

Pitfalls in the Diagnosis of Latex Allergy

Sujoy Khan^{1,2}, Steve Holding^{2,3}, Philip Doré^{2,4} and Carrock Sewell^{1,4}

ABSTRACT

Background: Screening patients for latex allergy prior to surgery is an important but intensive procedure. The appropriate testing strategy for diagnosing latex (*Hevea brasiliensis*) allergy involves *in-vitro* specific IgE or skin prick testing. The sensitivity and specificity of both tests are influenced by patient-specific factors or manufacturing processes that alter the clinically relevant allergens in skin testing solutions.

Methods: Total IgE and latex-specific IgE testing was introduced as a screening test. Skin prick testing was done on patients with a high probability of latex allergy and negative specific IgE with total IgE <100 kU/L. SDS-PAGE was done on the non-ammoniated latex (NAL) and newly introduced ammoniated latex (AL) reagents for the clinically relevant allergens.

Results: 51 patients had a total IgE <100 (range, 2.8-99.0 kU/L), and 10% had a positive skin test. 60% of positive skin tests would have been missed with lower total IgE cut-offs of 50 kU/L (6% of referrals). SDS-PAGE of the NAL solution showed 3 prominent bands with molecular weights of approximately 20, 24 and 42 kDa that correlated with Hev b 6, Hev b 3 and Hev b 7/13, respectively. In contrast, the AL solution showed 3 very faint higher molecular weights bands that did not correlate with clinically relevant antigens.

Conclusions: Increasing the cut-off value of total IgE for allergen-specific IgE testing increased the sensitivity of the specific IgE test. The NAL reagent had a greater number of clinically significant allergens at higher concentrations than AL, which may have implications for the clinical sensitivity of the newer AL reagent.

KEY WORDS

IgE, latex allergy, SDS-PAGE, skin prick test

ABBREVIATIONS

IgE, immunoglobulin E; SPT, skin prick testing; NAL, non-ammoniated latex; AL, ammoniated latex; HEP, histamine equivalence prick; SDS-PAGE, sodium-dodecyl sulphate polyacrylamide gel electrophoresis; MW, molecular weight; IUIS, International Union of Immunological Societies.

INTRODUCTION

Allergy to natural rubber latex is being increasingly recognized with important implications. While <1% of the general population is considered to be sensitized to latex, the figure rises to 17% in health care workers.¹ IgE tests are an alternative to skin tests in the diagnosis of type 1 hypersensitivity reactions,² and pre-operative screening for latex allergy accounts for a substantial workload in our practice. Challenge tests with latex are usually performed in cases negative for both specific IgE and skin prick tests in patients with

high prior probability of latex allergy. However, many questions remain unanswered in the *in-vitro* and skin testing strategies used for the diagnosis of latex allergy. We tried to address these questions in our cohort of patients referred from various departments for the diagnosis of latex allergy.

Is there a level of total IgE below which latex specific IgE becomes insensitive? Which latex skin testing solution, non-ammoniated latex [NAL] or the newly introduced ammoniated latex [AL] should be used for clinical diagnosis of latex allergy?

Following the replacement of NAL with AL re-

¹Path Links Immunology, Scunthorpe General Hospital, Scunthorpe, ²Department of Immunology, Hull Royal Infirmary, ³Post-graduate Medical Institute and ⁴Hull York Medical School, University of Hull, Hull, United Kingdom.

Correspondence: Dr Sujoy Khan, Path Links Immunology, Scunthorpe General Hospital, Cliff Gardens, Scunthorpe DN15 7BH,

United Kingdom.

Email: sujoykhan@gmail.com

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Table 1 The clinical symptoms and skin test results of patients with latex allergy

Patient	Clinical symptoms	Total IgE	Specific IgE	Latex SPT NAL solution weal (mm)
1	Urticaria, angioedema, rhinitis (exposure to balloons)	41	Negative	1 HEP 4 mm
2	Urticaria & angioedema (rubber gloves), reaction to condoms	65	Negative	10 HEP 4 mm
3	Reaction to condoms; lip angioedema (on exposure to balloons, eating peaches and watermelon)	79	Negative	100 HEP 2 mm Significant flare; itching++
4	Urticaria (contact with rubber)	82	Negative	10 HEP 4 mm
5	Urticaria & angioedema (rubber gloves), reaction to condoms	31	Negative	10 HEP 4 mm

Table 2 Analysis of various total IgE cut-offs

Total IgE cut-off (IU/ml)	% of positive SPT missed	% of referrals with missed positive SPT	Number needed to test
100	0% (0/5)	0% (0/51)	10
90	0% (0/5)	0% (0/51)	9
80	20% (1/5)	2% (1/51)	11
70	40% (2/5)	4% (2/51)	14
60	60% (3/5)	6% (3/51)	19
50	60% (3/5)	6% (3/51)	17

agents for skin prick testing (SPT) we observed a fall in the incidence of positive reactions. This was investigated by parallel SDS-PAGE of the old (NAL) and new (AL) SPT reagents to determine if the relevant proteins were present in both solutions.

METHODS

PATIENT SELECTION

Patients were referred to the Immunology Centre from many departments, including primary care and surgical pre-operative assessment clinics. All patients were interviewed by a Specialist Nurse and/or doctor using a structured questionnaire, examining atopic status, timing and nature of reactions, amount and type of triggering allergen and reactions with latex cross-reactive foods, and an estimate of pre-test probability of latex allergy was made from clinical experience.

All patients underwent total and latex-specific IgE blood tests. Those with an appropriate history (urticaria, angioedema, rhinitis, wheeze, or anaphylaxis) and a positive specific IgE result (>0.35 AU/ml) were termed 'latex allergic' and counselled accordingly. Those with a negative blood test and total IgE <100 kU/L were skin tested.

In addition, 627 consecutive unselected latex-specific IgE negative samples received by the laboratory were analysed to determine the effect of changing this cut-off on numbers of skin tests.

In-vitro TESTING

For *in-vitro* testing, we used an automated fluoroenzymeimmunoassay- ImmunoCAP (uniCAP 250; Pharmacia Diagnostics, Uppsala, Sweden) to

measure total IgE levels and latex specific IgE (non-ammoniated latex extract from *Hevea brasiliensis*-specification, k82).

SKIN-PRICK TESTING

For skin prick test (SPT), we used Soluprick SQ (non-ammoniated latex [NAL] extract) from ALK-ABELLÓ at 3 concentrations 1, 10 and 100 HEP. Positive controls (histamine) and negative controls (albumin) were used. A plastic lancet of 2 mm depth was used to puncture the surface of the skin after application of the allergen. Readings were performed after 15 minutes of application. Skin wheal ≥ 3 mm was taken as a positive result. The newly introduced ammoniated latex (AL) skin testing solution was not available at the time.

Data of total IgE, latex specific IgE and skin test results were collected. We analysed various cut-off levels of total IgE in the context of negative latex specific IgE and positive skin tests to determine a 'safe' cut-off of total IgE for interpretation of negative latex-specific IgE.

SDS-PAGE ON NAL AND AL SPT SOLUTIONS

18% SDS-PAGE pre-cast gel 8.6×6.8 cm and Tris/glycine/SDS buffer were obtained from Bio-Rad Laboratories Ltd (Bio-Rad House, Hemel Hempstead, Hertfordshire, UK). TCEP-HCl was used to reduce the 100 HEP NAL solution and AL solution. The Mini-PROTEAN 3 kit (Bio-Rad) was used for vertical protein electrophoresis. Different protein loading strengths were used and three sets of experiments were done to check for consistency of results. The gel was run at 200 V for 35 minutes.

Modified silver stain protocol was used to stain the protein bands as per manufacturer's instructions.³ Briefly, the electrophoresed gel was treated with fixative [40% methanol/10% acetic acid (v/v)] for a minimum of 30 minutes, followed by staining with oxidiser (potassium dichromate and nitric acid, 10-fold stock solution diluted) for 5 minutes. Large volumes of water were used to flush the oxidiser from the gel for a maximum of 15 minutes. This was followed by treatment with silver reagent for 20 minutes, and a quick water rinse of 30 seconds. Developer was then added for 30 seconds and changed as soon as a pre-

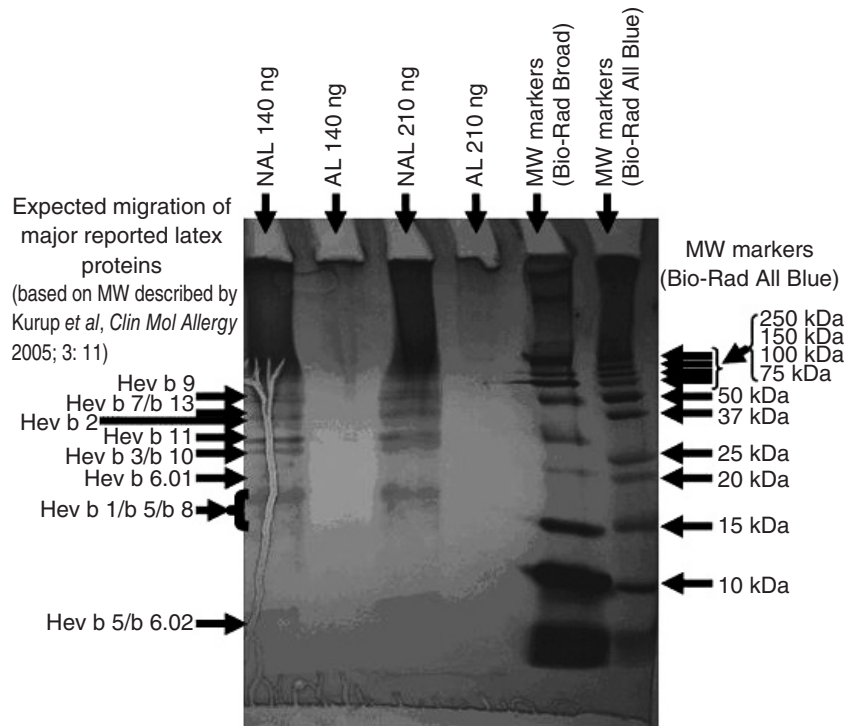


Fig. 1 18% SDS-PAGE gel photograph with molecular weight markers.

precipitate was formed until a desired staining intensity was obtained and further development stopped by addition of 5% acetic acid (v/v). Manufacturer reported sensitivity of protein intensity with this protocol was 1 ng/band. A pre-stained SDS-PAGE molecular weight standard was run at the same time.

This study was part of a service evaluation and all investigations performed were part of standardized testing procedures and skin testing were done with patient consent. The Research & Development Committee of the Trust approved the study.

RESULTS

LATEX SPECIFIC IgE TEST WAS NOT SUFFICIENTLY SENSITIVE WITH TOTAL IgE <100 kU/L

Pre-assessment clinic identified the patients with high pre-test probability of latex allergy who were offered SPT. 51 had a total IgE <100 (range 2.8-99.0 kU/L) with negative specific IgE were interviewed at the Nurse-led clinic and SPT was done. The clinical symptoms and skin test results are detailed in Table 1. Five of these 51 (10%) patients had a positive skin test (3-5 mm), and total IgE ranged from 31-82 kU/L. 20% (1/5) and 60% (3/5) of positive skin tests would have been missed using total IgE cut-offs of 80 and 50 respectively; equating to 2% and 6% of referrals (Table 2).

INCREASING TOTAL IgE CUT-OFF INCREASED SKIN TESTING WORKLOAD

Analysis on 627 consecutive negative latex specific

IgE samples showed an expected 40% increase in SPT workload on increasing the total IgE cut-off from 50 to 100 kU/L.

SDS-PAGE OF NAL SOLUTION HAD MORE RELEVANT ALLERGENS DETECTABLE THAN AL SOLUTION

SDS-PAGE of the NAL solution showed 3 prominent bands with molecular weights (MW) of approximately 20, 24 and 42 kDa when compared with the mobility of a standard range of 10 MW markers (Fig. 1). These correlated with the known *Hevea brasiliensis* allergens Hev b 6, Hev b 3 and Hev b 7/13, respectively. Another 5 faint bands were also evident. In contrast, the AL solution showed 3 very faint higher molecular weights bands (63, 94 and 124 kDa) seen also as faint bands in the NAL PAGE lane. These did not correlate with the MW of the known clinically relevant antigens (according to the IUIS Allergen Nomenclature Subcommittee).⁴

DISCUSSION

Our study showed that knowledge of total IgE levels was significant in the context of negative specific IgE in determining whether additional tests were required in patients with high pre-test probability of latex allergy. Best practice guidelines do not recommend routine total IgE testing in the diagnosis of allergy, and allergen-specific IgE testing are recommended based on the clinical indications.⁵ The inclusion of international reference standards for total IgE

in kits that are used to measure allergen-specific IgE has enabled the expression of specific IgE quantitatively in international units rather than semi-quantitatively in scores.

We believe that total IgE levels are useful in the interpretation of certain specific IgE tests, for example in anaphylaxis, because they permit the ascertainment of possible false-negative or false-positive results.⁶⁻⁸ It can be argued that the pre-test probability of latex allergy in general is low and that would not justify estimation of total IgE in all patients. However, this study showed that an increase in the total IgE cut-off limit from 50 kU/L to 100 kU/L led to an increase in specific IgE sensitivity, a factor that is not accounted for in most studies. We have adopted this policy of skin testing patients with high probability of latex allergy and negative specific IgE (total IgE <100 kU/L) for over 5 years and have not had any referral for serious reactions in those with negative specific IgE and total IgE >100 kU/L (Authors' observations, unpublished data). Total IgE level testing can therefore be recommended in the evaluation of predisposition to atopy, but further tests should be done in the context of clinical symptoms and negative specific IgE.

SDS-PAGE showed that the NAL SPT reagent contained the major latex proteins. The AL SPT reagent contained fewer proteins despite an identical protein load on electrophoresis. Previous studies have highlighted this as a potential issue for the AL preparation in comparison with others.⁹⁻¹¹ Our observation may be due to several factors: (1) loss of proteins during processing of the AL reagent, which may result in increased false negative SPT; (2) degradation of AL reagent more rapidly than NAL (but both were stored under similar conditions); and (3) interference by AL in the Bradford protein assay used to calculate the gel protein load. However, the dilutions required, based on the protein assay results, were similar for both SPT reagents; and there were other technical problems (4) with the SDS-PAGE such as failure to reduce proteins during the reducing step, or agglutination of proteins in the AL reagent.

In conclusion, we feel that specific IgE testing is a

good screening test, providing the total IgE is high enough to make the assay sufficiently sensitive. SPT alone may not be sufficient particularly with the newer reagents and challenge testing remains a possibility in certain cases. Estimation of pre-test probability in all cases of allergy is critical and influences the choice and combinations of tests used.

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