAC test and ImmunoCAP tests. This allowed analysis of sensitivities and specificities of a variety of tests, as well as analysis of positive predictive values, and Receiver Operating Characteristic (ROC) curve production for various diagnostic entities.

Lastly, the study is unique in that it analyses ethnic differences in food sensitisation and allergy patterns within the same defined geographical area. This study compares food sensitisation and allergy patterns in Xhosa patients with those of mixed race origin to assess whether there are clinically relevant differences.

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Chapter 2:

The Epidemiology of Food Allergy

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

sIgE: Specific IgE

PPV: Positive Predictive Value

2.1 Introduction

Food allergy represents a significant public health concern. The prevalence of food allergy varies significantly based on geographical region, allergens tested, criteria used for diagnosing food allergy, age of the population, setting of the population sample and concomitant atopic conditions.[1] Variations in the definition of food allergy and inconsistencies in study design make studies on food allergy prevalence difficult to compare. Self-report significantly over-estimates food allergy prevalence by up to 10-fold,[2] hence objective measurements are necessary to establish a true food allergy diagnosis. Similarly, sensitisation to foods is much higher than clinically relevant allergies, hence sensitisation should always be combined with more objective information to prove allergies. Recent large population based studies such as the EuroPrevall® study in Europe [2] and HealthNuts® study in Australia [3] have utilised food challenge testing and can be considered flagship studies of food allergy prevalence.

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2.2 Allergenic foods

Despite the large number of food that can cause IgE-mediated reactions, most prevalence studies have focused on the most common allergenic foods, namely cow's milk, hen's egg, peanut, tree nut, wheat, soya, fish and shellfish. Such allergens account for up to 90% of food allergy reactions. Other food allergens may be particularly prevalent in certain geographical areas, for example peaches in Mediterranean countries. The most common food allergens by age are depicted in table 2.1.

Table 2.1: Most common allergens by age

Most common allergens in children	**Most common allergens in adults**
Cow's milk	Fish
Hen's egg	Shellfish
Peanut	Peanut
Tree nut	Tree nut
Soya	Fruits
Wheat	Vegetables
Sesame	

2.3 Difficulties in Diagnosing Food Allergy

There are several diagnostic challenges in food allergy prevalence studies. The marked heterogeneity in diagnostic methodology and definitions of allergy make studies difficult to compare. A major pitfall is that sensitisation does not equate to allergy. Some of the inherent difficulties in diagnostic tests are mentioned briefly below:

- ***Skin Prick Testing (SPT)***: SPT is operator dependent and requires the patient to be off antihistamines. Ethnic differences in skin thickness and reactivity to histamine may influence outcomes.[5] The source of the allergen extracts may also influence results since these may not represent local allergens.[4,5] A study in the United Kingdom found Black patients to have lower SPT reactivity but higher specific IgE (ImmunoCAP) reactivity.[6]

- ***Food-Specific IgE (sIgE)***: Helminth infections may strongly amplify the IgE response and can lead to false elevation of specific IgE levels.[7]

- ***Cross reactivity*** between components of food allergens and environmental allergens are increasingly recognised. An example is grass pollen cross-reacting with cereals, soya and peanut by specific IgE to similar carbohydrate determinants.[8] Interpreting a specific IgE level for food without considering cross-reactivity can lead to false positives for true food allergy. This is an important consideration since aeroallergen sensitivity has increased significantly in South Africa in the last few decades.[9,10] Component resolved diagnostics has refined the art of cross-reactivity recognition, but high cost and poor availability make it prohibitive in many parts of South Africa.

- ***95% positive predictive value cut off levels*** for skin prick tests and specific IgE are commonly used to diagnose food allergy.[11,12] However, these cut-offs may be population specific and thus not necessarily applicable to each population.

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- ***Questionnaire-based diagnosis*** is influenced by local culture and language. Self-reporting of perceived food allergy reactions significantly overestimates allergies.[2]

- ***Oral Food Challenges*:** The definitive method of assessing the prevalence of IgE-mediated food allergy in young children is by oral food challenges.[13,14] The gold standard is considered the double blind placebo controlled food challenge. However, open challenges suffice in most cases, particularly in young children and when the reaction recapitulates the history of a prior reaction. However, food challenges demand resources, both financially and in terms of expertise. Travel limitations and parental expense are additional issues, particularly in the African setting. Non-participation or refusal to undergo food challenge may lead to over- or underestimation of allergy.

  Food challenges have been sorely underused in African countries to date and previous food prevalence studies have omitted them entirely.

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2.4 Prevalence of Food Allergy

Several meta-analyses and large cohort studies, mostly in the US, UK, Europe and Australia, have estimated food allergy prevalence, using different definitions and methodologies.

A meta-analysis of food allergy of 51 studies investigating food allergy to cow's milk, hen's egg, peanut, fish and shellfish in children up to 18 years showed self-reported food allergy to vary between 3 and 35%.[2] Challenge proven food allergy in these studies was between 1% and 10.8% (on average 0.9% to milk, 0.3% to egg, 0.3% to fish). A recently published European meta-analysis showed a challenge proven food allergy rate of 1% in children. [15] A German study assessing doctor-diagnosed food allergy prospectively reported a rate of 4.6% at 1 year, 6.6% at age 2 and 3.9% at ages 3-6. [16] A birth cohort study in the UK utilising oral food challenges showed cumulative incidence of 6% at 3 years.[17] A Danish birth cohort study showed that 3.7% of children had a positive food challenge by 6 years age,[18] and a similar Danish study on young adults showed a prevalence of 1.7%.[19] An Israeli study based on sensitisation and strong history reported a food allergy prevalence of 1.2% with a uniquely high proportion of sesame allergy. [20]

A study in the United States (US) analysing data from an infant feeding practices study showed probable food allergy (food-related symptoms or doctor-diagnosed allergy) in 6% of one year olds. [21]

A subsequent US national health survey estimated 3.9% of children to be affected by food allergy (based on specific IgE levels to foods); mostly milk (0.4%), egg (0.2%), peanut (1.3%) and shrimp

(1%).[22,23] The recently published HealthNuts® study from Australia showed more than 10% prevalence of challenge proven food allergy to common allergens of childhood in 1 year olds (3% to peanut, 8.9% to raw egg, 0.8% to sesame).[3]

Studies from developing countries show varying prevalence of food allergy. Recent studies from China show challenge-proven food allergy rates of 3.8-7.3%,[24,25] and a Thai study shows a challenge proven food allergy rate of 0.45%.[26] Food allergy prevalence from large population-based studies and meta-analyses are summarised in Table 2.2.

The prevalence of food allergy in an unselected population in South Africa is unknown and is currently being studied in the Western Cape in the South African Food Sensitisation and Food Allergy prevalence study (SAFFA study).

In summary, food allergy peaks in the first 2 years of life, then diminishes towards late childhood as tolerance to several foods develops over time. The true incidence of food allergy varies from 1% to over 10% depending on the geographical area and age of the patient studied. The prevalence of food allergy in South Africa is unknown and currently being studied.

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2.5 Allergies to specific foods

2.5.1 Cow's Milk

Cow's milk allergy peaks in the first year of life and can be either IgE-mediated, non-IgE-mediated or a combination of both. Based on several studies, the documented prevalence of cow's milk allergy is between 0.3% and 3.5% of young children (under the age of 5), [27-32] less than 1% in older children, and less than 0.5% in adults. [28,33]

Studies have indicated a generally good prognosis for cow's milk allergy, with 45-56% outgrowing the allergy by the age of one, 60-76% by age 2, 85-90% by age 3, and 97% at 15 years.[34,35] The prognosis in a tertiary referral centre study was, however, more guarded with 5% of children outgrowing the allergy by 4 years of age and 21% by 8 years.[36] The prognosis of cow's milk allergy may vary according to the mechanism of the allergy. A Finnish study on cow's milk allergic children showed that those with non IgE-mediated allergy have a tolerance of 64% by age 2, 92% at 3 years, and 96% by 4 years; versus 31, 53 and 63% of those with immediate reactions respectively.[37] Acquisition of tolerance is faster in those with:

- initial specific IgE < 2 kU/L
- delayed vs IgE-mediated reactions
- absence of asthma or allergic rhinitis. [38,39]

Table 2.2: Summary of food allergy prevalence from meta-analyses and large population-based studies

Location (year of publication)	**Study Size**	**Method of food allergy diagnosis**	**Age of patients**	**Prevalence of food allergy**
Europe (2007) [2]	Meta-analysis of 51 studies; 6 studies included food challenges	Oral food challenge	Children and adults	1-10.8% (average 3%)
Europe (2013)[15]	Meta-analysis	Multiple	Children 0-17 Adults	Symptom + IgE 2.7% Convincing history or challenge +ve 2.6% Challenge +ve 1% Symptom + IgE 2.2% Challenge +ve 0.9%
Germany (2010) [16]	1082	Doctor-diagnosed food allergy	1-6 years	4.6% (1 year) 6.6% (2 years) 3.9% (3-6 years)
UK (2008) [17]	969	Oral food challenge	3 years	6%
Denmark (2009) [18]	562	Oral food challenge	6 years	3.7%
Denmark (2009) [19]	1272	Oral food challenge	Young adults	1.7%
Israel (2002)[20]	9070	Food specific serum IgE with clinical symptoms	0-2 years	1.2%
USA (2008) [21]	2441	Questionnaire: food related symptoms or doctor diagnosed allergy	1 year	6%
USA (2010) [22,23]	8203	Food specific serum IgE	Children and adults	2.5% overall, 3.9% in children
China (2011)[24]	497	Food challenges	0-12 months	3.8%
China (2012)[25]	1604	Food challenges	0-2 years	5.5% - 7.3%
Thailand (2005)[26]	656	Questionnaire Food challenges	6 months – 6 years	6.25% by questionnaire 0.45% by food challenge

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2.5.2 Egg allergy

Egg allergy is more prevalent in children than in adults, and is usually IgE-mediated. The estimated prevalence is 0.5-5% in early childhood, [27-29, 32,33,40] dropping significantly to less than 0.5% in older children and adults. [28,32,41]

Previous studies have demonstrated a good prognosis for egg allergy, with around 50% of egg allergic children outgrowing the condition by the age of 3 years, and 66% by the age of 5.[42-43] However, figures in tertiary referral centres are more guarded, probably reflecting a more complex patient group. A study from the USA in a tertiary referral centre has shown that 11% of children outgrew their egg allergies by the age of 4, 26% by the age of 6 years, 53% by 10 years, and 82% by age 16. [44]

Factors associated with persistence of egg allergy include:

- high initial egg specific IgE,
- multiple food allergies,
- multiple atopic conditions,
- ovomucoid sensitivity
- slow decrease in specific IgE over time. [45]

2.5.3 Wheat allergy

Wheat allergy is self-reported in about 4.5% of the population[46] but confirmed in less than 1%.[29,31,32,33,40,41] Wheat allergy can manifest in both IgE-mediated and non-IgE mediated symptoms. The natural history of wheat allergy is less well studied, but the majority of patients tend to become tolerant by adolescence.[47] A study from the United States showed tolerance development in 29% by 4 years, 56% by 8 years, and 65% by 12 years.[48] Poorer outcomes in wheat allergy have been associated with:

- SPT wheal > 5 mm to gliadin
- Higher wheat serum IgE levels.

2.5.4 Fish and shellfish allergy

Fish allergy is one of the few allergies that may be more common in adulthood than childhood. A wide variety of fish and shellfish species have been implicated depending on availability and consumption patterns. A South African study of fish allergic patients showed yellowtail, hake, snoek and abalone to be most common proven seafood allergens in patients with fish allergy.[49] Interestingly, in that study,

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only 15-21% of those patients with a perceived fish allergy had proof of allergy by sensitisation or food challenge.[49,50]

Large US based studies have shown a prevalence of fish allergy in ≤ 0.2% of children and ≤ 0.5% of adults,[28,32,40,51,52] and shellfish in ≤ 0.5% of children and ≤ 2.5% of adults. [28,29,31-33,41,51,52] Seafood allergy is lifelong in the majority of cases.

2.5.5 Peanut allergy

Peanut allergy prevalence varies significantly between geographic regions: In The EuroPrevall study, the overall prevalence of peanut allergy was 2.6%,[53,54] with wide variation between countries from 0.06% (Israel)[20] to 5.9% (Sweden).[55] Prevalence of peanut allergy is about 0.8% of the population in the United States. [56] In a South African study of 212 Xhosa high school patients, 1.9% were sensitised to peanut but none reported allergic symptoms.[57]

Peanut allergy usually starts in early childhood and is outgrown in only a small proportion (20%) of patients.[58,59] A negative SPT or low level of peanut specific IgE make outgrowing the allergy more likely.

The prevalence of tree nut allergy in the US is approximately 0.5%.[53] The prevalence amongst European children in the EuroPrevall study varied from 0.2% (France)[60] to 1.4% (UK).[61] The prevalence may be higher in adolescents and adults as allergy to tree nuts can occur for the first time in adult life.

Allergy to tree nuts are outgrown in only about 10% of children, especially those with low specific IgE < 5kU/L. [59, 62]

2.5.6 Allergy to Plant Food

Allergic symptoms to various fruits and vegetables may be true allergies or cross reactivities (pollen fruit syndrome). In a recent review (EuroPrevall study), the prevalence of perceived allergy to any fruits varied between 0.4-11.5%, and challenge proven food allergy to fruits was 0.1-4.3%.[63]

Table 2.3 summarises the prevalence of allergy to individual food allergens, as well as the long term prognosis for outgrowing the allergy.

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Table 2.3: Summary of Food Allergy Prevalence to Individual Food Allergens

Allergen	Prevalence in Young Children	Prognosis
Cow's Milk	0.3-3.5% (< 0.5% in adults)	>80% outgrown by 16 years
Hen's Egg	0.5-8% (<0.5% in adults)	>80% outgrown by 16 years
Wheat	< 1%	Majority outgrow- 65% by 12 years
Fish	< 0.2% (children) and < 0.5% (adults)	Usually allergic for life
Shellfish	< 0.5% (children) and < 2.5% (adults)	Usually allergic for life
Peanut	0.06-5.9%	20% outgrown
Tree Nut	0.2-1.4%	10% outgrown
Plant food	0.1-4.3%	

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2.6. Food-related anaphylaxis and fatalities

Food allergy appears to be the most common trigger of anaphylaxis in the community. One third to half of anaphylactic episodes can be attributed to foods;[64,65] this proportion seems to be higher in children, in which food is responsible for up to 85% of anaphylactic reactions.[66] In adults, shellfish and nut are the most common triggers of food-induced anaphylaxis,[67] and in children, peanut, tree nut, milk and egg. [66]

The prevalence of food-induced anaphylaxis is difficult to estimate in view of varying definitions of diagnosis of anaphylaxis and different methodologies of acquiring data. However, there is evidence that anaphylaxis is increasing[64] and, by inference, the prevalence of food-induced anaphylaxis is increasing. In the US, comparison of results in a similar geographic region from 1983-1987 and 1993 to 1997 showed a 71-100% increase in anaphylaxis. [64,65] An Australian study which reviewed national databases for information on anaphylaxis from 1997-2005 showed an increase in food-induced anaphylaxis admissions of 350% over this period.[68] Data from Red Cross Hospital suggests a marked increase in cases seen at the allergy referral clinic with a diagnosis of anaphylaxis from 41 in 2008, to 171 in 2012 (unpublished data).

There are no studies which directly address the prevalence of food induced fatalities. By extrapolation, an estimate of 150 deaths per year in the USA due to food induced anaphylaxis has been proposed.[69]

Studies from the USA, UK and Australia investigating anaphylactic deaths have revealed the following risk factors for fatal reactions: [69-71]

- Adolescents and young adults
- Delayed use of adrenaline
- Comorbid moderate to severe persistent asthma, and poorly controlled asthma

2.7. Food allergy and co-morbid conditions

Food allergic patients are significantly more likely than non-allergic patients to have related atopic conditions. In patients with food allergy, studies have shown that: [72]

- 35-71% have evidence of atopic dermatitis
- 33-40% have evidence of allergic rhinitis
- 34-49% have evidence of asthma

Eczema, especially severe and early onset, is a major risk factor for food allergy. The prevalence of IgE-mediated food allergy in patients with moderate to severe atopic dermatitis is 30-40% based on studies in Europe and US, [73-76] and will be further explored in chapter 2. Our study aims to explore the prevalence of food allergy in South African children with moderate to severe atopic dermatitis.

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2.8 Increase in food allergy

Many studies have suggested a true rise in prevalence of true food allergies over the last 10-20 years[77] but further confirmation of this is required. This probable increase in food allergy requires urgent further investigation as it may be due to modifiable environmental factors. [77,78]

American data shows that, from 2004 to 2006, there was an increase from approximately 2000 to 10 000 hospital discharges per year of children under 18 years with a diagnosis related to food allergy.[72] Another American study showed that the number of visits to paediatric emergency department for food-induced anaphylaxis increased steeply from 14.9/10 000 visits in 2001 to 38 per 10 000 visits in 2006. [79] Random telephone surveys regarding peanut and tree nut allergy in the US using the same methodology showed an increase in reported peanut allergy from 0.4% to 1.4% between 1997 and 2008. [56] UK data reports peanut reactivity in a cohort of 4 year old children born between 1994-1996 at 1.5%, compared to reported peanut allergy of 0.5% in a cohort born in 1989.[80] Recent studies in the UK show that this increase may be levelling off. [81]

There is no South African data on changes in food allergy prevalence but a general consensus amongst allergologists that food allergy consultations are on the increase. Our study is the first study looking at prevalence of challenge-proven food allergy in South African children. The concern is that South Africa may be experiencing a current increase in food allergy, and that contributing environmental factors need to be identified.

2.9. Risk factors for food allergy

Below is a summary of risk factors for food allergy from population-based epidemiological studies to date.[82] The full implication of these risk factors and peri-natal exposure to allergens remains controversial and under active research.

1. Genetics: there is an increased risk in families of food allergy sufferers, certain HLA types and specific genes (eg filaggrin gene defects leading to a higher risk of ezema with food allergy).[82]
2. Associated atopic disease: Atopic dermatitis is a major risk factor for food allergy, and asthma is a risk factor for increased severity of reactions.[72,73]
3. Route of exposure: Exposure via the non-oral route during critical exposure periods (inhaled or via cutaneous route) may increase the risk of acquiring food allergy.
4. Timing of ingestion of allergen: recent studies have suggested that first ingestion of food allergens during a “window” between 4 and 7 months of age may be protective. [83,84]
5. Diet: reduced consumption of omega 3 fatty acids may be a risk factor, as are dietary differences in food preparation, e.g. roasted peanut is more allergenic than boiled peanut.
6. Vitamin D may be protective. [85]
7. Anti-acid medication may allow increased immune exposure to ingested allergens. [86]
8. Hygiene hypothesis: implies an advantage of diverse microbial exposure in children (reduced risk of food allergy in rural farming areas, families with pets and more siblings; increases risk with early antibiotic use and caesarean section).
9. Geographical location may affect patterns of allergen exposure and thus allergy rates. This may be due to a difference in food consumption patterns, e.g. early introduction of peanut in Israel may be associated with lower peanut allergy rates. [87] Moreover, urban-rural status may affect allergy rates, with higher odds of food allergy in urban areas. [88]

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2.10 Ethnicity and food allergy prevalence

Apart from geographical variation, ethnic differences in food allergy prevalence within a particular region may occur.[89] Studies in the UK and USA have suggested higher rates of IgE-mediated sensitisation in Black subjects,[6] but rates of clinical reactivity are less well documented. In a large, cross-sectional survey of households in the USA, Black and Asian children had significantly higher odds of reported food allergy than white children. [90]

Recent studies have suggested that non-whites may be at greater risk of food allergy, especially if they are living in a westernised environment. In the recent HealthNuts study, Asian children who were the "first generation" in Australia, whose parents were born in East Asia, had a higher prevalence of egg as well as peanut allergy than children with two Australian-born parents, even though their parents were less atopic.[91,92]

2.11 Conclusion

We find ourselves currently in the middle of a "food allergy epidemic", which may have already peaked in some countries, and certainly has made food allergy a major public health concern. Up to 10% of young children have at least one food allergy, most commonly to hen's egg, cow's milk or peanut. Severe and complex food allergies have also increased. Accordingly, a scale-up in allergy services has become a health priority in several countries. Food allergy prevalence is currently being studied in South Africa.

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Chapter 3:

The Relationship between Atopic Dermatitis and Food Allergy

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

sIgE: Specific IgE

EPAAC: Early Prevention of Asthma and Allergy in Children

3.1 Introduction

Atopic dermatitis (AD) is form of eczema which usually begins in early infancy, and is characterised by a typical distribution, extreme pruritis and a chronic relapsing course.[1] The prevalence of both AD and food allergies has increased in recent decades, especially in high socio-economic countries, at a rate too fast to be explained by genetic drift alone; it seems there may be a common environmental factor accounting for such an increase. In several westernised countries the prevalence of eczema is 10-30%, representing a significant chronic disease burden.

There are many triggers for AD, including *specific* immunological responses to inhalable respiratory allergens, defined food allergens, and inflammatory reactions to microbial agents; as well as *non-specific* responses to irritants, heat, humidity and stress. Moreover, skin barrier dysfunction and complex interplay of cells and mediators in the skin immune system all contribute to the clinical appearance of eczema (figure 3.1). [2,3]

The relationship between food allergies and eczema is complex and multifaceted, with often conflicting perspectives amongst clinicians, patients and families. There is increasing evidence, both clinical and laboratory, for a role of food hypersensitivity in the pathogenesis of AD in a subset of patients. Numerous studies have shown that the prevalence of clinically significant food allergy in children with moderate to severe AD is high at 30-40% (see table 3.1), yet not all food allergies lead to an exacerbation of eczema. The relationship between food allergy and eczema is important to identify as on the one hand food can be a trigger for moderate or persistent eczema, on the other

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hand unnecessary diets which are not based on proper diagnosis may lead to nutritional compromise and psychological distress.

Figure 3.1: Triggers of Atopic Dermatitis

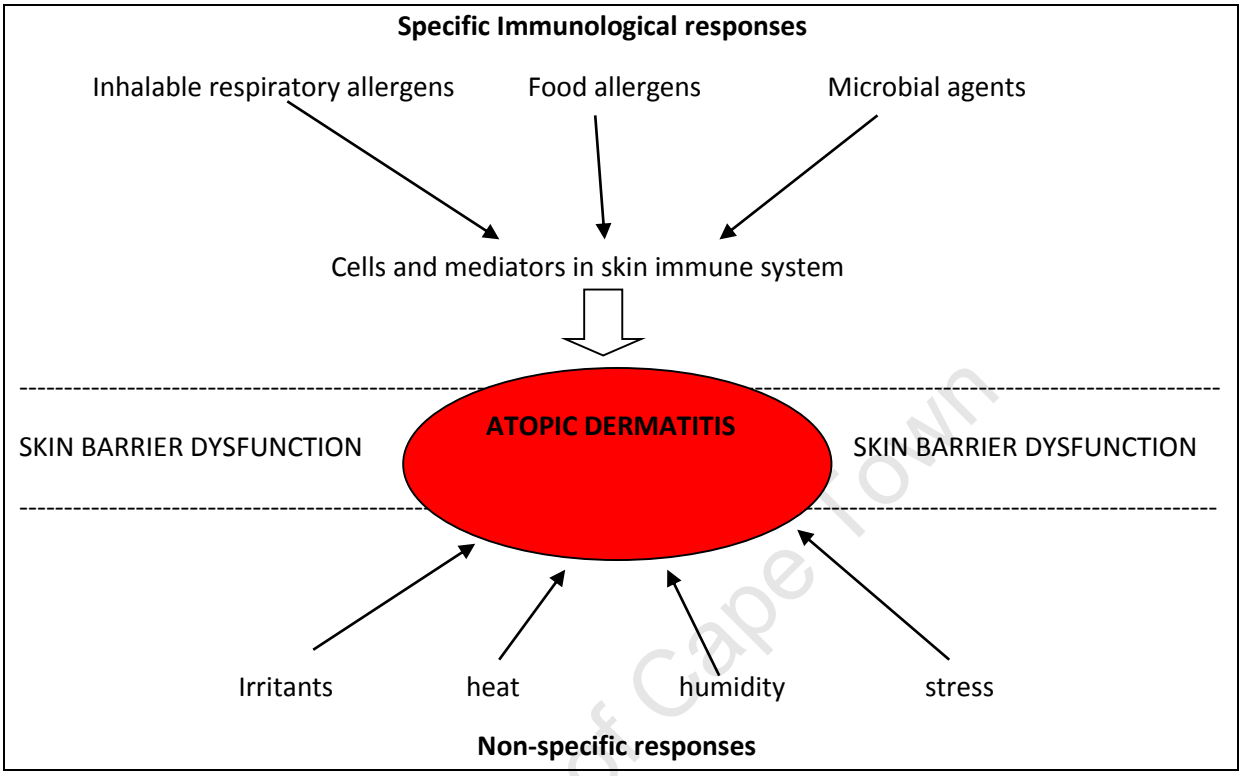

Table 3.1: Studies on Challenge-proven Food Allergies in Atopic Dermatitis

Study (year)	**Location (setting)**	**Number of Patients**	**Positive SPT or IgE**	**Food Allergy**
Sampson et al (1985)[10]	USA (allergy clinic)	113 (with severe atopic dermatitis)		56%
Burks et al (1998) [11]	USA (allergy clinic)	165 (mean age 48 months)	60% positive SPT	38.7%
Eigenmann et al (1998) [12]	USA (dermatology clinic)	63 (mean age 2.8 years)	65% positive IgE	37%
Eigenmann et al (2000) [13]	Switzerland (allergy/ dermatology clinic)	74 (median age 2.5 years)	59% positive IgE	33.8%
Garcia et al (2007) [14]	Spain (dermatology department)	44 (mean age 7.5 months)	61% positive SPT and/or IgE	27%
Kim et al (2013)[15]	Korea (dermatology clinic)	95 (mean age 6.85 years)	41% positive IgE	8.3%

SPT=skin prick test, IgE = serum food specific IgE

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As many food allergies are transient conditions that improve with increasing age, the close association between food allergy and eczema is of greater significance in the paediatric AD population than in adult patients. [4,5]

This article will explore the association between food allergies and eczema in children from 3 perspectives:

- The co-existence of food allergies in patients with eczema
- The possible role of food allergies in the pathogenesis of eczema
- The possible role of eczema in the pathogenesis of food allergies

Moreover the association between eczema and other atopic conditions will be discussed in brief.

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3. 2. Co-existence of food allergies and atopic dermatitis

3.2.1 Prevalence of food sensitisation and allergy in children with AD

When looking at the interplay between food allergies and eczema, the distinction between food *sensitivity* (a positive skin prick test or specific IgE to foods) and food *allergy* (a clinically significant reaction upon ingestion of the offending food protein) is a particularly important one. Several studies in the past 10-20 years have shown that children with AD have a propensity to both sensitisation and allergy to common food allergens:

The EPAAC™ study (Early Prevention of Asthma in Atopic Children) studied almost 2200 infants with eczema and looked at *sensitisation* patterns to common food and aeroallergens.[6] Globally, food sensitisation rates were as follows (South African figures in brackets):

Any food: 48.6%
Egg white: 41.9% (SA 47.1%)
Cow's milk: 27.4% (SA 28.4%)
Peanut: 24.4% (SA 26.8%)

The high rate of sensitisation to foods in mirrored in several other studies which generally suggest sensitisation to common food allergens in children with AD (mostly attending tertiary referral centres) the region of 60% (table 3.1). This is much greater than the overall prevalence of food sensitisation in the general population of around 16%.[7,8] In the EPAAC study, the number of children who were sensitised to the most common food allergens did not increase during the second year of life suggesting that the process of food sensitisation is completed by the first birthday. [9]

Numerous studies have shown that the prevalence of food allergy (positive responses to food challenge or recent history of significant reaction in a sensitised patient) in children with moderate to severe AD is 30-40%, as summarised in table 3.1. This is significantly higher than in the general population, in which food allergy peaks at 4-8% at one year of age (see chapter 2) and then falls progressively until late childhood, after which prevalence remains stable at 1-2%.[7]

In summary, children with AD are at high risk of food allergies but only about half of those sensitised to common foods are clinically allergic.

3.2.2 Types of food allergens

In children, classical food proteins most commonly cause food allergies in AD patients; and egg, milk, peanut, wheat and soy account for 90% of allergenic foods in children with AD.[5, 16] Overall, egg allergy is the most frequent cause of food induced eczematous symptoms, occurring in about two thirds of children with eczema who have food allergy. Children typically outgrow their clinical reactivity to egg, milk, wheat and soy despite persistently positive skin prick tests, whereas peanut, treenut, fish and shellfish allergy is typically lifelong. [17, 18]

In addition to classical food proteins, AD sufferers may more rarely react to foods which cross react with pollen (more commonly with adolescents and adults) or food additives/biogenic amines (usually non- IgE mediated food allergy). [1]

3.2.3 Patterns of clinical reactivity in food allergy in patients with AD

Patients with AD reacting to foods generally do so in one of three patterns: [19,20]

1. **Non-eczematous reactions (usually immediate):** cutaneous (pruritis, rashes, urticaria)/gastrointestinal (vomiting, diarrhoea) /respiratory symptoms/anaphylaxis. Such non-eczematous reactions make up 50% of cases and usually occur within 2 hours of food ingestion.
2. **Isolated eczematous reactions :** these occur in 10% of reactions and are usually delayed > 6 hours after food ingestion
3. **Combination of non- and eczematous reactions:** occurs in 40% of cases

The majority (at least 75%) of reactions occur within 2 hours of food ingestion; up to 25% of reactions occur after 2 hours (most commonly pruritis/ gastrointestinal symptoms/ eczema). [20-22]

75%- 94% of positive food reactions in AD patients involve cutaneous reactions, and include diffuse morbilliform or macular rashes, pruritis and urticaria.[12] Cutaneous reactions in food challenges are

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generally eruptions in sites affected by/predisposed to AD. [23] The pattern of cutaneous reactions may vary with disease activity: Sampson noted that children with food allergy who have AD may develop urticaria on food challenge when they are in remission but when their disease is active the same challenge can elicit morbilliform or eczematous reactions. [24]

In 90% of cases of clinically manifested food allergy the patient will have an IgE-associated response. During a positive immediate reaction such children have evidence of IgE response with higher plasma concentration of histamine, eosinophil granule products and eosinophil activation markers. [25, 26]

10% of cases of food allergy in AD are non-IgE mediated and not associated with food- specific IgE. Food specific T cells may play a predominant role in the pathogenesis of such reactions. [20] Non-IgE mediated food allergy is more commonly seen in wheat allergy as compared with cow's milk and egg, and is generally more difficult to diagnose. [27]

The characteristics of early and late cutaneous reactions to foods in AD are summarized in table 3.2 below:

Table 3.2: Early vs Late Cutaneous Reactions to Food in Atopic Dermatitis [21,22,27,28]

	Early reactions	**Late reactions (2-24 hours after food challenge)**
Typical cutaneous reactions	Frequently morbilliform rashes, pruritis or urticaria, often at sites of predilection for AD	Frequently eczematous
Positive SPT/specific IgE	Frequently	Not necessarily
Typically associated foods	Often hen's egg, cow's milk, peanut	Frequently wheat, less often soy and cow's milk
Frequency	In up to 90% of food induced reactions	Make up 10% of food induced reactions in isolation; although 40% of reactions have a combination of early and late reactions

3.3 The possible role of food allergies in the pathogenesis of eczema

Although the co-existence of food allergies and AD is high, the association is complex and not necessarily causal in all patients. There is, however, substantial clinical and laboratory evidence of a role of food allergy in AD causation in a subset of patients, as summarised below:

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3.3.1 Clinical evidence of causality

1. Several studies have shown that 60-80% of children with atopic dermatitis are sensitised to at least one common food allergen, and 30-40% have proven food allergies
2. Oral food challenges can reproduce skin symptoms.
3. Clinical studies have shown that at least 50% of the children with AD who react to certain foods will react with a worsening of eczema, either in addition to immediate food reactions (40%) or alone (10%). Eczema can be exacerbated in 2 ways:[29]

   i. either directly with IgE-mediated mast cell dependent development of new eczematous reactions [30], or

   ii. indirectly with early morbilliform rash/pruritis leading to itch -scratch cycle and secondary exacerbation of AD.
4. The presence of IgE to food and aeroallergens is associated with earlier onset and more severe AD. [9,31,32]
5. The greater the level of IgE and the earlier it is elevated, the more severe and persistent AD is likely to be.[32]
6. The strength of association between eczema and IgE mediated food allergy increases with increasing severity of AD. [33]
7. Appropriate dietary elimination results in significant improvement in atopic dermatitis in selected patients[34-36]

3.3.2 Histological evidence of causality

Patterns of cytokine expression found on lymphocytes infiltrating *acute* AD lesions are predominantly of the T helper cell type 2 (Th2) type (Interleukin (IL)4, IL5 and IL13) suggesting a role of the IgE antibody as well as Th2 cytokine mileau in the pathogenesis of the lesions.[36,37] Th2 cytokines promote eosinophil influx into legional skin lesions, upregulate vascular adhesion cells and upregulate high affinity IgE receptors on antigen presenting cells including Langerhans cells, thus also promoting chronic allergic inflammation. In addition to direct IgE-allergen activation of cutaneous mast cells, IgE seems to be involved in other mechanism such as high spontaneous basophil histamine release in patients with AD and food hypersensitivity.

3.4 Eczema and skin barrier defects as a risk factor for development of food allergies

The EPAAC study showed that patients with early onset AD < 3 months are at significantly greater risk of acquiring food allergies than those who develop AD after 12 months;[38] and in children whose

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eczema developed before 12 months of age, the frequency of high risk IgE-mediated sensitivity increased with increasing disease severity.

A recent explanation for the possible role of eczema in food allergy development relies on dysregulation of the epithelial barrier in AD, allowing for easier and earlier uptake of food and airborne allergens in the environment by the non-dietary route. Therefore, exposure of food proteins on AD skin may act as a risk factor for evasion of oral tolerance and development of food allergies. [39,40] For example, application of peanut containing skin creams on inflamed skin has been associated with an increased risk of sensitising children to peanuts. [41]

There has been recent identification of loss of function mutations in the gene encoding the protein filaggrin as possible causal mechanism in development of eczema.[42] Adequate functioning of skin barrier is dependent on the filaggrin-based keratin cytoskeleton. Patients with AD have increased frequency of the most common filaggrin null mutations (R501X and 2282del4) compared with healthy subjects. Filaggrin gene defects result in scaly itchy dry skin as well as increased permeability of the skin to proteins, representing a mechanism by which atopic dermatitis may be associated with increased risk of allergic sensitization.[43]

3.5 Risk factors for food allergy in eczema

Younger age, more severe eczema and early age of onset of eczema are major risk factors for food allergy and should be taken into account when selecting patients for food allergy screening.[39] The EPAAC study showed early onset eczema to be a risk factor for food sensitisation. [38] Age at the time of assessment also influences food allergy rates, which peak in young children at or below 2 years' age, and fall towards late childhood and adulthood, reflecting natural tolerance acquisition in a proportion of patients. [32]

3.6 Diagnosis of food allergy in atopic dermatitis patients

Evaluation for food allergy should be considered in:

1. Moderate to severe eczema, that does not respond to appropriate and adequate topical treatment.
2. Early onset eczema < 6 months
3. Eczema in the infant if accompanied by gut dysmotility
4. History of immediate-type food reaction
5. Convincing history of atopic dermatitis exacerbated by foods

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The aims of food allergy evaluation in AD should be differentiated, namely

i. Proving that food allergies result in non-eczematous type (usually immediate) reactions which may be of immediate danger to the patient
ii. Proving that food allergy results in delayed eczematous reaction that directly exacerbates AD.

An **accurate history** of what the patient eats, the condition of the skin, possible reactions (both atopic dermatitis and acute reactions) and extra-cutaneous symptoms can be useful to guide food related investigations.

The vast majority of food reactions in atopic dermatitis are IgE-mediated, hence tests for IgE sensitisation (SPT and specific IgE ImmunoCAP tests) are useful in the investigation of food allergy. However, there is no 100% reliable test for identifying which foods trigger atopic dermatitis. Sensitisation rates are far higher than clinically relevant allergy rates, hence frequently food allergy/tolerance needs to be proven by a provocation test (oral food challenge).

Tests with no or poor evidence to support their use include IgG testing, ELISA/ACT, applied kinesiology, ALCAT testing, analysis of hair samples, Vega testing, cytotoxic testing and others.

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3.6.1 Immediate (IgE mediated) hypersensitivity food reactions

The diagnosis of immediate type (IgE mediated) food allergy is made by taking a thorough history, looking for specific IgE sensitisation (SPT and ImmunoCAP), and performing oral food challenges if indicated.[1] Negative skin and ImmunoCAP tests are good for excluding an immediate type reaction, but cannot exclude a delayed type reaction. The presence of "positive" tests indicating sensitisation is not synonymous with food allergy. The predictive values for a history of a food reaction, positive SPT and positive food specific IgE in isolation are all poor for diagnosing food allergy in AD. The level of sensitisation must be interpreted in conjunction with the history, and in many cases where uncertainty remains, a food challenge test will be the best means to definitively prove food allergy or food tolerance.

Skin-prick tests have high negative predictive values and are a good predictor that subjects will not have an immediate type reaction on exposure but cannot exclude a delayed type reaction. However, positive predictive values are low,[44,45] hence a "positive" result does not equal clinical reactivity. Published "cut-off levels" for clinical relevance have been studied for selected allergens in the USA (child >2 years: milk ≥8mm, egg ≥7mm, peanuts ≥ 8mm; child <2 years: milk ≥6mm, egg ≥5mm, peanuts ≥4mm).[45] However, these values may be population-specific. As patients develop tolerance,

heightened SPT reactivity may lag behind reductions in specific IgE levels and may remain positive for years after a food has been successfully reintroduced into the diet.

ImmunoCAP testing for food specific IgE has high negative predictive values, but positive predictive values are low.[44,46] Published "cut-off levels" for clinical relevance have been studied for selected allergens. The values that achieved a 95% PPV are known for milk (≥15kU/L; ≥5kU/L if age <2), egg (≥7kU/L; ≥2kU/L if age <2), peanuts (≥14kU/L) and fish (≥20kU/L).[44,46] It is not currently known whether these results can be extrapolated to South Africa and other lower/middle income countries.

Atopy Patch Testing has not been shown to add significant information to a skin test as a diagnostic test for food triggers of acute or delayed reactions to foods.[47,48]

If the diagnosis of food allergy or tolerance is not absolutely clear or the clinical relevance of a positive food allergy test is not certain, a food challenge should be performed. The gold standard is the double-blind placebo-controlled food challenge.[49,50] This requires two separate challenges with a suitable vehicle, one with and one without the food under consideration, to avoid the patient and the operator from knowing which of the challenges contains the active food. For an open challenge the food is given in its usual form and therefore both the observer and the patient know the food is being ingested. Although this may be associated with false positive reactions it is acceptable in infants and young children with objective symptoms and as a preliminary screening of foods that are at a low level of suspicion as a negative challenge is definitive.

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3.6.2 Delayed eczematous food allergy reactions

The diagnosis of delayed eczematous reactions is more difficult than the diagnosis of immediate reactions.[1] In such cases specific IgE and skin prick tests may not correlate with the presence or absence of a delayed food reaction. In these cases an elimination-reintroduction diet is the only reliable way of determining whether or not a food is a trigger. Such diets must be done under supervision of a dietician. If patients respond to any dietary intervention, it is highly recommended that the food should be reintroduced to confirm the diagnosis. This may be a formal food challenge in hospital in the presence of any sensitisation or history of immediate reactions to the food(s), or a home challenge/reintroduction in the absence of sensitisation or a history of only delayed symptoms. If a formal food challenge is performed for atopic dermatitis, the schedule may need to be prolonged to observe the patient for up to six hours after the maximum dose for immediate and intermediate reactions. It is important to review the patient at 24 hours for scoring to formally document delayed-type worsening of atopic dermatitis. In cases of prolonged avoidance of a food, it is recommended to perform SPT or ImmunoCAP tests in patients prior to reintroduction of the food – even in cases where

there has not been a history of any immediate reactions- as immediate reactivity may have developed over time.[51] After prolonged avoidance of a food, or if there is any evidence of IgE-mediated sensitisation having occurred, food challenge should be performed under controlled circumstances.

The process of elimination-rechallenge testing for diagnosis of food allergy involves:[52]

- Removing all sources of the suspected food or foods for four to six weeks to bring about an improvement in the atopic dermatitis. If the atopic dermatitis does not improve within four weeks, it is unlikely that food allergy is a relevant trigger and oral food challenges are not necessary. In this case a normal diet should resume immediately.
- Even if the atopic dermatitis has resolved, foods should be reintroduced sequentially to assess for a return (or worsening) of the atopic dermatitis, prior to ascribing the improvement to the exclusion diet. This is because the improvement may be coincidental or reflect a placebo effect. Concomitant therapies and other environmental factors should not be changed during the period of assessment for food allergies. In addition, if multiple foods have been excluded it is imperative to see which of these foods is truly responsible and exclude only those foods, while allowing the return of non-contributory foods into the diet.
- Food reintroduction may be performed as a standard food challenge with a single food in incremental doses. If there is no immediate reaction, then give the food for three to four days successively and monitor atopic dermatitis scores daily. In selected cases where there has not been prolonged exclusion of the food and there was no immediate type reaction, a home challenge may be performed.
- Should the skin not react to the introduction of this food, challenge with a new food every three to four days.
- However, should the food exacerbate the atopic dermatitis, it may be considered a causal food allergen and be removed from the diet to bring about the improvement in the symptoms for the second time. Where doubt still exists, a second re-challenge may be necessary.

3.7 Therapeutic elimination diets

There is no specific diet for the treatment of unselected patients with atopic dermatitis so patients should not routinely be placed on exclusion diets.[53] Elimination diets are potentially harmful. Food allergy should only be considered in specific cases, and elimination diets reserved for those children who have been proven to be allergic and tailored to the individual after appropriate investigations, including challenge tests where necessary, have been performed to assess possible food triggers. They

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must be done under the supervision of a dietician and should always be combined with atopic skincare.

Removing foods from one's diet requires support and education, especially in cases where the food is common and present in many hidden sources. A dietician must be consulted to ensure the allergen is completely eliminated from the diet, as well as to provide alternatives to ensure nutritional adequacy of the residual diet.

3.8 Natural history of food allergy in eczema

The natural history of food allergy resolution is variable and may differ in those with and without atopic dermatitis. It varies between allergens, with milk, egg, soy and wheat resolving earlier, and more commonly than allergies to peanuts or tree nuts.[43] Allergy to fish and shellfish, which more commonly develops later, may be life-long. In atopic dermatitis, approximately 25% of patients will outgrow their food allergy after one year.[17,34] Patients with severe concomitant IgE mediated food allergy/anaphylaxis should be followed up very frequently, but all patients should be reassessed after 12 months. Repeat testing should be followed by food reintroduction in the form of a formal food challenge to reduce the risk of immediate reactions that may be present or may have developed, in order to restore a normal diet wherever possible.

3.9 Eczema as part of the atopic march

The concept of the atopic march describes the progression of atopic disorders from atopic dermatitis and food allergy in infants to allergic rhinitis and asthma in children.[54]

Clinical studies have shown that other atopic disorders (asthma and allergic rhinitis) develop in 50-80% of children with AD. [55,56] The main risk factors for progression to asthma are early onset AD, IgE sensitisation to common environmental allergens and severity of AD. Approximately 70% of patients with severe AD develop asthma compared with 20-30% with mild AD and 8% of the general population. [57,58]

In children with AD and egg allergy, respiratory allergies develop in approx 90%.[59] Early IgE responses to egg represent the most important infantile marker for atopy. [59,60]

Aeroallergen sensitisation is common in AD patients. In the recent EPAAC study 20-40% of AD patients were sensitised to house dust mite. [38] The presence of allergic sensitisation to aeroallergens in AD at one year was positively related to the occurrence of asthma. In contrast to food allergy sensitisation, aeroallergen sensitisation continued well beyond the first year of life.

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Environmental and genetic studies suggest that a defect in the epithelial barrier may contribute towards the progression of the atopic march, skin defects providing a site for inhaled antigen sensitization. [61,62]

3.10 Conclusion

A large proportion of children with moderate to severe AD (30-40%) will have a co-existing food allergy, mostly IgE-mediated. In approximately half of those AD patients who react to food, there will be a flare-up of eczema, mostly in combination with immediate symptoms, but sometimes in isolation. This means that in 15-20% of children with moderate to severe AD, food allergies play a role in the eczema pathogenesis.

In patients with moderate to severe eczema, or where there is a high suspicion of a food allergy, food allergies should be actively excluded. History, SPT and specific IgE are sensitive but not specific in AD sufferers, hence there is a crucial role for food challenges to confirm or refute clinically significant allergies. In cases in whom there are no symptoms of immediate allergies or no evidence of IgE sensitisation, elimination-rechallenge diets should be instituted if there is a high suspicion of food allergies exacerbating the eczema.

Early diagnosis of co-existing food allergies can lead to better management of food allergies and reduce the risk of exposure to food antigens. Blanket elimination diets are ineffective and may be nutritionally harmful, and all too often parents embark on an unsupervised elimination diet because of conflicting advice. Integrated treatment for eczema involves targeted elimination diets eliminating foods which the child is proven to be allergic to, combined with atopic skin care.

Both eczema and food allergies are an early part of the allergic march and have a close association with respiratory allergies. The child with eczema should be managed in an integrated manner taking into account the risks of associated allergies.

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Chapter 4:

Food Allergy Prevalence in South Africa

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

sIgE: Specific IgE

EPAAC: Early Prevention of Asthma and Allergy in Children

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4.1 Introduction

Current data on food allergy prevalence in South Africa is scanty, and studies have relied on patient report and sensitisation profiles, rather than oral food challenges. Indeed, the concept of the food allergy clinic, offering controlled food challenges, is a relatively new one in South Africa which was only introduced in the past 10 years. Anecdotally, and from the few existing previous studies, food allergy rates were thought to be extremely low in South Africa, especially in black South Africans.[1,2] In particular, peanut allergy, which has seen a significant increase in developed countries over the past few decades,[3,4] was virtually undiagnosed in Black South Africans until recently. However, a true estimation of food allergy prevalence in South Africa has not been performed in unselected populations or using food challenges for diagnosis.

This section summarises previous studies in South Africa which have included an investigation into food allergy.

4.2 Previous studies in South Africa of food sensitisation and allergy

In the early 1990's in Bloemfontein, Mercer et al studied 771 patients (aged 3 months-15 years) attending the paediatric allergy clinic.[5] Patients were predominantly Caucasian (personal communication, Dr Mercer). As part of allergic rhinitis evaluation, skin prick test (SPT) and specific IgE (sIgE) to foods were performed. sIgE in the entire group was positive for wheat (24.4%), milk (9.9%) and fish (5.9%). Similarly, SPT in 275 children over the age of 6 years showed sensitisation mainly to wheat (30.6%), milk (30.6%) and fish (26.2%). Some of the positive SPT/sIgE may be attributed to cross-

reactivities, e.g. wheat and grass cross-reacting.[6] None of the patients had symptoms of food allergy, although foods were reported as a possible trigger of allergic rhinitis in 3.5%.

In the mid 1990's in Gauteng, SPT to foods were performed in 58 of 468 Caucasian patients (aged 4-18 years) at a paediatric asthma clinic.[7] SPT positivity was defined as 2mm greater than the negative control. Sensitisation patterns in these selected patients were as follows: wheat 30.4%, peanut 18.2%, fish 15.1%, soy 12.7%, egg 6.9% and milk 5.4%. Only one patient had a history consistent with food allergy, but further details were not provided.

In 2005, 100 children (aged 2 months to 20 years) attending a tertiary asthma clinic in Pretoria underwent SPT to common foods.[8] By ethnicity, 67% were black Africans and the remainder Caucasian. Sporik's 95% positive predictive value cut off points for SPT,[9] which correlate with a high probability of food reactions, were exceeded for peanut (9%), egg white (7%), wheat (4%), fish (4%) and milk (3%). There was no mention of clinical reports of allergy.

In 2008, a study on an unselected group of 212 Xhosa high school patients in Cape Town (aged 15-24 years) showed positive SPT (≥3mm above the negative control) for food allergens in 5.4% of subjects, most commonly to egg white (3.3%), peanuts (1.9%) and milk (1.9%).[10] None of these students reported intolerance to these foods.

These studies are summarised in table 4.1 below. Marked heterogeneity in sensitisation rates reflect different age groups, intercurrent atopic conditions and aeroallergen cross-sensitisation rates. It is difficult to extrapolate actual food allergy rates from these studies since the focus is mainly on sensitisation with some patient reports of allergic symptoms. However, interestingly, of the total 645 patients who underwent SPT from these 4 studies, only 1 (0.2%) had symptoms suggestive of true food allergy.

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Table 4.1 Summary of previous food sensitisation/food allergy prevalence studies in South Africa

Date (place)	Population (age range)	Sensitisation	Confirmed Allergy
Early 1990's (Bloemfontein)[5]	761 children with allergic rhinitis (3 months-15 years)	sIgE positive for wheat 24.4%, milk 9.9% and fish 5.9%. SPT positive for wheat 30.6%, milk 30.6%, fish 26.2%.	No reported food allergy symptoms except possible trigger for allergic rhinitis in 3.5%
Mid 1990's (Gauteng)[7]	58 Caucasian children with asthma (4-18 years)	SPT positive for wheat 30.4%, peanut 18.2%, fish 15.1%, soy 12.7%, egg 6.9% and milk 5.4%.	One patient reported food allergy
2005 (Pretoria)[8]	100 patients (67 Black and 33 Caucasian) with asthma (2 months-20 years)	SPT over 95% Positive Predictive Value for allergy: peanut 9%, egg white 7%, wheat 4%, fish 4%, and milk 3%.	None
2008 (Cape Town)[10]	212 unselected Xhosa high school patients (15-24 years)	SPT positive for egg white 3.3%, peanuts 1.9%, milk 1.9%.	None

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4.3 Previous Studies in South Africa Looking at Food Allergy and Atopic Dermatitis

Atopic dermatitis (AD) is a particular risk factor for food allergy, with a complex (and not necessarily causative) relationship between these two entities, as described in chapter 3. Studies in high-income westernised countries have shown high rates of sensitisation (60-80%) as well as challenge proven food allergy (27-40%) in children with moderate to severe AD.[11-14] Two previous studies in South Africa have examined food allergy in patients with AD (table 4.2).

The first study in 1998 involved parental questionnaire-based perceptions of food reactions in 112 children with atopic dermatitis.[15] By recall, the commonest triggers of cutaneous symptoms were tomatoes, oranges, sweets, pineapple, chocolate, and sulphur dioxide containing soft-drinks. These foods were reported to cause symptoms in up to 49% of the children. The foods that commonly cause IgE mediated reactions, namely egg, fish, milk, and peanut, were reported to cause reactions in up to 25% of the children.

The second study formed part of the EPAAC™ (The Early Prevention of Asthma in Atopic Children) study, in which children aged 12-24 months with eczema underwent sIgE tests to egg, milk and peanut.[16] A heterogenous group of 161 South African infants from various centres was included. Rates of sensitisation were similar to those reported in westernised countries in the study, namely: egg 47.1%; cow's milk 28.4% and peanut 26.8%. Eleven infants (7.2%) in the South African cohort had peanut sIgE levels above 14 kU/L, more than the 95% predictive value for a positive food challenge.[16] However, there was no mention of clinical reports of symptoms of immediate food allergy.

True allergy rates in this population cannot be accurately extrapolated from these studies since the first study is questionnaire based, whilst the other made use of sensitisation patterns.

Table 4.2 Summary of food sensitisation/allergy prevalence studies in children with atopic dermatitis in South Africa

Study population	Food allergy diagnosis	Results
112 children with atopic dermatitis (1994)[15]	Questionnaire	Perceived allergy to tomatoes, oranges, sweets, pineapple, chocolate, soft drinks in up to 49%, and egg, fish, milk, and peanut, in up to 25%
161 children (12-24 months) with AD from various centres and ethnic backgrounds (2005)[16]	Sensitisation (specific IgE> 0.35 U/L)	Sensitisation to egg 47.1%; cow's milk 28.4% and peanut 26.8%. Peanut sIgE greater than 95% positive predictive value for allergy in 7.2%

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4.4 Concluding remarks

To date, very few studies in South Africa have looked into food allergy prevalence. They are based on sensitisation patterns and questionnaires; none to date have used food challenges in equivocal cases. These studies have shown significant sensitisation rates to commonly allergenic foods. Food allergy rates cannot be ascertained from these studies, however there have been extremely few reports of food allergy symptoms. This data, together with anecdotal experience in South African allergy clinics until the year 2010 when our study was commenced, suggested a low allergy rate, especially amongst black South Africans. This led to the hypothesis that black South Africans may have relative protection against food allergy, even in the face of a "food allergy epidemic" in other parts of the world, and prompted initiation of our study.

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Chapter 5:

Study aims, objectives and methodology

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

sIgE: Specific IgE

ISAC: Immuno Solid Phase Allergen Chip

ISU: ISAC Units

PPV: Positive Predictive Value

SCORAD: Scoring Atopic Dermatitis

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5.1 Study Title:

A prospective, descriptive study to determine the prevalence of IgE-mediated food sensitisation and allergy in South African children with atopic dermatitis attending a tertiary medical centre

5.2 Motivation for the study

With the background to the study described in chapters 2-4, it is evident from numerous studies in Europe and the USA that IgE-mediated food allergy has an important association with atopic dermatitis in a subset of patients. However, to date this has been inadequately studied in South Africa. Anecdotally, food allergy, particularly peanut allergy, is rare in South African children, particularly those of non-Caucasian decent. Our hypothesis driving the study was that food allergy is uncommon even in *high risk* South African children, such as those with atopic dermatitis (AD).

The aim of this study was to determine the prevalence of IgE-mediated food allergy in South African children with atopic dermatitis, presenting to a tertiary paediatric unit in an urban region in the Western Cape. We explored possible factors influencing sensitivity and allergy to foods, including ethnicity, dietary consumption and component allergen sensitisation in the case of peanut.

5.3 Objectives of the study

5.3.1 Primary objective

The primary objective was to determine the prevalence of IgE-mediated food allergy to any one or more of 7 common allergenic foods (hen's egg, cow's milk, peanut, tree nuts, soy, wheat and fish) in a South African urban population of children with atopic dermatitis.

5.3.2 Secondary objectives

1. To determine the rate of sensitisation to common food allergens in South African children with atopic dermatitis.
2. To explore the association between atopic dermatitis severity and the prevalence of food sensitisation and allergy.
3. To explore the association between age at the time of study entry, as well as age of onset of eczema, and the prevalence of food sensitisation and allergy.
4. To explore the association between ethnic origin and the prevalence of food sensitisation and allergy.
5. To explore patterns of peanut consumption in the study population.
6. To measure sensitisation to peanut component antigens Ara h1, 2, 3, 8 and 9, and to explore whether patterns of component antigen sensitisation are associated with tolerance versus clinical peanut allergy.
7. To explore whether the internationally derived 95% positive predictive values (PPV) levels for positive food challenge using skin prick tests (SPT) and specific IgE to cow's milk, egg and peanut are relevant to the study population.
8. To compare skin prick tests, specific IgE by ISAC test, and specific IgE by ImmunoCAP test as measures of detecting IgE-mediated food sensitivity and allergy in the study population.

5.4 Sample size

Sample size was calculated based on the estimate that in the South African population of children with AD the prevalence of food allergy would be approximately half of that demonstrated in westernised countries, i.e. 15% in comparison to 30% from previous studies in westernised countries. At 80% power and 5% significance level, this gave a sample size of 53. In order to include meaningful numbers from both Xhosa and mixed race groups, we aimed to include a total of 100 children in the study.

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5.5 Inclusion and Exclusion Criteria

5.5.1 Inclusion criteria

i. Children presenting to the paediatric dermatology clinic at Red Cross Hospital with atopic dermatitis

ii. Children of South African descent.

iii. Children in the age range 6 months to 10 years.

iv. Children for whom informed consent could be obtained from the parent or legal guardian.

5.5.2 Exclusion criteria

i. Children for whom no informed consent could be obtained from the parent or legal guardian.

ii. Children with significant chronic illness (apart from that related to their atopic predisposition, such as asthma).

iii. Unwillingness or inability to comply with study requirements or procedures including food challenge.

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5.6 Study definitions

5.6.1 Definition of atopic dermatitis (Williams 2005[1])

The diagnosis requires evidence of itchy skin (or parental report of scratching or rubbing) plus *three or more* of the following:

- History of involvement of the skin creases (e.g. fronts of elbows, backs of knees, fronts of ankles, and areas around the neck or eyes)
- History of asthma or hay fever (or history of atopic disease in a first-degree relative if the child is under four years of age)
- History of generally dry skin in the past year
- Onset in a child under two years of age (criterion not used if the child is under four years of age)
- Visible flexural dermatitis (including dermatitis affecting the cheeks or forehead and outer aspects of limbs in children under four years)

The diagnosis of atopic dermatitis was made by a paediatric dermatologist in all cases of study participants.

5.6.2 Positive Skin Prick Test

A SPT was considered positive if it resulted in a weal diameter of 3mm or greater than the negative control read at 15 minutes and ensuring adequate antihistamine washout.

5.6.3 Positive Specific IgE

A specific IgE ≥0.3 ISAC Units (ISU) for the ISAC 103 test, or ≥ 0.35 kU/L by standard ImmunoCAP test was considered positive.

5.6.4 IgE-mediated food sensitisation

Sensitisation was defined as a positive skin prick test and/or positive food specific IgE.

5.6.5 IgE-mediated food allergy

For the purposes of this study, food allergy was be defined as either:

- A convincing clinical history of significant type I allergic reactions after isolated ingestion of a food in the preceding 6 months, with significantly positive SPT/sIgE*; or
- Positive food challenge

*Significantly positive SPT/sIgE was at or above the internationally derived 95% positive predictive value for peanut, hen's egg and cow's milk allergy, either by SPT[2] (8mm for peanut, 7 mm for hen's egg, 8 mm for cow's milk) or specific IgE[3] (14 kU/L for peanut, 7 kU/L for hen's egg, 15 kU/L for cow's milk). Table 5.1 depicts 95% and 100% decision points that were being used in our allergy clinic at the time of the study.

Table 5.1 Summary of 95% and 100% Positive Predictive Values [2,3]

Allergen	**>95% PPV decision point for specific IgE (in kU/L-using Phadia ImmunoCAP method)**	**100% PPV for skin prick test diameter (mm)**
Egg White	≥ 2 (<2 yr) ≥7 (>2 yr)	≥5mm (<2 yr) ≥7mm (> 2 yr)
Cow's Milk	≥5 (<1 yr) ≥15 (>1 yr)	≥6mm (< 2 yr) ≥8mm (> 2 yr)
Peanut	14	≥4 mm (< 2 yr) ≥8 mm (> 2 yr)
Fish	20	-

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5.6.6. Outcome of food challenge

The outcome of food challenges was determined by evaluating the participant using the criteria in table 5.2 below.[4,5]

A **positive** food challenge was defined by the presence of either:

- One or more major criteria.
- Two or more minor criteria.

An **indeterminate** food challenge was defined by the presence of one minor criterion.

A **negative** food challenge was defined by the absence of major or minor criteria.

Table 5.2 Criteria for determining the outcome of food challenge

Major Criteria
1. Confluent erythematous pruritic rash 2. Respiratory signs (at least one of the following): • Wheezing • Inability to speak • Stridor • Dysphonia • Aphonia 3. ≥ 3 Non-confluent urticarial lesions 4. ≥ 1 Site of angioedema 5. Hypotension for age not associated with vasovagal episode 6. Evidence of severe abdominal pain (such as abnormal stillness or doubling over) that persists for ≥ 3 minutes
Minor Criteria
1. Vomiting 2. Diarrhoea 3. Persistent rubbing of nose or eyes that lasts for ≥ 3 minutes 4. Persistent rhinorrhea that lasts for ≥ 3 minutes 5. Persistent scratching that lasts for ≥ 3 minutes

All symptoms should be of new onset and not due to ongoing disease. Reactions during food challenge were defined as **immediate** if they occurred within 2 hours of consumption of the last administered dose of food, and as **delayed** if they occurred more than 2 hours after the last administered dose of the food.

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5.7 Design and setting

This prospective, observational study was conducted over 3 years, March 2010-March 2013 at Red Cross Children's Hospital, a paediatric university hospital in Cape Town. Patients were randomly recruited from the waiting area in the dermatology clinic, but not the allergy clinic, to minimise referral bias for food allergic patients.

5.8 Recruitment and assessment

5.8.1 Recruitment and enrolment

Recruitment took place at the paediatric dermatology clinic at Red Cross Children's Hospital. The study nurse had access to the pile of hospital folders of patients attending the clinic. By sequentially inspecting the folders, which were in random order, she identified the first 4 folders of patients with a diagnosis of AD, and invited those children to participate in the study. This continued on a weekly basis until 100 patients had been recruited. Parents were given written information on a sheet in the language of their choice: English, Xhosa or Afrikaans. Details of the study were discussed with the family, using an interpreter if necessary. If they agreed to participate, written informed consent was obtained from the parent/legal guardian for study participation in their language of choice. As far as was practically possible, the assessments were performed during the original hospital visit to avoid unnecessary travel and inconvenience to patients. An exception was the food challenges, which required patient selection after the allergy test results had been processed, and were planned for another day. In some cases skin tests had to be deferred to another day if the patient had recently taken antihistamines.

5.8.2 General and allergy assessment

An allergy questionnaire was completed, with a member of the study team going through each question with the parent/caregiver in their preferred language. This included the following details:

- age and date of birth
- ethnic origin and socio-economic status
- history of timing of introduction of cow's milk, hen's egg, wheat, soy, fish, peanut and tree nuts.
- detailed history of allergic reactions to the above foods or any other foods, including symptoms, severity and timing of reaction following consumption. Parents were specifically asked about the presence of rashes, flushing, itchy throat/clearing of throat, swelling, vomiting and diarrhoea, cough, wheeze, stridor and signs of circulatory compromise.
- history in the child of asthma or allergic rhinitis.
- other medical problems and current medication

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- detailed consumption history of peanut products
- weight and relevant general examination findings were also recorded

5.8.3 Atopic dermatitis severity assessment

The severity of atopic dermatitis was assessed at the initial visit using the SCORAD Index (SCORing Atopic Dermatitis),[6] which is a standardised clinical evaluation of atopic dermatitis, scored from 0 to a maximum total of 103 points. It consists of evaluating the extent of atopic dermatitis (body surface area of involved skin), and the intensity of atopic dermatitis based on scores for erythema, papulation, excoriation, oozing, lichenification and skin dryness. Subjective symptoms of pruritis and insomnia are assessed by visual analogue scale. Scoring was performed by a trained dermatology nurse and was recorded on the dedicated forms.

Severity of atopic dermatitis was defined as follows:

- SCORAD < 15: mild
- SCORAD 15-40: moderate
- SCORAD > 40: severe

5.8.4 Skin Prick Testing (SPT)

All patients underwent skin prick testing. SPT were performed using solutions from ALK Abello (ALK, Madrid, Spain) and ALK lancets, to egg white extract, cow's milk, soy, wheat (flour), fish (cod), peanut, positive (10mg/mL histamine) and negative (saline) controls. In addition, modified skin prick tests for egg and milk were performed using a drop of raw egg white and fresh 2% cow's milk.

The skin on the forearm was pricked through a drop of the extract. Results of the SPTs were read at 15 minutes, recorded as average weal diameter size in millimetres (mm) and deemed positive for sensitisation if the weal size was 3 mm or more above the negative control at 15 minutes.

5.8.5 Blood testing for food specific IgE

A blood sample was taken from all participating patients for IgE testing by the ImmunoCAP® ISAC test (Immuno Solid Phase Allergen Chip Test, Phadia™), using the103 allergen microarray chip. In patients with a positive skin prick test or ISAC test, ImmunoCAP® tests were also performed to those particular foods. An ISAC value at or above 0.3 ISAC Units or an ImmunoCAP test value at or above 0.35 kU/L was considered positive for sensitisation.

5.8.6 Food Challenges

All patients with evidence of sensitisation to the foods being tested underwent a food challenge unless the clinical history reported recent, unequivocal tolerance or allergy. If children were sensitised to

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more than one allergen, they underwent multiple food challenges on different days at least one week apart. All challenges were performed within 4 weeks of the original screening tests.

Food challenges were performed as open incremental supervised oral challenges at the Red Cross Hospital pediatric allergy department.[7] On the day of the food challenge, children were examined to ensure they were fit for the challenge, and the investigator ensured that they had been compliant with a histamine washout period. Before the start of the challenge, and before every dose escalation, the patient underwent a full set of observations including temperature, oxygen saturations, blood pressure, pulse rate and peak expiratory flow rate in the case of asthmatic children.

Foods were given in their native form, starting with a lip dose, increasing every 15 minutes over a further 6 doses, and ending with a full portion of the food (17 g peanut butter, 100 mL cow's milk or soya milk, 30 g scrambled egg, 20g wheat, 40g fish). A dosing schedule for food challenges is depicted in table 5.3.

The criteria for determining the outcome of a food challenge included only objective signs of new onset (table 5.2).

Food challenges were stopped if they met the positive criteria. Patients were treated according to the severity of their reaction with antihistamine syrup (for mild to moderate reactions) or intramuscular adrenaline (for severe reactions). Patients were observed for a minimum of 2 hours after a negative challenge and 2-4 hours after a positive challenge. If a reaction was equivocal, the challenge was continued until the top dose was reached or until symptoms satisfied criteria for a positive food challenge.

After a negative challenge, parents were encouraged to include the food regularly in the diet and to report any unexpected reactions on subsequent consumption. The investigator contacted the patient's family per telephone 48-72 hours after the food challenge to enquire about the patient's wellbeing and delayed symptoms.

5.8.7 Follow up of children with results suggestive of food allergy

If children had clinical history/food challenge suggestive of clinically significant food allergies, then appropriate food avoidance advice was given by a trained dietician. A written treatment plan was given to the family and relevant professionals. Further follow up was organised in the paediatric allergy clinic to monitor the allergy and the progress of the atopic dermatitis after dietary exclusion.

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Table 5.3: Doses of foods used for food challenges

FOOD	LIP CHALLENGE	DOSE 1 (20 MINUTES)	DOSE 2 (40 MINUTES)	DOSE 3 (60 MINUTES)	DOSE 4 (80 MINUTES)	DOSE 5 (100 MINUTES)	DOSE 6 (120 MINUTES)
Cow's milk	Rub drop of milk on lower lip	5 mL	10 mL	20 mL	40 mL	100 mL	200 mL
Egg (scrambled egg/omlette)	Rub small amount egg on lower lip	0.5g	2g	5g	10g	30g	
Soya Milk	Rub drop of milk on lower lip	5 mL	10 mL	30 mL	200 mL (or 160 g soya yoghurt)		
Cod (plain)	Rub small amount fish on lower lip	0.5g	2g	10g	40g		
Wheat (use weetabix) or maize	Rub small amount wheat/maize on lower lip	1g	3g	6g	20g		
Peanut Butter	Rub small amount on lower lip	0.3g	0.6g	2.3g	4.5g	11g	17g
Roasted Peanut	Rub small amount on lower lip	0.25g	0.5g	2g	4g	10g	15g
Tree Nuts	Rub small amount on lower lip	0.5g	2g	4g	15g		

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5.9 Ethics

The study was approved by the University of Cape Town's Faculty of Science Human Ethics committee (reference 426/2009). Informed consent was obtained from a parent/legal guardian for study participation, food challenges and blood testing.

5.10 Data Entry and Statistics

Data was entered into a clinical records form by the principal investigator, and subsequently checked and entered on to a computerised database using STATA version 11.1 (Stata Corp, College Station, Texas). Continuous variables were analysed using the Mann-Whitney test, and categorical variables were analysed using the chi-squared or Fisher's exact tests. In addition, the two sample test of proportion was used to test for statistical difference between proportions. A p- value of <0.05 was considered statistically significant.

5.11 Comparison between Ethnic Groups

The two main ethnic groups attending the dermatology and allergy clinics at the Red Cross Children's Hospital are Xhosa patients (the indigenous black Africans in the Western Cape) and mixed race patients (mixed black African and Caucasian). Food allergy patterns were thus compared between Xhosa and mixed race patients. Such an inter-ethnic comparison was aimed at determining:

- Whether there were inter-ethnic differences in the prevalence of food sensitisation and allergy
- whether any potential confounding factors might be responsible for inter-ethnic differences
- whether there were inter-ethic differences in the performance characteristics of diagnostic tests
- whether there may be any practical implications of differing food allergy patterns

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Chapter 6:

Overall results for food sensitisation and allergy, and risk factors for food allergy

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SPT: Skin Prick Test

sIgE: Specific IgE

ISAC: Immuno Solid Phase Allergen Chip

ISU: ISAC Units

PPV: Positive Predictive Value

SCORAD: Scoring Atopic Dermatitis

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6.1 Introduction

The complex relationship between atopic dermatitis (AD) and food allergy has been described in Chapters 2 and 3.[1,2] Eczema, particularly of early onset, is a known risk factor for food allergy.[3] The majority (around 90%) of food reactions in patients with AD are, at least in part, IgE-mediated. [4-7] Studies from high income countries have demonstrated a high degree of sensitisation (around 60%) as well as proven allergies (around 30-40%) to common foods in children with moderate to severe AD.[8-12] Geographical area and ethnicity may influence sensitisation and/or allergy rates, as discussed in chapter 2.[13-16]

The aim of this study was to determine the prevalence of IgE-mediated food allergy and sensitisation to common food allergens in children, with AD, presenting to a specialised paediatric unit in an urban region in the Western Cape, South Africa. Objectives and methodology have been described in the previous chapter, chapter 5. This chapter describes the overall results in terms of food sensitisation and allergy prevalence, as well as risk factors for food allergy. It also explores ethnic differences in food allergy patterns.

6.2 Background characteristics and demographics

100 patients participated in the study, 59 Xhosas and 41 of mixed race. Although eligible, there were no Caucasians or Asians amongst the study population, reflecting the demographics of the population attending this dermatology clinic. One patient was excluded from analyses pertaining to peanut and fish, as she defaulted her peanut and fish challenges. Thirteen potentially eligible patients refused study entry, mainly due to time constraints, hence 88% of patients approached participated in the study.

There were 54 males and 46 females. The age of participants ranged from 7-118 months, with a median age of 42 months, interquartile range 19-68 months. The median age of onset of eczema was 6 months, interquartile range 3-12 months. Fifty patients had moderate and 50 had severe eczema.

Total serum IgE ranged from 5 to over 5000 kiU/L (no further dilutions were performed), with a median of 1015 kU/L, interquartile range 220-2754 kU/L.

Gender distribution, age, age of onset of eczema, eczema severity and total IgE were comparable between the two ethnic groups with no statistically significant differences (Table 6.1). Monthly income was significantly lower in the Xhosa group, at a median of R2000 per month, compared with R3500 for the mixed race group (p=0.01).

6.3 Tests performed

All patients underwent skin prick tests (SPTs) to hen's egg, cow's milk, wheat (flour), fish (cod), soya and peanut. Cashew nut was unavailable as a SPT in South Africa at the time of the study. All 100 patients underwent Immuno-Solid Phase Allergen Chip (ISAC 103) tests. In those who were sensitised, or if there was a discrepancy between history and sensitisation, specific IgEs by ImmunoCAP® test were also performed (n= 69).

Seventy-one food challenges were performed in 47 patients (31 to egg, 25 to peanut, 5 each to cow's milk and fish, 3 to soya, and 2 to wheat). Thirty-two of the food challenges were positive (17 to peanut, 14 to egg, 1 to milk). There was one case of anaphylaxis during a peanut challenge, for which the patient required intramuscular adrenaline for stridor. The rest of the reactions were mild, mostly cutaneous, and treated with oral antihistamines.

All patients who reported immediate-type allergic symptoms but had a negative SPT were non-allergic based on food challenge.

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Table 6.1: Baseline characteristics of the study population

	Overall	**Xhosa**	**Mixed Race**	**Difference between ethnic groups**
Number of participants	100	59	41	
Gender distribution	54% males, 46% females	54% males, 46% females	54% males, 46% females	P=0.95*
Age (median in months)	42	40	48	P=0.09 #
SCORAD category	50% severe, 50% moderate	54% moderate, 46% severe	44% moderate, 56% severe	P=0.31*
Age of eczema onset (median in months)	6	6	6	Equal
Serum median total IgE (kU/L)	1015	656	1562	P=0.31 #
Symptoms of asthma	39%	36%	44%	P=0.40*
Median monthly household income (South African Rands)	R2500	R2000	R3500	P=0.01++

*= difference non-significant by chi-squared test
#= difference non-significant by Mann Whitney test
++ = difference significant by Mann Whitney test

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6.4 Food Sensitisation Patterns

Overall 66 (66%) patients were sensitised to at least one food. Sensitisation was detected in 61 cases by SPT (of which 45 were also ISAC positive), and in an additional 5 cases by ISAC. In the latter 5 cases which were missed by SPT, the patients were confirmed to be not allergic.

By ethnicity, 41/59 Xhosas (69%) and 25/41 of mixed race (61%) were sensitised to at least one of the foods tested; there was no significant difference between ethnic groups for overall sensitisation (p=0.37). The most common food to which patients were sensitised was egg (54%), followed by peanut (44%), milk (27%), fish (13%), soya (9%) and wheat (6%). No patients were sensitised to cashew nut by the screening ISAC test.

There were no significant ethnic differences for sensitisation rates to individual foods, apart from fish, which was significantly more positive in the mixed race group at 22% versus 7% in Xhosa patients, p=0.03 (Table 6.2).

Of those with a positive SPT, the median number of positive SPT overall was 2 (2 for Xhosas, 3 for Mixed Race patients, p=0.57 by Mann-Whitney test).

6.5 Food Allergy Patterns

Overall, 40% of patients in the cohort (34% of Xhosas and 49% of mixed race, p=0.1) had at least one IgE-mediated food allergy. There were no gender differences in food allergy rates, with 37% of male and 43% of female participants having food allergy (p=0.51).

Of the 55 cases of food allergy in 40 children, 32 were diagnosed by food challenge, and 23 by significant sensitisation (above the internationally derived 95% positive predictive values for food allergy) and recent past history of a reaction to the food. Table 6.4 at the end of the chapter describes all the cases of food allergy, symptoms and how the diagnosis was made.

All allergic patients were SPT positive to the food, 34/40 (85%) were ISAC positive. All cases of peanut allergy in Xhosa patients were challenge- proven. Of the 40 patients with allergy, 28 (70%) of patients had one allergy, 9 (22.5%) had 2 allergies and 3 patients (7.5%) had 3 different food allergies.

Overall, 14 patients reported a severe reaction with respiratory symptoms after previous exposure to peanut (7), tree nut (2) or egg (5). Two of these egg allergic patients were subsequently shown in the study to have outgrown their allergies. In addition, one severe reaction was elicited on food challenge (peanut). There were no parental reports of food allergy manifestations after a negative food challenge.

Foods most commonly implicated in food allergy were egg (25%) and peanut (24%) followed by tree nut (3%), cow's milk (2%) and fish (1%). Cow's milk allergy was low (2%) but 8 further patients reported that they had signs of allergy to dairy products in the past but now tolerated it. There were no confirmed allergies to wheat or soya.

The prevalence ratio for food allergy for mixed race relative to Xhosa patients was 1.44 (p=0.13). Allergy rates were not significantly different between ethnic groups for egg, cow's milk and fish allergies. However, the peanut allergy rate was significantly higher in the mixed race group at 38% versus 15% in the Xhosa group, p=0.01. Tree nut allergy was also significantly higher in the mixed race group (7% versus 0%), p=0.04.

Food sensitisation and allergy prevalence are summarised in Table 6.2 and figure 6.1. Ethnic differences in allergy sensitisation and allergy patterns are shown in figure 6.2.

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Table 6.2: Food Sensitisation and Allergy Patterns

	Overall sensitization and allergy to at least one food*		**Egg**		**Peanut**		**Cow's Milk**		**Soya**		**Wheat**		**Fish**		**Cashew Nut**	
	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy
Overall (n=100)	66%	40%	54%	25%	44%	24%	27%	2%	9%	0%	6%	0%	13%	1%	0	3%
Xhosa (n=59)	69%	34%	59%	24%	41%	15%	22%	0%	8.5%	0%	7%	0%	7%	1.6%	0	0%
Mixed race (n=41)	61%	49%	46%	27%	50%	38%	34%	5%	9.7%	0%	5%	0%	22%	0%	0	7%
Difference between ethnic groups #	Pr= 0.4	0.1	0.2	0.7	0.1	0.01$	0.2	0.1	0.8	1	0.7	1	0.03 $	0.7	1	0.04$

*By screening tests- Skin prick test (except cashew nut) or ISAC
# By chi-squared test
Sens= sensitised
$ = statistically significant

Figure 6.1 - Overall prevalence of sensitisation and allergy to egg, peanut, cow's milk and fish

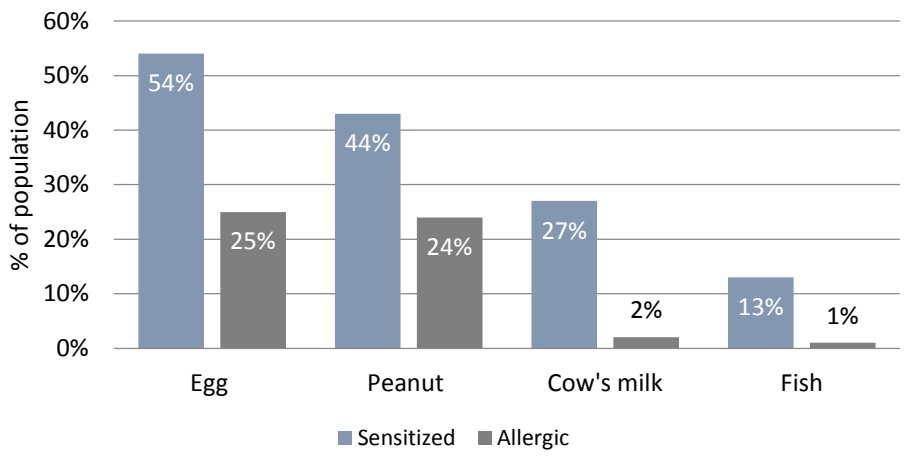
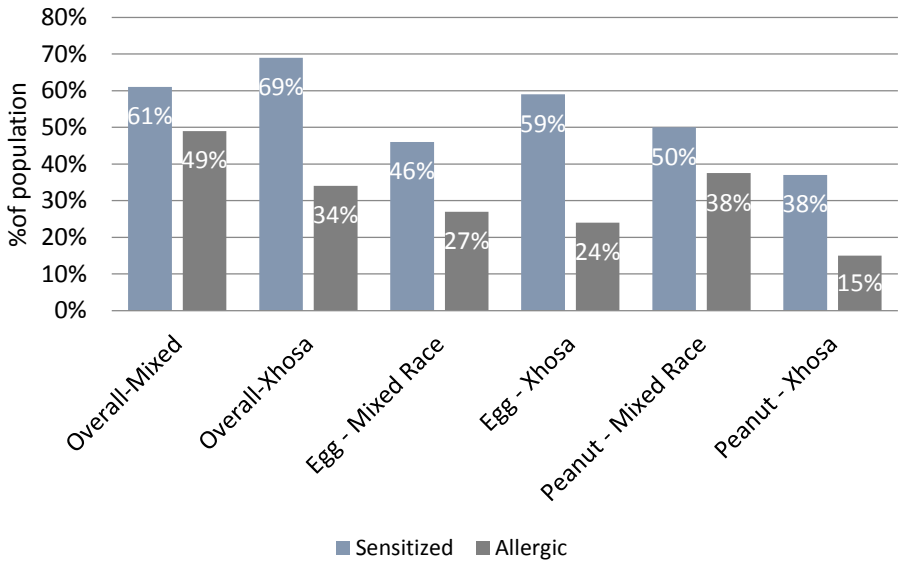


Figure 6.2 Food Sensitisation and allergy by ethnicity



6.6 Proportion of sensitised patients who were allergic

Overall, 66% of patients were sensitised to at least one food, and 40% of patients were allergic to at least one food. Therefore, 40/66 (60 %) of sensitised patients were confirmed as allergic. Out of 152 cases of sensitisation, 55 (36%) translated into allergies.

There was a significant ethnic difference in fall-off between sensitisation and allergy: in the mixed race patients, 25 were sensitised and 20 allergic, hence 80% of sensitised patients were allergic; in the Xhosa patients, 41 were sensitised and 20 allergic, hence 49% of sensitised patients were allergic, $p = 0.002$.

Overall 46% of egg-sensitised patients were egg-allergic (58% in mixed race and 40% in Xhosas, $p= 0.08$). Overall, 57% of peanut-sensitised patients were peanut-allergic (75% in mixed race and 39% in Xhosas, $p < 0.001$). 7% of milk sensitised patients were allergic (14% in mixed race and 0% in Xhosas, $p=0.003$).

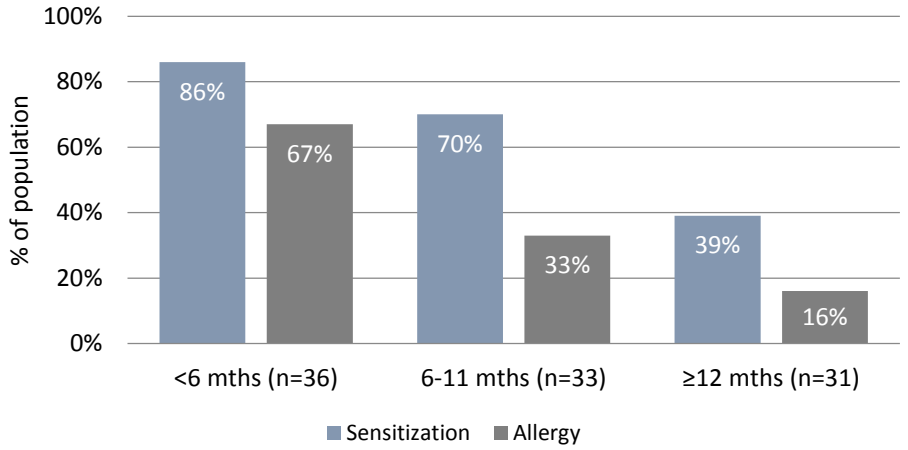
6.7 Risk factors for food allergy

6.7.1 Age of onset of eczema and food allergy rates

The median age of onset of eczema was 6 months in both ethnic groups (range 1-96 months). Fifty-six children had onset of eczema at or less than 6 months of age, of those 31 were allergic (55%); 44 children had onset of eczema after 6 months of age; of those 9 (20%) were allergic ($p< 0.001$).

This trend was similar in both ethnic groups: in Xhosa patients, 16/33 (48%) with early onset eczema (≤6 months) had allergies, compared with 4/26 (15%) with later onset (p=0.008). In mixed race patients, 15/23 (65%) with early onset eczema (≤ 6 months) were food-allergic, compared with 5/18 (28%) with later-onset eczema (p=0.017). The influence of age of onset of eczema (divided into tertiles) on food sensitisation and allergy rate is depicted in figure 6.3.

Figure 6.3 - Influence of Age of Onset of Eczema on Sensitisation and Allergy Rates



6.7.2 Eczema severity and food allergy rates

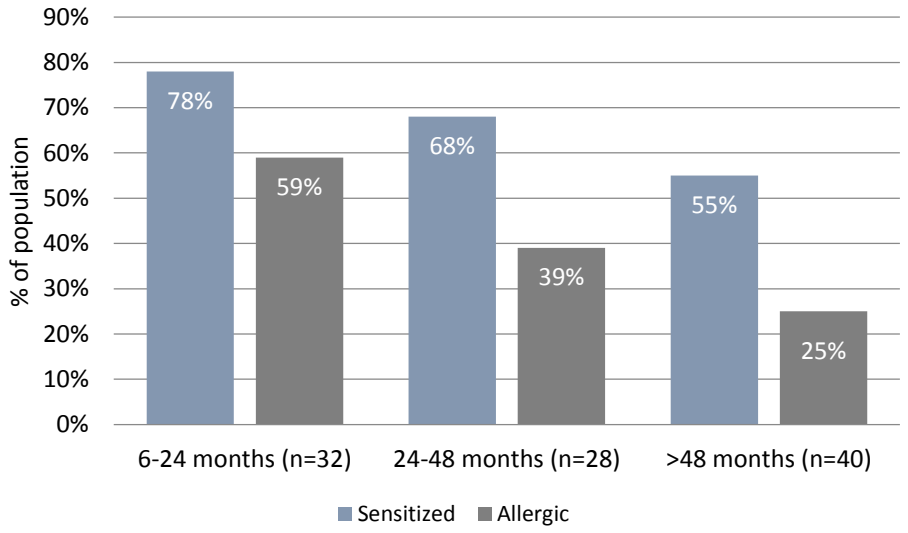
The SCORAD score was normally distributed with mean score 41 (range 16-71). In those with moderate eczema (SCORAD 15-40, n=50), 15 were allergic (30%). In those with severe eczema (SCORAD>40, n=50), 25 were allergic (50%) (p=0.04).

6.7.3 Age at time of assessment and food sensitisation and allergy rates

Sensitisation rates were higher in the younger age groups, implying that food sensitisation occurs early on. Similarly, allergy rates peaked in the 0-2 year old participants (59%) in comparison to the older age groups (25% in over 4 year olds), p=0.003. The proportion of allergic to sensitised patients was higher in the younger age groups, implying that a positive SPT is more likely to reflect a true allergy in the 0-2 year age group. Sensitisation and allergy rates by age are depicted in figure 6.4.

Egg allergy in particular was higher in the younger age groups (50% in under 2 year olds, 13% in over 2 year olds, p< 0.001). This is likely a reflection of the natural acquisition of tolerance to egg over time. Cow's milk allergy was low in both under 2 year olds (3.1%) and over 2 year olds (1.5%), p=0.59.

Figure 6.4 Influence of Age at Study Entry on Sensitisation and Allergy Rates



The trends in ISAC-determined sensitisation patterns at 1 year, 2 years, 4 years and 10 years for individual food allergens are depicted in table 6.3. This shows that sensitisation peaks by one year of age for cow's milk, hen's egg, soya and peanut. Fish sensitisation remained stable from 1 to 10 years, and wheat sensitisation increased over time in this population, likely reflecting cross-reactivity with grass pollens developing in the older children.

Table 6.3 ISAC-determined sensitisation patterns to individual foods over time

	Age in Years			
Food allergen	**1 year**	**2 years**	**4 years**	**10 years**
Egg	63% (5/8)	38% (12/32)	35% (21/60)	32% (32/100)
Cow's Milk	12.5% (1/8)	9% (3/32)	7% (4/60)	6% (6/100)
Peanut	63% (5/8)	34% (11/32)	33% (20/60)	30% (30/100)
Wheat	0%	0%	2% (1/60)	3% (3/100)
Fish	12.5% (1/8)	16% (5/32)	12% (7/60)	15% (15/100)
Soya	25% (2/8)	12.5% (4/32)	10% (6/60)	11% (11/100)

6.7.4 Ethnicity as a risk factor for food allergy

As discussed in section 6.5, mixed race children had a higher prevalence of peanut and tree nut allergy than Xhosa patients, and a greater chance of being food-allergic if sensitised. This was despite equivalent median age of onset of eczema, eczema severity and median age of the study population in mixed race and Xhosa groups (table 6.1), which therefore cannot be seen as confounding variables.

6.8 Food-induced flares in AD

Fifteen of the 40 patients (38%) with IgE mediated food allergy reported an eczema flare-up as part of their late symptomatology.

In addition, another 18 (18%) patients who were not sensitised to the food in question reported a late eczema flare (> 2 hours after ingestion) after eating the food. Whilst this may represent a non-IgE mediated allergy, these patients were not investigated any further as the focus of this study was on IgE mediated food allergy.

6.9 Previous diagnosis of food allergy

Only 7 of the 40 allergic children (18%) had previously been diagnosed with food allergy. Of the 18 food allergic children with intercurrent asthma symptoms, which puts them at greater risk of severe reactions, only 4 (22%) had doctor-diagnosed food allergy.

6.10 Peanut consumption patterns

Despite a difference in monthly household income (table 6.1), there were no significant differences in peanut consumption patterns between Xhosa and mixed race groups. Amongst Xhosa children, 78% had consumed peanut before, compared to 85% of mixed race children (p=0.4). In those who had introduced peanut into their diets, the median age of introduction was 19 months in the Xhosa group, and 12 months in the mixed race group (p=0.08). The median number of peanut servings per week in those consuming peanut was 3 for both ethnic groups. This will be discussed in more detail in chapter 12.

6.11 Discussion

This is the first study to investigate the rate of challenge-proven food allergy in children in Southern Africa. We targeted children with moderate-severe AD at high risk for food allergy, under the hypothesis that even in this high risk group, we would observe a low prevalence of food allergy, especially in the Xhosa group. However, we observed a high proportion of children sensitised and allergic to common food allergens, with equivalent rates to those reported in similar selected cohorts in westernised countries. This finding is significant, as food allergy prevalence in South Africa has previously been thought to be low, especially in Black Africans.

The overall sensitisation rate to common allergenic foods was 66%; highest for egg (54%), peanut (44%) and cow's milk (27%). The allergy rate was 40%; highest to egg (25%), peanut (24%) and tree nut (3%). Risk factors for food allergy included onset of eczema at an age ≤ 6 months, SCORAD score

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of > 40 ("severe eczema") as well as age below 2 years. These risk factors have similarly been described in previous AD/food allergy studies. [3,17-18]

Ethnic differences in sensitisation and allergy rates were evident between the indigenous black African Xhosas and the mixed race group. Food sensitisation was higher in the Xhosa population (69%) than in the mixed race cohort (61%). The allergy rate was surprisingly high in the Xhosas at 34%, yet lower than that in the mixed race cohort (49%). There were a significant number of cases with challenge proven peanut allergy in the Xhosa population (15% of Xhosa participants), in whom peanut allergy has been seldom reported previously.

Of particular interest was the proportion of truly allergic versus sensitised patients. This proportion was significantly lower in the Xhosa population compared with the Mixed race population (49% of sensitised Xhosas were allergic; versus 80% of mixed race patients). This was especially pronounced for peanut allergy, with only 39% of peanut-sensitised Xhosas having a proven allergy; versus 75% in the mixed race group (p=<0.001).

The common assumption that the high "false" sensitisation rates in black Africans may be caused by large levels of non-specific IgE as a result of helminthic or parasitic infection, [19-22] may not hold true in this study population. Although we did not assess for parasite infestation or sensitisation, the total IgE was found to be higher in the mixed race group (median 1562 kiU/L) than the Xhosa group (median 656 kiU/L). In peanut sensitised patients, the mixed race group had a higher total IgE (median 1703) than their Xhosa counterparts (median 1011). Moreover, a higher cross reactivity between peanut and Timothy grass [23] cannot explain the "falsely high" peanut sensitisation rate in Xhosa patients, nor can differences in peanut consumption patterns. This will be explored further in chapter 7, which focuses on peanut allergy patterns.

Therefore, the Xhosas seem genuinely highly sensitised to certain foods, but less allergic. There is a suggestion that, although allergies are now emerging strongly amongst Black South Africans, there may still be factor(s) providing relative "protection" in the Xhosa patients against an allergic manifestation. Such factors may include dietary patterns and microbial exposure, but require further exploration. Our concern is that, as the traditional lifestyle is replaced by a westernised lifestyle, the strong genetic propensity to food allergy may become unmasked via epigenetic changes, leading to further increases in allergy rates.[24]

In this study, the skin prick test was 100% sensitive in detecting food allergies, in both Xhosa and mixed race participants. Therefore, the lower skin prick test reactivity recently described in Black cohorts[15] has not been replicated in our study. However, only 60% of sensitised patients were allergic, which

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emphasises the high sensitivity but low specificity of allergy screening tests, and the vital role of food challenges in equivocal cases. Seventy-one food challenges were performed in 47 patients, with 32 positive challenges. This means that in almost half of the study population, history and screening investigations were not deemed adequate proof of allergy versus tolerance.

The ISAC test was used for plasma food-specific IgE in view of its high specificity, multiple allergen component testing on a small blood volume, and inclusion of aeroallergen sensitisation.[25] However, in this study the sensitivity of ISAC for detecting food allergy was 85% compared with 100% for SPT.

In this population, only a low percentage (18%) of food-allergic patients had previously had their allergies formally diagnosed. This means that a significant proportion of children with moderate to severe atopic dermatitis may have unrecognised food allergies which could cause immediate reactions or eczema flares. Moreover, those patients who had not been previously diagnosed would not have had access to emergency treatment plans and dietetic input.

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6.12 Conclusion

Food allergy rates in this cohort of South African children with moderate to severe AD are high and equivalent to westernised counterparts. There are ethnic differences, with Xhosas having equivalent sensitisation rates but lower allergy rates, than children of mixed race. In the case of peanut, egg and cow's milk, Xhosa children are less likely than children of mixed race to be truly allergic if they are sensitised. Further research should explore possible "protective" factors for food allergy in the Xhosa population, whilst a window of opportunity for possible intervention still exists.

Young age, early onset eczema (< 6 months) and severe eczema AD are significant risk factors for food allergies. In keeping with recent guidelines,[26-27] our study confirms the importance of early referral of young children with moderate to severe AD for allergy assessment.

Table 6.4 Summary of test results and clinical findings in patients with IgE mediated food allergy

Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
001	Mixed	46	Egg	Food challenge positive (diffuse urticarial, generalised pruritis)	Egg white extract (EWE) 9 mm Raw egg white (REW) 20 mm	n*Gal d 1*(Ovo-mucoid) 0.5	Egg white 0.21 Ovomucoid 1.33
004	Mixed	32	Egg	Food challenge positive (generalised pruritis and macular rash. Late exacerbation of eczema)	EWE 1 mm REW 20 mm	n*Gal d 1* 1.3	Egg white 0.56 Ovomucoid 11.7
			Peanut	Food challenge positive (> 5 wheals. Generalised pruritis, vomiting)	Peanut 20 mm	n*Ara h 1* 1.7 n*Ara h 2* 12 n*Ara h 3* 6	Peanut 15.2 r*Ara h 1* 0.72 r*Ara h 2* 16.9 r*Ara h 3* 3.54 r*Ara h 8* 0.08 r*Ara h 9* 0.09
005	Xhosa	44	Peanut	Food challenge positive (Wheals, facial flushing, generalised pruritis, irritability. Late worsening of eczema)	Peanut 13mm	n*Ara h 1* 0 n*Ara h 2* 9.4 n*Ara h 3* 0	Peanut 87.6 r*Ara h 1* 3.81 r*Ara h 2* 58.8 r*Ara h 3* 4.98 r*Ara h 8* 3.06 r*Ara h 9* 1.23
007	Mixed	90	Peanut	History: recent symptoms of itchy rash, tight throat and wheeze after peanut butter ingestion	Peanut 15mm	n*Ara h 1* 0.8 n*Ara h 2* 15 n*Ara h 3* 0	Peanut 19.3 r*Ara h 1* 0.1 r*Ara h 2* 41.7 r*Ara h 3* 0.27 r*Ara h 8* 0.26 r*Ara h 9* 0.17
			Treenut - cashew	History: recent symptoms of itchy rash, tight throat and wheeze after cashew nut ingestion	Not done	Negative	Cashew 30.2
010	Mixed	7	Egg	History: Background of wheeze and rash with egg. Recent symptoms still with immediate onset hives, pruritis and vomiting after scrambled egg	EWE 6mm REW 15mm	n*Gal d 1* 0.4 n*Gal d 2* 0.5 n*Gal d 3* 1.5 n*Gal d 5* 3.3	Egg white 8.38 Ovomucoid 1.85
			Peanut	Food challenge positive (itchy throat, clearing of throat, vomiting, abdominal cramps)	Peanut 20mm	n*Ara h 1* 1.8 n*Ara h 2* 1.0 n*Ara h 3* 3.7	Peanut 94.8 r*Ara h 1* 6.32 r*Ara h 2* 17.0 r*Ara h 3* 5.86 r*Ara h 8* 0.59 r*Ara h 9* 37.6
			Treenut	History: recent symptoms of diffuse urticarial rash and itchy throat/cough after cashew nut ingestion	Not done	Negative	Cashew 35.5
011	Mixed	88	Peanut	History: recent history itchy rash, flushing, itchy mouth and wheeze with peanut butter ingestion	Peanut 12mm	n*Ara h 1* 1.1 n*Ara h 2* 7.4 n*Ara h 3* 0.5	Peanut 14.5 r*Ara h 1* 14.9 r*Ara h 2* 4.76 r*Ara h 3* 0.36 r*Ara h 8* 0.21 r*Ara h 9* 0.22
			Egg	History: recent itchy rash, itchy mouth, clearing of throat and vomiting after cooked egg ingestion	EWE 8mm REW 7mm	n*Gal d 1* 38 n*Gal d 2* 12 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 44.6 Ovomucoid 63.2

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Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
012	Xhosa	40	Peanut	Food challenge positive (Red, itchy eyes, flushing of face and generalised pruritis, complained of itchy mouth and clearing of throat.)	Peanut 15mm	n*Ara h 1* 0 n*Ara h 2* 2.8 n*Ara h 3* 0	Peanut 6.66 r*Ara h 1* 2.14 r*Ara h 2* 6.43 r*Ara h 3* 0.04 r*Ara h 8* 0.01 r*Ara h 9* 0.0
015	Xhosa	24	Peanut	Food challenge positive (Generalised pruritis. Vomiting twice. Diarrhoea)	Peanut 16mm	negative	Peanut 1.12 r*Ara h 1* 0.03 r*Ara h 2* 0.32 r*Ara h 3* 0.02 r*Ara h 8* 0.05 r*Ara h 9* 0.0
019	Xhosa	86	Peanut	Food challenge positive (>3 urticarial lesions, oral itch, retching. Late eczema flare)	Peanut 4mm	n*Ara h 1* 3.1 n*Ara h 2* 0 n*Ara h 3* 0	Peanut 23.4 r*Ara h 1* 6.82 r*Ara h 2* 0.84 r*Ara h 3* 1.21 r*Ara h 8* 0.48 r*Ara h 9* 1.06
			Egg	History: recent itchy rash, itchy mouth, clearing of throat and vomiting after cooked egg ingestion	EWE 8mm REW 7mm	n*Gal d 1* 38 n*Gal d 2* 12 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 44.6 Ovomucoid 63.2
020	Xhosa	44	Egg	Food challenge positive (generalised pruritis, diffuse truncal macular rash, anxiety. Late worsening of eczema)	EWE 5mm REW10mm	n*Gal d 1* 2.5 n*Gal d 3* 3.2 n*Gal d 5* 3.5	Egg white >100 Ovomucoid >100
026	Mixed	52	Egg	History: itchy rash, angioedema and eczema flare-up after boiled egg ingestion	EWE 11mm REW 20mm	n*Gal d 1* 14 n*Gal d 2* 5.7 n*Gal d 3* 25 n*Gal d 5* 3.3	Egg white 53.6 Ovomucoid 22.7
			Peanut	History: recent itchy rash, angioedema, tight throat, light headedness and wheeze after peanut butter ingestion requiring IM adrenaline in emergency department	Peanut 12mm	n*Ara h 1* 5.1 n*Ara h 2* 53 n*Ara h 3* 3.6	Peanut >100 r*Ara h 1* 4.1 r*Ara h 2* 68.6 r*Ara h 3* 0.76 r*Ara h 8* 0.45 r*Ara h 9* 0.66
031	Mixed	10	Milk	Food challenge positive (generalised pruritis, facial flushing, vomiting x 2)	Fresh milk 17mm	nBosd8 0.7	Cow's milk 21.2 Casein 31.2
			Egg	Food challenge positive (multiple wheals 3 on trunk, pruritis)	EWE 6mm REW 20mm	n*Gal d 1* 0 n*Gal d 2* 0.7 n*Gal d 3* 1.2 n*Gal d 5* 0	Egg white 42.5 Ovomucoid 0.85
			Peanut	Food challenge positive (multiple wheals on face, generalised pruritis, flushing, anxiety)	Peanut 11mm	n*Ara h 1* 0.9 n*Ara h 2* 2.2 n*Ara h 3* 1.1	Peanut 62.5 r*Ara h 1* 6.68 r*Ara h 2* 16.2 r*Ara h 3* 7.6 r*Ara h 8* 0.1 r*Ara h 9* 0.1
033	Xhosa	12	Egg	Food challenge positive (generalised pruritis, macular rash trunk, vomiting)	EWE 4mm REW 8mm	n*Gal d 1* 22 n*Gal d 2* 5.8 n*Gal d 3* 21 n*Gal d 5* 38	Egg white>100 Ovomucoid > 100
034	Xhosa	16	Egg	History: recent itchy rash, vomiting and wheeze after egg ingestion	EWE 5mm REW 12mm	n*Gal d 1* 2.0 n*Gal d 2* 0 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 4.1 Ovomucoid 8.0

University of Cape Town

Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
038	Mixed	101	Peanut	History: itchy rash and clearing of throat/ cough and retching shortly after ingestion of peanut, recurrently	Peanut 7mm	Negative	Peanut 1.68 r*Ara h 1* 0.24 r*Ara h 2* 0.38 r*Ara h 3* 0.13 r*Ara h 8* 0.16 r*Ara h 9* 0.98
039	Mixed	17	Egg	Food challenge positive (Macular rash on trunk, generalised pruritis, anxiety)	EWE 6mm REW 8mm	n*Gal d 1* 0.7 n*Gal d 2* 0 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 0.43 Ovomucoid 0.27
040	Xhosa	10	Egg	Food challenge positive (pruritis, vomiting and copious diarrhoea; admitted overnight for observation)	EWE 3mm REW 25mm	n*Gal d 1* 1.6	Egg white 19.2 Ovomucoid 46.8
041	Xhosa	17	Egg	Food challenge positive (macular rash on trunk, pruritis, clingy, vomit x1)	EWE 4mm REW 7mm	All negative	Egg white 0.52 Ovomucoid 0.22
046	Xhosa	86	Fish	History: Recurrent itchy rash, hives and angioedema on ingestion of white fish. Whilst waiting for food challenge had a similar reaction hence challenge cancelled	Cod Fish 4mm	nCypc1 8.7 nGadc1 7.9	Cod 10.4
047	Xhosa	22	Egg	Food challenge positive (diffuse truncal and facial urticarial, itchy mouth, throat clearing, diarrhoea)	EWE 6mm REW 15mm	n*Gal d 1* 31 n*Gal d 2* 19 n*Gal d 3* 0 n*Gal d 5* 0	Egg white>100 Ovomucoid >100
052	Xhosa	16	Egg	History: Cough and vomit with itchy rash after ingestion of egg on several occasions; happened again accidentally whilst awaiting food challenge (hence challenge cancelled)	EWE 0mm REW 12mm	Negative	Egg white 7.7 Ovomucoid 3.6 r*Gal d 2* 5.94 r*Gal d 3* 0.08
053	Mixed	32	Egg	Food challenge positive (angioedema of lip, peri-oral and facial hives)	EWE 0 REW 10mm	n*Gal d 1* 0.6 n*Gal d 2* 1.1 n*Gal d 3* 0.6 n*Gal d 5* 0	Egg white 14.6 Ovomucoid 20.5
057	Mixed	10	Egg	Food challenge positive (macular rash on trunk, anxious, dry cough)	EWE 0mm REW 10mm	Negative	Egg white 12.0 Ovomucoid 0.15 r*Gal d 2* 19.5 r*Gal d 3* 0.29
			Peanut	Food challenge positive (flushing of face, wheals>3, pruritis)	Peanut 12mm	Negative	Peanut 1.89 r*Ara h 1* 0 r*Ara h 2* 0.08 r*Ara h 3* 0.21 r*Ara h 8* 0.02 r*Ara h 9* 0.01
059	Xhosa	37	Egg	Food challenge positive (itchy eyes and mouth, macular rash on trunk, blisters around mouth)	EWE 10 REW 14	n*Gal d 3* 0.4	Egg white 6.2 r*Gal d 1* 6.14 r*Gal d 2* 2.09 r*Gal d 3* 1.48
			Peanut	Food challenge positive (generalised pruritis, severe abdominal pain and vomiting)	Peanut 11 mm	n*Ara h 2* 0.6	Peanut 1.83 r*Ara h 1* 0 r*Ara h 2* 1.66 r*Ara h 3* 0.01 r*Ara h 8* 0.01 r*Ara h 9* 0.03

University of Cape Town

Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
060	Mixed	10	Egg	History: Recent reaction with flushing, hives and worsening of eczema	EWE 6mm REW 13mm	n*Gal d 1* 0.9 n*Gal d 2* 0 n*Gal d 3* 1.0 n*Gal d 5* 1.1	Egg white> 100 Ovomucoid 52.6
062	Xhosa	18	Egg	History: Recent history of diffuse urticarial rash within 30 minutes of egg ingestion, and late worsening of eczema. Reacted again whilst awaiting challenge	EWE 7mm REW 12mm	n*Gal d 1* 1.0 n*Gal d 2* 0.6 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 40 Ovomucoid 6.35
064	Xhosa	27	Peanut	Food challenge positive (multiple wheals on face, itchy mouth, irritability, late flare of eczema)	Peanut 17mm	n*Ara h 1* 1.6 n*Ara h 2* 0.6 n*Ara h 3* 0	Peanut 12.1 r*Ara h 1* 11.5 r*Ara h 2* 8.98 r*Ara h 3* 0.23 r*Ara h 8* 0 r*Ara h 9* 0
066	Xhosa	48	Peanut	Food challenge positive (Itchy eyes and mouth, generalised pruritis. Vomiting. Late exacerbation of eczema)	Peanut 16mm	n*Ara h 1* 0 n*Ara h 2* 3.8 n*Ara h 3* 0	Peanut 10.0 r*Ara h 1* 0.19 r*Ara h 2* 6.8 r*Ara h 3* 0.24 r*Ara h 8* 0.04 r*Ara h 9* 0.04
069	Xhosa	12	Egg	Food challenge positive (Macular rash, pruritis, erythema of left eye, vomiting. Worsening of eczema the next day)	EWE 7mm REW 11mm	n*Gal d 1* 0.5 n*Gal d 2* 0.7	Egg white 5.15 Ovomucoid 0.8
074	Mixed	19	Egg	History : recent urticaria, angioedema and flushing shortly after egg ingestion, and late eczema flare up	EWE 11mm REW 21mm	n*Gal d 1* 2.2 n*Gal d 2* 0 n*Gal d 3* 0 n*Gal d 5* 0	Egg white 48.7 Ovomucoid 45.0
			Peanut	History recent itchy rash, coughing, angioedema and flushing shortly after ingestion of peanut butter, and late eczema flare	Peanut 22mm	n*Ara h 1* 2.6 n*Ara h 2* 3.4 n*Ara h 3* 1.8	Peanut 52.7 r*Ara h 1* 6.8 r*Ara h 2* 25.5 r*Ara h 2* 1.59 r*Ara h 8* 0.12 r*Ara h 9* 0.13
075	Mixed	83	Peanut	History: immediate diffuse urticarial, clearing of throat, cough and late eczema flare up after peanut butter ingestion	Peanut 20mm	n*Ara h 1* 0 n*Ara h 2* 5.7 n*Ara h 3* 0	Peanut 3.36 r*Ara h 1* 0 r*Ara h 2* 3.97 r*Ara h 3* 0 r*Ara h 8* 0.05 r*Ara h 9* 0.94
079	Mixed	17	Treenut	History: recent history urticaria, flushing, angioedema and wheeze within 30 minutes of cashew nut ingestion.	Not done	negative	Cashew 1.61
086	Xhosa	21	Egg	Food challenge positive (Angioedema of lips, generalised pruritis, macular rash)	Ewe – Fresh egg white 12	n*Gal d 1* 7.7 n*Gal d 2* 4.4 n*Gal d 3* 2.2 n*Gal d 5* 4.7	Egg white > 100 Ovomucoid > 100
			Peanut	Food challenge positive (pruritis, urticarial rash and stridor requiring IM adrenaline)	Peanut 9mm	n*Ara h 1* 2.5 n*Ara h 2* 4.6 n*Ara h 3* 2.2	Peanut 88 r*Ara h 1* 23.2 r*Ara h 2* 80.2 r*Ara h 3* 8.26 r*Ara h 8* 0.05 r*Ara h 9* 0.05

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Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
087	Mixed	48	Milk	History: Positive recent symptoms with immediate urticarial rash, pruritis, vomiting and late flare of eczema. Whilst awaiting challenge had a further episode of angioedema and itchy rash after cow's milk ingestion	Milk extract 0 Fresh milk 6mm	nBosd4 1.0 nBosd5 0.9 nBosd8 0.4	Cow's milk 42.3 Alpha lactalbumin 49 Beta lactoglobulin 39.9 Casein 4.01
087	Mixed	48	Egg	Food challenge positive (Generalised pruritis, nausea and vomiting. Late exacerbation of eczema)	EWE 6mm REW 13mm	n*Gal d 1* 11 n*Gal d 2* 4.2 n*Gal d 3* 6.1 n*Gal d 5* 13	Egg white>100 Ovomucoid >100
			Peanut	History: recent itchy rash, tight throat, wheeze and vomiting shortly after peanut ingestion.	Peanut 10mm	n*Ara h 1* 12 n*Ara h 2* 11 n*Ara h 3* 1.1	Peanut 99.2 r*Ara h 1* 62 r*Ara h 2* 64.5 r*Ara h 3* 3.52 r*Ara h 8* 0.3 r*Ara h 9* 64.9
089	Mixed	90	Peanut	Recent and recurrent history itchy rash, flushing and late exacerbation of eczema	Peanut 15mm	n*Ara h 1* 0 n*Ara h 2* 8.9 n*Ara h 3* 0	Peanut 11.5 r*Ara h 1* 1.01 r*Ara h 2* 25.6 r*Ara h 3* 1.07 r*Ara h 8* 0.43 r*Ara h 9* 30.9
090	Mixed	43	Peanut	Food challenge positive (multiple wheals, itchy eyes, pruritis.)	Peanut 9mm	n*Ara h 1* 0 n*Ara h 2* 4.3 n*Ara h 3* 0	Peanut 8.03 r*Ara h 1* 1.35 r*Ara h 2* 14.3 r*Ara h 3* 0.65 r*Ara h 8* 0.15 r*Ara h 9* 0.18
091	Xhosa	18	Egg	History: Recent history diffuse urticarial rash, itching in mouth and coughing after cooked egg ingestion; followed by late eczema flare up.	EWE 15mm REW 15mm	n*Gal d 1* 0 n*Gal d 2* 0 n*Gal d 3* 0 n*Gal d 5* 0.6	Egg white 9.47 Ovomucoid 2.10
			Peanut	Food challenge positive (generalised pruritis, facial flushing, rhinorrhoea)	Peanut 13mm	n*Ara h 1* 0 n*Ara h 2* 2.2 n*Ara h 3* 0	Peanut 23.5 r*Ara h 1* 0.35 r*Ara h 2* 17.1 r*Ara h 3* 0.05 r*Ara h 8* 0.05 r*Ara h 9* 0.02
093	Mixed	59	Peanut	Food challenge positive (pruritis, macular rash, anxiety, retching and persistent vomiting)	Peanut 10mm	n*Ara h 1* 9.1 n*Ara h 2* 1.8 n*Ara h 3* 1.2	Peanut > 100 r*Ara h 1* >100 r*Ara h 2* 22.2 r*Ara h 3* 8.88 r*Ara h 8* 0.45 r*Ara h 9* 0.44

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Patient	Ethnicity	Age (in months)	Allergy	Clinical features	SPT (mm)	ISAC (ISAC U/L)	Specific IgE (immunocap) (kiU/L)
097	Xhosa	15	Egg	History: Recurrent urticaria, pruritis and angioedema on history; with late flare up of eczema. Called up for challenge but reacted whilst awaiting challenge	EWE 3mm REW 6mm	negative	Egg white 1.01 Ovomucoid 0.85
098	Mixed	7	Peanut	Food challenge positive (multiple> 3 urticarial lesions, pruritis, late worsening of eczema)	Peanut 10mm	n*Ara h 2* 0.8	Peanut 12.7 r*Ara h 1* 0.45 r*Ara h 2* 2.63 r*Ara h 3* 0.2 r*Ara h 8* 0.02 r*Ara h 9* 0.17

EWE= egg white extract
REW= raw egg white
n (eg n*Ara h 2*) =native component of *Ara h 2*
r (eg r*Ara h 2*)=recombinant component of *Ara h 2*
Note that *Gal d 1* and ovomucoid are equivalent

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Chapter 7:
Peanut Sensitisation, Allergy and Component Patterns in South African Children with Atopic Dermatitis

Abbreviations

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SCORAD: Scoring Atopic Dermatitis Score

SPT: Skin Prick Test

ISAC: Immuno Solid Phase Allergen Chip

PPV: Positive Predictive Value

NPV: Negative Predictive Value

ISU: ISAC Units

IQR: Interquartile range

ROC: Receiver Operating Characteristic

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7.1 Introduction

The increase in allergy to peanut in westernised countries [1-3] has sparked international interest in peanut allergy. Peanut allergy prevalence varies significantly between geographic regions: in the EuroPrevall study, the overall prevalence of peanut allergy was 2.6%,[4,5] with wide variation between countries from 0.06% (Israel)[6] to 5.9% (Sweden).[7]

Apart from geographical variation, ethnic differences in peanut allergy prevalence within a particular region may occur. Recent studies have suggested that non-whites may be at greater risk of food allergy, especially if they are living in a westernised environment. [8-10] In the recent HealthNuts study, Asian children who were the "first generation" in Australia, whose parents were born in East Asia, had a higher prevalence of peanut allergy than children with two Australian-born parents, even though their parents were less atopic.[11]

Sensitisation does not equate to allergy in peanut-sensitised patients, and food challenges may be required to differentiate between asymptomatically sensitised and truly allergic patients. 95% positive predictive values (PPVs) have been established to more reliably predict food allergies and reduce the number of labour-intensive and potentially hazardous food challenges. However, these predictive values may be population and age specific.[13,14]

Peanut components are prefixed "*Ara*" after the first 3 letters of the genus and first letter of the species of peanut, viz *Arachis Hypogaea.* Roman numerals after the preix are usually numbered in the order in which they were discovered. Component testing for peanut proteins helps differentiate between non-specific cross reactive components such as *Ara h 8* and *Ara h 9* and specific peanut components such as Ara1,2 and 3, which are heat resistant storage proteins. *Ara h 2* (2S albumin storage protein) has been shown to be the most important component in prediction of food allergy in several countries, including the UK,[14,15] Sweden,[16] France,[17,18] Japan[19] and the United States,[20] with a positive result (>0.35kU/L) to *Ara h 2* having a high predictive value for peanut allergy. In Mediterranean countries, the lipid transfer protein *Ara h 9* is an important peanut allergen.[21] *Ara h 8*, in the PR10 protein group of labile food allergens, is more prominent in those exposed to certain pollens such a birch, alder and Timothy grass.[16,22] Thus the pattern and relevance of peanut components may vary between geographical areas and possibly between ethnic groups.[23]

There is very little data on peanut allergy in South Africa. In a South African study of 212 Xhosa high school patients, 1.9% were sensitised to peanut (SPT≥3mm above the negative control) but none reported allergic symptoms.[24] Eczema, especially early onset and more severe in nature, is a significant risk factor for developing food allergy including peanut allergy.[25] In 2009, as part of the Early Prevention of Asthma and Allergies in Children (EPAAC) study, 114 South African infants with atopic dermatitis were shown to have a sensitisation rate of 26.8% to peanut.[26] This study did not, however, explore clinical food allergy.

The aim of this chapter is to describe sensitisation, allergy and component patterns in peanut allergy in our cohort of children with AD. It also aims to compare peanut sensitisation and allergy patterns between children of black South African origin (Xhosa) and children of mixed race origin. Patterns of peanut component sensitisation (*Ara h* 1,2,3,8 and 9) and the value of internationally derived 95% positive predictive values for peanut allergy will be explored in the two ethnic groups. It is the first study in South Africa to utilise oral food challenge tests in equivocal cases, and also the first to analyse peanut component patterns.

7.2 Methodology

The 100 children who took part in this study were screened for peanut allergy by allergy questionnaire, skin prick test (SPT) to peanut extract (Alk Abello, Madid Spain), and ImmunoCAP ISAC (103) test which tested for peanut components *Ara h 1*, 2, 3 and 8. The patients who were sensitised to peanut by SPT or ISAC test (n=44) futher underwent immunoCAP testing to whole peanut extract and components, r*Ara h 1*,2,3,8 and 9 (Phadia, Uppsalla, Sweden). In all patients in whom there was uncertainty

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regarding peanut allergy, an incremental open food challenge was performed as a day case at the Red Cross Children's Hospital. The challenge food was given in the form of peanut butter, starting with a lip challenge, them moving from 0.3g to 17 g of peanut butter over 2 hours with dose increments every 15-20 minutes.

7.2.1 Study definitions

IgE-mediated peanut sensitisation was defined as a positive SPT (3 mm or more above the negative control) and/ or positive food specific IgE by ISAC (≥ 0.3 ISAC units)

IgE-mediated peanut allergy was defined as either:

- A positive food challenge
- A convincing clinical history of significant type I allergic reactions after isolated ingestion of peanut-containing food in the preceding 6 months, with significantly positive SPT/sIgE above the internationally derived 95% positive predictive value for peanut of 8 mm for SPT and 14 kU/L for ImmunoCAP. [27,28]

7.3 Results

100 children were recruited into the study, 59 Xhosas and 41 of mixed race. One patient of mixed race did not complete her peanut challenge hence was excluded from the analyses. The median age at the time of participation was 42 months overall; 40 months in Xhosas and 48 months in mixed race patients (p=0.09). Other baseline characteristics which could affect peanut sensitisation and allergy patterns such as age of onset of eczema, SCORAD score for eczema severity, total IgE levels, sensitisation to Timothy grass as a cross reacting antigen, concomitant egg allergy and asthma, and median age of peanut introduction were not significantly different between the 2 ethnic groups. Such baseline characteristics are depicted in Table 7.1.

All 100 patients underwent SPT to peanut allergen; as well as ISAC testing to peanut components Ara1,2,3 and 8. Forty-four (44) patients who were sensitised by SPT or ISAC also underwent ImmunoCAP testing to whole peanut extract, and recombinant *Ara h 1*,2,3,8 and 9. Twenty-five patients underwent a peanut challenge, 16 Xhosa and 9 of mixed race. Of these 25 challenges, 64% (16) were positive: in 56% (9/16) Xhosa children and 78% (7/ 9) children of mixed race.

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Table 7.1. Inter-ethnic Comparison of baseline characteristics which could affect peanut sensitisation and allergy patterns

	Xhosa (n=59)	**Mixed Race (n=40)**	**Difference between ethnic groups (p-value)**
Median age at time of study entry	40 months	48 months	0.09++
Median age of peanut sensitised patients	31 months	52 months	0.27++
Median age of peanut allergic patients	37 months	52 months	0.26++
Median age of onset of eczema	6 months	6 months	1.0++
Median SCORAD (interquartile (IQ) range)	37 (32-45)	44 (33-52)	0.07++
Median SCORAD in peanut sensitised patients (IQ range)	36 (31-46)	44 (34-42)	0.13++
Total IgE (median in kU/L)	656	1562	0.31++
Total IgE in peanut sensitised patients (median in kU/L)	1059	1701	0.26++
Proportion of patients sensitised to Timothy grass	29%	46%	0.08*
Timothy grass sensitisation in peanut sensitised patients	33%	55%	0.24*
Co-existing asthma	36%	44%	0.4*
Co-existing egg allergy	24%	27%	0.73*
Median age of introduction of peanut	19 months	12 months	0.08*

++ by Mann-Whitney test

* by chi-squared test

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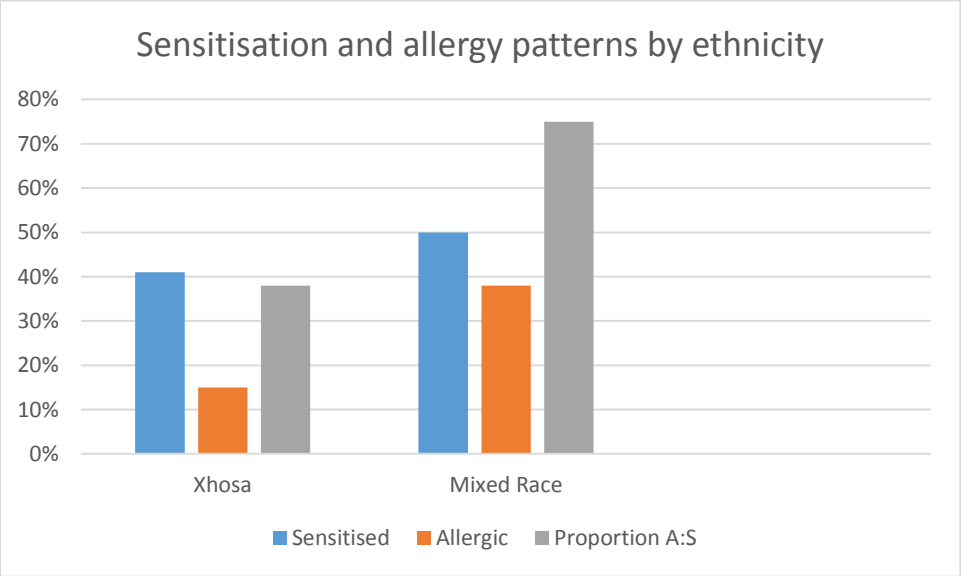
7.3.1 Peanut sensitisation and allergy patterns

Overall, 44% (44) patients were sensitised to peanut, 41% (24/59) of Xhosa patients and 50% (20/40) of mixed race patients (inter-ethnic difference in sensitisation non-significant p=0.1). Of those sensitised, 41 were skin prick test positive (of which 30 were ISAC positive) and an additional 3 were ISAC positive in the absence of a positive SPT. None of the patients with a positive ISAC but negative SPT were subsequently found to be allergic.

Overall, 25 % (25) of patients were peanut allergic: 15% (9/59) of Xhosa patients and 38% (15/40) of mixed race patients (inter-ethnic difference significant p=0.01). All of the 9 Xhosa patients who were classified as allergic were diagnosed by positive food challenge. In the mixed race group, 7 patients were diagnosed by positive food challenge, and 8 had a convincing recent history of a peanut allergy (4 of these had a history of anaphylaxis) and had skin prick test above the 8 mm level. 89% (21/24) patients with peanut allergy were positive by ISAC components 1, 2, 3 or 8. This means by using the ISAC test alone, 3 cases (11% of cases) of peanut allergy may have been missed.

Overall, 57% of peanut-sensitised patients were peanut-allergic; this ratio was 75% in mixed race and 38% in Xhosas, significantly different at p < 0.001. Sensitisation and allergy patterns are depicted in figure 7.1.

Figure 7.1 Chart showing proportion of patients with peanut sensitisation and allergy, by ethnicity



A:S=allergic:sensitised

Despite the differences in peanut allergy rates, median values for whole peanut ImmunoCAP, ImmunoCAP r*Ara h 2*, ISAC n*Ara h 2* and skin prick test diameter in peanut allergic and tolerant patients were not significantly different between the 2 ethnic groups, as depicted in table 7.2.

Table 7.2: Peanut sensitisation and allergy patterns in Xhosa and mixed race patients

	Xhosa (n=59)	**Mixed Race (n=40)**	**Difference between ethnic groups**
Sensitisation to peanut	41% (24/59)	50% (20/40)	0.1*
Allergy to Peanut	15% (9/59)	38% (15/40)	0.01*$
Proportion of sensitised to allergic patients	38%	75%	<0.001*$
ImmunoCAP whole peanut in peanut allergic patients (median in kU/L)	12.1 (6.7-23.5)	17.2 (9.7-97)	0.39++
ImmunoCAP whole peanut in peanut sensitised but tolerant patients (median in kU/L)	1.56 (0.13-9.1)	0.08 (0.01-3.4)	0.4++
ImmunoCAP r*Ara h 2* in peanut allergic patients (median in kU/L)	6.8 (1.66-17.1)	16.9 (3.97-25.6)	0.53++
ImmunoCAP r*Ara h 2* in peanut sensitised but tolerant patients (median in kU/L)	0.24 (0.1-1.22)	0.1 (0.1-0.12)	0.4++
ISAC n*Ara h 2* in allergic patients (median in ISAC Units)	2.2 (0.6-3.8)	5.7 (1.8-11)	0.08++
ISAC n*Ara h 2* in peanut sensitised but tolerant patients (median in ISAC units)	0 (0-0.6)	0 (0-0)	0.15++
SPT size in peanut allergic patients (median in mm)	13 (11-16)	12 (10-16)	0.9++
SPT size in peanut sensitised but tolerant patients (median in mm	4 (3-6)	5 (4-8)	0.42++
Proportion of peanut allergic patients with severe peanut allergy	11% (1/9)	27% (4/15)	0.35 *

++ by Mann-Whitney test
* by chi-squared test
$ statistically significant

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7.3.2. Risk factors for peanut allergy

Age at the time of assessment:

Peanut allergy was not significantly different in the younger age group (prevalence of peanut allergy 20% in under 2 year olds, 28% in over 2 year olds, p=0.52). The lower prevalence below the age of 2 years is likely a reflection of late timing of first oral exposure to peanut, frequently after the age of 2 years, as is described in chapter 11.

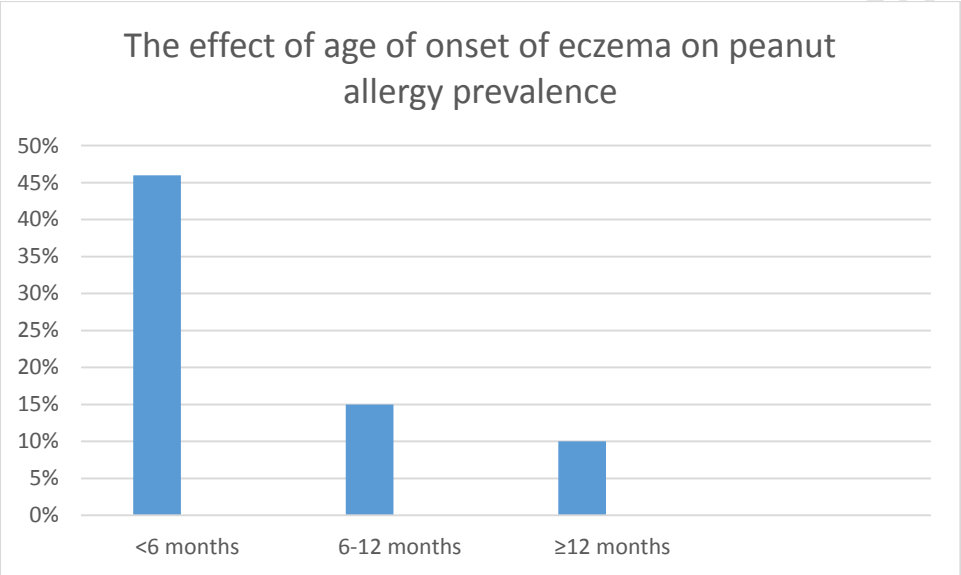
Eczema severity:

Of those patients in the moderate eczema severity category (SCORAD 15-40), 20% (10/49) had a peanut allergy; in those with more severe eczema SCORAD > 40, 28% (14/50) had a peanut allergy; this was not significantly different, p=0.48.

Age of onset of eczema:

Of those with early onset eczema (<6 months), 16/35 (46%) had peanut allergy; of those with intermediate onset eczema (6-12 months), 5/33 (15%) had peanut allergy and of those with later onset eczema (12 months and over), 3/31 (10%) had peanut allergy (p=0.001). The effect of age of onset of eczema on peanut allergy prevalence is depicted in figure 7.2.

Figure 7.2 Effect of age of onset of eczema on peanut allergy prevalence



7.3.3 Value of diagnostic tests in predicting peanut allergy in the study population overall (n=99)

7.3.3.1 Value of history of past reaction to peanut allergy in the diagnosis of peanut allergy

Overall, 23% (23) patients reported a reaction to peanut, of whom 16 were found to be allergic, therefore 70% (16/23) with a perceived peanut allergy had a true peanut allergy.

8 patients subsequently found to be allergic had never eaten peanut before.

By ethnicity, 19% (11/59) Xhosa patients reported a peanut allergy, of whom 64% (7/11) were found to be allergic; in mixed race patients, 29% (12/41) reported symptoms of peanut allergy, of whom 75%

(9/12) were found to be allergic. Difference between reported allergies and true allergies were not significantly different by ethnicity, as depicted in table 7.3.

With the reported reactions, 88% (21/24) were immediate-type occurring within 2 hours of ingestion. The median age at first perceived reaction was 24 months (interquartile range 18-36 months).

20 patients had *positive history plus sensitisation* to peanut, of whom 80% (16/20) were found to be allergic (77% of Xhosa and 81% mixed race); with no significant ethnic differences in these ratios. Table 7.4 summarises the symptoms that patients described in reaction to peanut and their predictive value for allergy.

Table 7.3 Value of history of peanut allergy in prediction of true peanut allergy

	Overall (n=100)	**Xhosa (n=59)**	**Mixed Race (n=41)**	**Difference between ethnic groups (p-value)***
Positive history for any reactivity to peanut	23% (23)	19% (11/59)	20% (12/41)	0.24
% of those with positive history who were found to have a true allergy	70% (16/23)	64% (7/11)	75% (9/12)	0.57
Positive history + sensitised to peanut	20% (20)	15% (9/59)	27% (11/41)	0.14
% of those with positive history + sensitisation who were found to have a true allergy	80% (16/20)	77% (7/9)	81% (9/11)	0.82

*= by chi-squared test

Table 7.4 Reported reactions to peanut and their predictive values

Symptom	**% of Patients with history of reaction to peanut**	**Proportion of Patients with this symptom who have been found to have peanut allergy**
Itchy rash	65% (15/23)	67% (10/15)
Angioedema	39% (9/23)	56% (5/9)
Exacerbation of eczema	39% (9/23)	56% (5/9)
"Doesn't like" the food	30% (7/23)	71% (5/7)
Flushing	22% (5/23)	80% (4/5)
Wheeze	22% (5/23)	100% (5/5)
Vomiting	17% (4/23)	75% (3/4)
Itchy mouth	13% (3/23)	100% (3/3)
Tight throat	13% (3/23)	100% (3/3)
Circulatory compromise (e.g. blue lips)	4% (1/23)	100% (1/1)
Diarrhoea	0%	-

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7.3.3.2 Sensitivity, specificity and Receiver Operating Characteristic (ROC) Curves for SPTs and ISAC tests as screening tests for peanut allergy (n=99)

All 100 patients underwent screening tests for peanut allergy by SPT to peanut and ISAC 103 test to components n*Ara h 1*, n*Ara h 2*, n*Ara h 3* and n*Ara h 8*. The results of 99 patients who completed the study were utilised for analyses.

Receiver Operating Characteristic (ROC) curves showed that the size of the SPT to peanut was superior in the prediction of peanut allergy (ROC area under curve (AUC) 0.98). This was followed by ISAC to *nAra h 2* with ROC AUC 0.90, n*Ara h 1* with ROC AUC 0.72 and n*Ara h 3* with ROC AUC 0.67. ROC curves for SPT and n*Ara h 2* did not differ significantly between ethnic groups; however the ROC AUC for n*Ara h 1* was significantly lower in the Xhosa group (0.5 in Xhosas versus 0.8 in mixed race, p=0.02) as was that for n*Ara h 3* (0.48 in Xhosas versus 0.77 in mixed race group, p=0.003). ROC curves for SPT and ISAC tests are depicted in table 7.5 and figures 7.3-7.6.

Table 7.5 ROC AUC for SPT and ISAC tests as screening test for peanut allergy (n=99)

	ROC Overall	**ROC area Xhosa**	**ROC area Mixed**	**Difference in area (p-value)**
SPT peanut	0.98 (0.96-1)	0.97 (0.93-1)	1 (1-1)	0.9
ISAC n*Ara h 1*	0.72 (0.61-0.82)	0.5 (0.29-0.73)	0.8 (0.67-0.93)	0.02*
ISAC n*Ara h 2*	0.9(0.82-0.98)	0.78 (0.59-0.98)	0.93 (0.84-1)	0.18
ISAC n*Ara h 3*	0.67 (0.57-0.77)	0.48 (0.34-0.62)	0.77 (0.64-0.89)	0.003*

*Statistically significant

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Figure 7.3 ROC Curves for SPT and ISAC components overall, n=99

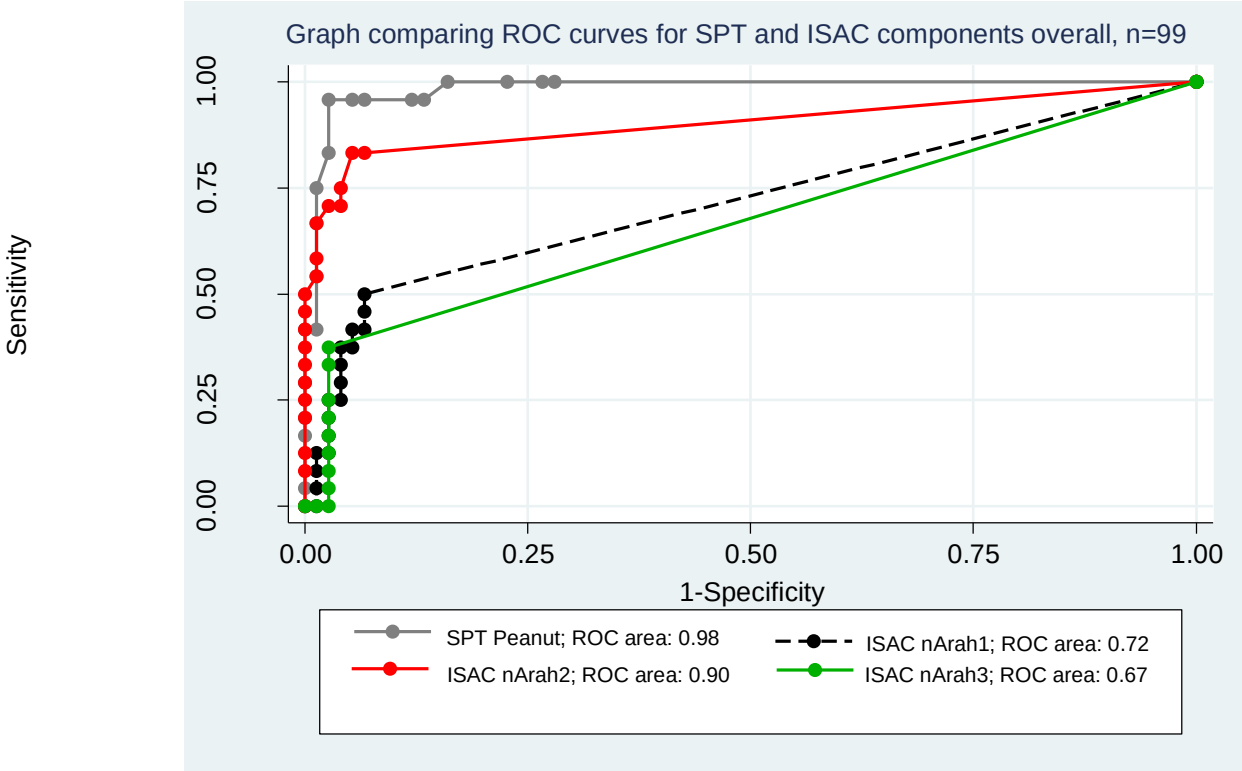


Figure 7.4 ROC Curves for SPT and ISAC components in Xhosa patients

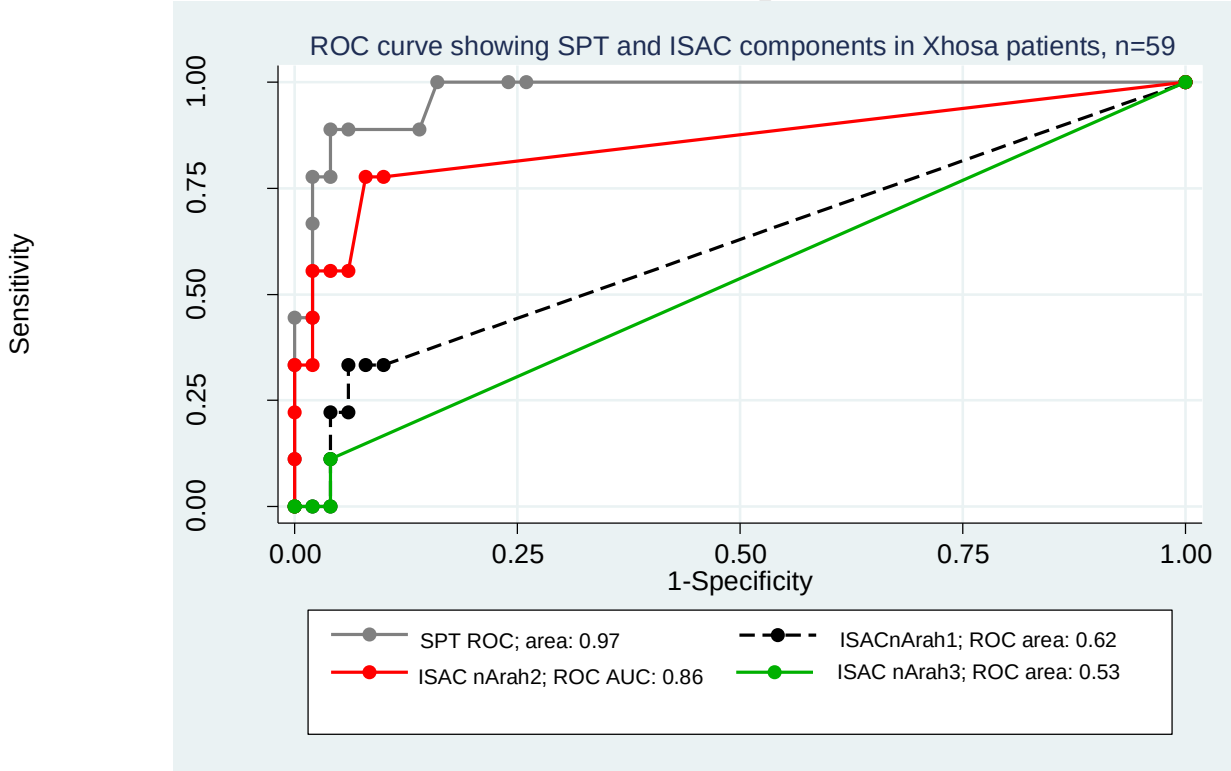


Figure 7.5 ROC Curves for SPT and ISAC components in mixed race patients

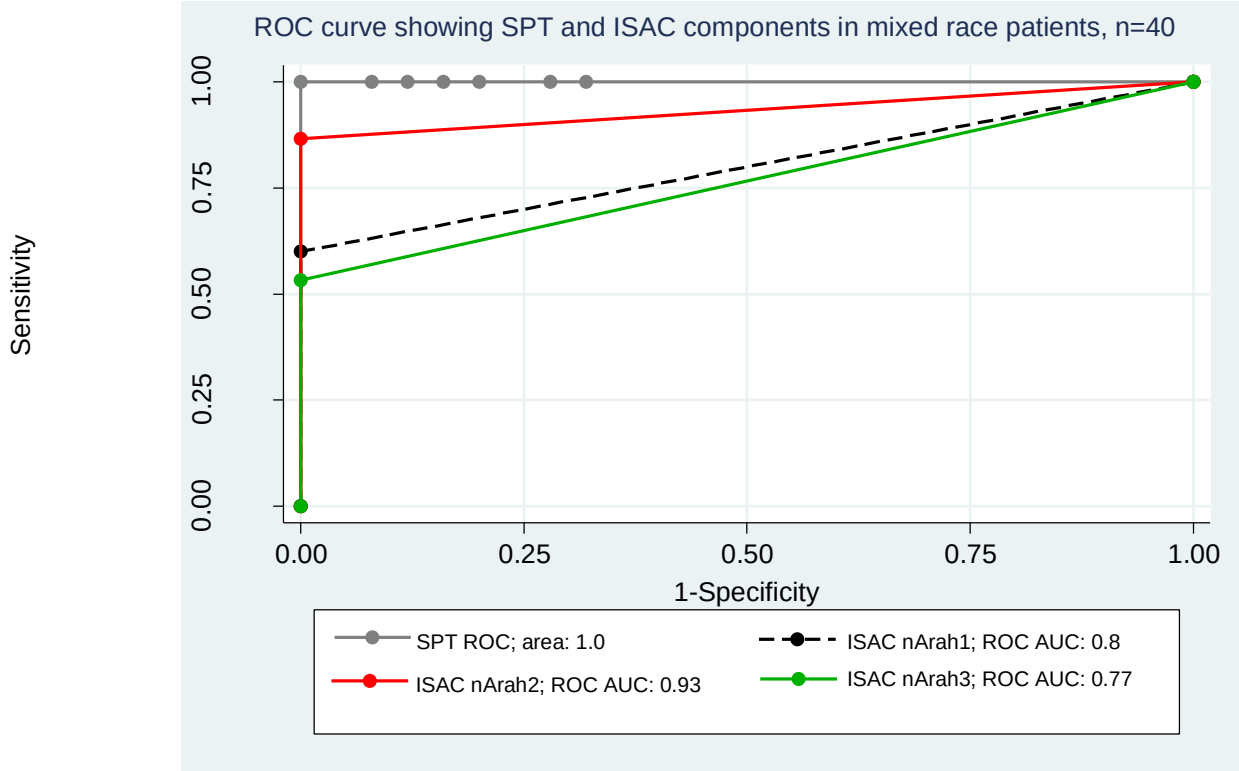
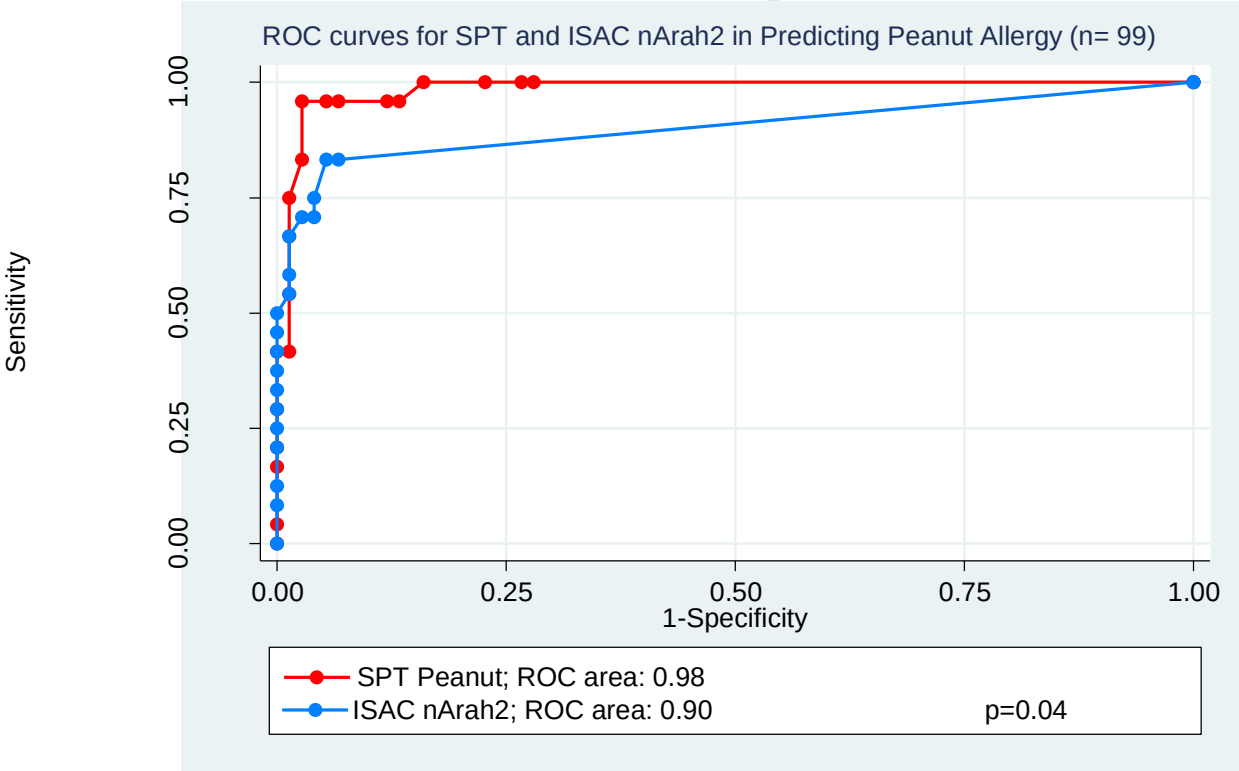


Figure 7.6 ROC for SPT and ISAC** Ara h 2 **in predicting peanut allergy



The SPT to peanut was the most sensitive screening test for peanut allergy (100% sensitive in both ethnic groups), but the PPV for peanut allergy was poor (53%). ISAC n*Ara h 2* had a reasonable sensitivity of 83% and PPV 80%, and a good specificity of 93%. Both ISAC n*Ara h 1* and 3 were specific (92% and 97% respectively) but not sensitive (50% and 38% respectively).

The overall trend for all 4 screening tests was towards a lower specificity and PPV in Xhosa patients, therefore the Xhosa patients had significantly more false positive results for these screening tests. Sensitivity, specificity, PPV and negative predictive value (NPV) are described in table 7.6 below, with ethnic breakdown.

Table 7.6. Sensitivities and specificities of screening tests for predicting peanut allergy

	Overall (n=99)	**Xhosa (n=59)**	**Mixed race (n=40)**	**Difference between ethnic groups (p-value)***
SPT peanut positive (≥3mm)				
Sensitivity	100%	100%	100%	1.0
Specificity	72%	70%	77%	0.44
PPV	53%	38%	71%	0.001**
NPV	100%	100%	100%	1.0
ISAC n*Ara h 1*≥0.3 ISU				
Sensitivity	50%	33%	60%	0.07
Specificity	92%	90%	96%	0.27
PPV	67%	38%	90%	<0.001**
NPV	85%	88%	81%	0.34
ISAC n*Ara h 2*≥0.3 ISU				
Sensitivity	83%	78%	87%	0.26
Specificity	93%	90%	100%	0.04**
PPV	80%	58%	100%	<0.001**
NPV	95%	96%	93%	0.51
ISAC n*Ara h 3*≥0.3 ISU				
Sensitivity	38%	11%	53%	<0.001**
Specificity	97%	96%	100%	0.2
PPV	82%	33%	100%	<0.001**
NPV	83%	86%	79%	0.36

*By chi-squared test
** statistically significant

7.3.3.3 Sensitivity, specificity and Receiver Operating Characteristic (ROC) Curves for SPTs and ISAC tests in differentiating peanut allergy from tolerance in peanut-sensitised patients (n=44)

Those patients who were found to be sensitised to peanut by the screening SPT/ISAC tests, underwent ImmunoCAP tests to whole peanut, and components r*Ara h 1*,2,3,*8* and *9* (n=44).

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ROC in peanut-sensitised patients showed the highest AUC for SPT to peanut (0.94), followed by ImmunoCAP *Ara h 2* and ISAC *Ara h 2* (both 0.86), then ImmunoCAP to whole peanut (0.80). The ROC AUC was significantly lower for *Ara h 1*, both by ISAC (AUC 0.62) and ImmunoCAP (AUC 0.68); and for *Ara h 3* for ISAC (AUC 0.62) and ImmunoCAP (AUC 0.64). The performance was poor for ImmunoCAP *Ara h 8* (AUC 0.56) and *Ara h 9* (AUC 0.51). Ethnic differences were seen only for ISAC *Ara h 1* and 3, which had a significantly higher AUC in the mixed race group. The ROC AUC results and ethnic comparisons for peanut-sensitised patients are tabulated in table 7.7 and in figures 7.7-7.10.

Table 7.7: ROC graphs for SPT, peanut ImmunoCAP and peanut components as predictors of peanut allergy in those who are peanut sensitised (n=44)

	ROC AUC (95% confidence interval): Overall (n=44)	**ROC AUC (95% confidence interval): Xhosas n=24**	**ROC AUC (95% confidence interval): Mixed race n=20**	**Difference in AUC between ethnic groups (p-value)***
SPT Peanut	0.94 (0.87-1)	0.91 (0.78-1)	1 (1-1)	0.15
ImmunoCAP Peanut	0.80 (0.66-0.94)	0.76 (0.55-0.96)	0.87 (0.69-1)	0.40
ISAC n*Ara h 1*	0.62 (0.47-0.77)	0.50 (0.29-0.72)	0.8 (0.67-0.93)	0.02**
ISAC n*Ara h 2*	0.86 (0.76-0.97)	0.79 (0.59-0.98)	0.93 (0.84-1)	0.18
ISAC n*Ara h 3*	0.62 (0.49-0.75)	0.48 (0.34-0.62)	0.77 (0.64-0.89)	0.003**
ImmunoCAP r*Ara h 1*	0.68 (0.48-0.89)	0.64 (0.37-0.91)	0.77 (0.55-0.99)	0.48
ImmunoCAP r*Ara h 2*	0.86 (0.74-0.98)	0.85 (0.69-1)	0.91 (0.76-1)	0.60
ImmunoCAP r*Ara h 3*	0.64 (0.44-0.84)	0.53 (0.27-0.8)	0.8 (0.49-1)	0.26
ImmunoCAP r*Ara h 8*	0.56 (0.37-0.76)	0.43 (0.37-0.89)	0.63 (0.37-0.89)	0.31
ImmunoCAP r*Ara h 9*	0.51 (0.31-0.71)	0.35 (0.08-0.61)	0.7 (0.19-1)	0.22

*By chi-squared test
**Statistically significant by chi-squared test

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Figure 7.7 ROC Curves for ImmunoCAP tests and SPT in all peanut-sensitised patients (n=44)

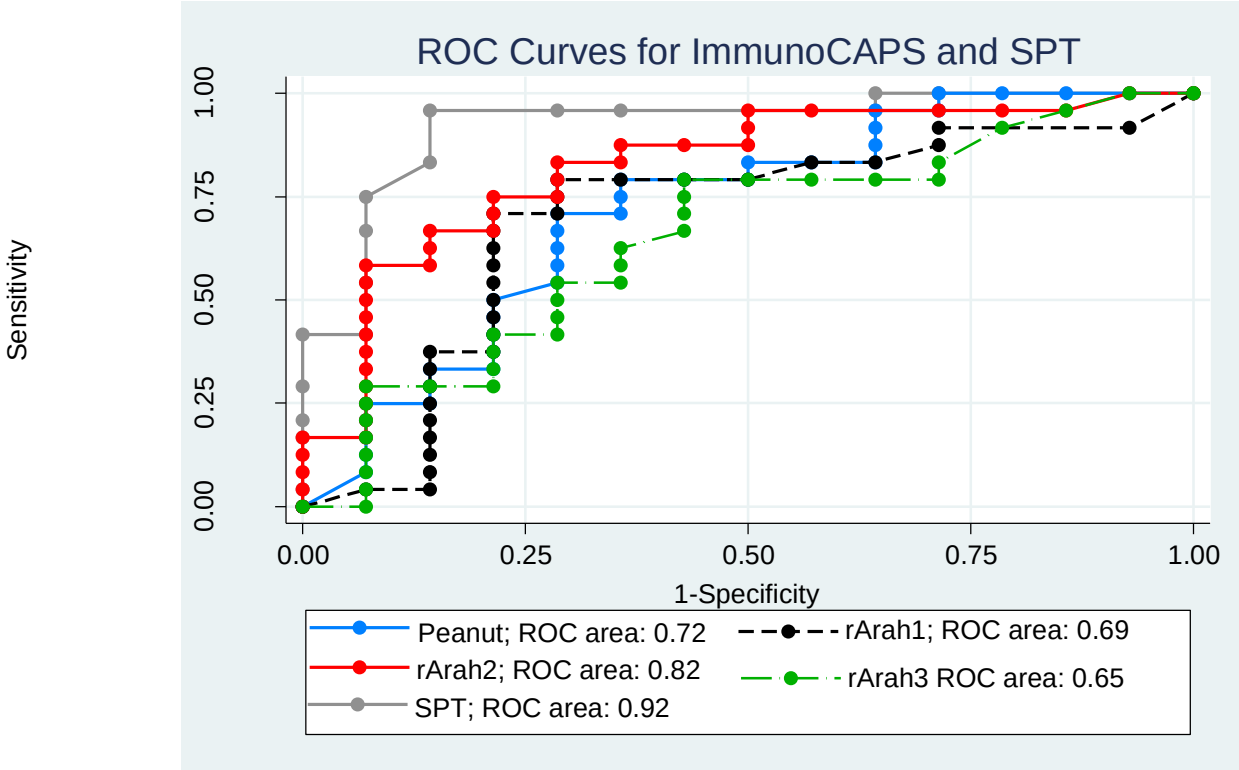


Figure 7.8 ROC Curves for ImmunoCAP tests and SPT in peanut-sensitised Xhosa patients (n=24)

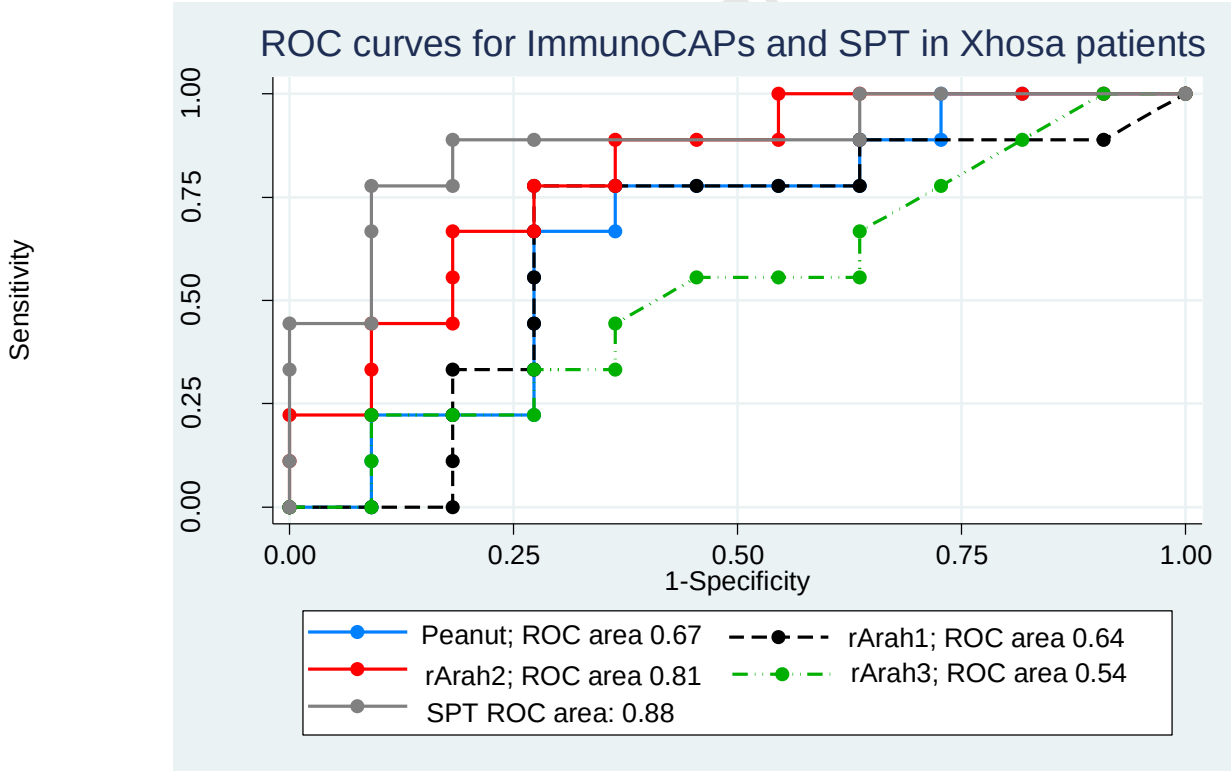


Figure 7.9 ROC Curves for ImmunoCAP tests and SPT in peanut-sensitised mixed race patients (n=20)

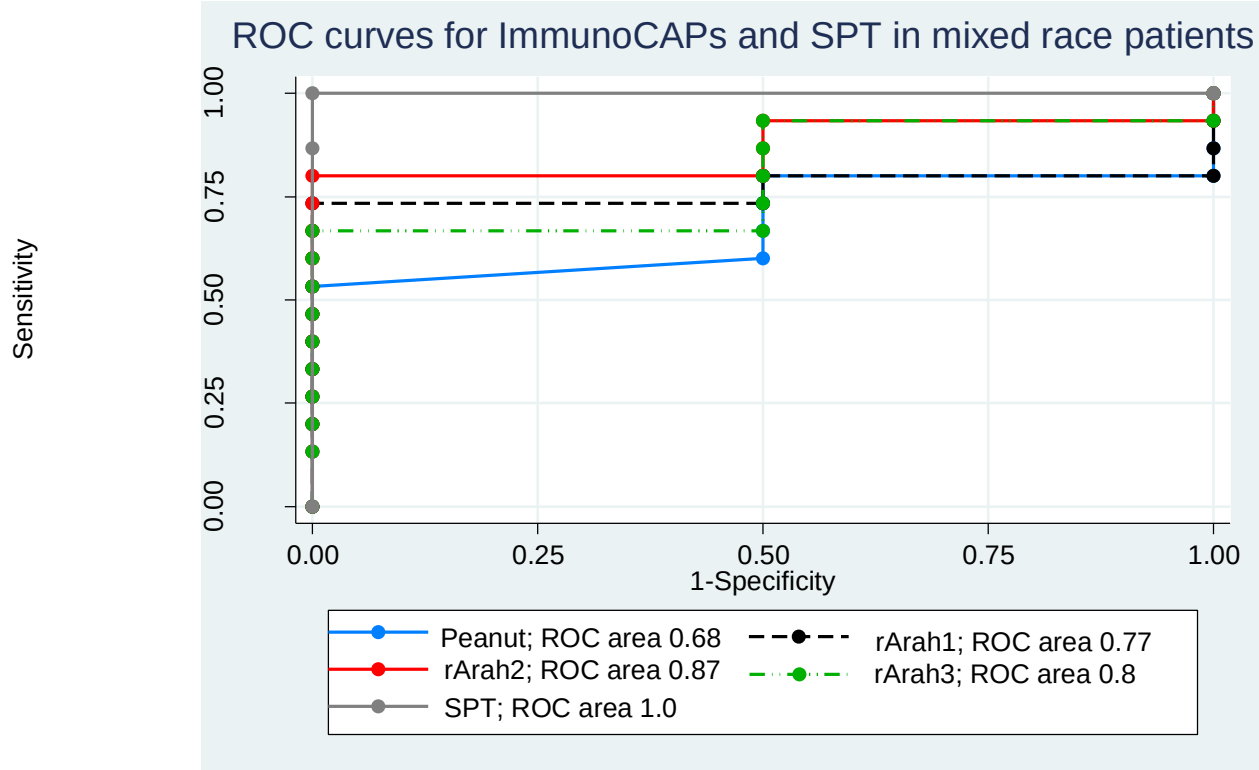
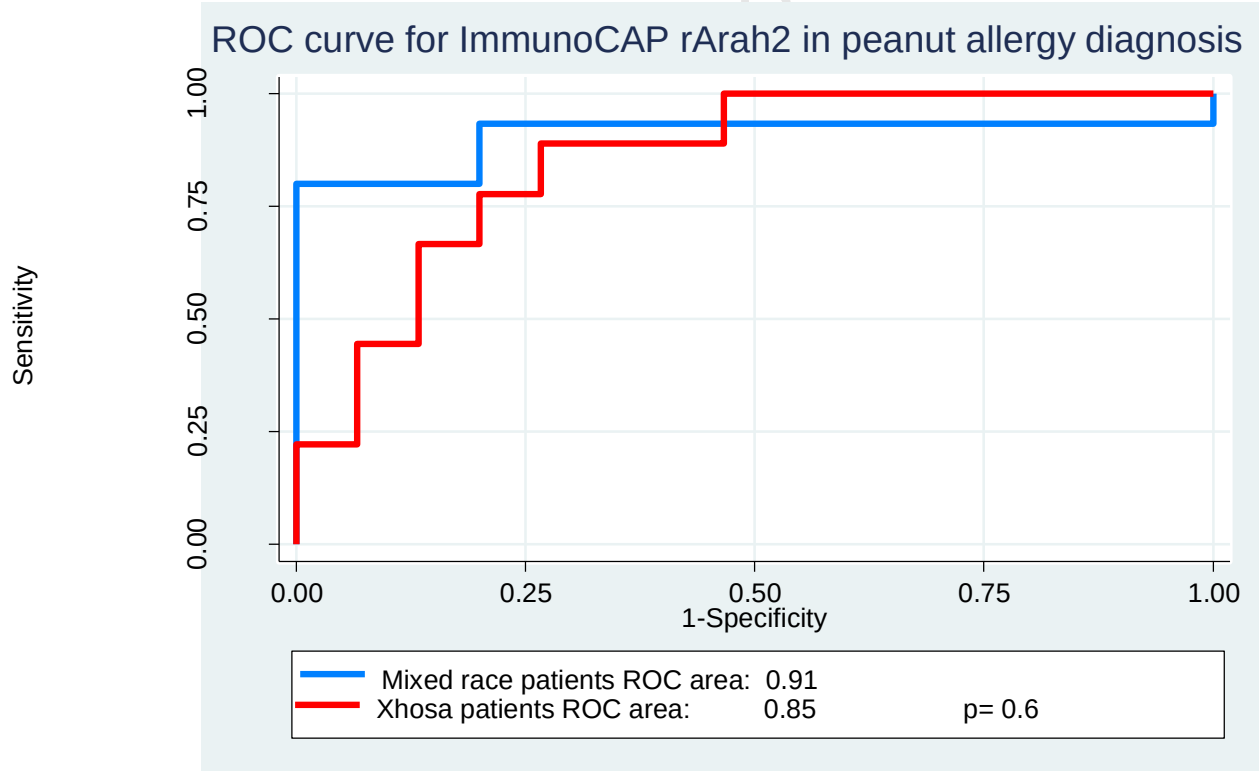


Figure 7.10 Inter-ethnic comparison for ROC Curves for ImmunoCAP rAra h 2



In the 44 peanut-sensitised patients, who underwent further ImmunoCAP tests, the highest sensitivity for diagnosis of peanut allergy was achieved by both the SPT and ImmunoCAP test to whole peanut, at 100% in both ethnic groups. However, the specificity of these tests and the PPV was poor: for SPT specificity 73% and PPV 55% and for ImmunoCAP peanut specificity 40% and PPV 67%. Component testing for *Ara h 2* by both ISAC test and ImmunoCAP test produced lower sensitivities than the SPT but higher specificities and PPV: For ISAC *Ara h 2* ≥ 0.3 ISU, sensitivity for peanut allergy diagnosis was 83%, specificity 75% and PPV 80%, and for ImmunoCAP to *Ara h 2* sensitivity was 92%, specificity 60% and PPV of 73%. Sensitivities, specificities and predictive values for peanut-sensitised patients are depicted in table 7.8.

The trend for all of the above screening tests was towards a lower specificity and PPV in the Xhosa patients, as depicted in table 7.8 below. For example, the PPV for an ImmunoCAP *Ara h 2* ≥0.35 kU/L was 53% for Xhosa patients and 93% in mixed race patients.

Table 7.8 Sensitivities and specificities of screening tests for predicting peanut allergy in peanut-sensitised patients

	Overall (n=44)	**Xhosa (n=24)**	**Mixed race (n=20)**	**Difference between ethnic groups (p-value)***
SPT peanut positive (≥3mm)				
Sensitivity	100%	100%	100%	1.0
Specificity	73%	70%	80%	0.45
PPV	55%	38%	75%	0.01**
NPV	100%	100%	100%	1.0
ImmunoCAP Peanut ≥0.35 kU/L				
Sensitivity	100%	100 %	100%	1.0
Specificity	40%	33%	60 %	0.07
PPV	67%	47%	88%	0.004**
NPV	100%	100%	100%	1.0
ISAC n*Ara h 2* ≥ 0.3 ISU				
Sensitivity	83%	78%	87%	0.44
Specificity	75%	67%	100%	0.005**
PPV	80%	58%	100%	0.001**
NPV	79%	83%	71%	0.34
ImmunoCAP *Ara h 2* ≥0.35 kU/L				
Sensitivity	92 %	89%	93%	0.65
Specificity	60%	53 %	80%	0.06
PPV	73%	53 %	93%	0.004**
NPV	86%	89 %	80%	0.41

*by chi-squared test ** statistically significant difference

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7.3.3.4 Baseline characteristics and median values of SPT, ISAC and ImmunoCAP tests in tolerant versus allergic patients who are peanut-sensitised

There was no significant difference in age at the time of the study, age of onset of eczema, gender distribution, concomitant egg allergy, asthma, allergic rhinitis, or total IgE between peanut tolerant and peanut allergic patients in those who were peanut-sensitised (table 7.9). The mean SCORAD was higher in the allergic group (44.5 versus 36, p=0.06).

The median value for SPT size, ImmunoCAP peanut, ImmunoCAP *Ara h 2* and ISAC *Ara h 2* were significantly higher (p<0.001) in the allergic group: for SPT, 13 mm in the allergic group versus 4.5 mm in the tolerant group; for ImmunoCAP peanut, 14.9 kU/L versus 4.5 kU/L; for ImmunoCAP *Ara h 2*, 15.25 kU/L versus 0.21 kU/L, and for ISAC *Ara h 2*, 3.6 ISU versus 0 ISU. These values as well as the baseline characteristics are depicted in table 7.9 below.

Table 7.9 Baseline characteristics and median values for screening tests in peanut allergic versus tolerant patients with peanut-sensitisation

	Sensitised and allergic to peanut (n=24)	**Asymptomatic sensitisation to peanut (n=20)**	**Difference (p-value)**
Age (median in months)	43	30	P=0.32[++]
Gender	Male 55%	Male 50%	0.74*
SCORAD (median)	44.5	36	0.06[++]
Proportion with Egg allergy	46% (11/24)	30% (6/20)	0.28*
Proportion with Asthma	46% (11/24)	50% (10/20)	0.78 *
Proportion with Hayfever	54% (13/24)	55% (11/20)	0.71*
Total IgE (median in kU/L)	1362	1222	0.7[++]
Age onset eczema (median in months)	3	6	0.11[++]
SPT peanut size (median in mm)	13	4.5	P<0.001[++$]
Peanut specific IgE (median in kU/L)	14.9	1.32	P<0.001[++$]
ImmunoCAP r*Ara h 2* (median in kU/L)	15.25	0.21	P<0.001[++$]
ISAC n*Ara h 2* (median in ISU)	3.6 (0.7-9.15)	0 (0-0.25)	P<0.001[++$]

[++] by Mann-Whitney test
* by Chi-squared test
$ statistically significant

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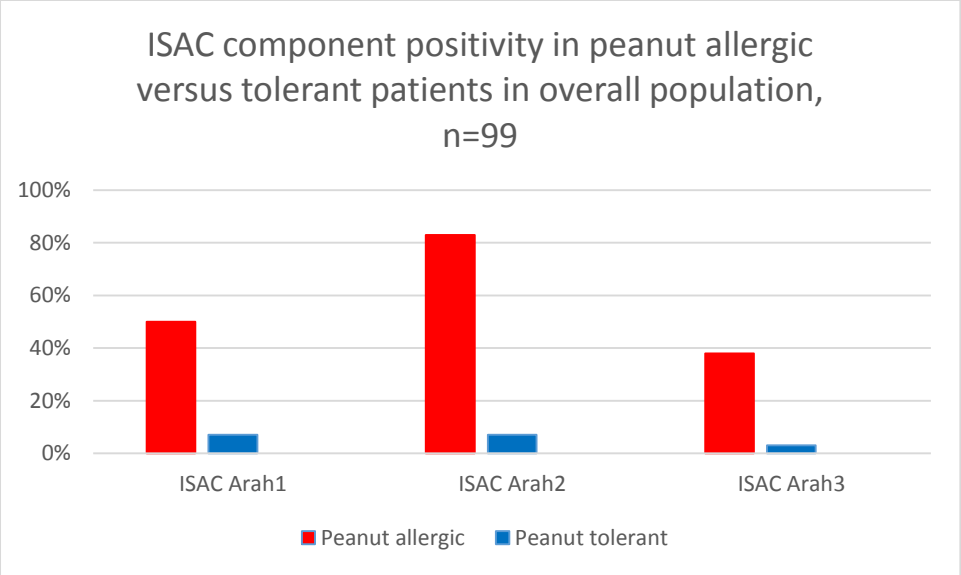
7.3.4 Peanut component patterns

7.3.4.1 Peanut component testing in in the overall study population (n=99)

The most common peanut component in this population was ISAC n*Ara h 2* (25%), followed by ISAC n*Ara h 1* (18%) and ISAC n*Ara h 3* (11%). There were no cases of ISAC positivity for *Ara h 8*. When breaking down these components in the overall group by allergy versus tolerance to peanut, the proportion of patients was significantly higher for all three ISAC components in peanut allergic patients. For ISAC n*Ara h 2*, 83 % of peanut allergic patients were positive, versus 7 % of peanut tolerant patients, p<0.001. For ISAC n*Ara h 1*, 50 % of peanut allergic patients were positive, versus 7% of peanut tolerant patients, p<0.001. For ISAC n*Ara h 3*, 38 % of peanut allergic patients were positive, versus 3 % of peanut tolerant patients, p<0.001. These results for ISAC components in the overall study population are depicted in figure 7.11.

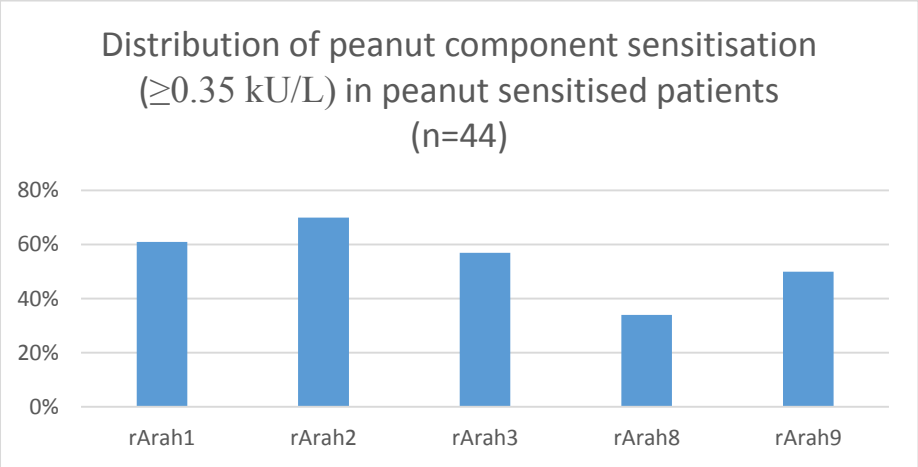
Figure 7.11 ISAC components in peanut allergic versus tolerant patients in the overall study population



7.3.4.2 Peanut component testing in peanut sensitised patients (n=44)

In the 44 sensitised patients, the most common peanut components by ImmunoCAP were r*Ara h 2* (69%), r*Ara h 1* (62%), r*Ara h 3* (58%), r*Ara h 9* (49%) and r*Ara h 8* (33%) as depicted in figure 7.12 below.

Figure 7.12 Distribution of peanut components by ImmunoCAP test in peanut-sensitised patients



The proportion of patients with sensitisation to *Ara h 2* and *Ara h 3* by the ISAC test was significantly higher in allergic patients as depicted in table 7.10. For ISAC *Ara h 2*, 83% of patients with peanut allergy were positive, versus 25% of patients without a peanut allergy despite sensitisation (P<0.001). Similarly, For ImmunoCAP *Ara h 2*, peanut-allergic patients were 92% positive, and peanut-tolerant patients were 40% positive, significantly higher in allergic patients with p<0.001. The components ISAC *Ara h 1*, ImmunoCAP *Ara h 1*, ImmunoCAP *Ara h 3*, ImmunCAP *Ara h 8* and 9 were not significantly different between allergic and tolerant patients in this peanut-sensitised group. Peanut component distribution in allergic and tolerant patients is depicted in table 7.10 and figure 7.13.

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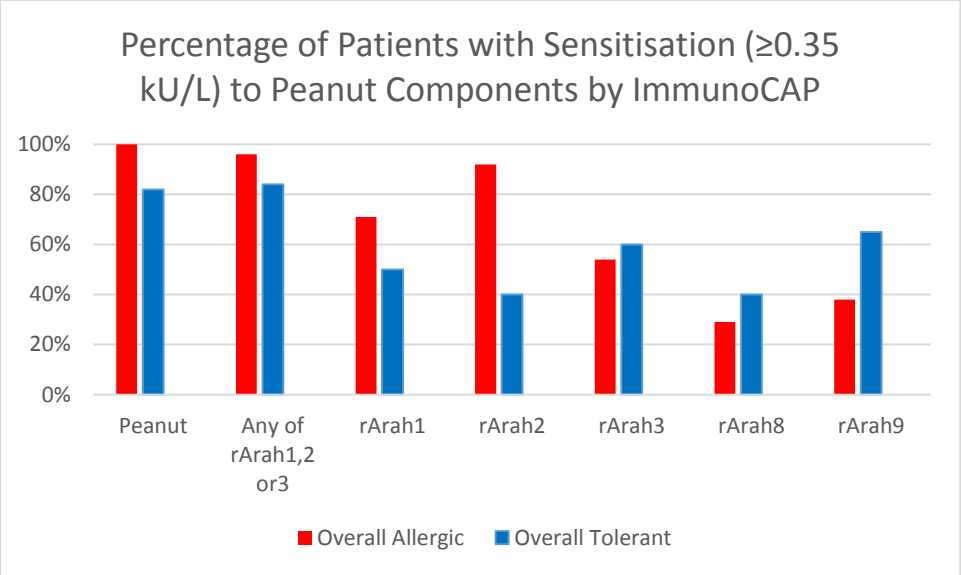
Table 7.10 Peanut component sensitisation in peanut-allergic versus tolerant patients in peanut-sensitised patients

% Patients with Positive component test (≥0.3 ISU for ISAC or ≥0.35 kU/L for ImmunoCAP)	**Overall allergic % (n=24)**	**Overall tolerant % (n=20)**	**Difference between allergic and tolerant patients (p-value)***
ISAC n*Ara h 1*	50 %	25%	0.09
ImmunoCAP r*Ara h 1*	71%	50%	0.16
ISAC n*Ara h 2*	83%	25%	<0.001**
ImmunoCAP r*Ara h 2*	92%	40%	<0.001**
ISAC n*Ara h 3*	38%	10%	0.04**
ImmunoCAP r*Ara h 3*	54%	60%	0.7
ImmunoCAP r*Ara h 8*	29%	40%	0.45
ImmunoCAP r*Ara h 9*	38%	65%	0.07

*By chi-squared test

**Statistically significant

Figure 7.13 Peanut component distribution in peanut allergic and tolerant patients who are peanut-sensitised



7.3. 4. 3 Peanut component patterns in peanut-sensitised patients (n=44) by ethnicity

In both ethnic groups, the component r*Ara h 2* by ImmunoCAP test was significantly more frequently positive in allergic versus tolerant patients. In Xhosa patients, 89% of allergic vs 47% of tolerant patients were *Ara h 2* positive (p=0.04), and in mixed race patients 93% of peanut allergic and 20% of tolerant patients were *Ara h 2* positive (p= 0.001). The other components by ImmunoCAP (Ara1, 3, 8 and 9) were not significantly higher in allergic versus tolerant patients (table 7.11). In all cases of peanut allergy with *Ara h 1* and/or 3 positivity, *Ara h 2* was also positive. *Ara h 8* and 9 were higher in tolerant than allergic patients in both ethnic groups, representing the broad cross reactivity of these components. In the Xhosa group, *Ara h 9* was significantly more frequent in non-allergic patients (p=0.04). In all but one case of *Ara h 8* positivity, *Ara h 9* was also positive, hence in this population *Ara h 9* seems to be the most useful test in assessing tolerance by cross-reactivity.

ISAC patterns to *Ara h 2* were similar to ImmunoCAP patterns with both ethnic groups having a significantly higher positive rate in allergic patients (ISAC *Ara h 2* was positive in 78% of allergic patients versus 33% of tolerant patients in Xhosas, p=0.04; and ISAC *Ara h 2* was positive in 87% of allergic versus 0% of tolerant patients in mixed race, p= < 0.001). In mixed race patients only, ISAC *Ara h 1* and *Ara h 3* were also significantly more positive in allergic patients (table 7.12)

Despite the overall superiority of *Ara h 2* in differentiating allergy from tolerance in both ethnic groups, the Xhosa patients had a significantly higher false positive rate. The probability of being peanut allergic given a positive ImmunoCAP *Ara h 2* was significantly lower in Xhosa patients (53%) than mixed race (93%), p= 0.01. Similarly for a positive ISAC *Ara h 2* test, the probability of peanut allergy was 58% for Xhosa patients, versus 100% for mixed race patients (p=0.009).

Peanut component patterns in sensitised patients by ethnicity are depicted in tables 7.11 and 7.12, and figures 7.14-7.15.

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Table 7.11: Frequency of Positive Components by ImmunoCAP Test in Peanut Allergic versus Tolerant Patients

Immuno-CAP component (positive defined as ≥0.35 kU/L)	**Xhosa allergic (n=9) % (n)**	**Xhosa tolerant (n=15) % (n)**	**Difference between Xhosa allergic and tolerant patients (p-value)***	**Probability of Peanut Allergy in Xhosa patients if Immuno-CAP positive**	**Mixed race allergic (n=15)**	**Mixed race tolerant (n=5)**	**Difference between mixed race allergic and tolerant patients (p-value)***	**Probability of Peanut Allergy in mixed race patients if Immuno-CAP positive**	**Difference between ethnic groups in probability of peanut allergy ***
ImmunoCAP r*Ara h 1*	67% (6)	47% (7)	0.34	46%	73% (11)	60% (3)	0.57	79%	0.08
ImmunoCAP r*Ara h 2*	89% (8)	47% (7)	0.04**	53%	93% (14)	20% (1)	0.001**	93%	0.01**
ImmunoCAP r*Ara h 3*	33% (3)	53% (8)	0.34	27%	67% (10)	80% (4)	0.57	71%	0.03**
ImmunoCAP r*Ara h 8*	22% (2)	33% (5)	0.56	29%	33% (5)	60% (3)	0.29	63%	0.19
ImmunoCAP r*Ara h 9*	22% (2)	67% (10)	0.04**	17%	47% (7)	60% (3)	0.61	70%	0.01 (for tolerance)
ImmunoCAP r*Ara h 1* and *2*	56% (5)	27% (4)	0.16	56%	73% (11)	0% (0)	0.005**	100%	< 0.001**

Table 7.12 Frequency of Positive Components by ISAC in Peanut Allergic versus Tolerant Patients

ISAC Component (Positive defined as ≥0.3 ISAC U/L)	**Xhosa allergic (n=9) % (n)**	**Xhosa tolerant (n=15) % (n)**	**Difference between Xhosa allergic and tolerant patients (p-value)***	**Probability of Peanut Allergy in Xhosa patients if ISACpositive**	**Mixed race allergic (n=15)**	**Mixed race tolerant (n=5)**	**Difference between mixed race allergic and tolerant patients (p-value)***	**Probability of Peanut Allergy in mixed race patients if ISAC positive**	**Difference between ethnic groups in probability of peanut allergy ***
ISAC n*Ara h 1*	33% (3)	33% (5)	1.0	38%	60% (9)	0% (0)	0.02**	100%	0.005**
ISAC n*Ara h 2*	78% (7)	33% (5)	0.04**	58%	87% (13)	0% (0)	0.00**	100%	0.009**
ISAC n*Ara h 3*	11% (1)	13% (2)	0.87	33%	53% (8)	0% (0)	0.035**	100%	0.01**
ISAC *Ara h 8*	0% (0)	0% (0)	1	N/A	0% (0)	0% (0)	1	N/A	-
ISAC n*Ara h 1* and *2* positive	22% (2)	13% (2)	0.56	50%	60% (9)	0% (0)	0.02**	100%	0.02**
Bromelin (CCD)	0% (0)	13% (2)	0.56	N/A	33% (5)	20% (1)	0.58	N/A	-
Glym5	22% (2)	13% (2)	0.55	N/A	40% (6)	0% (0)	0.09	N/A	-

For both tables: * by chi-squared test ** statistically significant

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Figure 7.14 Component patterns by ImmunoCAP test in peanut-sensitised Xhosa patients

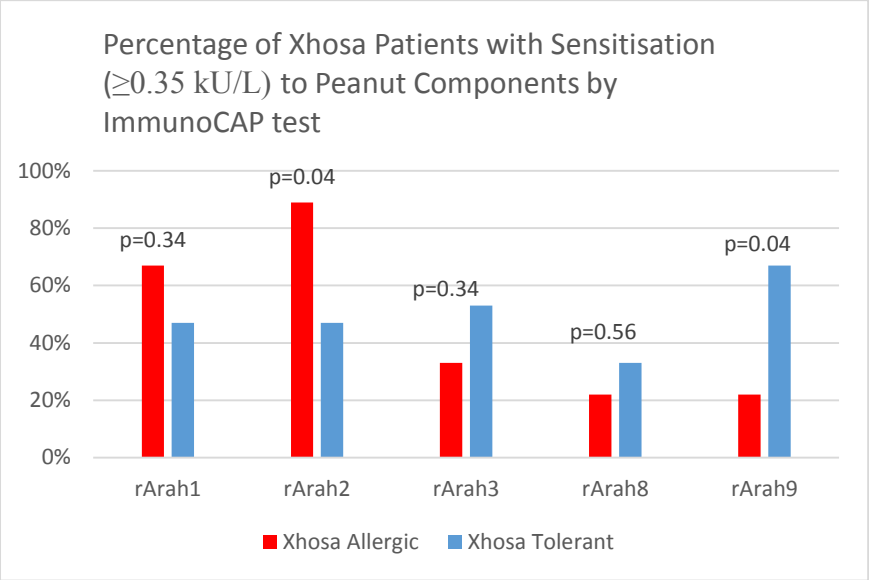
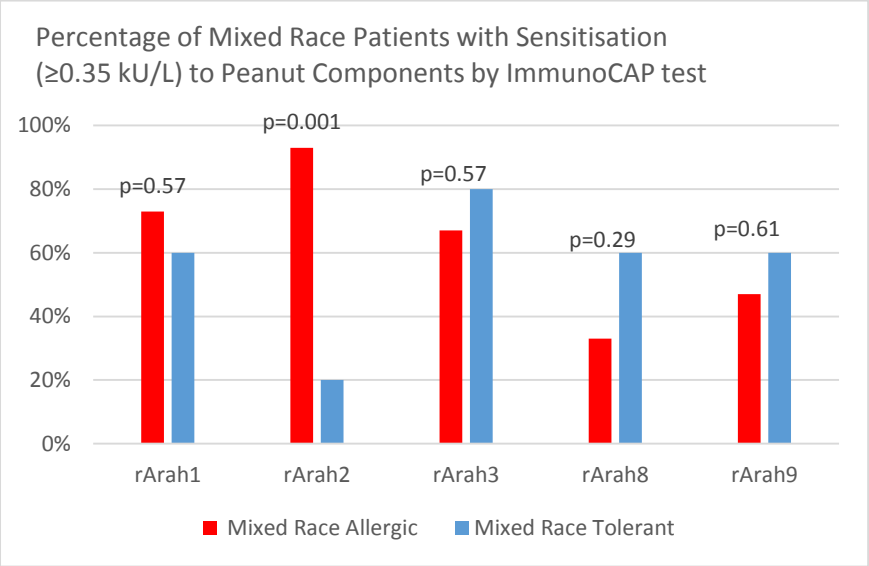


Figure 7.15 Component patterns by ImmunoCAP test in peanut-sensitised mixed race patients



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7.3.5 Cross Reactivity Patterns

ImmunoCAP *Ara h 8* was significantly more positive in Timothy grass sensitised patients: *Ara h 8* was positive in 53% (10/19) with Timothy grass sensitisation and in 16% (4/25) of those without Timothy sensitisation (p=0.009).

There was no significant difference in reactivity to bromelin (a marker of a cross-reactive carbohydrate determinant (CCD)) between peanut allergic and tolerant patients in both Xhosa and mixed race patients (table 7.12).

7.3.6 Comparing ISAC versus ImmunoCAP tests for peanut allergy

The ISAC test proved less sensitive but more specific for peanut allergy in comparison with the ImmunoCAP tests. In the peanut sensitised subgroup (n=44), in which both ISAC and ImmunoCAP test were performed, these 2 tests could be compared. For peanut allergic patients, 88% tested positive to at least one ISAC components, and 83 % for ISAC *Ara h 2*; whilst 96% tested positive to any ImmunoCAP component, and 92 % to ImmunoCAP *Ara h 2* (p=0.35). For peanut tolerant patients, 40% tested positive to at least one ISAC component and 25% to ISAC n*Ara h 2*, whilst 84% tested positive to any ImmunoCAP component and 40 % to ImmunoCAP *Ara h 2* (p=0.31). With comparison of proportions positive by ISAC versus ImmunoCAP, the only statistically significant result was attained for *Ara h 3*, for which tolerant patients were positive in 10% of cases for ISAC *Ara h 3*, and in 60% of cases for ImmunoCAP *Ara h 3*, p<0.001. Testing by ISAC components alone would have missed 3 cases (12.5%) of peanut allergy.

Median values for ImmunoCAP component levels were significantly higher than ISAC values for both allergic and tolerant patients for both *Ara h 2* and *Ara h 3*; for *Ara h 1* the difference was only significant in tolerant patients (table 7.13). The median value for *Ara h 2* by ImmunoCAP in peanut allergic patients was 15.25 kU/L, versus 3.6 ISU by the ISAC test (p<0.001). In tolerant patients, the median value for *Ara h 2* by immunoCAP patients was 0.21 kU/L, versus 0 ISU by the ISAC test (p<0.001).

In our study, the ISAC and ImmunoCAP tests are therefore not directly comparable.

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Table 7.13 Median values of RAST versus ISAC values in peanut-sensitised patients

	Immuno-CAP r*Ara h 1*: median value in kU/L (IQR)	**ISAC n*Ara h 1*: median in ISU (IQR)**	**Difference between CAP and ISAC (p -value)***	**Immuno-CAP r*Ara h 2*: median in kU/L (IQR)**	**ISAC n*Ara h 2* median in ISU (IQR)**	**Difference (p -value)***	**Immuno-CAP r*Ara h 3*: median in kU/L (IQR)**	**ISAC n*Ara h 3*: median in ISU (IQR)**	**Difference (p-value)***
Overall Allergic	1.73 (0.22-6.8)	0.4 (0-2.15)	p<0.001**	15.25 (3.3-25.5)	3.6 (0.7-9.15)	p<0.001**	0.51 (0.17-3.53)	0 (0-1.15)	0.007**
Overall Tolerant	0.11 (0.01-0.29)	0 (0-0.5)	0.21	0.21 (0.1-0.97)	0 (0-0.25)	p<0.001**	0.11 (0.04-0.95)	0 (0-0)	0.02**

IQR: Interquartile Range

* By Mann-Whitney Test ** statistically significant

7.3.7 Epitope diversity and peanut allergy

Diversity of IgE binding to peanut allergens has been proposed as being more predictive of peanut allergy and severe peanut allergy.[30] In our study, the combination of *Ara h 1* and 2 positivity gave the best overall predictive value for peanut allergy at 80% (56% for Xhosas and 100% in mixed race patients). If *Ara h 1* and 2 were positive, and *Ara h 9* was negative, the predictive value became 100% for peanut allergy in both ethnic groups (table 7.14)

Table 7.14 Combination of components giving best predictive values

	Overall: % (n)	**Xhosa: % (n)**	**Mixed Race: % (n)**
***Ara h 2* positive (with any other combination)**	73% (22/30)	53% (8/15)	93% (14/15)
***Ara h 1* and 2 positive**	80% (16/20)	56% (5/9)	100% (11/11)
***Ara h 1* and 2 positive, 8 and 9 negative**	100% (9/9)	100% (3/3)	100% (6/6)
***Ara h 1* and 2 positive, 9 negative**	100% (9/9)	100% (3/3)	100% (6/6)
***Ara h 2* positive and *Ara h 9* negative**	82% (14/17)	70% (7/10)	100% (7/7)
***Ara h 2* positive and *Ara h 8* and/or 9 positive**	64% (9/14)	33% (2/6)	88% (7/8)
***Ara h 2* negative and *Ara h 8* and/or 9 positive**	0% (0/9)	0% (0/6)	0% (0/3)

7.3.8 Severe peanut allergy

5 patients (4 of mixed race, 1 Xhosa) had symptoms of severe peanut allergy. All 5 were ImmunoCAP *Ara h 2* and ISAC *Ara h 2* positive, as well as ISAC *Ara h 1* positive; 80% (4/5) were, in addition, ImmunoCAP *Ara h 1* positive and ImmunoCAP *Ara h 3* positive, and 80% (4/5) were ISAC *Ara h 3* positive. Only 40% (2/5) were *Ara h 8* and/or 9 pos. The presence of ISAC *Ara h 1* and ISAC *Ara h 3* in addition to a positive ISAC *Ara h 2* significantly increased the likelihood of the allergy being severe, as depicted in table 7.15.

The median value for ImmunoCAP to whole peanut was significantly higher in those with a severe peanut allergy in comparison to those with an allergy but no anaphylactic symptoms (88 kU/L versus 12.1 kU/L, p=0.04). Similarly, the median value for ImmunoCAP *Ara h 2* was significantly higher (64.5 kU/L versus 8.98 kU/L, p=0.01) and the median ISAC to *Ara h 2* was significantly higher (11.0 ISU versus 2.2 ISU, p=0.009) in the patients with severe peanut allergy.

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Table 7.15 Component patterns and median values in patients with severe peanut allergy

	Severe peanut allergy	**Peanut allergy, no severe reaction**	**Difference (p- value)**
ISAC *Ara h 2* positive	100% (5/5)	78% (15/19)	0.26*
ISAC *Ara h 1* positive	100% (5/5)	36% (7/19)	0.012*$
ISAC *Ara h 3* positive	80% (4/5)	26% (5/19)	0.027*$
ImmunoCAP *Ara h 1* positive	80% (4/5)	68% (13/19)	0.61*
ImmunoCAP *Ara h 2* **positive**	100% (5/5)	94% (18/19)	0.6*
ImmunoCAP *Ara h 3* positive	80% (4/5)	47% (9/19)	0.19*
ImmunoCAP *Ara h 8* positive	40% (2/5)	26% (5/19)	0.55*
ImmunoCAP *Ara h 9* positive	40% (2/5)	36% (7/19)	0.9*
ImmunoCAP *Ara h 1* and 2 positive	80% (4/5)	68% (13/19)	0.61*
ImmunoCAP Arah 1,2 and 3 positive	80% (4/5)	47% (9/19)	0.19*
Median ImmunoCAP to peanut (kU/L)	88 (IQR 19-99)	12.1 (IQR 3.3-53)	0.04++$
Median ImmunoCAP *Ara h 2* (kU/L)	64.5 (IQR 41.7-68.8)	8.98 (IQR 1.66-17.1)	0.01++$
Median ISAC *Ara h 2* (ISU)	11.0 (IQR 7.4-15)	2.2 (IQR 0.6-5.7)	0.008++$
Median SPT size (mm)	12 mm	13 mm	0.45++

* by chi-squared test

++ by Mann Whitney test

$ statistically significant

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7.3.9 Value of internationally derived 95% positive predictive values in each ethnic group

The sensitivities, specificities and positive predictive values in diagnosing peanut allergy were analysed by ethnicity at the levels of internationally derived SPT and specific IgE widely used as 95% predictive for peanut allergy: these levels were 8mm for SPT to peanut extract, [27] 14kU/L for ImmunoCAP to Peanut,[28] and 0.35 kU/L for ImmunoCap r*Ara h 2*[15] (table 7.16). Overall, these cut-off values proved useful in the mixed race population (PPV 88%, 93% and 100% respectively for SPT 8mm, ImmunoCAP peanut 14 kU/L and *Ara h 2* 0.35 kU/L) but of significantly less predictive value in the Xhosa population (80%, 57% and 53% respectively).

For the skin prick test to whole peanut extract, the cut-off point producing the highest combination of sensitivity and specificity, and thus the highest rate of correct classification into allergic versus tolerant, was 9mm for patients of mixed race (100% sensitivity and specificity), and 11 mm for Xhosa patients (at which level sensitivity was 78%, specificity was 98%, and 95% of patients were correctly classified). In analysing the cut-off points producing the highest PPV for allergies, at a SPT of 9mm, the PPV was 100% for mixed race patients and 80% for Xhosas; at a SPT of 11mm the PPV in Xhosas was 88% and at 15 mm, it was 100%.

For peanut specific IgE, best performance overall for Xhosa patients was at 6.66 kU/L (sensitivity 78%, specificity 73%, correctly classifying 75% of patients) and for mixed race patients at 1.68kU/L (sensitivity 100%, specificity 60%, correctly classifying 90%). Looking at PPVs, in mixed race patients the PPV was 90% at a level of 14 kU/L and 100% at 15 kU/L (versus 57% in Xhosas for both levels). For Xhosas, the maximum PPV of 66% was attained at a level of 65 kU/L.

For ImmunoCAP r*Ara h 2*, in Xhosas the optimal cut-off point was 6.43 kU/L, which had sensitivity of 67%, specificity of 87% and correctly classified 79% of patients. For mixed race patients, the optimal cut-off came at 0.38 kU/L, which had sensitivity 93%, specificity 80%, and correctly classified 90%. At a level of 0.38 kU/L, in the mixed race population this produced a 93% PPV, versus 53% in Xhosas; for the Xhosa population the maximum PPV of 80% was attained at a level of 8 kU/L.

For ISAC *Ara h 2*, in Xhosas the optimal level was 2.2 ISAC units/L (sensitivity 56%, specificity 98%, correctly classifying 92%) and for mixed race patients 0.8 ISAC units/L (sensitivity 87%, specificity 100%, correctly classifying 95%). Taking a cut-off level of 0.3 ISAC units/L, the PPV value in the mixed race population was 100% versus 58% in Xhosas. For the Xhosa population, a maximum PPV of 83% was attained at a level of 1.8 ISAC U/L.

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Table 7.16: Comparison of use of widely used 95% PPVs in Xhosa versus mixed race patients

	Sensitivity	**Specificity**	**Positive Predictive Value for Allergy**
Skin Prick Test to Peanut ≥8mm			
Xhosa	89%	96%	80%
Mixed Race	100%	92%	88%
Difference (p-value)*	0.03**	0.4	0.29
ImmunoCAP Peanut≥14 kU/L			
Xhosa	44%	80%	57%
Mixed Race	60%	80%	90%
Difference (p-value)*	0.29	1.0	0.02**
ImmunoCAP r*Ara h 2* ≥0.35 kU/L			
Xhosa	89%	53%	53%
Mixed race	93%	80%	93%
Difference (p-value)*	0.65	0.06	0.004**
ISAC n*Ara h 2* ≥0.3 ISAC U/L			
Xhosa	78 %	90 %	58%
Mixed Race	87%	100 %	100%
Difference (p-value)*	0.44	0.04**	0.001**

*by chi-squared test

** statistically significant

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7.4 Discussion

This is the first study in South Africa to explore challenge-proven peanut allergy, as well as component patterns. The study was performed in children with moderate to severe atopic dermatitis, a population at higher risk of peanut allergy than the general population. Overall, sensitisation and allergy rates in both ethnic groups were higher than previously shown in this setting, being equivalent to or higher than those in studies of peanut allergy in patients with moderate to severe eczema from developed countries.[31-33] Early onset eczema was a risk factors for peanut allergy. The 5 patients with severe peanut allergy tended to be sensitised to multiple storage proteins (*Ara h 1*, 2 or 3) and had higher median values for specific IgE to whole peanut and *Ara h 2*, but not for SPT size.

Allergy rates were significantly higher in mixed race patients (38%) as compared with Xhosa patients (15%), despite similar sensitisation rates.

The reasons for the lower peanut allergy rates despite similar sensitisation rates in Xhosa patients are unclear. Previous population studies have postulated cross reactivity to Timothy grass[34] as a cause of falsely raised peanut specific IgEs. In our population of peanut sensitised patients, timothy grass sensitisation was actually higher in the mixed race group (55%) versus Xhosas (33%), so this does not provide a plausible explanation for ethnic differences. Moreover, our results do not concur with a recently published study in Ghanaian school children in which IgE against peanut was strongly correlated with IgE against CCD, concluding that parasite- induced IgE against CCD may account for high levels of asymptomatic peanut sensitisation in this population.[35] Conversely, in our study there were no significant ethnic differences in reactivity to CCD amongst peanut sensitised patients, and indeed no difference in CCD levels between sensitised but tolerant versus peanut allergic patients. Total IgE levels, and timing of peanut introduction and peanut consumption patterns, did not differ significantly between ethnic groups.

Thus the reasons for the relative "protection" of Xhosa patients against expression of allergy are not known, but the concern is that the protective factors may be waning. The rapid increase in peanut allergy prevalence over one generation in Asian children in the HealthNuts study is a concerning demonstration of the potential to epigenetic changes which may be secondary to modifiable lifestyle factors. African populations are at risk of epigenetic influences as they take on a westernised diet and lifestyle.

Components measured in this study included *Ara h 1,2,3,8* and *9*. *Ara h 1*-3 are peanut storage proteins, shown in previous studies to be the major allergens among allergic patients.[36] *Ara h 8* is a Betv1 homologous PR 10 protein, the major allergen in concurrent peanut and birch allergies or grass sensitisation (Cynd1, Phlp1 and Phlp12.) [34] *Ara h 9* is a lipid transfer protein which is a pan-allergen.

In this study, the patterns of component reactivity between the 2 ethnic groups were similar, with *Ara h 2* being the superior component in both ethnic groups for differentiating true allergy from tolerance. Our study concurs with previous studies that *Ara h 2* seems to be the most important peanut allergen.[14-20,36] *Ara h 2* by ImmunoCAP as well as ISAC test differed significantly between asymptomatic and symptomatically sensitised patients in both ethnic groups with respect to frequency of positivity and level. The proportion of patients who were ImmunoCAP *Ara h 2* positive was 89% in peanut allergic Xhosas and 93% in allergic mixed race patients; similar to recent studies in

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China[37] which showed 87% positivity and Japan[19] which showed 88% positivity to *Ara h 2.* However, the component reactivity amongst asymptomatically sensitised patients was significantly higher in the Xhosa population than the mixed race patients. Therefore in Xhosa patient sensitised to *Ara h 2*, the probability of having a peanut allergy was significantly lower (53% if *Ara h 2* positive by ImmunoCAP and 58% if *Ara h 2* positive by ISAC) compared with the mixed race group (93% by ImmunoCAP and 100% by ISAC). Food challenges may therefore be of particular importance in Xhosa patients with sensitisation to peanut.

Little additional benefit was shown from ImmunoCAP *Ara h 1*, and *Ara h 3*, which were not significantly higher in allergic patients than tolerant patients in either ethnic group. ISAC *Ara h 1* and *Ara h 3* were significantly higher in allergic patients in the mixed race group only. However, if *Ara h 1* and *Ara h 2* were both positive, the risk of true peanut allergy versus asymptomatic sensitisation was higher. ImmunoCAP *Ara h 8* or 9 reactivity in the absence of *Ara h 2* reactivity was highly suggestive of tolerance despite positive skin prick test or ImmunoCAP to peanut.

Ninety-five percent positive predictive values (95% PPV) have been developed as a surrogate to oral food challenges to avoid laborious and sometimes potentially dangerous food challenges; also to minimise the over- diagnosis of food allergy based on laboratory results alone.[38] Although there is some variation in the international literature of PPVs for peanut allergy, a specific IgE level of ≥14 kU/L is commonly used, [28] as is a SPT value of over 8 mm.[27] In a recent British study, an excellent performance of r*Ara h 2* ImmunoCAP was attained with 97.5% of the 80 included peanut allergic patients correctly classified as peanut allergic versus tolerant at a cut off of 0.35kU/L. [14,15]

However, 95% PPVs may be age- and population-specific. [12,13] The HealthNuts study, performed in infants in Australia, recently showed that a SPT of 8 mm had a PPV of 95% in this population, similar to previous studies, however the serum IgE with a 95% PPV for peanut allergy was higher than previously quoted, at 34 kUa/L. [13]

Our study shows that 95% PPV for peanut may differ significantly between ethnic groups in the same geographical area. Cut off levels for SPT of 8mm, ImmunoCAP Peanut of 14 kU/L and ImmunoCAP *Ara h 2* of 0.35 kU/L produced good PPVs in the mixed race group: 88%, 93% and 100% respectively. However, at the same levels, PPV in the Xhosa group were far lower at 80%, 57% and 53% respectively. These findings suggest that 95% PPVs may have to be tailored to the ethnicity of the patient, and larger studies in unselected population may be needed to determine 95% PPVs amongst different ethnic groups.

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The use of ISAC technology offers a wider sensitisation profile for each patient, and may have significant impact on patient management in terms of risk assessment and diagnosis of cross-reactivity. However, our study shows that the ISAC technology may be less sensitive than ImmunCAPs in peanut allergy although these two technologies have in the past shown high concordance in the measurement of IgE to peanut allergens.[29] Our results show that the ISAC ImmunoCAP test has lower sensitivity (87.5% vs 100%), higher specificity (60% v 40%) and generally lower values than the ImmunoCAP test, and missed 12.5% cases of peanut allergy. We therefore advocate that ISAC and ImmunoCAP tests are not equivalent or interchangeable.

7.5 Conclusion

Peanut allergy may be increasing in South African children, including Xhosa patients, in whom peanut allergy was previously thought to be rare. However, despite equivalent sensitisation rates, peanut allergy rates are still lower in Xhosa than in mixed race patients, for reasons which are incompletely understood. In Xhosa patients, sensitisation to peanut (including *Ara h 2*) is significantly less likely to equate to true allergy than in mixed race patients. Internationally derived 95% PPVs for peanut allergy perform sub-optimally in Xhosa patients. The component *Ara h 2* is the most valuable for differentiating sensitisation from allergy in both ethnic groups; little added benefit is derived from measuring *Ara h 1* and 3; and *Ara h 8* and 9 are associated with tolerance.

Further exploration of ethnic differences in peanut allergy rates and possible factors influencing allergy patterns are currently being explored in a large, unselected population of South African children. [39]

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CHAPTER 8:
Egg Sensitisation, Allergy and Component patterns in South African Children with Atopic Dermatitis

ABBREVIATIONS

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SCORAD: Scoring Atopic Dermatitis Score

SPT: Skin Prick Test

ISAC: Immuno Solid Phase Allergen Chip

PPV: Positive Predictive Value

NPV: Negative Predictive Value

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8.1 Introduction

Egg allergy is one of the most common allergies in infants and young children. The estimated prevalence of egg allergy is 0.5-5% in early childhood. [1-6] Owing to the high degree of natural acquisition of tolerance to egg over time, the prevalence of egg allergy is lower in older children and adults at less than 0.5%.[1,4,7]

Atopic dermatitis (AD) is a major risk factor for egg allergy, and in a previous series of food challenges in children with AD, egg allergy was the most common associated allergy, with 2/3 of positive food challenges being to egg. [8]

Previous studies in Europe and the United States have shown the prevalence of egg sensitisation in patients with AD to be 35-73%, and egg allergy 16-22%. [9-12]

Egg allergy and sensitisation have in past studies been shown to be risk factors for aeroallergen sensitisation, especially indoor perennial allergens such as house dust mite, as well as asthma. [13-15] Therefore egg allergy may be an important association with the propagation of the atopic march.

The major allergenic proteins in hen's (*Gallus domesticus*) egg have been designated *Gal d 1-Gal d 5* Most of the allergenic proteins are found in egg white, including: [16, 17]

- Ovomucoid *(Gal d 1):* makes up 11% of the proteins in egg white
- Ovalbumin *(Gal d 2):* makes up 54% of the proteins in egg white
- Ovotransferrin *(Gal d 3):* makes up 12% of the proteins in egg white
- Lysozyme *(Gal d 4):* makes up 3.4% of the proteins in egg white

Gal d 5 is the major allergen in egg yolk.

Measurement of specific IgE antibodies to individual egg white components has been shown to predict different clinical patterns of egg allergy.[18] Ovomucoid (*Gal d 1*) is a heat and protease-stable antigen and is the dominant allergen in egg allergy.[19,20] High IgE levels to ovomucoid are associated with persistence of egg allergy.[21] Ovalbumin (*Gal d 2*) is heat labile, and children who are mainly sensitised to ovalbumin tend to tolerate the "baked" (heat denatured) form of egg. [22,23] Sensitisation to a high diversity of egg allergen components has also been found to be correlated with an increased chance of egg allergy.[24]

Quantitative measurements of specific IgE to egg white, either by ImmunoCAP test or by skin prick test (SPT), have been used to create 95% positive decision points for egg allergy. Internationally derived and widely used decision points were described by Sampson (for specific IgE by ImmunoCAP test) [25] and Sporik (for SPT).[26] However, such decision points may be age-dependent and may not be applicable to all populations.[27] Table 8.1 shows a summary of previous studies showing positive predictive values (PPVs) for various levels of SPT, and Table 8.2 shows a summary of previous studies of PPVs for specific IgE (by ImmunoCAP test) to egg white.

Table 8.1: Diagnostic decision points for SPT diameter (egg white) in predicting egg allergy

Study	**SPT (mm)**	**Positive predictive value**
Sampson et al [28]	≥ 3mm	85%
Sporik et al [26]	≥ 7 mm (over 2 year olds) ≥ 5 mm (under 2 years)	100%
Boyano Martinez et al [29]	≥ 3 mm (< 2 years)	93%
Hill et al [30]	≥ 7 mm (over 2 year olds) ≥ 5 mm (under 2 years)	100%
Verstege et al [31]	≥ 13 mm (overall) ≥ 13.3mm (≥ 1 year) ≥ 11.2mm ≤1 year)	95%
Peters et al [32]	≥ 4mm	95%

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Table 8.2: Diagnostic decision points for ImmunoCAP to egg white in predicting egg allergy

Study	ImmunoCAP egg white (kiU/L)	Positive predictive value
Sampson et al [28]	≥ 6.0 kU/L	95%
Sampson et al [25]	≥ 2.0 kU/L ≥ 7.0 kU/L	95%
Boyana-Martinez et al [29]	≥ 0.35 kU/L (< 2 years)	94%
Roehr et al [33]	≥ 17.5 kU/L	100%
Osterballe et al [34]	≥ 1.5 kU/L	95%
Celik-Bilgilli et al [35]	≥ 12.6 kU/L	95%
Komato et al [36]	≥ 25.5 kU/L	95%
Benhamou et al [37]	≥ 7 kU/L	100%
Ando et al [38]	≥ 7.4 kU/L	95%
Peters et al [32]	≥ 1.7 kU/L	95%

The prevalence of egg allergy in South African children with atopic dermatitis is unknown. The EPAAC™ study (Early Prevention of Allergy and Asthma in Children), which looked at sensitisation patterns in children with AD, showed that of 117 South African children participating, 49% were egg-sensitised. [12] This study did not, however, explore challenge-proven egg allergy. Moreover, the egg component patterns in South African children have not been explored previously, and the value of commonly used PPV have not been explored.

The aims of this part of the study were to determine:

- egg sensitisation and allergy patterns
- component patterns in egg allergic and tolerant patients
- the value of positive predictive values (PPVs) for SPT and specific IgE in egg allergy diagnosis
- ethnic difference in egg allergy and sensitisation patterns
- ethnic differences in performance characteristics of tests of food allergy

8.2 Methodology

This was part of the wider study investigating food sensitisation and allergy patterns in South African children with atopic dermatitis, including 59 children of Xhosa origin, and 41 of mixed race origin.

Screening tests for egg allergy were performed in all 100 patients by taking a thorough history of previous reactions, performing SPT to commercial egg white extract (ALK), a modified SPT to fresh

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(raw) egg white, and an Immuno Solid Phase Allergen Chip (ISAC 103®, Phadia) test, which included components *Gal d 1*, 2, 3 and 5. Please see chapter 5 for full study methodology.

In those children who were sensitised to egg by the screening tests (n=54), bloods were sent for ImmunoCAP (Phadia) egg white and ovomucoid (*Gal d 1*). In all patients in whom there was uncertainty regarding egg allergy, an incremental open food challenge was performed as a day case at the Red Cross Children's Hospital. The challenge food was given in the form of scrambled egg, starting with a lip challenge, then incremental increasesevery 15-20 minutes from 0.5g to 30g scrambled egg over 2 hours.

8.2.1 Study definitions

IgE-mediated egg sensitisation was defined as a positive skin prick test to egg white extract or fresh egg white (3 mm or more above the negative control) and/ or positive egg specific IgE by ISAC (≥ 0.3 ISAC units)

IgE-mediated egg allergy was defined as either:

- Positive food challenge
- A convincing clinical history of significant type I allergic reactions after isolated ingestion of egg-containing food in the preceding 6 months, with significantly positive SPT/sIgE above the internationally derived 95% positive predictive value for egg of 7mm for SPT[26] and 7 kU/L for Immuncap.[25]

8.3 Results

All of the patients completed the screening tests and food challenges where indicated, hence screening data for SPT and ISAC components from all 100 participants was utilised. Of the 54 patients who were egg-sensitised, blood from one patient was insufficient, thus ImmunoCAP results to egg and ovomucoid were obtainable from 53 patients.

8.3.1 Egg Sensitisation and Allergy Patterns

Overall, 54 (54%) patients were sensitised to egg, 35/59 (59%) Xhosas and 19/41 (46%) mixed race children, p=0.2. Of the 54 sensitised patients, 48 were sensitised by SPT to fresh egg white, 40 by SPT to egg white extract and 32 to any ISAC component for egg.

Overall, 25 patients (25%) were found to be allergic to egg: 14/59 (24%) Xhosas and 11/41 (27%) mixed race patients (p=0.7). All 25 cases of egg allergy had a positive SPT to fresh egg white. Twenty out of twenty-five (80%) of patients with egg allergy had a positive SPT to egg white extract, hence SPT to egg white extract missed 5/25 (20%) cases of egg allergy. 21/25 (84%) patients with egg allergy

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were positive to any one or more ISAC components to egg, hence ISAC missed 16% of egg allergy cases.

Thirty-one patients underwent an egg challenge, of whom 14 were positive and 17 negative. Eleven patients diagnosed with egg allergy did not have a challenge but had a significant recent history of a reaction and a SPT or specific IgE above the frequently used 95% PPV of 7mm for SPT and 7 kU/L for ImmunoCAP (please refer to table6.4 in chapter 6 for a description of results and reactions of patients who did not undergo an egg challenge).

The median age of patients with an egg allergy was 18 months (interquartile range 15-37 months).

8.3.2 Proportion of egg-sensitised patients who were found to be allergic

Overall, 46% of egg-sensitised patients were found to be allergic. This proportion was 58% in mixed race and 40% in Xhosas, a difference which was considerable but did not reach statistical significance (p=0.08).

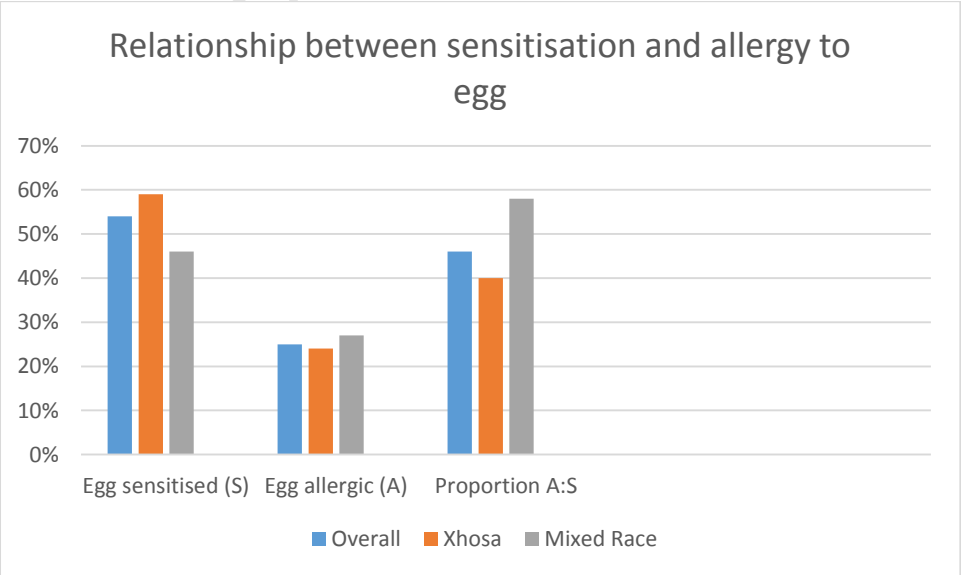
Table 8.3 and figure 8.1 depict egg sensitisation and allergy patterns.

Table 8.3: Egg sensitisation and allergy patterns by ethnicity

	Sensitised to egg	**Allergic to egg**	**Ratio of allergic: sensitised**
Overall (n=100)	54%	25%	46%
Xhosa (n=59)	59%	24%	40%
Mixed race (n=41)	46%	27%	58%
Difference between ethnic groups (p-value)*	0.2	0.7	0.08

*by chi-squared test

Figure 8.1: Egg sensitisation and allergy patterns by ethnicity



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8.3.3 Risk factors for egg allergy

8.3.3.1 Age at the time of assessment:

Egg allergy was significantly higher in the younger age groups (50% in under 2 year olds, 13% in over 2 year olds, p< 0.001). This is likely a reflection of the natural acquisition of tolerance to egg over time.

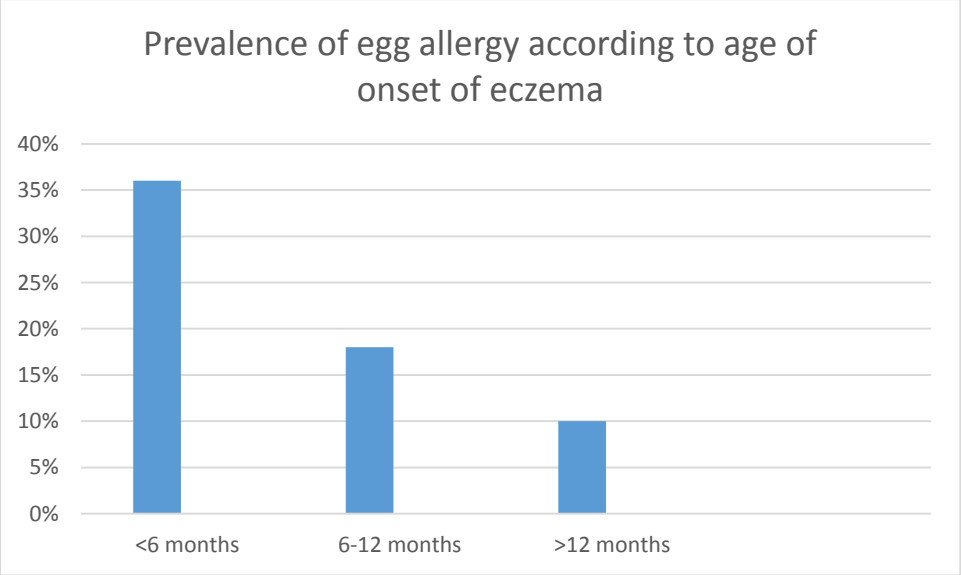
8.3.3.2 Eczema severity:

Of those patients in the moderate eczema severity category (SCORAD 15-40), 6/50 (12%) had an egg allergy; in those with more severe eczema SCORAD > 40, 19/50 (38%) had an egg allergy (p=0.003)

8.3.3.3 Age of onset of eczema:

Of those with early onset eczema (<6 months), 16/36 (44%) had egg allergy; of those with intermediate onset eczema (6-12 months), 6/33 (18%) had egg allergy and of those with later onset eczema (12 months and over), 3/31 (10%) had egg allergy (p=0.001). The effect of age of onset of eczema on egg allergy prevalence is depicted in figure 8.2.

Figure 8.2: Effect of age of onset of eczema on egg allergy prevalence



8.3.4 Value of diagnostic tests in predicting egg allergy in the population overall (n=100)

8.3.4.1 Value of history of past reaction to egg in diagnosis of egg allergy

Forty-three patients (43%) reported one or more symptoms of a reaction to egg (25/59 Xhosas (42%) and 18/41 mixed race (44%), p=0.88). Of these, 24/43 (56%) were found to be allergic, hence in those who gave a history of having reacted to egg, just over half were found to be truly allergic. This ratio of

truly allergic: history positive was 56% for both ethnic groups (14/25 Xhosa and 10/18 mixed race). Five patients (5% overall) reported a severe reaction to egg, of whom 2 were subsequently found to have outgrown their egg allergy. Out of 21 patients who reported "not liking" egg, 12 were found to be allergic (57%).

The vast majority of perceived reactions (39/43: 91%) were immediate or had an immediate component followed by late eczema. The median age at reaction was 12 months (interquartile range 8-18 months).

The most commonly reported symptoms of a reaction to egg, as well as their association with true egg allergy, are described in table 8.4. Itchy mouth, wheeze and tight throat, though uncommon symptoms, were most closely associated with true egg allergy.

Table 8.4: Most Commonly Reported Reactions to Egg

Symptom	**% with this symptom amongst those patients with a history of reaction to egg (n=43)**	**Proportion of patients with this symptom with true egg allergy**
Itchy rash	53% (23/43)	65% (15/23)
"Doesn't like" the food	49% (21/43)	57% (12/21)
Exacerbation of eczema	33% (14/43)	57% (8/14)
Flushing	23% (10/43)	60% (6/10)
Angioedema	19% (8/43)	62.5% (5/8)
Vomiting	16% (7/43)	71% (5/7)
Itchy mouth	14% (6/43)	83% (5/6)
Wheeze	9% (4/43)	75% (3/4)
Tight throat	2% (1/43)	75% (3/4)
Circulatory compromise (blue lips, shock)	0%	
Diarrhoea	0%	

8.3.4.2 Value of history together with positive sensitisation in prediction of egg allergy

36 patients (36%) had an egg-positive history as well as positive sensitisation to egg white, of whom 24 (67%) were found to be allergic; this ratio was 14/22 (64%) in Xhosas and 10/14 (71%) in mixed race patients, p=0.66. Therefore, in the population overall, if one had a positive history of a reaction to egg accompanied by sensitisation to egg, there was a 67% chance of an egg allergy.

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Table 8.5 shows the value of history, as well as history together with sensitisation to egg, in the diagnosis of egg allergy.

Table 8.5 Value of history of reaction to egg, coupled with sensitisation, in prediction of true egg allergy

	Overall (n=100)	**Xhosa (n=59)**	**Mixed Race (n=41)**	**Difference between ethnic groups (p-value)**
Positive history of any reactivity to egg	43% (43)	42% (25/59)	44% (18/41)	0.84
% of those with positive history who were found to have a true allergy	56% (24/43)	56% (14/25)	56% (10/18)	1.0
Positive history + sensitised to egg	36% (36)	37% (22/59)	34% (14/41)	0.76
% of those with positive history + sensitisation who were found to have a true allergy	67% (24/36)	64% (14/22)	71% (10/14)	0.66

8.3.4.3 Sensitivity, specificity and Receiver Operating Characteristic (ROC) Curves for SPTs and ISAC tests as screening tests for egg allergy (n=100)

All 100 patients participating in the study underwent screening tests to egg allergy by SPT to egg extract and fresh egg white, as well as ISAC 103 test to egg components *Gal d 1,2,3* and *5.*

Receiver Operating Curves (ROC curves) showed that the size of the SPT to fresh egg white was superior in the prediction of egg allergy (ROC area under the curve (AUC) 0.92).This was followed by ISAC to *Gal d 1* (ROC AUC 0.84) and SPT to egg white extract (ROC AUC 0.79), with poor performance of components *Gal d 2*, 3 and 5 (ROC AUC 0.69, 0.67 and 0.66 respectively). The difference in ROC AUC between fresh egg white and egg white extract SPT was significant (p=0.005). There was no significant ethnic difference in the pattern of the ROC prediction curves.

ROC curves for various screening tests in the overall study population are depicted in table 8.6 and figures 8.3, 8.4 and 8.5.

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Table 8.6: ROC area under curves for SPT and ISAC components in predicting egg allergy in all patients (n=100)

	Overall (n=100)	**Xhosa (n=59)**	**Mixed Race (n=41)**	**Difference between ethnic groups (p-value)**
SPT fresh raw egg white	0.92	0.90	0.94	0.36
SPT egg white extract	0.79	0.76	0.83	0.47
ISAC n*Gal d 1*	0.84	0.80	0.90	0.26
ISAC n*Gal d 2*	0.69	0.71	0.67	0.7
ISAC n*Gal d 3*	0.67	0.60	0.76	0.15
ISAC n*Gal d 5*	0.66	0.61	0.71	0.35

Figure 8.3: ROC curves for SPT and ISAC components in overall population (n=100)

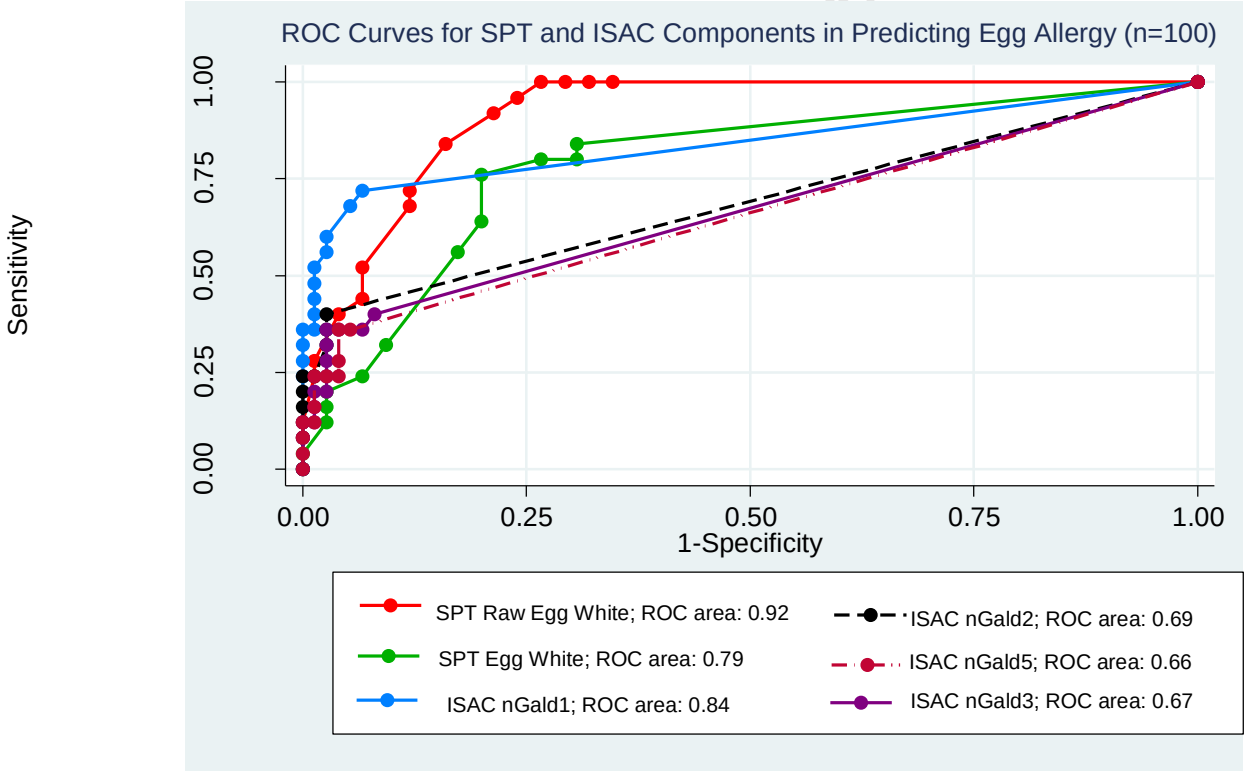


Figure 8.4: ROC curves for SPT and ISAC components for Xhosa patients (n=59)

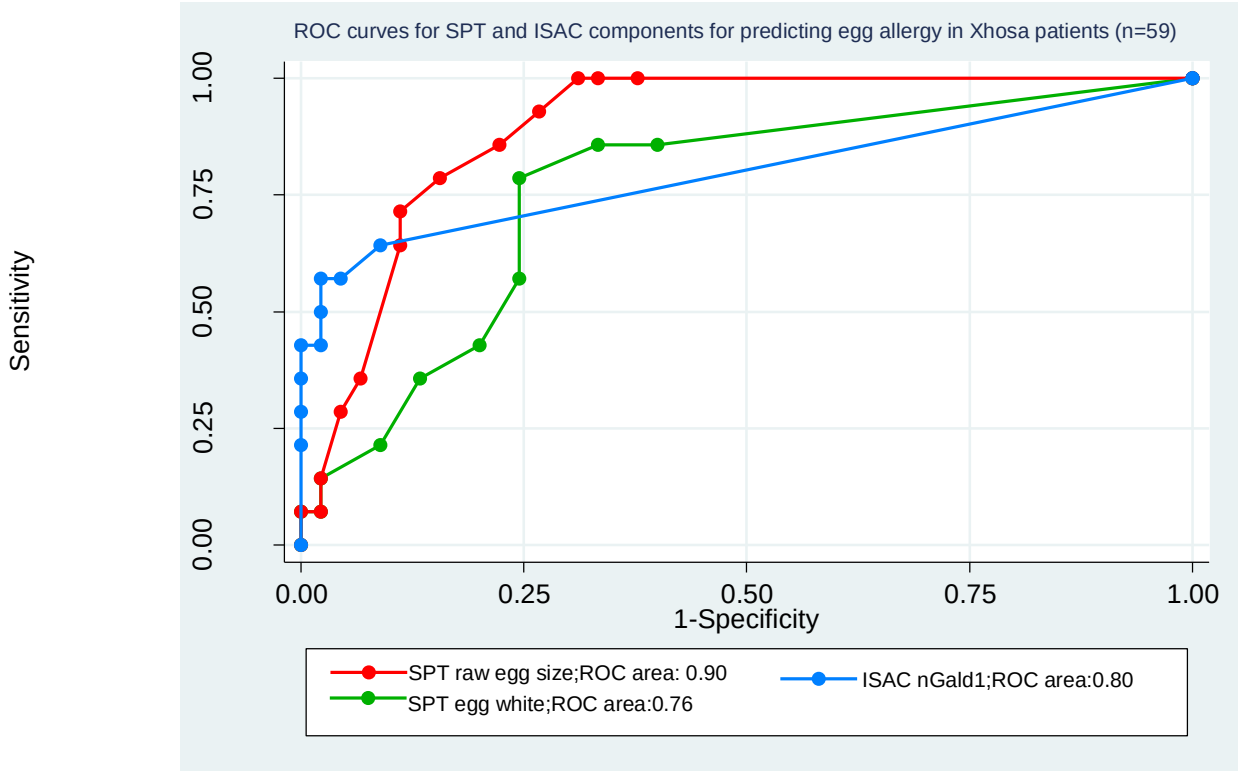
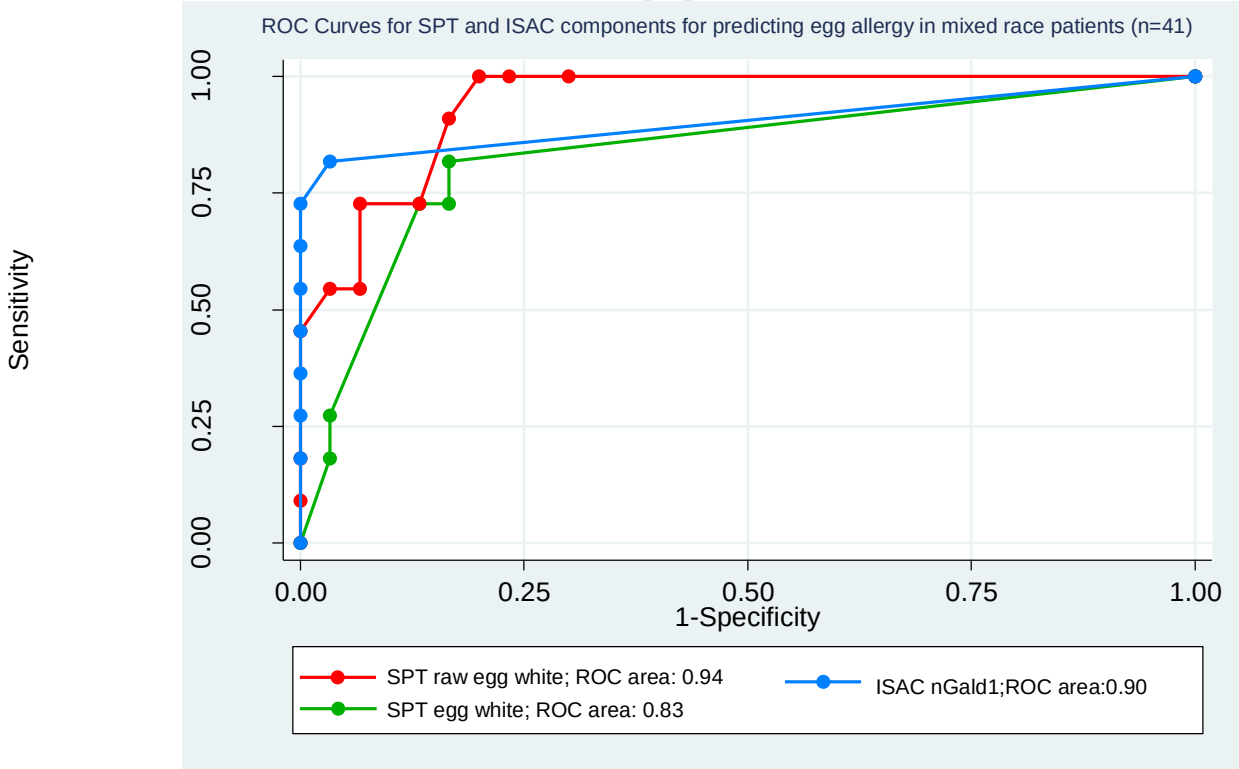


Figure 8.5: ROC curves for SPT and ISAC components for mixed race patients (n=41)



The SPT to fresh egg white was the most sensitive screening test for egg allergy (100% sensitive in both ethnic groups), but positive predictive value (PPV) for egg allergy was poor (PPV 52 % overall). SPT to egg white extract performed sub-optimally in both sensitivity (80%) and specificity, with PPV for egg allergy 50%. ISAC to *Gal d 1* had poor sensitivity (72%) but good specificity (93%), and the best PPV of all the screening tests (78% overall, 69% in Xhosas and 90% in mixed race patients).

The overall trend for all 3 screening tests was towards a lower specificity and PPV in Xhosa patients, thus Xhosa patients had more false positive results. Sensitivity, specificity as well as PPV and negative predictive values (NPV) for SPTs and ISAC *Gal d 1* are summarised in table 8.7.

Table 8.7: Overall sensitivities and specificities in predicting egg allergy

	Overall (n=100)	**Xhosa (n=59)**	**Mixed race (n=41)**	**Difference (p-value)***
SPT egg white extract positive (≥3mm)				
Sensitivity	80%	86%	73%	0.11
Specificity	73%	67%	83%	0.08
PPV	50%	44%	62%	0.08
NPV	92%	94%	89%	0.37
SPT fresh egg white positive (≥3mm)				
Sensitivity	100%	100%	100%	1.0
Specificity	69%	67%	73%	0.52
PPV	52%	48%	58%	0.34
NPV	100%	100%	100%	1.0
ISAC *Gal d 1* positive (≥ 0.3 ISAC U/L)				
Sensitivity	72%	64%	82%	0.052
Specificity	93%	91%	97%	0.24
PPV	78%	69%	90%	0.01**
NPV	91%	89%	94%	0.39

*By chi-squared test

** statistically significant

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8.3.4.4 Sensitivity, specificity and Receiver Operating Curves (ROC) for SPTs and ISAC tests in predicting egg allergy in egg-sensitised patients (n=54)

Those patients who who found to be egg-sensitised by screening SPT/ISAC tests underwent ImmunoCAP screening to Egg White and Ovomucoid (*Gal d 1*). Of the 54 egg-sensitised patients, 53 completed these additional tests and will be included in the analyses of egg-sensitised patients.

Receiver Operating Curves (ROC curves) in egg-sensitised patients (n=53) showed that the size of the ImmunoCAP test to *Gal d 1* was superior in the prediction of egg allergy (ROC area under the curve 0.83). This was followed closely by ISAC to *Gal d 1* (ROC area under curve 0.81), SPT to raw egg white (0.78), and ImmunoCAP to egg white (0.77). The difference in ROC AUCs for ImmunoCAP *Gal d 1*, ISAC *Gal d 1*, SPT raw egg and ImmunoCAP egg white was not significant at p=0.81, hence these tests had comparable value in differentiating egg allergy from tolerance in sensitised patients. SPT to egg white extract performed poorly (AUC 0.60), statistically significantly lower than the other parameters (p=0.005). There were no significant ethnic difference in the pattern of the ROC prediction curves.

ROC curves for various tests in predicting egg allergy in the egg-sensitised population are depicted in table 8.8 and figure 8.6.

Table 8.8 ROC area under curves for SPT, ImmunoCAP and ISAC components size in predicting egg allergy in egg-sensitised patients (n=53)

	Overall (n=100)	**Xhosa (n=59)**	**Mixed Race (n=41)**	**Difference between ethnic groups (p-value)**
SPT fresh egg white	0.78	0.75	0.80	0.66
SPT egg white extract	0.6	0.55	0.68	0.41
ISAC n*Gal d 1*	0.81	0.77	0.89	0.24
ImmunoCAP *Gal d 1*	0.83	0.85	0.83	0.96
ImmunoCAP Egg White	0.79	0.84	0.74	0.51

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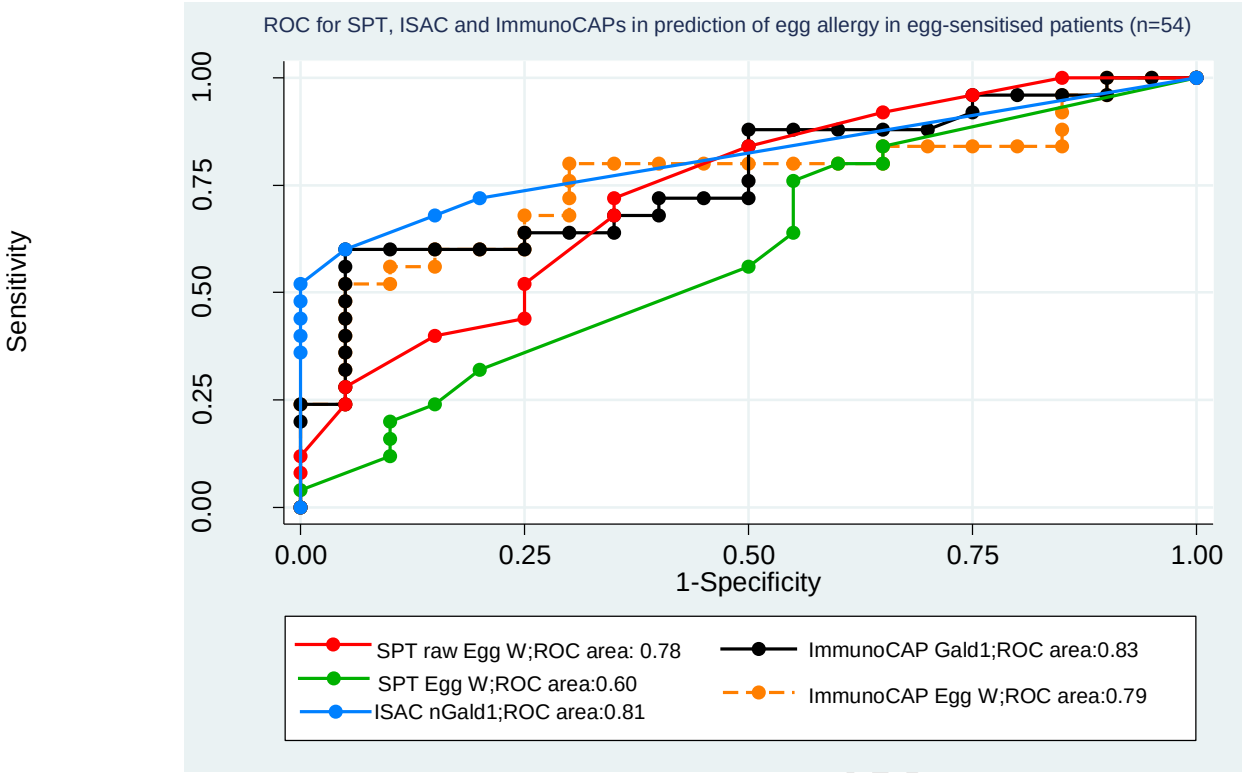
Figure 8.6: ROC curves for SPT and ISAC components in egg-sensitised patients



In the 53 egg-sensitised patients who underwent further ImmunoCAP tests, the SPT to fresh egg showed the highest sensitivity in diagnosing egg allergy (100% in both ethnic groups) but poor specificity, and a PPV of 54%. A positive ImmunoCAP to egg white also showed high sensitivity of 96% (100% in Xhosas and 91% in mixed race) but poor specificity in both ethnic groups. ISAC test to *Gal d 1* showed poor sensitivity (72%) but the highest specificity (82%) and PPV (78%).

Again, the overall trend for all 3 screening tests was towards a lower specificity and PPV in Xhosa patients. Table 8.9 shows sensitivities and specificities of various SPT, ISAC and ImmunoCAP tests in predicting egg allergy amongst those patients who are egg-sensitised.

Table 8.9: Sensitivities and specificities of tests in predicting egg allergy in patients who are egg-sensitised (n=53)

	Overall (n=53)	**Xhosa (n=34)**	**Mixed race(n=19)**	**Difference (p-value)***
SPT egg white extract ≥3mm				
Sensitivity	80%	86%	73%	0.24
Specificity	39%	32%	56%	0.09
PPV	54%	48%	67%	0.18
NPV	69%	75%	63%	0.36
SPT fresh egg white ≥3mm				
Sensitivity	100%	100%	100%	1.0
Specificity	25%	21%	33%	0.34
PPV	54%	48%	65%	0.23
NPV	100%	100%	100%	1.0
ImmunoCAP egg white≥0.35 kU/L				
Sensitivity	96%	100%	91%	0.08
Specificity	18%	16%	22%	0.59
PPV	51%	47%	59%	0.4
NPV	83%	100%	67%	<0.001**
ImmunoCAP *Gal d 1* ≥0.35 kU/L				
Sensitivity	88%	93%	82%	0.22
Specificity	50%	42%	67%	0.08
PPV	61%	54%	75%	0.13
NPV	82%	89%	75%	0.18
ISAC *Gal d 1* > 0.3 ISAC Units/L				
Sensitivity	72%	64%	82%	0.17
Specificity	82%	79%	89%	0.36
PPV	78%	69%	90%	0.08
NPV	77%	75%	80%	0.68

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8.3.4.5 Median values of SPT, ISAC and ImmunoCAP tests in tolerant versus allergic patients who are egg -sensitised

The median values in allergic versus tolerant egg-sensitised patients were significantly higher for all tests except SPT egg white extract (Table 8.10). For SPT fresh egg white, the overall median value in allergic patients was 13mm, in comparison to that of 8mm in tolerant patients (p<0.001). For ISAC *Gal d 1*, median values in allergic and tolerant patients were 0.9 and 0.0 ISAC units respectively (p<0.001); for ImmunoCAP *Gal d 1* median values were 8 kU/L and 0.32 kU/L respectively (p<0.001), and for ImmunoCAP to egg white median values were 14.6kU/L and 1.54 kU/L respectively (p= 0.003). These trends were similar between ethnic groups with no statistically significant differences. In both ethnic groups, the SPT to egg white extract was not significantly different between allergic and tolerant patients (6mm and 5 mm respectively, p=0.21).

The median SPT to fresh egg was significantly higher than the median SPT value to egg white extract (p<0.001 by Wilcoxon sign-rank test). Moreover, when comparing ImmunoCAP and ISAC median values, the median ImmunoCAP *Gal d 1* was found to be significantly higher than the median ISAC levels (p<0.001 by Wilcoxon sign rank test) in both tolerant and allergic patients. These results suggest that ISAC and ImmunoCAP to egg are not directly comparable.

Table 8.10: Median values of SPT, ISAC and ImmunoCAP tests in allergic versus tolerant patients who are egg-sensitised

	Overall (n=53) Median (interquartile range)	**Xhosa (n=34) Median (interquartile range)**	**Mixed race (n=19) Median (interquartile range)**	**Difference between ethnic groups (p-value)***
SPT egg white in egg-allergic patients	6 mm (4-7mm)	5 mm (4-7mm)	6 mm (1-9mm)	0.68
SPT egg white in egg-tolerant patients	5 mm (0- 6.5mm)	5mm (0-7mm)	0mm (0-6mm)	0.31
Difference between allergic and tolerant patients (p-value)	0.21	0.61	0.15	
SPT fresh egg white in egg- allergic patients	13mm (10-17mm)	12 mm (10-15mm)	15 mm (10-20mm)	0.14
SPT fresh egg white in egg-tolerant patients	8 mm (2.5-12mm)	8 mm (5-12mm)	10 mm (0-12mm)	0.7
Difference between allergic and tolerant patients (p-value)	P<0.001**	0.01**	0.02**	
ImmunoCAP egg white in egg- allergic patients	14.6 kU/L (5.15-53.6 kU/L)	14.34 kU/L (5.15-100 kU/L)	14.6 kU/L (0.56-53.6 kU/L)	0.87
ImmunoCAP egg white in egg-tolerant patients	1.54 kU/L (0.68-3.72 kU/L)	1.51 kU/L (0.65-3.57 kU/L)	1.79 kU/L (0.7-8.5 kU/L)	0.92
Difference between allergic and tolerant patients (p-value)	0.003**	0.001**	0.06	
ImmunoCAP *Gal d 1* in egg- allergic patients	8 kU/L (1.33-52.6 kU/L)	7.2 kU/L (2.1-100 kU/L)	11.7 (0.85-45)	0.43
ImmunoCAP *Gal d 1* in egg- tolerant patients	0.32 kU/L (0.01-2.06 kU/L)	0.53 kU/L (0.01-3.48 kU/L)	0.17 kU/L (0.02-2 kU/L)	0.57
Difference between allergic and tolerant patients (p-value)	P<0.001**	P<0.001**	0.001**	
ISAC n*Gal d 1* in egg-allergic patients	0.9 ISU/L (0-2.5 ISU/L)	1.3 ISU/L (0-7.7 ISU/L)	0.7 ISU/L (0.4-2.2 ISU/L)	0.76
ISAC n*Gal d 1* egg-tolerant patients	0 ISU/L (0-0)	0 ISU/L (0-0)	0 ISU/L (0-0)	0.44
Difference between allergic and tolerant patients (p-value)	P<0.001**	0.004**	0.002**	

*by Mann-Whitney test ** statistically significant

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8.4 Egg component patterns

8.4.1 Egg component patterns in overall study population (n=100)

In the population overall (n=100), the most common egg component detected by ISAC (103) analysis was *Gal d 1* (23%), followed by *Gal d 3* (16%), *Gal d 5* (13%) and *Gal d 2* (12%). This pattern was similar between both ethnic groups (table 8.11).

In the overall population, the proportion of patients with positive reactivity to the component *Gal d 1* was significantly higher in egg allergic (72%) versus egg tolerant patients (7%), p<0.001. Significant differences between allergic and tolerant patients (p<0.001) were also found for all of the other egg components: for *Gal d 2* 40% versus 3%; for *Gal d 3* 40% versus 8%; for *Gal d 5* 36% versus 5%. (table 8.12)

Table 8.11: Egg component patterns by ISAC test in the population overall (n=100)

	Overall (n=100) % positive (n)	**Xhosa (n=59) % positive (n)**	**Mixed (n=41) % positive (n)**	**Difference between ethnic groups (p-value by chi^2 test)**
ISAC n*Gal d 1*	23% (23/100)	22% (13/59)	24% (10/41)	0.78
ISAC n*Gal d 2*	12% (12/100)	12% (7/59)	12% (5/41)	0.96
ISAC n*Gal d 3*	16% (16/100)	14% (8/59)	20% (8/41)	0.43
ISAC n*Gal d 5*	13% (13/100)	12% (7/59)	15% (6/41)	0.69

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Table 8.12: Egg component patterns amongst egg allergic versus tolerant patients in the population overall (n=100)

	Overall (n=100) % positive (n)	**Xhosa (n=59) % positive (n)**	**Mixed race (n=41) % positive (n)**	**Difference between ethnic groups (p-value)***
ISAC n*Gal d 1* in egg-allergic patients	72% (18/25)	64% (9/14)	82% (9/11)	0.33
ISAC n*Gal d 1* in egg-tolerant patients	7% (5/75)	9% (4/45)	3% (1/30)	0.35
Difference between allergic and tolerant patients (p-value)	P<0.001**	P<0.001**	P<0.001**	
ISAC n*Gal d 2* in egg-allergic patients	40% (10/25)	43% (6/14)	36% (4/11)	0.74
ISAC n*Gal d 2* in egg-tolerant patients	3% (2/75)	2% (1/45)	3% (1/30)	0.77
Difference between allergic and tolerant patients (p-value)	P<0.001**	P<0.001**	0.004**	
ISAC n*Gal d 3* in egg-allergic patients	40% (10/25)	29% (4/14)	55% (6/11)	0.19
ISAC n*Gal d 3* in egg-tolerant patients	8% (6/75)	9% (4/45)	7% (2/30)	0.73
Difference between allergic and tolerant patients (p-value)	P<0.001**	0.06	0.001**	
ISAC n*Gal d 5* in egg-allergic patients	36% (9/25)	27% (4/15)	45% (5/11)	0.38
ISAC n*Gal d 5* in egg-tolerant patients	5% (4/75)	7% (3/45)	3% (1/30)	0.53
Difference between allergic and tolerant patients (p-value)	P<0.001**	0.03**	0.009**	

*by chi-squared test
** statistically significant

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8.4.2 Egg component patterns in egg sensitised patients (n=54)

The pattern of component sensitivity were similar in egg sensitised patients (n=54) to that in the population overall, as described in section 8.4.1 above. In egg sensitised patients, the most common positive component was *Gal d 1* (43%), followed by *Gal d 3* (28%), *Gal d 5* (25%) and *Gal d 2* (23%) (figure 8.7). There were no significant ethnic differences in this pattern profile (table 8.13).

Figure 8.7: Egg component patterns in egg-sensitised patients (n=54)

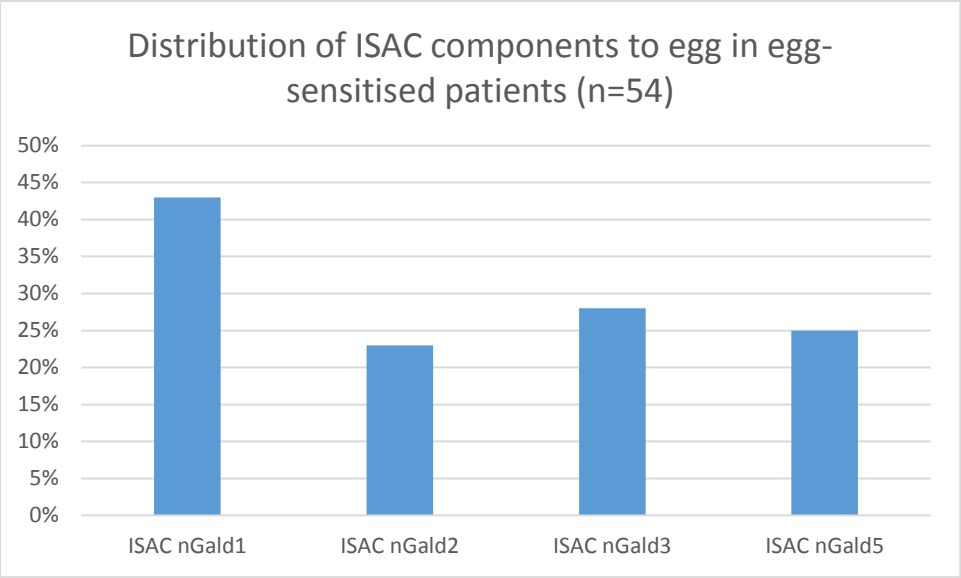

Table 8.13: Egg component patterns in egg-sensitised patients (n=54) by ethnicity

	Overall (n=54) % positive (n)	**Xhosa (n=34) % positive (n)**	**Mixed (n=20) % positive (n)**	**Difference between ethnic groups (p-value) chi²**
ISAC n*Gal d 1*	43% (23/53)	38% (13/34)	53% (10/19)	0.31
ISAC n*Gal d 2*	23% (12/53)	21% (7/34)	26% (5/19)	0.63
ISAC n*Gal d 3*	28% (15/53)	21% (7/34)	42% (8/19)	0.10
ISAC n*Gal d 5*	25% (13/53)	21% (7/34)	32% (6/19)	0.37

In the egg-sensitised population, the proportion of patients with positive reactivity to the component *Gal d 1* was significantly higher in egg allergic (72%) versus egg tolerant patients (18%), p<0.001. This difference in proportion with *Gal d 1* positivity was mirrored by ImmunoCAP *Gal d 1*, which was positive in 88% of those with egg allergy, and 50% of those with tolerance (p=0.03). *Gal d 2* positivity was also significantly higher in egg allergic patients 40% versus 7%. (p=0.004). *Gal d 3* and *Gal d 5* were higher amongst allergic patients, but the difference did not reach statistical significance (40% versus 18% for *Gal d 3*, and 36% versus 14% for *Gal d 5*, p=0.07 in both cases).

There were no significant ethnic differences in component reactivity in egg allergic versus tolerant patients (table 8.14 and figure 8.8).

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Table 8.14: Egg component patterns amongst egg allergic versus tolerant patients who are egg sensitised (n=54)

	Overall (n=53) % positive (n)	**Xhosa (n=34) % positive (n)**	**Mixed (n=19) % positive (n)**	**Difference between ethnic groups (p-value)***
ISAC n*Gal d 1* in egg-allergic patients	72% (18/25)	64% (9/14)	82% (9/11)	0.33
ISAC n*Gal d 1* in egg-tolerant patients	18% (5/28)	20% (4/20)	13% (1/8)	0.64
Difference between allergic and tolerant patients (p-value)	P<0.001**	0.009**	0.003**	
ISAC n*Gal d 2* in egg-allergic patients	40% (10/25)	43% (6/14)	36% (4/11)	0.72
ISAC n*Gal d 2* in egg-tolerant patients	7% (2/28)	5% (1/20)	13% (1/8)	0.49
Difference between allergic and tolerant patients (p-value)	0.004**	0.007**	0.25	
ISAC n*Gal d 3* in egg-allergic patients	40% (10/25)	29% (4/14)	55% (6/11)	0.19
ISAC n*Gal d 3* in egg-tolerant patients	18% (5/28)	15% (3/20)	25% (2/8)	0.53
Difference between allergic and tolerant patients (p-value)	0.07	0.32	0.19	
ISAC n*Gal d 5* in egg-allergic patients	36% (9/25)	29% (4/14)	45% (5/11)	0.41
ISAC n*Gal d 5* in egg-tolerant patients	14% (4/28)	15% (3/20)	12.5% (1/8)	0.86
Difference between allergic and tolerant patients (p-value)	0.07	0.32	0.13	
ImmunoCAP *Gal d 1* in egg-allergic patients	88% (22/25)	93% (13/14)	82% (9/11)	0.4
ImmunoCAP *Gal d 1* in egg-tolerant patients	50% (14/28)	55% (11/20)	38% (3/8)	0.4
Difference between allergic and tolerant patients (p-value)	0.03**	0.02**	0.047**	

*chi-squared test **statistically significant

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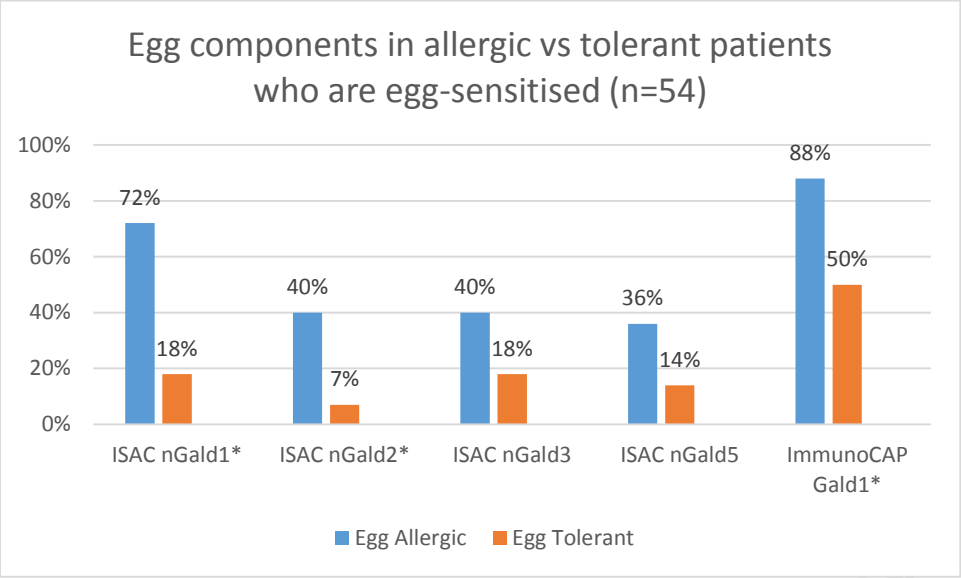
Figure 8.8: Egg component patterns amongst egg allergic versus tolerant patients who are egg sensitised (n=54)



*Denotes statistically significant difference (Chi^2 test)

8.5 Epitope diversity and egg allergy

Our study demonstrates that a greater number of positive ISAC components increases the probability of egg allergy: if any one component was positive, the probability of an egg allergy was 73%; if two or more components were positive, the probability of egg allergy was 85%. If three or more components were positive, there was a 92% probability of egg allergy, and if all 4 ISAC components were positive, there was a 100% probability of egg allergy (figure 8.9). These results are detailed in table 8.15.

Figure 8.9: Epitope diversity and egg allergy

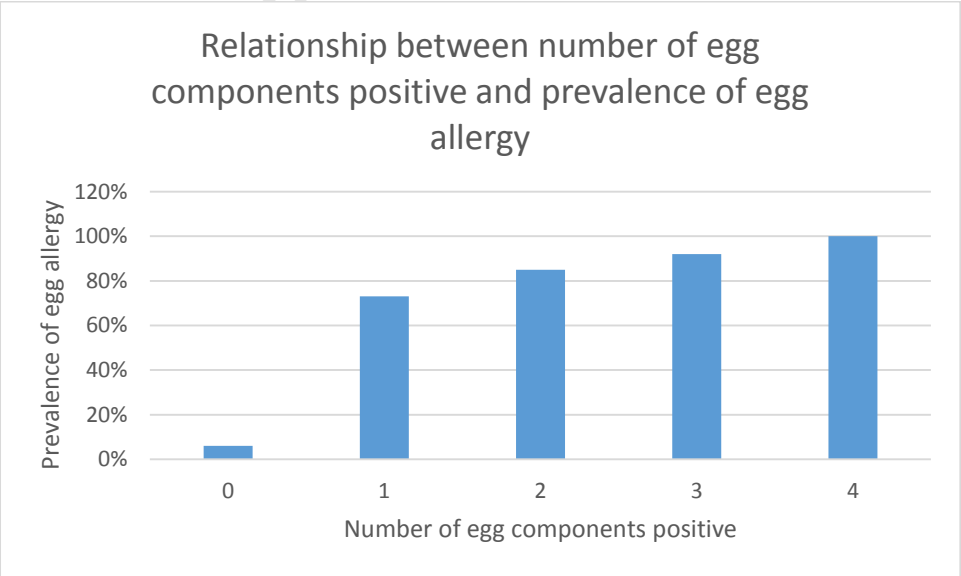


Table 8.15: Role of egg-component diversity in predicting egg allergy

	Proportion of patients with egg allergy if component positive % (n)	
ISAC n*Gal d 1* positive	78% (18/23)	
ISAC n*Gal d 2* positive	83% (10/12)	
ISAC n*Gal d 3* positive	63% (10/16)	
ISAC n*Gal d 5* positive	69% (9/13)	Likelihood of egg allergy if one or more components positive: **73% (47/64)**
***Gal d 1* and 2 positive**	90% (9/10)	
Gald 1 and 3 positive	89% (8/9)	
***Gal d 1* and 5 positive**	100% (8/8)	
Gald 2 and 3 positive	75% (6/8)	
Gald 2 and 5 positive	83% (5/6)	
***Gal d 3* and 5 positive**	73% (8/11)	Likelihood of egg allergy if 2 or more components positive: **85% (44/52)**
***Gal d 1*,2 and 3 positive**	83% (5/6)	
Gald 1,2 and 5 positive	100% (5/5)	
Gald 1,3 and 5 positive	100% (8/8)	
Gald 2,3 and 5 positive	83% (5/6)	Likelihood of egg allergy if 3 or more components positive: **92% (23/25)**
Gald 1,2,3 and 5 positive	100% (4/4)	Likelihood of egg allergy if all 4 components positive: **100% (4/4)**

8.6 Value of 95% Positive Predictive Values (PPVs)

Internationally derived 95% PPVs for a positive food challenge to egg were described by Sporik for SPT[26] and Sampson for specific IgE.[25] Both differentiate between younger children under the age of 2 years (SPT≥ 5mm or specific IgE to egg ≥ 2 kU/L) and older children over 2 years (SPT≥7mm or specific IgE to egg ≥7 kU/L). These values are used widely in South African clinics as guidelines for egg allergy prediction. In this study we have chosen to use the higher of these cut off values: 7mm for SPT and

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7 kU/L for specific IgE to egg white, to assess their value in egg allergy prediction in our study population.

For the population overall, a SPT egg white extract of ≥7 mm had a positive predictive value of 53% (46% in Xhosas and 75% in mixed race patients); a SPT to fresh raw egg white of ≥7mm had a PPV of 57% (52% in Xhosas and 65% in mixed race); and an ImmunoCAP of ≥7 mm had a PPV of 74 % (75% in Xhosas and 73% in mixed race) (table 8.16).

Further analysis looking into optimal values for this population to establish local 95%+ PPV showed for SPT, an optimal PPV of 100% was reached at 12 mm for egg white extract. Overall, for SPT fresh raw egg, the value of 17 mm achieved the highest positive predictive value for the overall study population of 87.5% (at this level PPV was 67% in Xhosas and 100% in mixed race). By ethnic group, maximal PPVs were achieved at values of 21 mm in Xhosas (100%) and 16 mm in mixed race group (100%).

For ImmunoCAP to egg, a value of 13 kU/L obtained a PPV of 93% overall (PPV 88% for Xhosas and 100% for mixed race at this level).

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Table 8.16: Sensitivities, specificities and Positive Predictive Values of tests in predicting egg allergy in egg-sensitised patients (n=53)

	Overall	**Xhosa**	**Mixed race**	**Difference (p-value)***
SPT egg white extract ≥7mm				
Sensitivity	32%	36%	27%	0.5
Specificity	75%	68%	89%	0.09
PPV	53%	46%	75%	0.04**
NPV	55%	59%	50%	0.53
SPT fresh egg white ≥7mm				
Sensitivity	96%	93%	100%	0.24
Specificity	36%	37%	33%	0.77
PPV	57%	52%	65%	0.34
NPV	91%	88%	100%	0.12
RAST egg white≥ 7 kU/L				
Sensitivity	68%	64%	73%	0.50
Specificity	79%	84%	67%	0.15
PPV	74%	75%	73%	0.87
NPV	73%	76%	67%	0.88

*chi-squared test ** statistically significant

8.7 Discussion

In this study of food allergy in children with atopic dermatitis, hen's egg was the most common allergen overall causing sensitisation (54% overall) and allergy (25%), comparable with previous results from westernised countries. [2-9] Similar to previous studies,[8] egg allergy was seen in approximately 2/3 of children with moderate to severe AD with a food allergy (25/40, 63%). Analysis by ethnic group showed that sensitisation rates did not differ significantly between Xhosa and mixed race patients (59% and 46% respectively), neither did allergy rates (24% in Xhosa and 27% in mixed race). Prevalence of egg allergy, specifically in Xhosa patients, was unexpectedly high.

Of all the food allergens tested, egg was the most common cause of sensitisation as well as allergy in Xhosa patients. In mixed race patients, egg was the second most common cause of sensitisation and allergy, with peanut the most common (see chapter 6).

Young age at the time of assessment (< 2 years), age of onset of eczema below 6 months and severe eczema were significant risk factors for egg allergy.

Overall, egg sensitisation by SPT or ISAC test significantly overestimated true egg allergy, with 46% of patients who are sensitised to egg actually having a clinically significant IgE mediated food allergy. This proportion was even lower in Xhosa patients (40% versus 58% in mixed race patients) but the ethnic difference did not reach statistical significance (p=0.08). A patient-reported history of symptoms of egg allergy also significantly overestimated egg allergy. Of the 43% of patients who reported symptoms of an egg allergy, 56% were found to be allergic. Thirty-one patients with egg sensitisation required an egg challenge for clarification of their allergy status (57%). These figures emphasise the risk of over-diagnosing egg allergy significantly by relying on screening allergy tests or patient history alone. Over half of sensitised cases may require a food challenge for confirmation.

As a screening test for egg allergy, a skin prick test to fresh raw egg white was significantly more sensitive than a skin prick test to commercial egg white extract (100% versus 80% respectively). This is in agreement with previously published findings that fresh foods may be more effective for detecting sensitivity to food allergens.[39] A positive ISAC to the egg component *Gal d 1* was specific for egg allergy (specificity 93%) but not adequately sensitive as a screening test (sensitivity 72%). In prediction of egg allergy in the population overall, SPT to fresh raw egg white had the highest receiver operating curve (ROC) area under the curve in both ethnic groups.

In patients who were sensitised to egg (54 of the 100 participants), SPT to raw egg as well as ImmunoCAP to egg white showed the highest sensitivity (100% and 96% respectively), but low specificity and poor positive predictive value (PPV 54% and 51% for egg allergy for SPT raw egg and

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ImmunoCAP egg white respectively). Similar to the analysis in the whole study population, ISAC to *Gal d 1* was specific (82%) but not sensitive (sensitivity 72%) for egg allergy.

In prediction of egg allergy in the egg-sensitised population, ImmunoCAP to *Gal d 1* showed the greatest area under the ROC curve (0.83), followed closely by ISAC *Gal d 1* (0.81), SPT raw egg (0.79) and ImmunoCAP egg white (0.78), with an insignificant difference between these parameters. SPT egg extract performed sub-optimally with a significantly lower predictive value by ROC curve.

Median values for SPT raw egg, ISAC egg *Gal d 1*, ImmunoCAP *Gal d 1* and ImmunoCAP egg white were significantly higher in allergic versus tolerant egg-sensitised patients, for both ethnic groups. In both ethnic groups, SPT egg extract was not significantly different between allergic and tolerant patients.

Egg component pattern analysis was similar between ethnic groups, and found that egg-sensitised patients, *Gal d 1* was the most common component, followed by *Gal d 3*, *Gal d 5* and *Gal d 2*. Even though *Gal d 2* is the most abundant protein in egg white, it is one of the least allergenic in this population. Ovomucoid (*Gal d 1*) is a heat and protease-stable antigen and is the dominant allergen in egg allergy.[19,20] The proportion of positive components in allergic versus tolerant patients was higher for all of the tested components, significantly so for *Gal d 1* and *Gal d 2*.

In this study, the greater the number of positive egg components in a patient, the greater was the probability of egg allergy. This is in keeping with previous studies of component reactivity which have shown that increased component diversity (i.e. larger number of components bound) is a risk factor for egg allergy. [24]

The commonly used PPV of ≥7mm in SPT to egg white produced poor results overall for both egg white extract (53%) and fresh raw egg white (57%). These PPVs were even poorer for Xhosa patients (for egg white extract SPT≥7mm, PPV was 46% for Xhosas and 75% for mixed race patients; for fresh raw egg white SPT≥7mm, PPV was 52% for Xhosas and 65% for mixed race patients). For ImmunoCAP egg white ≥7 kiU/L, the PPV did not vary significantly by ethnicity, but were still suboptimal at 75% for Xhosa and 73% for mixed race patients.

These findings suggest that PPVs may be population specific and even race specific and may require further exploration in the local context to ascertain optimal local 95% PPVs. Furthermore, the vast difference in values producing “optimal PPVs” between skin prick to commercial egg extract and fresh egg white suggest that studies to determine population-specific 95% PPVs must take into account whether fresh or commercial extracts are used.

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8.8 Conclusion

In this population of South African children with moderate to severe AD, the prevalence of egg allergy is high in both Xhosa and mixed race patients, and equivalent to those in westernised countries. [9-12] Egg allergy is significant in South African children, including Xhosa patients, in whom food allergy was previously thought to be rare. SPT to fresh raw egg white is significantly more sensitive than SPT to commercial egg white extract in the diagnosis of egg allergy, thus addition of fresh raw egg white is recommendable in a screening SPT panel for food allergy.

The component *Gal d 1* (ovomucoid) is the superior component in differentiating asymptomatic sensitisation from egg allergy. Internationally derived widely used 95% PPVs for egg allergy perform sub-optimally in both Xhosa and mixed race patients and may need to be revised for our population.

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Chapter 9:

Sensitisation and Allergy Patterns to Cow's Milk, Soya, Wheat, Fish and Tree Nut in South African Children with Atopic Dermatitis

ABBREVIATIONS

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SCORAD: Scoring Atopic Dermatitis Score

SPT: Skin Prick Test

ISAC: Immuno Solid Phase Allergen Chip

PPV: Positive Predictive Value

NPV: Negative Predictive Value

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9.1 Introduction

In this study, hen's egg and peanut were by far the most common sources of sensitisation and proven food allergy, as described in chapters 7 and 8. This chapter serves to explore the sensitisation and allergy patterns of the other foods tested in this study of 100 children of South African origin with atopic dermatitis (AD), namely cow's milk, soya, wheat (flour), fish (cod) and tree nut (cashew). The chapter also describes the characteristics and value of perceived reactions to these foods, and the role of diagnostic tests and component patterns.

Cow's milk allergy is one of the most common allergies in early childhood. The tendency to natural acquisition of tolerance to cow's milk over time accounts for a far lower prevalence of cow's milk allergy in older children and adolescents.[1] The documented prevalence of cow's milk allergy is between 0.3% and 3.5% of young children (under the age of 5),[2-7] less than 1% in older children, and less than 0.5% in adults. [3,8] Cow's milk allergy, along with egg and peanut allergy, is one of the most common allergies in children with AD. Rates of cow's milk sensitisation (19-27%) and allergy (11-15%) were found to be significantly above that of the general population in previous studies of children with AD from Europe and the USA. [9-11]

Cow's (*Bos Domesticus*) milk consists of casein and whey proteins.[12,13] Casein (*Bosd8*) makes up approximately 80% of the total protein in cow's milk and is relatively heat stable. The whey proteins are α-lactalbumin (*Bos d 4*), β-lactoglobulin (*Bos d 5*), bovine serum albumin (*Bos d 6*) and bovine immunoglobulin (*Bos d 7*). The whey proteins make up about 20% of cow's milk protein and are heat

sensitive. Milk-allergic children are often sensitised to several cow's milk proteins. Casein and beta-lactoglobulin specific IgE antibodies are associated with persistent allergy to milk, including heated milk, in milk allergic patients; while undetectable levels indicate tolerance to baked milk products.[14]

Studies have shown that in children over the age of 2 years, a SPT wheal of ≥8mm[15] or milk specific IgE by ImmunoCAP test ≥15ku/L[16] convey a 100% and 95% likelihood respectively that the child will have a positive milk challenge. In children 2 years and younger the corresponding values are an SPT ≥ 6mm and IgE ≥5kU/L. However, these values may vary depending on age, concomitant disease and geographical differences, and have not yet been evaluated in South Africa. [15,17,18] Skin prick tests are excellent at ruling out the diagnosis with a negative skin test having a negative predictive value of ≥95%.[19]

Soya allergy: The prevalence of allergy to the soybean is lower than that to cow's milk, and estimated in the region of 0.4-1.2% of children. [20-21] In children with AD, previous studies in the USA and Europe have found a sensitisation rate to soya of around 5% and allergy rate 0-1%, [9,10,11] similar to studies in unselected populations.[21]

Tolerance acquisition to soya is common in late childhood. Soya (*Glycine Max*) consists of 3 main components, *Gly m 4*, *Gly m 5* and *Gly m 6. Gly m 5* (β-conglycinin) and *Gly m 6* (glycinin) are the most important markers of soya allergy. [22] *Gly m 4* is a *Bet v 1* (birch) homologue and is associated with cross-reactivity. Previous studies have shown that, in patients who have antibodies to the peanut allergen *Ara h 2*, up to 60% have IgE antibodies to soya as a cross-reactive phenomenon, and most of these patients are soya tolerant. [23,24]

Wheat Allergy: Wheat allergy is confirmed in less than 1% of the general population, [6-8,25] however, symptoms to wheat allergy are self-reported in about 4.5% of the population.[26, 27] In studies of children with AD, the prevalence of wheat sensitisation was in the region of 5-10% and the prevalence of wheat allergy around 5%. [9-11]

Wheat (*Triticum Aestivum*) consists of several components, of which *Tri a 19* (omega-5-gliadin) is the most sensitive indication of genuine wheat allergy. [14,28] Other components are *Tri a 18* (Agglutinin), *Tri a gliadin* (crude gliadin), and *Tri a aA* (alpha amylase). There is significant cross-reactivity between wheat and grass pollen, which may lead to over-diagnosis of wheat allergy based on sensitisation alone.[29]

Fish allergy: The prevalence of fish allergy in children is ≤ 0.2% in unselected populations, and in the majority of cases the fish allergy is persistent.[30,31] Fish allergy is one of the few allergies that can develop in adulthood. Studies in children with AD have shown the prevalence of fish sensitisation to

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be around 5% and fish allergy to range from 0-9%,[9-10] so fish allergy generally seems significantly less common than allergies to egg, peanut and cow's milk in AD patients.

The prevalence of fish allergy is grossly overestimated based on history of reactions alone: a South African study showed that only 15-21% of those patients with a perceived fish allergy had proof of allergy by sensitisation or food challenge.[32,33]

The major allergen in cod (*Gadus Callarias*) is the parvalbumin *Gad c 1*. Parvalbumins are typically stable to heat and digestive enzymes, and show a high degree of cross reactivity between fish species.[14] In our study, the screening SPT and ISAC test (*Gad c 1*) tested for cod, which cross reacts frequently with carpfish, salmon, herring, pollack and hake. Hake is one of the most common species associated with fish allergy in South Africa.[32] Hake and cod are closely related as members of the *Gadiformes* group.[34]

Treenut allergy: The prevalence of tree nut allergy varies from 0.2% to 1.4% depending on geographical area.[35,36] Cashew nut is one of the most allergenic tree nuts, and was thus chosen for analysis in this study. Most cases of tree nut allergy are persistent, with only 10-20% outgrowing the allergy. [37] The main allergens in cashew nut (*Anacardium occidentale*) are the *Ana o 1* allergen of the 2S albumin family and the *Ana o 2* legumin-like protein.

9.2 Methodology

This was part of the wider study investigating food sensitisation and allergy patterns in South African children with atopic dermatitis, including 59 children of Xhosa origin, and 41 of mixed race origin.

Screening tests for cow's milk, soya, wheat, fish and cashew nut allergy were performed in all 100 patients. This included taking a thorough history of previous reactions, performing skin prick tests to commercial extract (ALK) to cow's milk, soya and cod fish, a modified SPT to fresh cow's milk, and an Immuno Solid Phase Allergen Chip (ISAC 103®, Phadia) test. The ISAC test included components *Bos d 4, Bos d 5, Bos d 6* and *Bos d 8* for cow's milk; *Glym 4, 5 and 6* for soya; *Tri a gliadin*, *Tri a 18*, *Tri a 19* and *Tri a αA* for wheat, *Gad c 1* for cod and *Ana o 2* for cashew nut. Please see chapter 5 for full study methodology.

In selected patients further blood was sent for ImmunoCAP tests for specific IgE: 15 to whole cow's milk, 10 to casein (*Bos d 8*), 10 to soya, 1 to wheat, 12 to cod and 3 to cashew nut.

In all patients in whom there was uncertainty regarding food allergy, an incremental open food challenge was performed as a day case at the Red Cross Children's Hospital. The challenge food was given in the form of native food: 2% cow's milk, soya milk, pure wheat cereal, grilled hake or whole

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roasted cashew nuts. Food challenges started with a lip challenge, then moved through gradual dose increments every 15-20 minutes to a final dose of 100 mL (cow's milk or soya), 20 g wheat cereal, 40g hake or 15g cashew nut.

9.2.1 Study definitions

IgE-mediated food sensitisation was defined as a positive skin prick test to the food in question (3 mm or more above the negative control) and/ or positive food-specific IgE by ISAC (≥ 0.3 ISAC units)

IgE-mediated food allergy was defined as either:

- Positive food challenge
- A convincing clinical history of significant type I allergic reactions after isolated ingestion of the food in the preceding 6 months, with significantly positive SPT/sIgE; in the case of cow's milk above the internationally derived 95% positive predictive value for cow's milk of 8mm for SPT[15] and 15 kU/L for Immuncap.[16]

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9.3 Results

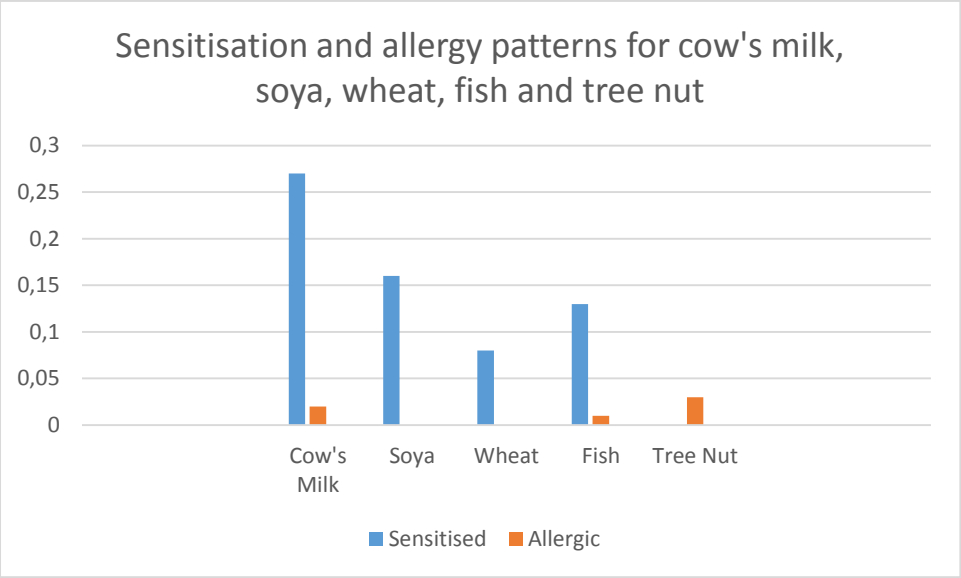
All of the patients completed the screening tests and food challenges where indicated, hence screening data for SPT and ISAC components from all 100 participants was utilised. Sensitisation and allergy patterns, with ethnic breakdown, are depicted in Table 9.1 as well as in figure 9.1.

Table 9.1: Sensitisation and Allergy Patterns for cow's milk, soya, wheat, fish and cashew nut

	Cow's Milk		**Soya**		**Wheat**		**Fish**		**Cashew Nut**	
	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens	Allergy	Sens+	Allergy
Overall (n=100)	27%	2%	16%	0%	8%	0%	13%	1%	0	3%
Xhosa (n=59)	22%	0%	12%	0%	8%	0%	7%	1.6%	0	0%
Mixed race (n=41)	34%	5%	22%	0%	7%	0%	22%	0%	0	7%
Difference between ethnic groups*	0.2	0.1	0.18	1	0.8	1	0.03**	0.7	1	0.04**

* By chi-squared test
Sens= sensitised
** statistically significant
\+ Cashew nut sensitisation evaluated by ISAC only, not SPT

Figure 9.1: Sensitisation and allergy patterns for cow's milk, soya, wheat, fish and cashew nut



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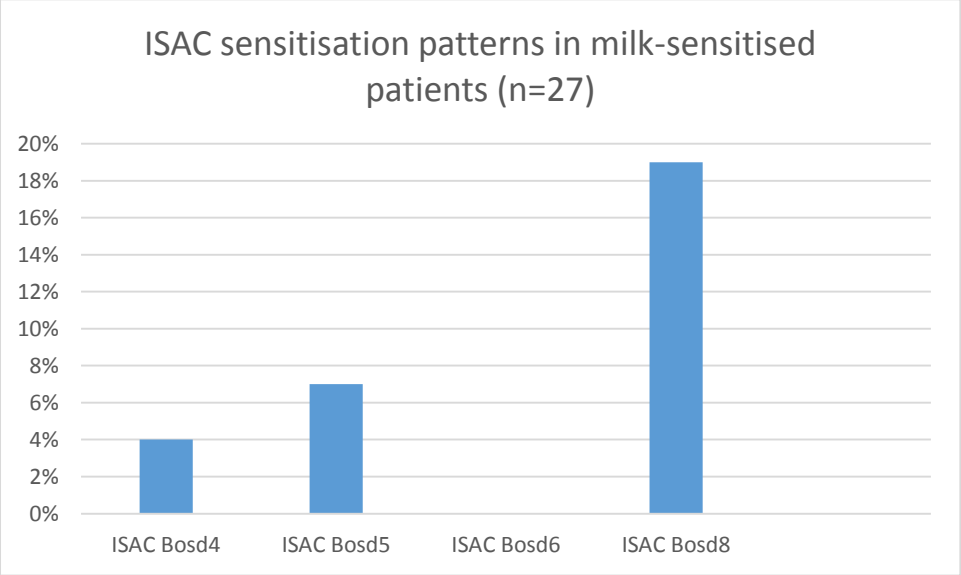
9.3.1 Cow's Milk

9.3.1.1 Sensitisation and Allergy Patterns

Twenty-seven patients were sensitised to cow's milk. Of these, all 27 had a positive SPT to fresh cow's milk, and only 2 to cow's milk extract (both of whom were also fresh cow's milk positive). Overall 5 patients showed sensitisation to cow's milk by ISAC component tests, all of whom were SPT positive to fresh cow's milk. Of the 5 patients who were ISAC positive, 1 was positive to *Bos d 4* (4% of all cow's milk-sensitised patients), 2 were positive to *Bos d 5* (7% of all cow's milk-sensitised patients), and 5 were positive to *Bos d 8* (19% of all cow's milk-sensitised patients)–as depicted in figure 9.2.

Figure 9.2: Sensitisation patterns to cow's milk components by ISAC Test



Two patients were found to be allergic to cow's milk at the time of the study. Both patients were SPT positive to fresh milk but negative to cow's milk extract. One was diagnosed by a positive challenge, one had a highly suggestive history of a reaction, sensitisation above the 95% PPV for SPT and ImmunoCAP to cow's milk, and a reaction whilst awaiting milk challenge.

A further 8 patients were assessed as likely to have outgrown a previously described IgE-mediated cow's milk allergy; of these, 5 were SPT negative and were consuming cow's milk regularly, 1 was SPT (fresh milk) and ImmunoCAP positive and was challenged (negative), and 2 were still SPT positive (to fresh milk) but ImmunoCAP negative, and were consuming cow's milk products regularly without immediate reactions.

Fifteen of the 27 patients with sensitisation to cow's milk underwent an ImmunoCAP test to whole cow's milk, of who 13 were positive (≥0.35 kU/L) and 2 were found to be allergic. Ten of the 27 patients with cow's milk sensitisation underwent an ImmunoCAP test to casein, of who 6 were positive (≥0.35 kU/L) and 2 were found to be allergic. Therefore, both children diagnosed with milk allergy were ImmunoCAP whole cow's milk, ImmunoCAP *Bos d 8* (casein), ISAC *Bos d 8* and SPT fresh milk positive, but both were negative to SPT cow's milk extract. The number of allergic children was too low (n=2) to allow for meaningful ROC curve analysis. Moreover, the low number of cow's milk allergic children meant that the value of traditionally used PPVs was not possible to analyse for most parameters except SPT to fresh cow's milk. Using SPT to fresh milk ≥8mm as a cut off, the overall PPV of this SPT size in determining cow's milk allergy was only 17% in this population.

The sensitivities and specificities of the various tests to diagnose cow's milk allergy are summarised in table 9.2.

Table 9.2: Value of SPT milk extract, SPT fresh milk, ISAC Bos d 8 and ImmunoCAP tests in predicting cow's milk allergy

	SPT Cow's Milk extract (n=100)	**SPT Fresh Cow's Milk (n=100)**	**ISAC *Bos d 8* (n=100)**	**ImmunoCAP *Bos d 8* (n=10)**	**ImmunoCAP cow's milk (n=16)**	**SPT fresh cow's milk ≥8 mm**	**ImmunoCAP Cow's milk ≥15 kU/L**
Sensitivity	0%	100%	100%	100%	100%	50%	100%
Specificity	96.9%	75%	96%	50%	13%	95%	85%
PPV	0%	7.4%	33%	33%	13%	17%	50%
NPV	98%	100%	100%	100%	100%	99%	85%

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9.3.1.2 Reported reactions to cow's milk or dairy

Fourteen patients (14%) reported a reaction to cow's milk, 6/59 Xhosas (10%) and 8/41 mixed race (20%). Overall, of the 14 patients with reported cow's milk symptoms, only 2 were found to have an ongoing IgE-mediated allergy (14%).This ratio was 0/6 (0%) in Xhosas and 2/8 (25%) in mixed race patients. There were no significant inter-ethnic differences in these ratios (table 9.3).

Of the 14 patients who reported a reaction to cow's milk, 5 (36%) reported an immediate reaction (within 2 hours of ingestion), and 9 (64%) reported a delayed reaction, more than 2 hours post ingestion. Of the 14 patients with reported reaction to cow's milk, 85% reported a worsening of eczema, 50% reported an itchy macular rash, 29% reported flushing, 14% reported angioedema and 7% reported each of diarrhoea, vomiting and itchy throat.

Of the 14 patients with reported cow's milk allergy symptoms, only 6 were sensitised to cow's milk; and of those 6 with symptoms and sensitisation, 2 were found to be allergic (33%).

The prevalence and value of reported reactions to milk are summarised in table 9.3, and the value of individual symptoms in table 9.4.

Table 9.3 Table showing value of patient-reported symptoms of cow's milk allergy in prediction of true cow's milk allergy

	Overall (n=100)	**Xhosa (n=59)**	**Mixed Race (n=41)**	**Difference between ethnic groups (p-value)***
Positive history for any reactivity to milk	14% (14/100)	10% (6/59)	20% (8/41)	0.84
% of those with positive history who were found to have a true allergy	14% (2/14)	0% (0/6)	25% (2/8)	0.19
Positive history + sensitised to milk	6% (6)	2% (1/59)	12% (5/41)	0.04**
% of those with positive history + sensitisation who were found to have a true allergy	33% (2/6)	0%	40% (2/5)	Insufficient observations

*by chi-squared test ** statistically significant

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Table 9.4: Most Commonly Reported Reactions to Cow's Milk

Symptom	**% with this symptom amongst those patients reporting a reaction to milk (n=14)**	**Proportion of patients with this symptom with true cow's milk allergy**
Exacerbation of eczema	86% (12/14)	8 % (1/12)
Itchy rash	50% (7/14)	29% (2/7)
Flushing	29% (4/14)	15% (1/4)
Angioedema	14% (2/14)	50% (1/2)
Diarrhoea	14% (2/14)	0% (0/2)
Vomiting	7% (1/14)	100% (1/1)
Itchy mouth	7% (1/14)	0% (0/2)
Wheeze	0%	-
Tight throat	0%	-
Circulatory compromise (blue lips, shock)	0%	-
"Doesn't like" cow's milk	0%	-

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9.3.2 Soya

Overall, 16 patients were found to be sensitised to soya, 9 by SPT to soya extract, 11 by ISAC test (4 to both SPT and ISAC). Of those 11 with a positive result on the ISAC test, 9 were positive to the component *Gly m 5*, 8 to *Gly m 6* and none to *Gly m 4*. By ethnicity, 12% (7/59) Xhosas and 22% (9/41) mixed race patients were sensitised to soya, p= 0.18. There were no cases of soya allergy, despite a high median result of 25.6kU/L (interquartile range 12.2-29.7) in the 10 patients who underwent soya ImmunoCAP testing.

2 patients reported reactivity to soya, both had a soya challenge and were found to be soya tolerant.

9.3.3 Wheat

Overall, 8 patients were sensitised to wheat, 5 by SPT to wheat extract, and 3 by ISAC test (2 to *Tri a αA* and 1 to *Tri a gliadin*, none to *Tri a 19*). Sensitisation rates by ethnicity were 8% (5/59) in Xhosa patients and 7% (3/41) in mixed race patients, p=0.83.

2 patients had a perceived allergy to wheat, both were challenged and found to be wheat tolerant.

9.3.4 Fish

Overall, 13% (13) patients were sensitised to white fish (cod): 7% in Xhosas (4/59) and 22% of mixed race children (9/41), with a significantly higher sensitisation rate in the mixed race group (p=0.03). Of the 13 sensitised patients, 11 were positive by SPT to fish extract, and 6 by ISAC (4 were both ISAC and SPT positive). Only one patient was found to be fish allergic.

Although cod was the fish tested for, there is a high degree of cross reactivity between fish species, hence in the questionnaire reactions were asked for "to any fish species." 21 (21%) patients reported a reaction to any fish species, 15 Xhosas (25% of Xhosas) and 6 of mixed race (14%). The median age at first reported reaction was 24 months. The most common complaints were itchy rash (in 71% of those who reported a reaction), facial flushing (in 33% of those who reported a reaction) and eczema flare (29% of those who reported a reaction). Only 2 patients with perceived food allergy were SPT positive, of whom 1 was found to be allergic. Therefore, overall only 5% (1/21) of those with a perceived reaction to fish were found to have an IgE-mediated reaction to white fish.

9.3.5 Cashew Nut

Three patients reported allergic symptoms in response to cashew nut (all mixed race), all immediate reactions. All 3 were found to be allergic. The median age at the time of the first reported reaction was 42 months. None of those found to be allergic were ISAC positive (to the cashew nut component *Ana o 2*), but all were positive to the ImmunoCAP to cashew nut, which was performed in those with a reported allergy.

9.3.6 Overall summary of reported reactions to foods

Table 9.5 summarises the percentage of patients reporting a reaction to each food tested; the percentage of those patients found to be truly allergic, the percentage of those reported reactions classified as immediate reactions, and the median age at the time of the first reaction. Overall, 42% of of all cases of patient-reported food reactions were a true IgE-mediated food allergy.

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Table 9.5: Perceived Reactions to the Foods Studied

Food	% of all patients with a perceived allergy (n=100)	Age at time of first reaction (median and IQ Range, in months)	% of patients with immediate reactions (<2 hours)	% of those with a perceived allergy who were found to be truly allergic
Any Food	109 cases			42% (46 cases)
Peanut	23%	24 (18-36)	88% (21/24)	70%
Hen's Egg	43%	12 (8-18)	91% (39/43)	56%
Cow's Milk	14%	12 (9-24)	36% (5/14)	14%
Soya	2%	2.5 (1-4)	100% (2/2)	0%
Wheat	2%	7.5 (6-9)	100% (2/2)	0%
Fish	21%	24 (12-36)	68% (13/19)	5%
Tree Nut	3%	42 (26-60)	100% (3/3)	100%

9.4 Discussion

Egg and peanut allergy were by far the most prevalent allergies in this cohort of South African children with atopic dermatitis. Smaller numbers of children were, at the time of the study, allergic to cow's milk, fish and tree nut, and none were found to be allergic to wheat and soya. Moreover, there were no reported cases of sesame allergy and no sensitisation to sesame by ISAC test. This is unlike in countries where the consumption of sesame is high and the prevalence of sesame allergy relatively high, such as in Israel. [38]

The prevalence of patient-reported reaction to foods far exceeded the true prevalence of food allergy, hence patient history on its own is a poor predictor of food allergy. Overall, of 109 reported cases of allergic reactions to the 7 foods studied, 46 (42%) were found to be truly allergic.

The prevalence of sensitisation to cow's milk (CM) in our study (27%) was similar to that in studies of children with moderate to severe AD from Europe and the USA (19-27%).[9-11] The EPAAC study, which studied the prevalence of food sensitisation in children with AD, analysed data from 2154 patients and found the prevalence of sensitisation to cow's milk to be 27%, equivalent to that in our study. However, cow's milk allergy in our study was low (2%) in comparison to other studies of food allergy in AD, which showed prevalence of 11-15%.[9-11] There were no significant ethnic differences between sensitisation and allergy rates for cow's milk. Possible reasons for the lower than expected prevalence of allergy to cow's milk include the relatively high median age of the study population (42 months), by which time some children may have outgrown their cow's milk allergy. Indeed, in 8 cases there was a

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fairly convincing history of a past reaction to cow's milk which had been outgrown, but this past history of cow's milk allergy could not be confirmed retrospectively. Moreover, many symptoms of cow's milk allergy such as worsening of eczema, reflux or severe colic represent non-IgE mediated cow's milk allergy, which was not explored in this study. The possible large contribution of non-IgE mediated mechanisms of cow's milk allergy was evidenced by the finding that 64% (9/14) of the reported reactions to cow's milk were not within 2 hours of ingestion.

The rate of asymptomatic sensitisation to cow's milk was high in this study, in both ethnic groups, with a high fall off between sensitisation (27%) and IgE-mediated allergy to cow's milk protein (2%). SPT to fresh milk had a greater sensitivity (100% sensitive) in diagnosing cow's milk allergy in comparison to SPT to cow's milk extract (which was not positive in either of the 2 allergic children); therefore a SPT to fresh milk should be recommended as part of a SPT panel to assess cow's milk allergy.[39] Widely used positive predictive values (PPVs) for cow's milk allergy, derived from other studies in high socio-economic settings, may not be applicable to this population: a SPT to fresh milk ≥ 8mm gave a PPV of 17% and an ImmunoCAP to cow's milk of ≥ 15 kU/L gave a PPV of 50%. Larger studies are needed to establish more appropriate PPVs for our population.

There were 3 cases of tree nut allergy in this study, all of whom also had a peanut allergy, and all of whom described symptoms of cashew allergy. The ISAC performed poorly as a screening test for cashew nut allergy and was negative in all 3 cashew-allergic patients. All 3 patients with cashew allergy had a positive ImmunoCAP to cashew nut.

Sensitisation to cod fish was high in this study (13%) and significantly higher in mixed race patients, but allergy rates were low (1%), which mirrors the fish allergy prevalence in the general population rather than in a high-risk population. This was despite a high reported rate of reactions to fish.

The reason for the low allergy rate may be explained partly by the fact that only codfish was used as a screening test for fish, whereas the consumption of many other fish species (eg the "snoek" fish, similar to Jack Mackerel) are common in the Western Cape and may have a different allergy pattern. Moreover, several patients (32% of those reporting a reaction to fish) described a delayed (≥ 2 hours post consumption) rash to fish with no immediate component, mostly in response to tinned pilchards. This may represent a reaction to the spices or preservatives in the tinned pilchards rather than a reaction to the fish itself. Other mechanisms of reaction to fish could include scromboid reactions and reactions to the fish parasite, anisakis.

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There was a high rate of sensitisation to soya (16% overall) but no cases of proven soya allergy, similar to other studies of food allergy in patients with AD. Moreover, there were no cases of wheat allergy in our study, despite a significant sensitisation of 8%, whereas studies in the UK and USA show around 5% prevalence of wheat allergy in children with AD. [9-11] However, of the 8 patients sensitised to wheat, 5 (63%) were also sensitised to Timothy grass, which cross-reacts with wheat, and could account for asymptomatic sensitisation to wheat.

9.5 Conclusion

Sensitisation rates to cow's milk, cod fish, soya and wheat were significantly higher than the general population and equivalent to those from studies of children with AD in Europe and the USA. Allergy rates were higher than the general population for cashew nut (3%), but lower than expected to cow's milk (2%), and were low for cod fish (1%), wheat and soya (0%). However, several patients may have already outgrown their cow's milk allergies at the time of study entry due to the natural history of tolerance acquisition in a significant proportion of patients with cow's milk allergy by the age of 3-4 years, which was the median age of the study population. The ISAC test fared well as a screening test for allergies to cow's milk and fish, but poorly for cashew nut. SPT to fresh cow's milk was superior to cow's milk extract in the diagnosis of cow's milk allergy. Widely used internationally derived 95% PPV for SPT and ImmunoCAP cow's milk fared poorly in this population and may need to be investigated in large population studies in infants and young children. Patient history of a reaction to any food significantly overestimates true food allergy rates.

University of Cape Town

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Chapter 10:

Allergic co-morbidity of patients with atopic dermatitis and food allergy

ABBREVIATIONS

AD : Atopic Dermatitis

IgE : Immunoglobulin E

SCORAD: Scoring Atopic Dermatitis Score

SPT: Skin Prick Test

ISAC: Immuno Solid Phase Allergen Chip

PPV: Positive Predictive Value

NPV: Negative Predictive Value

DerP: Dermatophagoides Pteronyssinus

DerF: Dermatophagoides Farinae

ISAAC: International Study on Asthma and Allergies in Children

TSLP: Thymic stromal lymphopoietin

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10.1 Introduction

Food allergy is associated with several co-morbid conditions, which can increase morbidity and influence risk for severity of reactions. Atopic dermatitis (AD) in children has also been related to multiple co-morbid conditions and increased healthcare utilisation. [1]

Eczema and food allergy

The complex and multi-directional association between food allergy and AD is described in chapter 5. Food allergies can exacerbate eczema in a proportion of patients, most often together with typical IgE-mediated reactions. On the other hand, eczema is a major risk factor for food allergies. Epicutaneous sensitisation on a disrupted skin barrier has been recognised as a potential factor increasing the development and persistence of food allergy.[2] Filaggrin loss of function mutations are associated with atopic dermatitis, but also with food allergy in childhood and adolescence.[2] Atopic dermatitis is the main risk factor for food sensitisation in exclusively breastfed infants, and the risk increases as disease severity increases.[3] The mechanism for epicutaneous sensitisation on a disrupted skin barrier is potentially through a thymic stromal lymphopoietin (TSLP) basophil axis. [4] Expansion

of TSLP-elicited basophils in the skin promote antigen specific Th2 cytokine response, antigen-specific serum IgE levels and accumulation of mast cells in the intestine, promoting the development of intestinal food allergy. [4]

Early onset eczema under 12 months of age, severe eczema and younger age at the time of assessment have been identified as significant risk factors for food allergy in eczema. [5]

Atopic dermatitis and aerollergen-related disorders

AD is considered to be one of the first manifestations in the atopic march. On the basis of longitudinal studies, approximately a third to half of AD patients will develop asthma, and two thirds or more will develop allergic rhinitis.[6-11] Epicutaneous sensitisation to aeroallergens has been thought to be responsible, with subsequent migration of sensitised T cells into the nose and airways, causing upper and lower airway disease.[12-13] Aeroallergen sensitisation is common in AD patients, and in the EPAAC (Early Prevention of Asthma and Allergies in Children) study, 20-40% of AD patients were sensitised to house dust mite.[14] The presence of allergic sensitisation to aeroallergens in AD at one year was positively related to the occurrence of asthma. In contrast to food allergy sensitisation, aeroallergen sensitisation continued well beyond the first year of life in the EPAAC cohort.[14]

However, the classical sequence of atopic dermatitis followed by aeroallergen sensitisation then asthma is not always followed: in the Multicenter Allergy Study, a German birth cohort study following 1314 children from birth to age 7 years, in many of the asthmatic children, wheezing manifested before or with the onset of AD. Children with AD and wheeze may have a marked loss in lung function, suggesting that they may have a distinct phenotype rather than a progressive development from AD to asthma.[15]

Studies have shown that the severity of AD, as well as co-morbidity of AD and food allergy, are particular risk factors for asthma and allergic rhinitis.[16] In a study looking at the natural history of children with *both* AD and food allergy attending a tertiary allergy clinic, 75% of children had another atopic condition as follows: [16]

•44% had allergic rhinitis and asthma

•27% had allergic rhinitis

•4% had asthma, without another atopic condition

Early responses to egg represent an important infantile marker for atopy. In children with AD and egg allergy, respiratory allergies develop in up to 90% of cases.[17]

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In a 7 year follow-up study of children with AD, 80% of the children became sensitised to airborne allergens and 75% of them noticed symptoms when exposed. Family history of atopy and eczema, sensitisation to hen's egg, and early onset of eczema imparted an increased risk of becoming sensitised.[18] Another prospective study on eczema and co-morbid conditions showed that egg sensitisation and severity of AD were positively related to the occurrence of asthma.[19]

AD is a major risk factor for propagation of the allergic march, which is the typical sequence of IgE responses and clinical symptoms which appear in atopic people. Studies have suggested that early intervention in AD may reduce further manifestations of the allergic march.[20]

Food allergy and asthma

Food allergy, has been found to be an independent risk factor for asthma and allergic rhinitis. Early sensitisation to food allergens, especially hen's egg, has been shown to be a valuable predictor of subsequent sensitisation to inhalant allergens.[6] Co-existence of food allergy with asthma may be a risk factor for hospitalisation for severe asthma exacerbations.[21]

Asthma, on the other hand, is a risk factor for severe reactions to foods, and a high prevalence of asthma is reported amongst patients with life-threatening or fatal allergic food-allergy reactions.[22]

Respiratory symptoms as part of a food-allergic reaction are a risk factor for persistence of food allergy: patients with both skin and respiratory tract symptoms on exposure to a food are less likely to have their food allergy resolve than patients with only skin or gut symptoms.[16]

This chapter explores the co-morbidity between AD, food allergy, aeroallergen sensitisation, asthma and allergic rhinitis. Age-dependent patterns of aeroallergen sensitisation are explored, and indoor versus outdoor aeroallergens are differentiated with respect to risk profiles for co-morbid conditions.

10.2 Methodology

This was part of the wider study investigating food sensitisation and allergy patterns in South African children with atopic dermatitis, including 59 children of Xhosa origin, and 41 of mixed race origin.

Only children with atopic dermatitis were included in this study, and the severity of the atopic dermatitis was assessed using the SCORAD index as described in chapter 5. Patients were assessed for food allergy by questionnaire, skin prick tests, ISAC 103 test and controlled open oral food challenge where indicated.

Aeroallergen sensitisation was tested using the ImmunoCAP ISAC (103) test. Patients were assessed for the following:

House dust mite Dermatophagoides pteronyssimus *(DerP):* components *nDer p 1* and *nDer p 2*

House dust mite Dermatophagoides Farinae *(DerF): nDer f 1* and *rDer f 2*

Storage Mite: *rEur m 2*

Bermuda Grass*: nCyn d 1*

Timothy Grass: *r Phl p 1,2,4,5 6, 7, 11, 12*

Tree Pollen: Olive tree *nOle e 1, nOle e 2*

Plane tree *rPla a1, rPla a2*

Cypress *nCup a 1*

Japanese Cedar *nCry j 1*

Birch *rBet v1,2 and 4*

Alder *rAln g 1*

Alernaria Mould*: rAlt a 1, rAlt a 6*

Dog: *rCan f 1 and 2*

Cat: *rFel d 1 and 4*

Symptoms of asthma and allergic rhinitis were elicited using a questionnaire modified from the ISAAC study questions: [23]

1. *For asthma:* Has your child ever had symptoms of asthma (such as wheeze, persistent cough at night or when exercising, shortness of breath)? If yes, was the asthma diagnosed by a doctor, nurse or self-diagnosed?
2. *For allergic rhinitis:* Has your child ever had symptoms of hayfever (such as itchy runny eyes, itchy runny nose, blocked nose, frequent sneezing)? If yes, was the hayfever diagnosed by a doctor, nurse or self-diagnosed?

Furthermore, all patients were examined for signs of asthma and allergic rhinitis, and a list of all their preventer and reliever medications for asthma and allergic rhinitis was recorded.

10.2.1 Statistical analysis

The prevalence of asthma and allergic rhinitis symptoms was determined, and differences between those with or without food allergy were analysed by the chi squared test. Aeroallergen sensitisation patterns were described and analysed for their association with asthma, allergic rhinitis and food allergy.

As egg allergy has been deemed a major risk factor for respiratory allergies, a separate analysis was performed for egg allergy and asthma, as well as aeroallergen sensitisation patterns. The association

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between peanut allergy and egg was also explored in view of the substantial co-sensitisation between egg and peanut.

10.3 Results

10.3.1 Asthma

10.3.1.1 Prevalence of asthma in this study cohort of patients with AD

Overall, 39% of patients described symptoms of asthma; 36% (21/59) in the Xhosa group and 44% (18/41) in the mixed race group. The interethnic difference in symptoms of asthma was not significant, p=0.4. Overall, 29% of patients had doctor-diagnosed asthma, 25% (15/59) of Xhosas and 34% (14/41) of mixed race patients, p=0.34. Of those with doctor-diagnosed asthma, 48% (14/29) were on a regular preventer and 86% (25/29) were on a regular reliever. By ethnicity, 27% (4/15) Xhosas with doctor-diagnosed asthma were taking a regular preventer and 71% (10/14) of mixed race patients; controller medication use was significantly lower in the Xhosa patients p=0.02. However, the difference in reliever medication use was insignificant between ethnic groups, with 80% Xhosas (10/14) and 93% (13/14) of mixed race patients having a reliever medication, p=0.3.

Asthma prevalence increased with age: 22% (7/32) children under the age of 2 years had asthma symptoms, 43% (12/28) children between 2-4 years and 50% (20/40) of children above the age of 4 years had asthma symptoms.

10.3.1.2 Food allergy and asthma

Of the 40 patients with food allergy, 18 had symptoms of concurrent asthma (45%), and of the 60 without food allergy 21 had asthma symptoms (35%), p= 0.31.

For doctor-diagnosed asthma, 35% (14/40) with food allergy had doctor-diagnosed asthma versus 25% (15/60) without food allergy, p=0.28. By ethnicity, in Xhosa patients 35% (7/20) with food allergy had doctor-diagnosed asthma versus 21% (8/39) without asthma (p=0.23), and in mixed race patients, 35% (7/20) with food allergy had doctor-diagnosed asthma versus 33% (7/21) without food allergy, p=0.9. The most common food allergens associated with asthma were egg and peanut. In those patients with doctor diagnosed asthma, 38% (11/29) had egg allergy and 38% (11/29) had peanut allergy. There was no significant relevant relationship between multiple food allergies and asthma.

Therefore, in this cohort of children with moderate to severe atopic dermatitis, the co-existence of food allergy was not associated with a higher prevalence of asthma. Moreover, increasing eczema severity was not associated with higher asthma rates: 46% (23/50) with moderate eczema had symptoms of asthma versus 32% (16/50) with severe eczema (p=0.15), these ratios were 30% (15/50)

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and 28% (14/50) for doctor-diagnosed asthma, p=0.8. Age of onset of eczema similarly did not influence asthma prevalence: 28% (10/36) with early onset eczema under the age of 6 months had asthma symptoms versus 45% (29/64) with later onset eczema (p=0.08); for doctor diagnosed asthma, paradoxically early onset eczema had a lower incidence of doctor-diagnosed asthma then later onset eczema: 14% (5/36) with early onset eczema had doctor-diagnosed asthma, versus 38% (24/64) with later onset eczema (p=0.01). However, the median age of patients with early onset eczema at the time of study participation was 28.5 months, versus 52.5 months in those patients with later onset eczema, hence this may bias asthma prevalence, which tends to increase with age.

However, age at study entry had a significant influence on asthma prevalence: in those under the age of 42 months at study entry (n=49), 24% (12/49) had symptoms of asthma, versus 53% (27/51) in those who entered the study at or above 42 months (p=0.004). This reflects the finding that asthma prevalence increases with age in this high-risk population, with the median age at study entry of those with asthma symptoms being 51 months.

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10.3.2 Allergic Rhinitis

10.3.2.1 Prevalence of allergic rhinitis in this study cohort of patients with AD

53% of patients described symptoms of allergic rhinitis, 53% in Xhosas (31/59) and 54% (22/41) in mixed race patients, p=0.9. Overall, 28% of patients had doctor-diagnosed allergic rhinitis, 25% (15/59) Xhosa patients and 32% (13/41) mixed race patients, p=0.41.

Age at study entry had a significant influence on allergic rhinitis prevalence: in those under the age of 42 months at study entry (n=49), 37% (18/49) had symptoms of allergic rhinitis, versus 69% (35/51) in those who entered the study at or above 42 months (p=0.001). This reflects the finding that allergic rhinitis prevalence increases with age in this high-risk population: 31% (10/32) children under the age of 2 years had allergic rhinitis symptoms, 53% (15/28) children between 2-4 years and 70% (28/40) of children above the age of 4 years.

10.2.2.2. Food allergy and allergic rhinitis

Overall, 48% (19/40) of patients with food allergy had symptoms of allergic rhinitis (9 cases egg, 13 cases peanut), and 57% (34/60) of those without food allergy had symptoms of allergic rhinitis (p=0.37). By doctor-diagnosed allergic rhinitis, 28% (11/40) with food allergy had allergic rhinitis and 28% (17/60) without food allergy had allergic rhinitis. (p=0.93). Therefore, the presence of food allergy was not associated with an increased prevalence of allergic rhinitis in this cohort of patients with co-existing eczema.

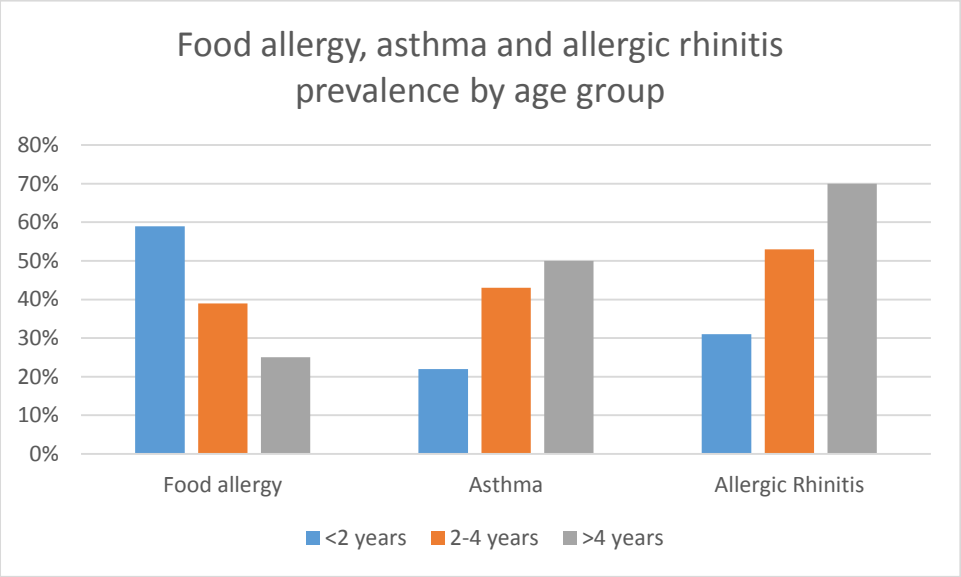
Of the 28 cases of doctor-diagnosed allergic rhinitis (who therefore had access to treatment), 64% (18/28) were on an intranasal corticosteroid; by ethnic breakdown 53% (8/15) in Xhosas and 77% (10/13) in mixed race patients, p=0.19. Overall, 86% (24/28) of patients with doctor-diagnosed allergic rhinitis were on antihistamines; 80% (12/15) in Xhosas and 92% (12/13) in mixed race patients, p=0.37. Patterns of asthma and allergic rhinitis in the cohort of 100 patients with AD are summarised in table 10.1. Age-related changes in food allergy, asthma and allergic rhinitis prevalence are shown in figure 10.1.

Table 10.1: Prevalence patterns of asthma and allergic rhinitis

	% Patients overall	**% of Xhosa patients**	**% of mixed race patients**	**Difference between Xhosa and mixed race children (p-value)***	**% in patients with food allergy**	**% in patients without food allergy**	**Difference between food-allergic and non-allergic children (p-value)***
Asthma symptoms	39%	36%	44%	0.4	45%	35%	0.31
Allergic Rhinitis symptoms	53%	53%	54%	0.9	48%	57%	0.37

*Chi-squared test

Figure 10.1: Age related changes in food allergy, asthma and allergic rhinitis prevalence



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10.3.3 Sensitisation Pattern to Aeroallergens

Overall, 89% of patients tested positive to at least one aeroallergen on the ISAC test. The median number of positive aeroallergen tests was 3.5. Aeroallergen sensitisation was most common to Dermatophagoides Pteronyssinus (Der P) at 81%, followed by Dermatophagoides Farinae (Der F) at 78%, storage mite at 51%, Timothy Grass at 36%, cat at 35%, Bermuda Grass at 30%, dog at 18%, tree pollen at 17% and *alternaria* mould at 16%. Aeroallergen sensitisation patterns are depicted in table 10.2 and figures 10.2 and 10.3. Sensitisation to tree pollen, cat dander, Bermuda grass and dog dander was significantly more common in the mixed race group in comparison to the Xhosa group.

Table 10.2 Aeroallergen sensitisation patterns

	% sensitised overall	**% of Xhosas sensitised**	**% of mixed race sensitised**	**Difference between ethnic groups (p-value)***
Any aeroallergen sensitisation	89%	86%	93%	0.33
DerP	81%	80%	83%	0.68
DerF	78%	78%	78%	0.99
Storage Mite	51%	54%	46%	0.51
Timothy Grass	36%	29%	46%	0.08
Cat	35%	27%	46%	0.05**
Bermuda Grass	30%	20%	44%	0.011**
Dog	18%	10%	29%	0.014**
Tree pollen	17%	10%	27%	0.03**
Alternaria	16%	14%	20%	0.42

*by chi-squared test
**Statistically significant

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Figure 10.2 Overall prevalence of aeroallergen sensitisation

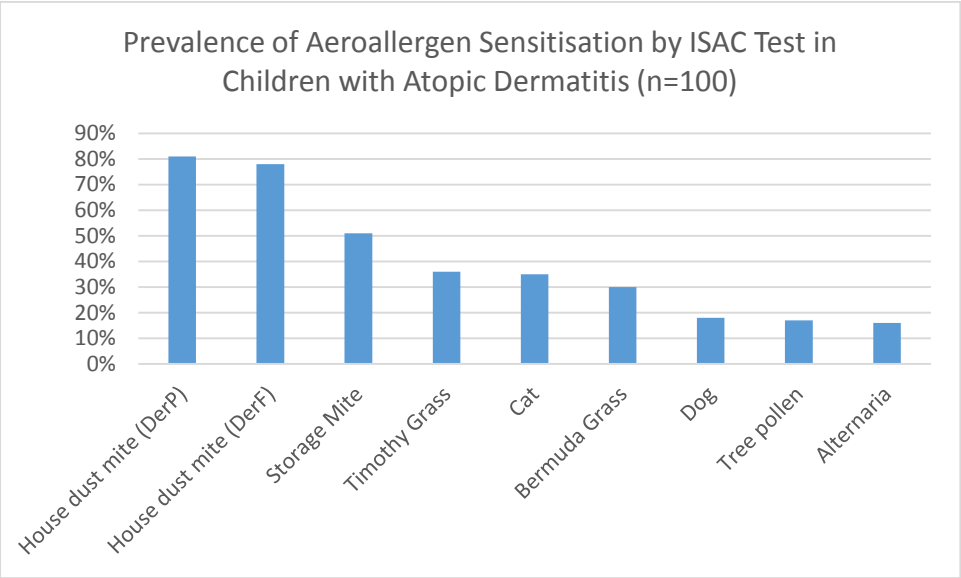
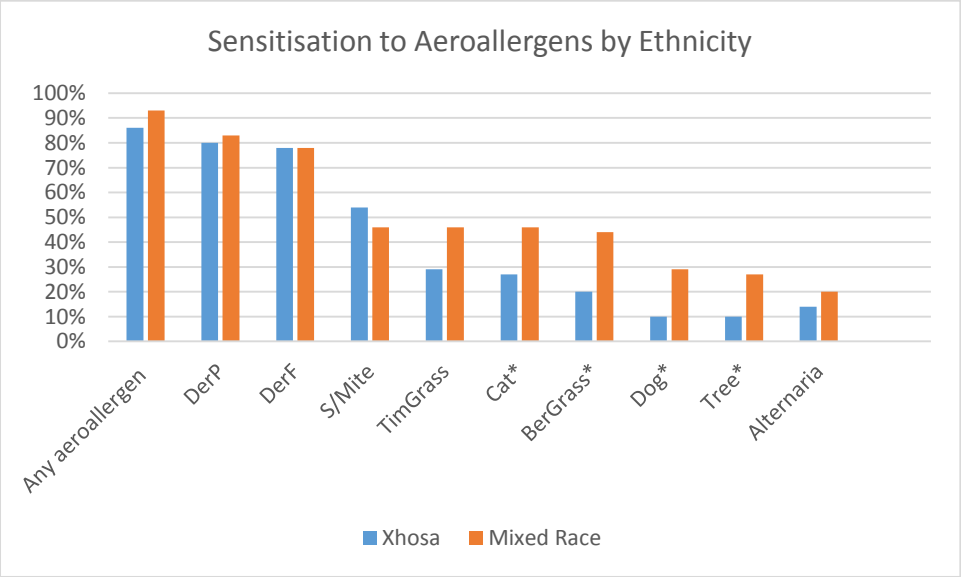


Figure 10.3: Prevalence of aeroallergen sensitisation by ethnic group



*Statistically significant difference by Chi-squared test

Der P= Dermatophagoides Pteronnysinus Der F=Dermatophagoides Farinae S/mite=storage mite

10.3.3.1 House dust mite sensitisation:

Dermatophagoides Pteronyssinus (DerP)

Overall, 81% of patients were sensitised to DerP, 80% (47/59) in Xhosa patients and 83% (34/41) in mixed race patients, p=0.68. Of these 81 patients with DerP sensitisation, 73 (90%) were sensitised to

the component *Der p 1* and 65 (80%) to *Der p 2*. In those patients sensitised to DerP, The median value for *Der p 1* was 10 ISU, and for *Der p 2* 5.8 ISU.

There was no significant increase in asthma or allergic rhinitis symptoms in those patients with DerP sensitisation: 87% (34/39) of patients with asthma symptoms were DerP sensitised, compared with 77% (47/61) of non-asthmatics (p=0.21). 83% (44/53) of patients with symptoms of allergic rhinitis were DerP sensitised versus 79% (37/47) without allergic rhinitis symptoms (p=0.59). There was, however, a significant association between DerP sensitisation and food allergy: 93% (37/40) with food allergy were DerP sensitised versus 73% (44/60) without food allergy, p=0.02.

Dermatophagoides Farinae (DerF)

Overall, 78% of patients were sensitised to DerF, 78% (46/59) in Xhosa patients and 78% (32/41) in mixed race patients, p=0.99. Of these 78 patients with DerF sensitisation, 71 (91%) were sensitised to the component *Der f 1* and 62 (79%) to *Der f 2*. The median value for *Der f 1* was 7.15 ISU, and for *Der f 2* 7.5 ISU.

There was no significant increase in asthma or allergic rhinitis symptoms in those patients with DerF sensitisation: 82% (32/39) of patients with asthma symptoms were DerF sensitised, compared with 75% (46/61) non-asthmatics (p=0.43). Eighty-three percent (44/53) of patients with symptoms of allergic rhinitis were DerF sensitised versus 72% (34/47) without allergic rhinitis symptoms (p=0.2). There was, however, a significant association between DerF sensitisation and food allergy: 90% (36/40) of children with food allergy were DerF sensitised versus 70% (42/60) without food allergy, p=0.02.

77/78 (99%) patients with sensitisation to DerF were also DerP sensitised, and 77/81 (95%) of patients with sensitisation to DerP were also DerF sensitised; hence there was a near complete overlap between house dust mite species.

10.3.3.2 Storage Mite Sensitisation

Overall 51% of patients were sensitised to storage mite, 54% (32/59) Xhosas and 46% (19/41) in mixed race patients (p=0.51). The median level in the positive group was 3.8 ISU. Fifty of the 51 patients with storage mite sensitisation (98 %) were co-sensitised with DerP.

There was a significant increase in asthma symptoms in those patients with storage mite sensitisation: 67% (26/39) of patients with asthma symptoms were storage mite sensitised, compared with 41%

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(25/61) of non-asthmatics (p=0.015). There was a trend towards higher storage mite sensitisation in those patients with allergic rhinitis symptoms, but it did not reach statistical significance: 60% (32/53) of patients with symptoms of allergic rhinitis were storage mite sensitised versus 40% (19/47) without allergic rhinitis symptoms (p=0.06). There was no significant association between storage mite sensitisation and food allergy: 60% (24/40) with food allergy were storage mite sensitised versus 45% (27/60) without food allergy (p=0.16).

10.3.3.3 Timothy Grass Sensitisation

Overall, 36% of patients were sensitised to at least one of the Timothy Grass antigens, 29% (17/59) Xhosas versus 46% (19/41) in mixed race patients, p=0.08. In those who were Timothy grass sensitised, the median level was 12 ISAC units. 83% (30/36) patients with Timothy grass sensitisation were also Bermuda grass sensitised.

There was a significant increase in asthma symptoms in those patients with Timothy grass sensitisation: 51% (20/39) of patients with asthma symptoms were Timothy grass sensitised, compared with 26% (16/61) non-asthmatics (p=0.008). Timothy grass sensitisation was also significantly associated with allergic rhinitis symptoms: 51% (27/53) of patients with symptoms of allergic rhinitis were Timothy grass sensitised versus 19% (9/47) without allergic rhinitis symptoms (p=0.001). There was no significant association between Timothy grass sensitisation and food allergy: 38% (15/40) with food allergy were Timothy grass sensitised versus 35% (21/60) without food allergy, p=0.73.

10.3.3.4 Cat sensitisation

Overall, 35% of patients were sensitised to at least one of the cat allergens tested, significantly higher in mixed race patients at 46% (19/41) versus Xhosa patients at 27% (16/59), p=0.047. Forty percent (14/35) of patients with cat sensitisation were also dog-sensitised. Of those who were sensitised to cat, the median level was 3.6 ISU.

There was no significant increase in asthma or allergic rhinitis symptoms in those patients with cat sensitisation: 41% (16/39) of patients with asthma symptoms were cat sensitised, compared with 31% (19/61) non-asthmatics (p=0.31). Thirty-six percent (19/53) of patients with symptoms of allergic rhinitis were cat sensitised versus 34% (16/47) without allergic rhinitis symptoms (p=0.85). There was, however, a significant association between cat sensitisation and food allergy: 58% (23/40) of patients with food allergy were cat sensitised versus 20% (12/60) of patients without food allergy, p< 0.001.

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10.3.3.5 Bermuda grass sensitisation

Overall, 30% of patients were Bermuda grass sensitised. Bermuda grass sensitisation was significantly higher in the mixed race group 44% (18/41) versus Xhosas 20% (12/59), p=0.011. In those who were Bermuda grass sensitised, the median ISAC value was 10.3 ISU. All patients with Bermuda grass sensitisation were also Timothy grass sensitised, hence there was significant co-sensitisation with the grasses.

There was a significant increase in asthma symptoms in those patients with Bermuda grass sensitisation: 46% (18/39) of patients with asthma symptoms were Bermuda grass sensitised, compared with 20% (12/61) non-asthmatics (p=0.005). Bermuda grass sensitisation was also significantly associated with allergic rhinitis symptoms: 43% (23/53) of patients with symptoms of allergic rhinitis were Bermuda grass sensitised versus 15% (7/47) without allergic rhinitis symptoms (p=0.002). There was no significant association between Bermuda grass sensitisation and food allergy: (33% (13/40) with food allergy were Bermuda grass sensitised versus 28% (17/60) without food allergy, p=0.66).

10.3.3.6 Dog sensitisation

Overall, 18% of patients were dog sensitised; significantly higher in mixed race patients at 29% (12/41) versus Xhosa patients at 10% (6/59), p=0.014. Of those who were positive to dog, the median value was 7.3 ISU. Seventy-eight percent (14/18) of patients with dog sensitisation were also cat sensitised.

Dog sensitisation was not significantly associated with asthma or allergic rhinitis symptoms. Overall, 23% (9/39) of patients with asthma were dog sensitised, compared with 15% (9/61) in non-asthmatics (p=0.3). 19% (10/53) of patients with symptoms of allergic rhinitis were dog sensitised versus 17% (8/47) without allergic rhinitis symptoms (p=0.81). There was a significant association between dog sensitisation and food allergy: 38% (15/40) of those with food allergy were dog sensitised versus 5% (3/60) of those without food allergy, p< 0.001.

10.3.3.7 Tree Pollen Sensitisation

17% of patients were sensitised to any of the tree pollens tested, the percentage being significantly higher in mixed race patients: 10% (6/59) in Xhosas and 27% (11/41) in mixed race, p=0.03.

There was a trend towards an increase in asthma symptoms in those patients with tree pollen sensitisation, but it did not reach statistical significance: 26% (10/39) of patients with asthma symptoms were tree pollen sensitised, compared with 11% (7/61) non-asthmatics (p=0.07). Tree

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pollen sensitisation was not significantly associated with allergic rhinitis symptoms: 23% (12/53) of patients with symptoms of allergic rhinitis were tree pollen sensitised versus 11% (5/47) without allergic rhinitis symptoms (p=0.111). There was no significant association between tree pollen sensitisation and food allergy: 25% (10/40) with food allergy were tree pollen sensitised versus 12% (7/60) without food allergy, p=0.08.

10.3.3.8 Alternaria mould sensitisation

Overall, 16% of patients were *Alternaria* mould sensitised, 14% (8/59) in Xhosas versus 20% (8/41) in the mixed race group, p=0.42. The median level of Alternaria in those who were sensitised was 4.75 ISU.

Alternaria sensitisation was not significantly associated with asthma or allergic rhinitis symptoms. Twenty-three percent (9/39) of patients with asthma were *Alternaria* sensitised, compared with 11% (7/61) of non-asthmatics (p=0.12). Thirteen percent (7/53) of patients with symptoms of allergic rhinitis were *Alternaria* sensitised versus 19% (9/47) without allergic rhinitis symptoms (p=0.42). There was no significant association between *Alternaria* sensitisation and food allergy: 15% (6/40) of those with food allergy were *Alternaria* sensitised versus 17% (10/60) without food allergy, p=0.82.

Aeroallergen sensitisation pattern in patients with asthma, allergic rhinitis and food allergy in this population of children with AD is demonstrated in table 10.3 and figures 10.4, 10.5 and 10.6.

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Table 10.3: Aeroallergen sensitisation patterns in concomitant asthma, allergic rhinitis and food allergy

	% of asthmatics sensitised	% non-asthmatics sensitised	Differ-ence (p-value)	% with allergic rhinitis sensitised	% without allergic rhinitis sensitised	Differ-ence (p-value)	% with food allergy sensitised	% without food allergy sensitised	Differ-ence (p-value)*
Any aeroallergen	92%	87%	0.4	91%	87%	0.6	93%	87%	0.36
DerP	87%	77%	0.21	83%	79%	0.59	93%	73%	0.02**
DerF	82%	75%	0.43	83%	72%	0.2	90%, 70%,	70%	0.02**
Storage Mite	67%	42%	0.015**	60%	41%	0.06	60%	45%	0.16
Timothy Grass	51%	26%	0.008**	51%	19%	0.001**	38%	35%	0.73
Cat	41%	31%	0.31	36%	34%	0.85	58%	20%	<0.001**
Bermuda Grass	46%	20%	0.005**	43%	15%	0.002**	33%	28%	0.66
Dog	23%	15%	0.3	19%	17%	0.81	38%	5%	<0.001**
Tree pollen	26%	11%	0.07	23%	11%	0.111	25%	12%	0.08
Alternaria	23%	11%	0.12	13%	19%	0.42	15%	17%	0.82

*by chi-squared test
** statistically significant

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Figure 10.4 Pattern of aeroallergen sensitisation in patients with asthma

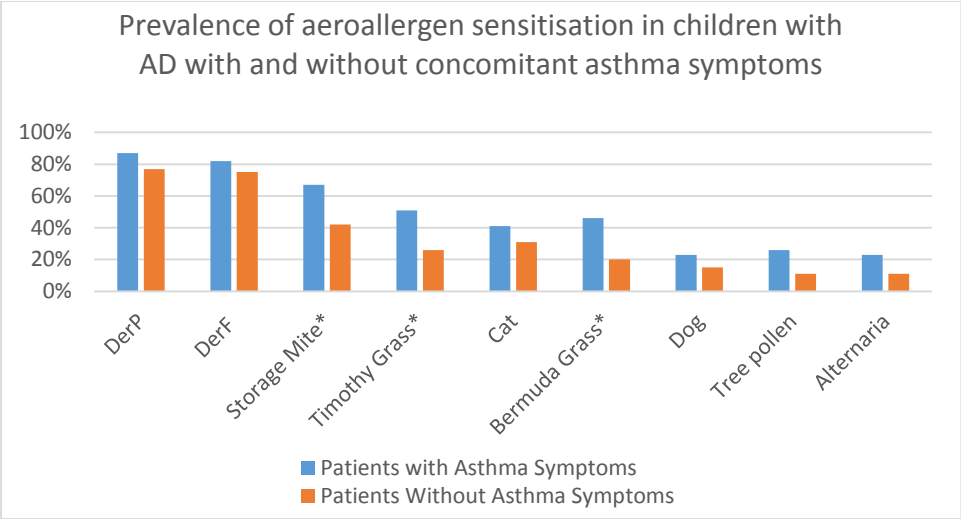


Figure 10.5 Pattern of aeroallergen sensitisation in patients with allergic rhinitis

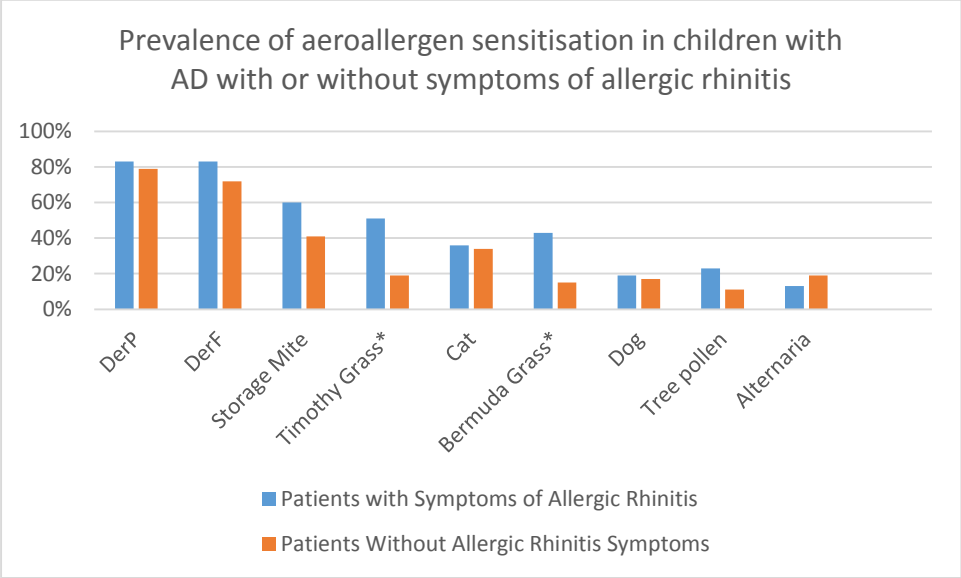
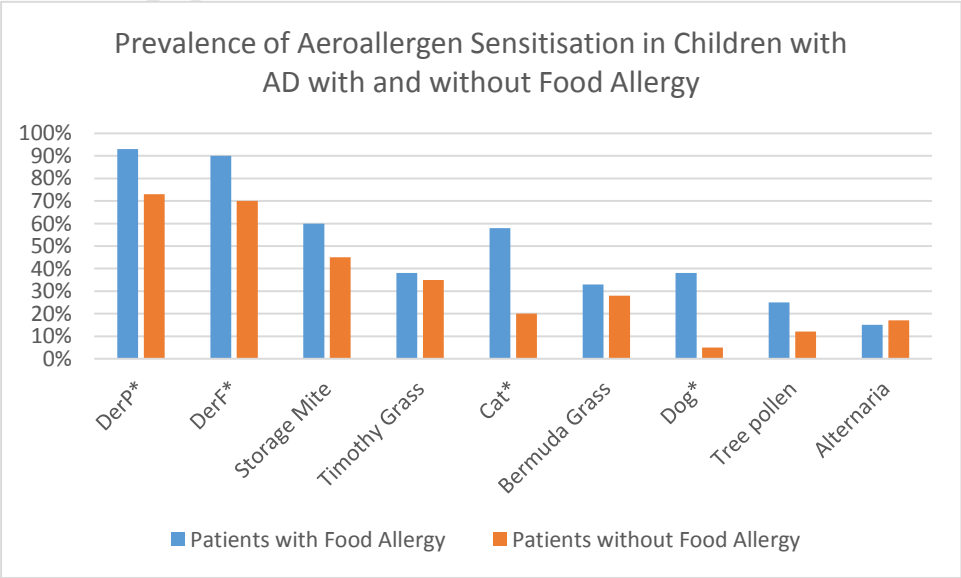


Figure 10.6 Pattern of aeroallergen sensitisation in patients with food allergy



10.3.4 Age and aeroallergen sensitisation patterns

The sensitisation pattern to aeroallergens by age is depicted in table 10.4 and figure 10.7. For the indoor allergens, including house dust mite, cat and dog, the sensitisation rates were similar in the younger children (even at one year of age) compared to the older children, suggesting that this sensitisation occurs early on in life to these allergens. Timothy grass, Bermuda grass, storage mite and tree pollen sensitisation increased with age, suggesting an ongoing sensitisation process far beyond the first year of life to certain aeroallergens. The mean number of aeroallergens the patient is sensitised to was 2.25 in children under the age of 2 years; 2.8 at or below 4 years and 3.63 at or below 10 years. This suggests that aeroallergen sensitisation may continue beyond 4 years of age.

Table 10.4 Aeroallergen sensitisation by age

	Age in Years			
	1 year	**2 years**	**4 years**	**10 years**
Any aeroallergen	88%	78%	85%	89%
DerP	88%	72%	77%	81%
DerF	75%	72%	75%	78%
Storage Mite	13%	22%	37%	51%
Timothy Grass	0%	3%	18%	36%
Cat	38%	31%	32%	35%
Bermuda Grass	0%	3%	12%	30%
Dog	25%	19%	15%	18%
Tree pollen	0%	0%	5%	17%
Alternaria	0%	0%	10%	16%

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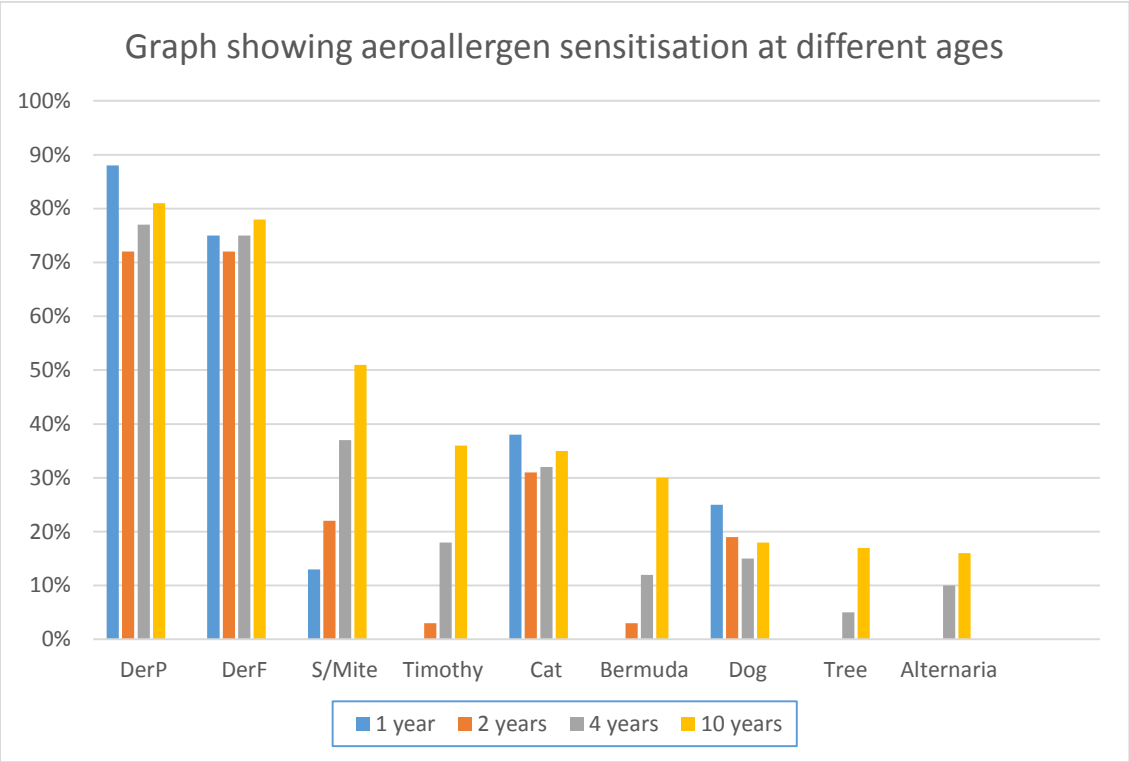
Figure 10.7 Aeroallergen sensitisation by age



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10.3.5 Egg Allergy and Co-Morbidity

10.3.5.1 Egg allergy and asthma

Forty-four percent (11/25) of children with egg allergy had symptoms of asthma, compared with 37% (28/75) without egg allergy (p=0.55). This finding was similar between ethnic groups: 50% (7/14) of Xhosa patients with egg allergy had asthma, and 36% (4/11) of mixed race patients with egg allergy had asthma symptoms, p=0.49. There was a tendency for the older children (over 24 months) with egg allergy to have more asthma than the younger children with egg allergy, but this did not reach statistical significance: 31% (5/16) under 24 month's age with egg allergy had asthma symptoms; and 67% (6/9) of children over 24 month's age with egg allergy had asthma, p=0.08.

10.3.5.2 Egg allergy and allergic rhinitis

Thirty-six percent (9/25) of children with egg allergy had symptoms of asthma, compared with 59% (44/75) without egg allergy; paradoxically this resulted in egg allergic children having a lower risk of allergic rhinitis, p=0.049. This finding was similar between ethnic groups: 36% (5/14) Xhosa patients with egg allergy had allergic rhinitis, and 36% (4/11) of mixed race with egg allergy had allergic symptoms, p=0.99.

10.3.5.3 Egg sensitisation/allergy and aeroallergen sensitisation

Overall, there was a high rate of aeroallergen sensitisation which was not statistically different in those with or without egg sensitisation or allergy. Eighty-one percent (44/54) of patients with egg sensitisation were sensitised to at least one aeroallergen, and 96% (45/47) of those without egg sensitisation had aeroallergen sensitisation (p=0.41). Ninety-two percent (23/25) of patients with egg allergy were sensitised to at least one aeroallergen, compared with 88% (66/75) without egg allergy (p=0.58).

Moreover, in the population overall, there was not a significant difference in house dust mite sensitisation rates between egg sensitised patients and those not sensitised to egg. Seventy-eight percent (42/54) of patients with egg sensitisation were sensitive to DerP, and 85% (39/46) of those without egg sensitisation were sensitised to DerP (p=0.11). Ninety-two percent (23/25) of patients with egg allergy were sensitised to DerP, compared with 77% (58/75) of those without egg allergy (p=0.11).

However, there was an interethnic difference in the association between house dust mite and egg sensitisation, with a stronger association in the mixed race group. In the Xhosa group, 79% (23/29) with egg sensitisation were also sensitised to DerP, compared with 80% (24/30) without egg sensitisation (p=0.95). In the mixed race group, 100% (19/19) of egg sensitised patients were also DerP sensitised, compared with 68% (15/22) who were not egg-sensitised, p=0.007. The interethnic difference in proportion of egg sensitised patients who were are also DerP sensitised was significant at 0.034.

10.3.5.3 Egg Allergy and Peanut allergy

In this study, 44% (11/25) of children with egg allergy had a peanut allergy, versus 17% (13/75) without egg allergy (p=0.005). By ethnic breakdown, this relationship between egg allergy and peanut allergy was significantly stronger in the mixed race group, in whom 64% (7/11) with egg allergy had peanut allergy versus 27% (8/30) without egg allergy (p=0.014). In the Xhosa group with egg allergy, 29% (4/14) had peanut allergy; compared with 11% (5/45) of those without egg allergy (p=0.113).

10.4 Discussion

The significant comorbidity between atopic dermatitis, food allergy, asthma and allergic rhinitis reflects the process of the allergic march, with epicutaneous sensitisation to allergens via a broken epithelial barrier in patients with AD being a major risk factor.

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Allergic co-morbidity was significant in our cohort of children with moderate to severe atopic dermatitis. The prevalence of asthma symptoms, at 39%, is significantly higher than in the general population, estimated at 14% in South Africa adolescents based on data from the ISAAC study.[23] Although the ISAAC study was based on adolescents, it is the only data for respiratory allergies that we have from a large unselected population in South Africa. The prevalence of reported allergic rhinitis symptoms of 53% is higher than the figure of 39% in South African adolescents in the ISAAC study. There were no significant ethnic differences in asthma and allergic rhinitis prevalence, and no significant difference between food allergic and food tolerant children in terms of asthma and allergic rhinitis prevalence. Although egg sensitisation was significantly associated with house dust mite sensitisation in mixed race children, egg sensitisation and allergy rates were not overall associated with an increase in asthma or AR symptoms. AD per se rather than food allergy therefore seems to be the more important risk factor for progression to respiratory diseases in this cohort.

The prevalence of both asthma and allergic rhinitis increased with age, in contrast to the prevalence of food allergy, which fell with age. This reflects the progression of the allergic march, with eczema and food allergy peaking earlier than asthma and allergic rhinitis.

Aeroallergen sensitisation was extremely common in this population, and the pattern was age-dependent. 81% of patients were sensitised to house dust mite DerP. Sensitisation to indoor allergens such as house dust mite, cat and dog dander peaked already by the age of 2 years, whereas sensitisation to grass pollen, tree pollen, *Alternaria* mould and storage mite increased with age. Unlike food allergy sensitisation, which peaked by 2 years and then declined steadily, aeroallergen sensitisation occurred well after 2 years, hence a negative aeroallergen test at a young age should be repeated at an older age in high risk patients. Also, the aeroallergen pattern evolves over time with the seasonal allergens becoming more important in the older children.

Sensitisation to Timothy grass, Bermuda grass and storage mite was significantly higher in asthmatic than non-asthmatic patients, and sensitisation to Timothy and Bermuda grass was significantly higher in patients with allergic rhinits symptoms. The indoor allergens DerP, DerF, cat and dog were not significantly higher in asthma or AR. It may be that the induction of specific IgE responses to certain aeroallergens and the development of childhood asthma are, at least partly, determined by independent factors.[24]

The indoor allergens DerP, DerF, cat and dog were, however, significantly higher in children with food allergy. As these are the allergens which peak in sensitisation at a young age, this may reflect early-onset epicutaneous sensitisation to a number of food and aeroallergens with which the young child is in contact.

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Food allergies can also co-exist in patterns, and co-sensitisation with egg and peanut allergy was common in this population, especially in those of mixed race. Overall, 44% of patients with egg allergy also had a peanut allergy, and 64% in the mixed race subgroup.

Poor control of one atopic condition can lead to increased morbidity as result of another atopic condition, e.g. poor asthma control is a significant risk factor for a more severe food-allergic reactions.[22] This particularly concerning in light of the low rates of controller medication use reported by asthmatic patients in this cohort: only 27% of Xhosa patients with doctor- diagnosed asthma took a regular controller.

On the other hand, improved control of atopic conditions may reduce the progression to or severity of associated atopic conditions, e.g. better control of atopic dermatitis may reduce the risk of food allergy or asthma. An Italian study following up 176 children with AD suggested that early diagnosis and improved management of AD may lead to a reduction in percentage of children evolving towards asthma from 29% to 15%.[25] Integrated management of the allergic patient is therefore essential, treating each allergic manifestation well to try an avoid progression to or severity of associated allergic manifestations.

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Chapter 11:

Patterns of Introduction of Complementary Feeding and Solids

11.1 Introduction

The optimal timing of the introduction of solids to minimise the risk of food allergies is currently under study. Some immunological and epidemiological evidence has suggested that early exposure of the immune system to allergens, especially via the enteral route, may enhance immunological tolerance.[1]

Delaying the introduction of solids beyond 6 months has not been shown to have any significant effect on reduction in allergies; indeed, recent studies for wheat, egg, cow's milk and fish allergy have shown that delayed introduction may lead to a higher incidence in allergies.[2-5]

Several large prospective studies are currently underway to investigate whether early or late exposure to highly allergenic foods such as peanut and egg results in a lower rate of allergies to those foods. [6-8]

This chapter explores the timing of introduction of solids and peanut dietary intake patterns of children with atopic dermatitis and their potential influence on allergy rates.

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11.2 Methodology

As part of the study investigating food allergy prevalence in 100 children with atopic dermatitis, parents were asked about dietary introduction patterns by questionnaire. They were asked about the timing of the first introduction of peanut, egg, cow's milk or dairy, soya, wheat, fish, tree nuts and sesame seed. For peanut, there was an additional questionnaire to ascertain the average weekly consumption of peanut and peanut-containing foods.

11.3 Results

11.3.1 Peanut introduction and consumption patterns

Overall, 81 (81%) of patients in the study had consumed peanut: 78% (46/59) of Xhosa children and 85% (35/41) of mixed race children (p=0.4). Overall, 62% of patients were still eating peanut on a regular basis, this was equal in both ethnic groups at 62% each. Despite a difference in monthly household income (median R2000 in Xhosa patients and R3500 in mixed race patients, p=0.01 by Mann-Whiney test), there were no significant differences in peanut consumption patterns between Xhosa and mixed race groups. In those who had introduced peanut into their diets, the median age of introduction was 18 months overall: 24 months in the Xhosa group, and 12 months in the mixed race

group (p=0.08). The median number of peanut servings per week in those consuming peanut was 3 for both ethnic groups.

Only 7 of the Xhosa patients (12%) had introduced peanut before 12 months of age, and 8 of the mixed race patients (20%), p=0.28.

Of the 13 Xhosa patients who had never consumed peanut before, 2 were found to be allergic on challenge (15%), and of the 6 mixed race patients who had never consumed peanut, 4 were subsequently found to be allergic on challenge (67%). The median age at the time of the first reaction to peanut was 24 months.

11.3.1.1 Age of Peanut Introduction and Allergy Risk

In those patients who had introduced peanut before the age of one year (n=15), 5 were found to have a peanut allergy (33%), compared to those who introduced peanut butter after a year (n=84), in whom 21 were found to be allergic (25%), p=0.51. Therefore, in this cohort there was not an obvious protective effect of peanut introduction before a year of age in comparison to after a year of age. However, with age of introduction of peanut below 8 months, the trend was towards a lower prevalence of peanut allergy: in those who introduced peanut below 8 months (n=8), only 1 had an peanut allergy (12.5%), compared to those introducing peanut at or after 8 months (n=92) in whom 23 were allergic (25%); however, this did not reach statistical significance (p=0.42).

11.3.2 Hen's egg introduction patterns

Overall, 96 (96%) of patients had eaten egg before, this was 98% in Xhosa patients (58/59) and 93% (38/41) in mixed race patients, p=0.21. The median age of egg introduction was 12 months in both ethnic groups. Of those who had never eaten egg before, 2 were sensitised to egg and were challenged, of whom one was positive. Of all the patients with an egg allergy, the median age of the first allergic reaction was 12 months.

Overall, 36 (36%) of patients introduced egg before a year of age: 34% (20/59) of Xhosas and 39% (16/41) of mixed race origin introduced egg before a year of age, p=0.6.

In those patients who introduced egg before a year of age (n=36), 11 were found to have an egg allergy (31%), compared to those who introduced egg after a year (n=64), in whom 14 were fund to be allergic (22%), p=0.32. Therefore, in this cohort there was not an obvious protective effect of egg introduction before a year of age in comparison to after a year. However, with introduction of egg below the age of 8 months, the trend was towards lower prevalence of egg allergy: in those who introduced egg below 8 months (n=12), only 1 had an egg allergy (8%), compared to those introducing egg at or after

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8 months (n=88) in whom 24 were allergic (27%). However, this did not reach statistical significance (p=0.15).

11.3.3 Cow's milk and dairy products introduction

Overall, 89 (89%) of patients had consumed a cow's milk based formula: 92% (54/59) of Xhosas and 85% (35/41) of mixed race patients, p= 0.27. The median age of introduction of cow's milk formula was 3 months in both ethnic groups.

Whole cow's milk had been introduced in 86 (86%) cases: in 89% (50/56) of Xhosas and 88% (36/41) of mixed race patients, p=0.9. The median age of introduction of whole cow's milk was 18 months overall: 13.5 months in Xhosa patients and 18 months in mixed race patients.

Overall, 96 (96%) patients had consumed dairy products: 95% (56/59) of Xhosas and 98% (40/41) of mixed race patients, p=0.44. These were introduced at a median age of 8 months overall, 8 months in Xhosa patients and 12 months in mixed race patients.

The median age at first reported reaction to cow's milk was 12 months, and to dairy products 18 months.

11.3.4 Soya Introduction

Overall, 26 patients (26%) had consumed a soya formula, 27% (16/59) in Xhosa patients and 24% (10/41) in mixed race patients, p=0.77. The median age of soya introduction was 6 months overall: 6.5 months in Xhosa patients and 3 months in mixed race patients. The median age at the time of perceived soya allergy was 2.5 months, but none of the patients with perceived reactions were subsequently found to have an IgE-mediated allergy to soya at the time of the study.

11.3.5 Wheat introduction

All 100 patients in this cohort had consumed wheat. The median age of wheat introduction was 6 months overall, 7 months in Xhosa patients and 6 months in mixed race patients. The median age at first perceived reaction to wheat was 7.5 months, but at the time of study no patients were found to have IgE-mediated wheat allergy.

11.3.6 Fish introduction

Overall, 87 (87%) of patients had consumed fish at the time of the study: 83% (49/59) Xhosa patients and 93% (38/41) mixed race patients, p=0.14. The median age at fish consumption was 24 months overall: 24 months in Xhosa patients and 15 months in mixed race patients. The median age at first reported reaction to fish was 24 months.

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11.3.7 Tree Nut introduction

Only 30 patients (30%) had knowingly consumed tree nuts. Tree nut consumption was significantly more common in the mixed race patients: 22% (13/59) of Xhosas and 41% (17/41) of mixed race patients had introduced tree nut into their diet, p=0.04. Of those who consumed tree nuts the average age of introduction was 24 months in both ethnic groups. The average age at the time of first reaction to tree nuts was 42 months.

11.3.8 Sesame introduction

Overall, 16 (16%) of patients had knowingly consumed sesame. Sesame consumption was significantly higher in mixed race patients: 8% (5/59) of Xhosas had consumed sesame and 27% (11/41) of mixed race patients had consumed sesame, p=0.01. The median age at introduction of sesame was 24 months in both ethnic groups.

Complementary food introduction patterns are summarised in table 11.1

Table 11.1: Table showing age of introduction of complementary feeds

	% of patients who had introduced the food into their diet	**Median age of introduction overall (months)**
Peanut	81%	18
Hen's Egg	96%	12
Cow's Milk-based formula	89%	3
Whole Cow's Milk	86%	18
Dairy products	96%	8
Wheat	100%	6
Soya	26%	6
Fish	87%	24
Tree Nuts	30%	24
Sesame	16%	24

11.4 Discussion

In this cohort of patients with atopic dermatitis, consumption patterns of complementary feeding did not differ significantly between ethnic groups. The tendency was towards relatively late introduction of peanut (median 24 months), egg (median 12 months), tree nut (24 months) and fish (24 months).

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These foods are known to be allergenic; and atopic dermatitis patients may well deliberately delay the introduction of such solids to try and minimise allergic reactions. This is despite the increasing evidence that a delay in introduction of allergenic foods does not reduce allergy risk. Despite the delayed introduction of these solids, the prevalence of egg and peanut allergy in this cohort was particularly high. Recall bias could have influenced the reported age of solids introduction in the older children, reporting introduction either earlier or later than occurred in true life. Those children who had not yet introduced a certain feed were omitted from the analysis of that particular food.

Of particular interest is a possible shift in peanut consumption patterns amongst the Xhosa group.
In a dietetic study in 2006, mothers (n = 198) of Black infants aged 4 – 36 months in Cape Town were interviewed about their infants' peanut intake using a peanut consumption questionnaire.[9] In that study, the mean age of introduction of peanuts and peanut products was found to be 10 months, and 64% of subjects started eating peanuts before one year of age. The median total peanut intake was 12 g/day (1-2 portions per day). In comparison, in the 59 Xhosa patients from our study cohort, the median age of introduction of peanut was 24 months, and only 12% of children had consumed peanut before the age of one. Of those consuming peanut, the average consumption was 3 portions per week.

Caregivers of patients with eczema may deliberately delay the introduction of peanuts to the child's diet because of a growing public knowledge about peanut allergy or because of the advice of a healthcare practitioner. This may lead to reverse causation, with the eczema actually leading to later introduction of peanut. However, the cautious introduction of allergenic solids is a potential concern with mounting evidence that delaying the introduction of allergenic solids is not beneficial in allergy prevention. [1-5]

In our study, there was a trend towards lower allergy rates in those who had introduced egg and peanut earlier (younger than 8 months), however, this did not reach statistical significant and requires larger studies for accurate results.

Wheat products were consumed in all children at the time of study and were introduced relatively early at a median age of 6 months. There was no IgE-mediated wheat allergy in this cohort of children.

Cow's milk formula had been consumed in a majority of the population (89%) at a median age of 3 months, once again relatively early. The prevalence of cow's milk allergy in this population was lower than expected (2%). Although dairy products were introduced by 8 months in the majority, fresh whole

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cow's milk was ingested at a reasonably late age, 18 months, suggesting prolonged formula or breastfeeding well past a year of age before switching to fresh whole cow's milk.

The consumption of tree nuts (30%) and sesame (16%) was low, especially in Xhosa patients.

Overall, this study suggests the trend towards late introduction of certain allergenic foods, particularly peanut. The outcome of studies investigating the ideal time of enteral introduction of allergenic foods such as peanut and egg is awaited,[6-8] and will help guide our patients as to the best strategy to try and minimise allergies. This is particularly important in children at high risk of food allergies, such as those with atopic dermatitis. The trend towards later introduction of solids does not seem to benefit allergy reduction.

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Chapter 12

Conclusion: Clinical Application of Study Findings and the Increasing Burden of Food Allergy in South Africa

12.1 Summary and Clinical Application of Study Findings

This was the first study in South Africa designed to investigate the prevalence of IgE-mediated food allergy in a defined population, and to include the use of controlled food challenges. Several modalities of screening for food allergy were performed and could be compared: patient history, skin prick test (SPT) results, specific IgE components by the ISAC (Immuno Solid Phase Allergen Chip) 103 test, ImmunoCAP tests to whole foods as well as components, as well as results from controlled incremental food challenges. Patient history alone significantly overestimated true allergy prevalence, and of those cases of reported allergies, only 42% were found to be truly allergic.

The prevalence of sensitisation (66%) as well as confirmed food allergy (40%) in our cohort of South African children with atopic dermatitis was unexpectedly high, in both Xhosa children as well as those of mixed race. It mirrored that of previous studies in high socio-economic areas, mainly Europe and the USA.[1-3] Egg (25%) and peanut (24%) allergies were by far the most common. However, although sensitisation rates were comparable between the 2 ethnic groups, allergy rates were generally higher in the mixed race group than in Xhosa patients, significantly so for peanut (38% versus 15%, p=0.01) and cashew nut (7% versus 0%, p=0.04). Therefore, although food allergy prevalence is high in this selected population, the Xhosa patients may still have a relative protection from manifesting as allergic when they are sensitised.

Overall, 60% of sensitised patients had a true allergy, this ratio was significantly lower in the Xhosa group: 49% versus 80% in mixed race patients (p=0.002). This means that less than half of Xhosa patients who are sensitised to one or more food are actually truly allergic, indicating a high rate of false positives. Once again, this was especially evident for peanut allergy, in which 75% of peanut-sensitised mixed race patients but only 39% of peanut-sensitised Xhosa patients were found to be allergic (p<0.001). This emphasises the need for further confirmatory tests in the form of the controlled food challenges, especially so in the Xhosa population.

Risk factors for food allergy included early age of onset of eczema (< 6 months), young age at the time of assessment (<2 years) and higher eczema severity (SCORAD score in the severe level). Children with

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any of these risk factors together with moderate to severe eczema, especially if inadequately controlled by topical skin care, should thus be screened for food allergy.

In the diagnosis of peanut allergy, out of all the tests performed (SPT, ISAC components, ImmunoCAP peanut and ImmunoCAP peanut components) the diameter of the SPT was the superior test in differentiating allergy from tolerance to peanut. Although the widely used positive predictive value (PPV) of 8 mm[4] for the peanut SPT is applicable to the mixed race group (88% PPV at SPT diameter ≥8 mm), it performed slightly less well in the Xhosa population (80% PPV at SPT diameter ≥8 mm), who fared better at a higher SPT diameter (88% PPV at SPT diameter of 11 mm). Thus, although the SPT to peanut remains a sensitive test for peanut allergy, its specificity may be increased by revising the 95% PPV levels for the prediction of peanut allergy in our local population, especially in the Xhosa subgroup.

Peanut components measured in this study included *Ara h 1*, 2, 3, 8 and 9. Component patterns were similar amongst ethnic groups. *Ara h 2* was the superior component in both ethnic groups for differentiating true allergy from tolerance. However, the component reactivity amongst asymptomatically sensitised patients was significantly higher in the Xhosa population than the mixed race patients, leading to higher false positives in the Xhosa group. For a positive ImmunoCAP to *Ara h 2* (≥0.35 kU/L), the chances of a peanut allergy were high (93%) for mixed race patients but significantly lower (53%) in Xhosa patients. Once again, the role of food challenges is crucial to differentiate tolerance from allergy in sensitised patients. In this study, the component *Ara h 9* (a lipid transfer protein, which is a significant allergen in Mediterranean countries[5]) was associated with tolerance, representing a cross-reactive component. The greater the number of peanut storage proteins (*Ara h 1*, 2 and 3) positive, the greater was the chance of peanut allergy and also more severe peanut allergy. Positivity to *Ara h 8* and 9 actually reduced the chances of a true allergy.

Importantly, in our study there was no evidence of parasitaemia or reactivity with cross-reactive carbohydrate determinants causing false positive sensitisation in the Xhosa group in comparison to the mixed race group.[6] Moreover, SPT reactivity and peanut component reactivity were high amongst our Black participants, unlike findings from other regions which suggest low SPT positivity but high specific IgE reactivity to whole peanut amongst the Black population.[7]

Egg allergy was the most common allergy overall (25%) and peaked in the younger age group under the age of 2 years. As a screening test for egg allergy, the SPT to raw egg white was 100% sensitive, in comparison to the SPT to egg extract which was 80% sensitive and thus missed 20% of cases of egg allergy. Moreover, In prediction of egg allergy in the population overall, SPT to fresh raw egg white had the highest Receiver Operating Characteristic (ROC) area under the curve in both ethnic groups.

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Egg component pattern analysis was similar between ethnic groups, and found that egg-sensitised patients, *Gal d 1* (ovomucoid) was the most common component, followed by *Gal d 3*, *Gal d 5* and *Gal d 2*. In differentiating egg allergy from tolerance in egg-sensitised patients, the component *Gal d 1* gave best results with the highest ROC area under the curve. The higher the number of egg components positive, i.e. the greater the epitope diversity, the greater was the chance of egg allergy. Widely used 95% PPV cut-offs for SPT to egg as well as ImmunoCAP[4,8] to egg white performed sub-optimally in this population, more so in the Xhosas, and may need to be revised.

The prevalence of cow's milk allergy was surprisingly low (2%) in comparison to cohorts from westernised countries. This may reflect the higher median age of our study population (42 months, by which time many children have outgrown their cow's milk allergy) or it may truly indicate that IgE-mediated cow's milk allergy is less common in the South African population, and deserves further investigation. SPT to fresh cow's milk was superior to cow's milk extract in the diagnosis of cow's milk allergy, and was 100% sensitive for cow's milk allergy, whilst SPT to cow's milk extract was negative in both cases of cow's milk allergy. Therefore, in the case of both egg and cow's allergy screening, SPT to fresh raw egg white and fresh milk should be included in the screening panel of SPTs. Widely used 95% PPV for SPT and ImmunoCAP cow's milk[4,8] fared poorly in this population and may need to be investigated in large population studies in infants and young children.

Allergic co-morbidity was significant, with 39% of children reporting symptoms of asthma, and 53% of children reporting symptoms of allergic rhinitis. 89% of participating patients were sensitised to at least one aeroallergen, and over 80% to a house dust mite species. Sensitisation to house dust mite, cat and dog allergens already peaked by 2 years of age. Sensitisation to grass and tree pollen increased with age and was associated with an increased risk of asthma and allergic rhinitis. Asthma controller medications and intranasal corticosteroids for allergic rhinitis were underused, which is concerning in view of the fact that poor asthma control can increase the risk of severe food allergy reactions. Epicutaneous sensitisation to food as well as aeroallergens is a result of the defective skin barrier in eczema, which should be addressed at an early stage in order to try and dampen the progression of the allergic march.

In this cohort of patients with atopic dermatitis, consumption patterns of complementary feeding did not differ significantly between ethnic groups. However, there was a trend towards late introduction of certain allergenic foods, especially peanut, which was introduced at a median age of 18 months (24 months in Xhosa, 12 months in mixed race group). This introduction of peanut is more conservative than in a previous study amongst Xhosa patients,[9] in whom peanut has traditionally been a commonly used, nutritious food supplement. Suggestions that later introduction of allergenic foods may led to

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an increase in allergy rates have led to several studies which are currently investigating the ideal time of solids introduction, including amongst high-risk patients with eczema.[7] Results of these studies are awaited, and will allow us to advise our patients more appropriately on the optimal time of solids introduction.

The ImmunoCAP ISAC test has many advantages in terms of small volume of blood required, multiple food and aeroallergen components tested simultaneously and identification of co- and cross reactivity patterns.[10] However, we found it to be less sensitive in the diagnosis of food allergy than skin prick tests and traditional ImmunoCAP tests, and it missed 15% of cases of food allergy. Thus, the skin prick test has proven to be an excellent screening test for food allergy, and more complex and costly tests such as the ISAC test should be reserved for those with complex or multiple allergies.

Co-existing food allergies in children with moderate to severe AD are currently being underdiagnosed in South African children, and co-ordinated interaction between dermatology and allergy clinics is needed. In this study, 47% of patients required at least one food challenge to correctly identify their allergies, therefore, a full allergy service offering food challenges is needed as part of a multidisciplinary team offering comprehensive care for atopic children.

The unexpectedly high prevalence of food allergy in this cohort of South African children has led to the concern that food allergy may be on the increase in South Africa, amongst all ethnic groups, and that the local buden of food allergy may be significant. Although this cannot be extrapolated to an unselected population, the general trend amongst allergy clinics in South Africa, including Red Cross Hospital, has been an increase in case load of food allergy patients over the past 5-10 years.[11] In addition, previously rare food allergy manifestations such as eosinophilic oesophagitis have been increasingly identified in South Africa in the past few years.[12]

Indeed food allergies may also be on the increase in other African countries. In a recent study in Ghanaian school children, 11% of 1407 children reported adverse reactions to foods, and 5% of 1431 children showed positive SPT reactivity.[13] Food challenges were not performed as part of this study.

Protective and aggravating factors which affect allergy expression, possibly by epigenetic mechanisms, need to be identified urgently.

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12.2 What Factors Could be Driving an Increase in Food Allergy in South Africa?

Food allergy is usually associated with other atopic disorders and hence likely shares many common risk factors. However, unique food allergy considerations such as the delay between the "respiratory allergy" epidemic and the "food allergy epidemic", the potential life-threatening nature of food allergy and related public health measures make the identification of specific risk factors a priority. Two factors which deserve further mention in allergy causation are early life influences, and migration of populations.

12.2.1 Early life influences

The distinct sequence of the allergic march suggests that certain individuals are prone to manifesting their atopic conditions under the influence of environmental factors within a particular timeframe. [14] Expression of food allergy in very early infancy dictates that early post-natal events and likely antenatal events play a critical role in abrogating the normal default response of tolerance to food allergens. [15] Factors which could play a role in the expression of food allergy include:[14,16,17]

1. Allergen exposure
2. Gut microflora
3. Gastric acid
4. Changes in diet
5. Infections
6. Eczema and other atopic conditions
7. Genetic and epigenetic factors

12.2.2 Migrant Populations

The rapid urbanisation, adoption of a westernised lifestyle and diet in South Africa simulate the characteristics of a large "migrant" population. In migrant populations, the change in environment and lifestyle may affect genotype expression, thus leading to the manifestation of an allergy. Three factors are important in migrant populations: [18]

1. The population must have an innate genetic predisposition to development of allergies
2. Environment or lifestyle factors in their place of birth actively prevented/ suppressed the expression of allergy
3. Exacerbating factors in an altered environment actively allow the genetic potential for allergy to become unveiled.

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The following section discusses some of the factors influencing the expression of allergy and their change in South Africa over the last decade or two.

12.2.3 Factors influencing the expression of allergy

12.2.3.1 Allergen Exposure

Whether a person becomes sensitised or tolerant to an allergen depends on multiple factors such as the timing, dose, route of exposure and possibly the nature of the allergen.[19] Allergen exposure via the inhaled or cutaneous route may abrogate the tolerance normally afforded via gut exposure.

There seems to be a window of opportunity for optimal allergenic solid food introduction between 4-6 months of age. Delayed introduction of allergenic foods such as wheat, egg and cow's milk has been associated with higher risk of food allergy in some studies.[20,21]

In South Africa, patterns of utilisation, consumption and exposure of foods need to be explored in depth as urbanisation occurs. Consumption patterns, for example with peanut, may be changing. Furthermore, the presence of potentially allergenic food components in non-food items, such as peanut oil in emollients, need further clarification.

12.2.3.2 Gut Microflora

There is a delicate balance between bacteria and the immune system. Early colonisation of the intestinal tract by appropriate microbiota is important for healthy maturation of the immune system, especially appropriate programming of oral tolerance to dietary antigens.[22,23] Differences in certain bacterial populations between allergic and non-allergic infants have been noted.[24]

In South Africa, an increase in the rate of caesarian sections, changing diet in childhood, more frequent use of antibiotics and a changing maternal intestinal microbial milieu could all impact on a child's microflora. Much of the microbiome may be shaped by early exposures to environmental soil/dust by hand to mouth transmission (personal communication Christine Cole, Detroit USA). As the environmental dust and maternal microflora change in a migrant population, so too does the microbiome of children, which may affect immune responses to allergens.

12.2.3.3 Gastric Acid

Most food allergens are susceptible to acid digestion in the stomach. This has raised the concern that changes in the stomach acid content may make subjects more susceptible to allergen sensitisation.

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Indeed, the increasing use of anti-acids in young babies with presumed gastro-oesophageal reflux disease has paralleled the rise in food allergies.[25]

12.2.3.4 Changes in Diet

In African countries, considerable changes in diet have occurred. Notably, the intake of plant foods has diminished, with increasing ingestion of animal products, and a decrease in fibre intake.[26] Anti-inflammatory immunomodulating factors in the diet such as prebiotics, fat soluble vitamins and polyunsaturated fatty acids may be reduced in westernised diets, making them more "inflammatory".[27] An increase in "fast food" consumption is a concern: the ISAAC study (International Study on Asthma and Allergy in Children) showed that consuming 3 or more portions of fast food per week increased the risk of asthma significantly, whilst fruit consumption more than 3 times a week was protective.[28]

Large urban-rural differences in diet do not only affect allergen exposure to food allergens, but have been found to affect skin sensitivity to common aeroallergens; in other words they can shift the entire allergic "make up."[29]

The influence of maternal diet during pregnancy and lactation on allergy expression in the infant remains controversial and under study.

12.2.3.5 Burden of Infection

Developing countries including South Africa are frequently plagued by poor sanitation and housing, overcrowding and lack of access to clean water. It seems that early exposure to such environments with high microbial and endotoxin levels, can be protective against allergic conditions (the hygiene hypothesis).[30] However, the degree of protection depends on the intensity and timing of the infections.

Certain helminthic infestations may protect an individual from allergic predisposition by mechanisms that are incompletely understood. These may include IL-10 related suppression of the allergic response.[31]

In South Africa, the intensity of infection exposure may be lessening with urbanisation, more ready access to medical treatment and smaller family size. In addition, helminthic infections are treated more readily and regularly.

University of Cape Town

An interesting theory is that the increase in allergic burden in Black Africans may be even more dramatic as parasitic infections could have primed the immune system for IgE responses.[30,32]

12.2.3.6 Genetic Factors

A family history of atopy is a strong risk factor for developing atopic diseases later in childhood. Rising rates of maternal allergy, which is a strong direct determinant of allergic risk, may have implications for allergic burden in generations ahead. [15]

The rapidity of the increase in food allergies worldwide suggests that epigenetic changes modify disease expression under the influence of environmental changes. Epigenetic changes may be heritable, and even amplified across generations. It has been suggested that the new generation of food allergy patients is more likely to have a more severe or persistent clinical course. [15]

Moreover, evidence suggests that development of allergy in non-White populations is heightened in a westernised environment, over and above that of the local western population.[32,33] This suggests a strong genetic propensity that is amplified in a western environment. This is of particular concern in the Black South African population as urbanisation and movement away from traditional lifestyles occur.

12.3 Future Directions

The results of this study suggest a significant burden of food allergy in South Africa. As opposed to other allergic conditions, when considering food allergies, a critical time and dose of the food, in conjunction with the delicate balance between exacerbating and protecting factors are vitally important. The question remains as to whether there are as yet unidentified factors influencing the food allergy epidemic, or whether it is simply an amplification of allergic manifestations from one generation to the next.

Following the surprising burden of food allergy revealed by this study, we have now embarked on a large food allergy prevalence study in an unselected population of 1-3 year olds in South Africa.[34] The aim is to examine rural-urban differences and ethnic patterns in food allergy prevalence. The results of this forthcoming study will provide important data on the possible food allergy epidemic in South Africa, allowing more targeted and informed planning of healthcare services. Moreover, by exploring factors which may influence the current food allergy epidemic, we hope to identify early determinants of food allergy which can potentially be modified.

University of Cape Town

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University of Cape Town

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University of Cape Town

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University of Cape Town