In vivo and in vitro diagnosis of latex allergy at Groote Schuur Hospital

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Objective. The aim of this study was to evaluate the diagnostic utility of skin-prick tests, radio-allergosorbent tests (CAP RASTs), basophil histamine release, sulphidoleukotriene release and Western blotting in the diagnosis of latex allergy at Groote Schuur Hospital.

Design. Patients with a history suggesting latex hypersensitivity were recruited via staff health and allergy clinics at Groote Schuur Hospital. A clinical assessment was followed by laboratory investigation and skin-prick testing. A control group consisted of laboratory and hospital staff who had regular latex exposure but were asymptomatic.

Setting. Hospital-based cohort at Groote Schuur Hospital.

Participants. Twenty-three patients with suspected latex allergy; 10 control subjects exposed to, but not clinically sensitive to, latex.

Main outcome. Skin-prick testing was more sensitive than in vitro diagnostic tests for the diagnosis of latex allergy.

Results. Eighteen of 21 (85.7%) of the patients tested had a positive skin-prick test with a commercial latex solution (Allerbioprick) and 17/21 (80%) tested skin-prick-positive with an in-house glove extract. CAP RASTs were positive in 13/23 patients (66.6%), sulphidoleukotriene release was positive in 10/23 (43%), histamine release assay was positive in 10/23 (43%) and Western blots were positive in 8/23 (34.7%). All patients with only urticaria were Western blot-negative and CAP RAST-negative, suggesting that they have very little circulating latex-specific IgE. Although patients who were Western blot-positive tended to have multi-organ involvement, both patients with anaphylaxis were Western blot-negative.

Conclusion. Latex allergy is a significant clinical problem at Groote Schuur Hospital. Titrated skin-prick testing performed in a controlled environment can safely and reliably confirm the diagnosis in patients who do not give a history of anaphylaxis. The CAP RAST was the most sensitive in vitro test for latex allergy locally available, but lacks sensitivity in patients presenting with urticaria only.

Immediate hypersensitivity to products containing natural rubber latex poses a major occupational health hazard to health workers. Published reports of the prevalence of latex hypersensitivity among health care personnel have varied between 10% and 17%. With increasing reports of latex allergy appearing in the literature, health care workers in South Africa are becoming increasingly aware of the existence of latex hypersensitivity, but the prevalence of latex allergy at South African hospitals is, as yet, unknown.

Latex hypersensitivity is a typical IgE-mediated immediate hypersensitivity. Symptoms range from urticaria, rhinoconjunctivitis, asthma and angio-oedema, to sometimes fatal anaphylaxis. Delayed hypersensitivity contact dermatitis reactions due to other additives in gloves, e.g. thiurams and carbamates, which are used in the manufacturing process of latex products, were previously more commonly encountered than true latex hypersensitivity. Delayed reactions have become less frequent due to improvements in modern glove manufacturing processes which reduce carbamates and thiuram content.

The diagnosis of true latex allergy can be elusive at times, but it is important to make a specific diagnosis, since continued exposure to latex in a sensitised patient may have serious or even life-threatening consequences. Confirmation of the diagnosis of latex allergy may be made by direct challenge tests, skin-prick testing or in vitro testing.

Skin testing with latex extracts is a sensitive test of latex hypersensitivity, but has been associated with severe adverse reactions in highly sensitive subjects. The major allergens in natural latex have not been well characterised and there are, as yet, no standardised or reference latex allergen extracts available for skin testing. In vitro tests have the advantage that they are safe and could be useful in screening. To date, several in vitro methods have been developed, but all have been found to have disadvantages. Immunoblotting has been shown by some workers to be a sensitive means of in vitro diagnosis, but is too time-consuming to be used for routine diagnostic purposes. The basophil histamine release assay has been shown to be a sensitive method of demonstrating latex hypersensitivity in vitro. However, the lengthy procedures and the requirement for fresh cells make it an unsuitable test for routine diagnostic purposes. The radio-allergosorbent test (CAP RAST) is a readily available and standardised in vitro assay available to diagnose latex hypersensitivity, and has been reported as having a sensitivity ranging from 40% to 70%.

The determination of sulphidoleukotriene release from basophils has not previously been evaluated as a diagnostic test for latex allergy. In addition to its ability to detect the existence of specific hypersensitivity, it may also reflect the intensity of allergen hypersensitivity.

The leucocyte histamine release test (LHRT) is an advance on the standard histamine release assay. This assay utilises a unique glass fibre which binds histamine with high affinity and selectivity. In a recent study of the LHRT assay in latex-allergic patients the LHRT had a sensitivity of 61%, compared with the CAP RAST, which had a sensitivity of 37%.

The aim of this study was to compare the diagnostic utility of skin-prick testing, SLT, LHRT, CAP RAST assays and Western blotting in a cohort of patients with a clinical history of latex hypersensitivity at Groote Schuur Hospital.
Patients and methods

Patients
Twenty-three patients with a history of latex hypersensitivity were recruited via the Staff Health and Allergy Clinics at Groote Schuur Hospital. Ten patients (laboratory and hospital staff) who had regular exposure to latex but were asymptomatic, were recruited as control patients. Patients underwent skin-prick testing and donated blood for Western blotting, LHRT, CAP RAST assays, and SLT release assays.

In-house latex extract
The 'in-house' glove extract was prepared as follows: latex gloves (Latex Surgical Products) were cut into 1 cm x 1 cm squares and incubated in phosphate-buffered saline (1:5 w/v) overnight at 4°C. The extract was centrifuged and the supernatant sterile-filtered. The protein concentration was adjusted to 2 mg/ml as determined by the BCA (Pierce) protein assay.

Skin-prick tests
Patients and controls were skin-tested with the Allerbioprick latex extract, the 'in-house' latex extract, and a panel of six common inhalant allergens including house-dust mite, cat, dog, grasses, mould and feathers (Dome Holister Steer). Two patients who had a clear history of anaphylaxis were not skin-tested. Patients who reported systemic reactions (bronchospasm, generalised urticaria and angio-oedema) to latex were skin-tested, starting with 1:5000 dilutions of both commercial and the in-house latex extracts. A wheal of > 3 mm appearing after 20 minutes, in the presence of a positive histamine and negative saline control, was regarded as positive.

Sulphidoleukotriene assays
Reagents for the SLT assays were supplied by Buhlmann (Basel). Five millilitres of blood were collected into a tube containing 0.1M EDTA and the assay was performed within 3 hours of blood collection. Briefly, red blood cells were separated by means of dextran and leucocytes centrifuged and re suspended in an incubation buffer containing interleukin-3. The cell suspension was incubated in duplicate with either incubation buffer (background), stimulation control (positive control anti-lgE) or a latex allergen, in a microtitre plate (Nunc). Cell suspensions were centrifuged after 30 minutes' incubation and the supernatants assayed with an ELISA for sulphidoleukotrienes (Buhlman). Results (in pg/ml) were expressed as a stimulation index (SI) obtained by dividing the latex stimulation value by the background value, or as a stimulation yield (SY) obtained by subtracting the background value from the latex stimulation value. An SI value of 2, or an SY value of 300 pg, was interpreted as a positive result.

Leucocyte histamine release test
Materials were supplied by Refilab (Copenhagen, Denmark). Ten millilitres of venous blood was collected into tubes containing lithium heparin. The blood was processed within 24 hours of collection. Briefly, the samples were centrifuged and the plasma was replaced with PIPES buffer. PIPES buffer was also added to the latex LHRT strips. The LHRT strips and blood samples were pre-incubated for 30 minutes. After pre-incubation, 50 μl of blood per well was added to the LHRT strips and incubated for 60 minutes. Anti-lgE LHRT strips were used as a positive control. LHRT strips were washed with distilled water, air-dried overnight and then assayed for histamine concentration by the manufacturer. Positive results are graded as 0 - 6+. Grades 1 - 6 represent decreasing concentrations of latex on the LHRT strip. The grade of a positive result corresponds with the lowest concentration of latex on the LHRT strip which causes release of > 10 ng/ml of histamine.

Western blots
Ammoniated rubber latex extract was diluted 1/5 and electrophoresed in a 12% reducing polyacrylamide gel. The gel was blotted onto PVDF membrane (Amersharm) using a semi-dry system. The membrane was blocked with 1% polyvinylpyrrolidine (PVP) and incubated with patient's sera overnight (diluted 1/25). Latex-specific IgE was then detected using monoclonal anti-lgE antibodies (prepared in our laboratory), biotinylated rabbit anti-mouse (DAKO), streptavidin-peroxidase (DAKO) and enhanced chemiluminescence (ECL) substrate.

CAP RAST
CAP RAST assay was performed according to the manufacturer's (Pharmacia — Uppsala, Sweden) specifications. A value of > 0.35 kU/l was regarded as positive.

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

Results
Patients
Twenty-three patients with a history suggestive of immediate hypersensitivity to latex were investigated. The mean age of patients was 36.2 years. Of the 23 patients 19 (82%) were atopic, as defined by their having a positive skin-prick test for one or more of the inhalant allergens. Sixteen of the 23 (70%) were female. Urticaria was present in 21 patients, rhinoconjunctivitis in 12 patients, bronchoconstriction in 8 patients, angio-oedema in 3 patients and anaphylaxis in 2 patients. In all but 2 patients, the initial presentation of latex sensitivity was a contact urticaria following exposure to latex products (gloves). All of the patients with urticaria were regularly using powered surgical latex gloves. The 2 patients who did not experience contact urticaria presented with symptoms of rhinoconjunctivitis. Of the 2 patients who presented with anaphylaxis, 1 was a surgeon who had donned a latex glove over an open hand wound, and the other a paraplegic patient who had lost consciousness after using a latex glove for a manual faecal removal. Five of the patients had symptoms severe enough to warrant removal from their working environments. Results of the skin tests, LHRT, CAP RAST, SLT and Western blot assays are shown in Table I.
Table I. Results of skin tests, LHRT, CAP RAST, SLT and Western blot assays

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<th>No.</th>
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<th>Sex</th>
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<th>SPT extr</th>
<th>RAST</th>
<th>SLT</th>
<th>LHRT</th>
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SPT comm = commercial skin-prick test; SPT extr = in-house skin-prick test; RAST = CAP radio-allergosorbent test; SLT = sulphidoleukotriene result; LHRT = leucocyte histamine release test; Biot = Western blot; Band kDa = IgE binding to molecular weight band in kilodaltons; U = urticaria; RC = rhinoconjunctivitis; BC = bronchoconstriction; ANA = anaphylaxis; ND = not done; TE = technical difficulties.

Skin-prick tests
The 2 patients with a history of anaphylaxis were not skin-tested with latex extracts. Of the 21 patients who were skin-tested, 18 (85.7%) were positive with the commercial extract and 17 (80.9%) were positive with the in-house glove extract. Wheal size varied from 4 mm to > 10 mm. The patients who were tested with the 1:1 000 dilution of the latex extracts responded immediately and further testing with undiluted extract was therefore unnecessary. Of the 3 patients who were negative on skin testing, 2 were CAP RAST (class 1+) positive and 1 negative in all the in vitro assays. All 10 control patients were negative on skin testing. None of the patients tested had any systemic reactions during or after skin testing.

Sulphidoleukotriene assays
The SLT assay was positive in 10/23 (43.4%) patients. In 8 of the patients tested, we observed extremely high background levels of SLTs, i.e. > 1 000 pg/ml. These 8 patients were all highly atopic and clinically highly sensitive to latex. The SLT assay was repeated in these 8 patients and the results were found to be reproducible. One control patient was found to be positive on SLT assay.

Leucocyte histamine release tests
The LHRT was positive in 10/22 (45%) patients. The LHRT was not performed in 1 patient because of technical difficulties. In addition, 4 patients were found to be anti-IgE negative. These 4 patients also had background values > 1 000 pg/ml on SLT assay. One of the control patients was positive on LHRT assay.

Western blots
Specific IgE binding to latex allergens on Western blots was demonstrated in 8/23 patients (34.7%). Seven different IgE-binding profiles were observed (Fig. 1). Patients in whom the Western blotting was positive are indicated in Table I and the molecular weight fractions identified are shown. The 40 kDa band was present in each of the 8 patients and a 32 kDa band was present in 7/8 patients. In each of the patients who were Western blot-positive (except patient 4), the RAST was positive. Other than in patients 8 and 19, the SLT assay was positive when the Western blot was negative. Six of the 8 patients with a positive SLT test had bronchoconstriction and multiple organ involvement. All patients presenting with urticaria alone were Western blot-negative. Both patients with anaphylaxis were Western blot-negative. One of the control patients was weakly positive on the Western blot.

Fig. 1. Western blots showing 7 different specific IgE-binding profiles to ammoniated latex in clinically sensitive patients (lanes 1 - 7). Lanes 8 - 15 show absence of specific binding in 5 negative controls.
**Discussion**

Latex allergy is a significant occupational hazard at Groote Schuur Hospital. Sensitisation must be diagnosed at an early stage to prevent progression to more severe symptoms. At the time of recruitment, only 2 of the patients we tested were aware that their symptoms were due to latex allergy. Our study emphasises the need for education among health care personnel about the symptoms, hazards and prevention of latex allergy. It also highlights the need for sensitive screening procedures among health care personnel to diagnose latex allergy early and to institute latex avoidance measures.

The most sensitive confirmatory test for latex allergy was the skin-prick test. Skin testing with latex extract is safe, provided that patients are carefully selected and that the procedure is done by competent medical personnel, with full facilities on standby for resuscitation. In the case of highly sensitive individuals it is advisable to perform titrated skin testing initially with high dilutions of commercial latex extracts. The evidence from other studies that adverse reactions can occur with skin testing cannot be ignored. The CAP RAST test was positive in 57% of the subjects. In view of its safety and simplicity it serves as a useful screening assay in patients with multiple organ involvement. The CAP RAST was highly specific but its lack of sensitivity was a disadvantage. Its sensitivity was particularly low in patients who presented only with a history of urticaria.

The SLT test was found to have a low positivity of 43%. Technically the assay was easy to perform and requires little time. We found that 8 of our clinically highly atopic patients had extremely high background levels of SLT which made interpretation of results, using the formula provided by the manufacturers, difficult at times. In several cases these high background values differed very little from the anti-IgE and latex allergen stimulation values. If patients with such high background values were also high-risk patients, given their frequent exposure to latex products. We regard these 2 patients as being sensitised to latex and having the potential to become clinically affected in the future. Careful observation and follow-up evaluation are advisable in such cases.

An important difficulty in the development of ideal in vitro assays for latex allergy lies in the fact that relatively little is known about the nature of the different latex allergens. Different assays may use latex from different sources and a fair comparison of different in vitro assays would be difficult unless a standardised allergen is used. We believe that the difference in the potency of allergens used in the tests we have evaluated may partially account for some of the differences we have observed in our results. Our Western blotting techniques have demonstrated that different patients recognise different molecular weight fractions in latex extracts. Profiles of IgE binding on Western blots are also variable and dependent upon the latex extract used for the gels. The 40 kDa protein was recognised by all the patients who were positive on the Western blots. Although patients with positive Western blots invariably had multiple organ pathology, it is of interest that both patients with a history of anaphylaxis were Western blot-negative. Patients with a history of only urticaria were invariably Western blot-negative and CAP RAST-negative. Patients with only urticaria appear to have very little circulating specific IgE and therefore have negative Western blots and RASTs. They clearly have latex-specific IgE bound to cutaneous mast cells and to basophils.

The development of better in vitro screening tests will not be possible until detailed biochemical and immunological characterisation of the important latex allergens or epitopes has been achieved. Our data indicate that depending on the clinical presentation of the patients, the reliability of certain current in vitro tests will vary. For example, if patients with only urticaria are excluded, the CAP RAST would be positive in 13/16 (81%).

Differences in the sensitivity and specificity of in vitro tests which measure in vitro biological function, e.g. histamine or SLT release, compared with tests which measure antibody production, e.g. specific IgE, may also reflect differences in the mechanisms underlying clinical sensitivity and organ specificity in patients with latex allergy. Until improvements in the sensitivity of available in vitro tests have been
realised, careful, controlled skin-prick testing is currently the most sensitive and reliable technique available to clinicians for confirmation of the diagnosis of latex allergy.

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REFERENCES


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