

Original article

Is there a role for immunoblots in the diagnosis of latex allergy? Intermethod comparison of *in vitro* and *in vivo* IgE assays in spina bifida patients

Background: The best diagnostic method for latex allergy is still controversial. This investigation was designed to evaluate the diagnostic efficiency of immunoblotting in comparison with established *in vitro* and *in vivo* test systems.

Methods: A total of 108 spina bifida patients were investigated by questionnaire and skin prick test (SPT). Specific serum IgE to latex was analyzed by the Pharmacia CAP FEIA immunoassay, DPC AlaSTAT microplate immunoassay, and DPC AlaBLOT immunoblot. Patients were regarded as latex allergic if they reacted positively to challenge by the latex glove wearing test.

Results: Thirty-four patients reacted positively to challenge. The sensitivity rates were 97% (SPT), 94% (immunoblot, CAP), 74% (AlaSTAT), and 35% (clinical history). The specificity rates were 92% (clinical history), 88% (AlaSTAT), 77% (SPT), 76% (CAP), and 69% (immunoblot). If two methods were combined, efficiency rates were highest for SPT combined with CAP (sensitivity 94%, specificity 82%), with AlaSTAT (sensitivity 74%, specificity 92%), or with immunoblot (sensitivity 91%, specificity 84%). The sera of challenge-positive patients recognized more immunoblot bands than challenge-negative patients, and the severity of symptoms correlated with the number of recognized bands.

Conclusions: The diagnostic efficiency of immunoblotting is not superior to that of SPT. However, immunoblotting may serve as an additional tool to increase slightly the specificity of SPT and specific serum IgE tests.

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Recently, latex allergy has been recognized as an increasingly prevalent health problem. Patients with spina bifida have been identified as the major risk group. Prevalence rates have been reported to range from 34% to 65% for sensitization (1–6), and in a study of latex provocations in spina bifida patients, 63% of those sensitized to latex were found to be symptomatic (7).

Proper diagnosis of latex allergy is a prerequisite for appropriate prevention measures and treatment. However, the diagnostic approach of choice is still controversial, and limited information is available on the clinical relevance of test results. Patient history has been reported to miss about 50% of patients found to be symptomatic in a latex provocation test (7, 8). The diagnostic capacity of the skin prick test (SPT) is highly dependent on the protein extract used, it is difficult to perform in very young patients, it may be confounded in patients under antiallergic medication, and it carries the risk of severe adverse reactions (9). *In vitro* testing of sensitization by analysis of specific serum IgE to latex is safe and shows good sensitivity at the cost of specificity

(7, 10). *In vivo* provocation testing may be regarded as the reference standard, but it is time-consuming and may be not advisable in patients with a history suggestive of anaphylactic reactions.

Immunoblotting has been used in research settings for the identification of latex sensitization and is now commercially available. It offers the advantage of identifying sensitization to particular components of the allergen extract and could therefore be more specific than a test measuring global sensitization to the whole extract. However, we have found little in the literature about the diagnostic capacity of this test in clinically relevant latex allergy.

This study was designed to evaluate the diagnostic efficiency of immunoblotting in comparison with SPT and two established *in vitro* test systems for the analysis of specific serum IgE in spina bifida patients subjected to latex provocation. The DPC AlaSTAT system is a liquid-phase assay. It was the first to offer analysis of latex-specific IgE and was chosen because in some latex studies its sensitivity was found to be better than that of the CAP system (11, 12). The Pharmacia CAP system is

a solid-phase assay and showed excellent diagnostic values for various other allergens (13). Both systems are widely used.

Methods

Patients and study design

A total of 108 consecutive patients (60 female, 48 male) from the Berlin area with spina bifida were included in the study from April to October 1997. Ages ranged from 12 months to 33 years (mean 10.8 years). Eighteen patients had a history of typical immediate-type reactions to products containing latex.

All patients were provoked with latex gloves and given SPT. Coded serum aliquots of all patients were kept frozen at -20°C until IgE analysis and immunoblotting. Two patients with a history of anaphylactic reactions to latex were excluded. The study protocol was approved by the local ethics committee, and parents or patients gave their written consent.

Questionnaire

The questionnaire included questions concerning clinical symptoms on contact with latex in the daily environment (e.g., balloons, mattresses) and medical supplies (e.g., latex gloves, bladder catheter), surgical procedures, and complications during anesthesia or surgical intervention.

Specific and total IgE

Specific IgE against latex was determined by two FDA-cleared immunoassays, a solid-phase immunoassay (FEIA), the Pharmacia CAP System (Kabi-Pharmacia, Uppsala, Sweden), and a liquid-phase immunoassay, the AlaSTAT Microplate system (DPC, Los Angeles, CA, USA). If not stated otherwise, in both test systems, samples with specific IgE concentrations of ≥ 0.35 kU/l were regarded as positive. Total IgE was measured in the CAP system.

To establish atopic status, sera were screened for specific IgE against inhalant and nutritional allergens by two multiallergen panels (14). SX1 CAP FEIA contains the allergens timothy, birch, mugwort, house-dust mite, cat, dog, and *Cladosporium herbarum*; fx5 CAP FEIA contains the foods cow's milk, hen's egg, fish, soy, wheat, and peanut. None of these allergens are known to cross-react with latex. Atopy was defined as a positive response to SX1, fx5, or both (>0.35 kU/l).

Immunoblotting

Immunoblotting was performed with the AlaSTAT AlaBLOT specific IgE test system (DPC, Los Angeles, CA, USA) according to the manufacturer's instructions. Briefly, latex extract was electrophoresed by SDS-PAGE, electrotransferred to nitrocellulose mem-

brane strips, and then incubated with diluted patient serum overnight at 6°C room temperature. As reporter enzyme, alkaline phosphate-labeled anti-IgE monoclonal antibody was immobilized at sites where IgE was bound to latex extract on the membrane strip. By adding a chromogenic substrate for the reporter enzyme, latex bands recognized by a patient's specific IgE were visualized. The color development was stopped after 10 min. Nonlatex gloves were used during the procedure. If not stated otherwise, an immunoblot was considered positive if any band was visualized. A band was defined as positive if two investigators independently could identify them by visual inspection. Bands of coded sera were read blinded to the allergic status of the patients. The code was broken after identification of bands. Molecular masses were estimated by measuring the distance in millimeters between the center of the determinant band and the bottom edge of a dye front. A lot-specific formula provided by the manufacturer was used to calculate the base-10 logarithm of the determinant's molecular mass in kilodaltons (kDa). Proteins smaller than 14 kDa were excluded since weight markers below this threshold are not in the linear region of the calibration curve.

Skin prick tests

SPT was performed with low ammoniated raw and unfinished natural rubber latex milk (Ansell, Munich, Germany). SPT responses were regarded as positive if the maximum wheal diameter was ≥ 3 mm without reaction to negative control (normal saline), and the ratio of the allergen wheal diameter to the histamine wheal diameter (positive control) was above 0.6.

Latex provocation test

After having washed their hands, patients put one wet hand into a latex glove (Sempermed classic, Lot YE5F7; Semperit, Vienna, Austria), and, as a control, put the other wet hand into a neoprene glove (Dermaprene; Ansell, Munich, Germany). After 30 min (or on showing clinical symptoms), the gloves were taken off, and possible clinical reactions were assessed by a physician. Hands were washed again. The single-blind test response was assessed as positive if typical immediate-type clinical reactions (urticaria, Quincke's edema, rhinoconjunctivitis) occurred without reactions to the control glove.

According to Baur et al. (15) the protein content of the Sempermed classic latex glove (determined by a modification of the Lowry method with ovalbumin as the standard) was $1034 \mu\text{g/g}$ glove, and the allergen content (measured by competitive RAST immunoinhibition with the CAP system) was $626 \mu\text{g/g}$.

Statistical analysis

Sensitivity was defined as the fraction of true positives among challenge-positive individuals, specificity as the fraction of true negatives among challenge-negative individuals, positive predictive value as the fraction of challenge-positive individuals among test positives, negative predictive value as the fraction of challenge-negative individuals among test negatives, and efficiency as the fraction of tested individuals correctly classified by the test. For statistical comparisons, the chi square test for dichotomous variables, the Mann–Whitney U-test for ordinal and continuous variables, and Spearman's rank test for correlations were used. Values ($\pm$) indicate standard errors of the mean.

Results

Among the 108 spina bifida patients investigated, 34 (31.5%) reacted positively in the latex glove exposure test. Clinical symptoms were local urticaria, except for one patient who had mild generalized urticaria. Three patients had additional symptoms of allergic rhinoconjunctivitis. In all, 65 patients (60.2%) were sensitized according to one or more tests: 50 patients (46.3%) were sensitized according to the SPT, 50 patients (46.3%) to CAP immunoassay, 34 patients (31.5%) to AlaSTAT immunoassay, and 58 patients (53.7%) to immunoblotting. However, the subpopulations identified varied. The numbers of patients overlapping were 45 (SPT and CAP), 31 (SPT and AlaSTAT), 43 (SPT and AlaBLOT), 34 (CAP and AlaSTAT), 47 (CAP and AlaBLOT), and 31 (AlaSTAT and AlaBLOT). Among the 18 patients, those with a positive clinical history overlapping with a positive test result numbered 14 (SPT), 15 (CAP), 12 (AlaSTAT), and 15 (AlaBLOT).

The diagnostic performance values of patient history, SPT, determination of specific IgE, and immunoblotting are shown in Table 1. Challenge-positive patients

Table 1. Evaluation of predictive capacity (%) of diagnostic tools for clinically relevant latex allergy in spina bifida patients asymptomatic ($n=74$) or symptomatic ($n=34$) upon latex challenge. For combinations, patients are classified as test positive if all tests are positive

Tests	Sensitivity	Specificity	PPV	NPV	Efficiency
Clinical history	35.3	91.9	66.7	75.6	74.1
SPT	97.1	77.0	66.0	98.3	83.3
CAP	94.1	75.7	64.0	96.6	81.5
AlaSTAT	73.5	87.8	73.5	87.8	83.3
AlaBLOT	94.1	68.9	58.2	96.2	76.9
SPT/AlaBLOT	91.2	83.8	72.1	95.4	86.1
SPT/CAP	94.1	82.4	71.1	96.8	86.1
SPT/AlaSTAT	73.5	91.9	80.6	88.3	86.1
AlaSTAT/AlaBLOT	70.6	90.5	77.4	90.5	84.3
AlaSTAT/CAP	73.5	87.8	73.5	87.8	83.3
CAP/AlaBLOT	91.2	78.4	66.0	95.1	82.4
CAP/AlaSTAT/AlaBLOT	70.6	90.5	77.4	87.0	84.3

PPV: positive predictive value; NPV: negative predictive value; SPT: skin prick test.

were almost all identified by SPT, CAP immunoassay for specific serum IgE, and immunoblots, whereas the AlaSTAT immunoassay missed about a quarter of the challenge-positive patients, and patient history about two-thirds of them. In contrast, the identification of challenge-negative patients was more complete with history or AlaSTAT immunoassay than with SPT, CAP immunoassay, or immunoblotting. Although the correlation of test results obtained with the two immunoassay systems was acceptable ($r_s=0.86$), the AlaSTAT system tended to produce lower results and consequently classified fewer patient sera as positive for latex IgE than the CAP system (data not shown).

The analytic sensitivities of immunoblots as measured by the fraction of blot-positive among *in vitro* assay positive sera were 91% and 94% for the AlaSTAT and CAP systems, respectively. In turn, among the immunoblot-positive sera, 47% (AlaSTAT) and 19% (CAP) had a latex-specific IgE level of <0.35 kU/l.

We identified 46 bands of different molecular masses on immunoblots (Fig. 1). The pattern of the bands was similar for challenge-negative and -positive patients. Except for a single band of one challenge-negative patient, no bands were identified in challenge-negative patients that were not found in challenge positives. However, the prevalence of particular bands was significantly lower in challenge-negative patients. In challenge negatives, the bands identified were found in less than 20% of the sera. Among challenge-positive patients, 23 bands were found in $\geq 20\%$ and 14 bands in $\geq 50\%$ of the patients. The difference between both groups in the prevalence of these 14 bands was statistically highly significant (Table 2). No particular band was found in all challenge-positive patients.

The number of stained bands was higher in patients with a more severe clinical reaction to latex challenge (challenge-negative, 1.7 ± 0.4 bands; local urticaria, 12.0 ± 1.7 bands; allergic rhinoconjunctivitis, 10.7 ± 4.2 bands; generalized urticaria, 23 bands; $r_s=0.615$; $P<0.001$). A band estimated at 40 kDa was stained by 8% of challenge-negative patients' sera, by 43% of sera from patients who reacted with local urticaria, and by 100% of sera from patients who reacted with allergic rhinoconjunctivitis or generalized urticaria.

The total number of bands was higher in the sera of the 78 patients with a ventriculoperitoneal shunt than in the 29 patients without a shunt (6.17 ± 0.92 bands vs 2.28 ± 1.08 bands; $P=0.003$). The number of bands correlated with the number of past surgical procedures ($r_s=0.444$; $P<0.001$). In addition, the number of bands (5.01 ± 1.53) correlated well with the specific IgE titer as measured by the CAP (6.57 ± 1.53 kU/l; $r_s=0.878$; $P<0.001$) or AlaSTAT systems (1.97 ± 0.64 kU/l; $r_s=0.659$; $P<0.001$).

Total IgE was significantly higher in latex-sensitized ($n=50$) than nonsensitized ($n=58$) patients ($214 \pm$

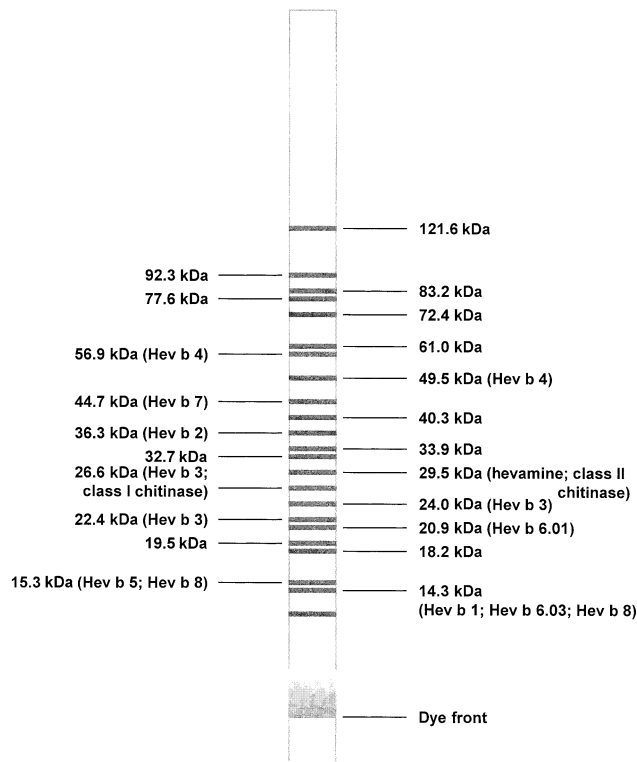


Figure 1. Topography of frequent latex immunoblot bands in symptomatic latex-allergic spina bifida patients and possible corresponding latex epitopes.

39 kU/l vs 74 ± 24 kU/l; $P < 0.0001$). In immunoblot-positive, challenge-positive patients ($n = 32$), total IgE was significantly higher than in immunoblot positive, challenge negative ($n = 26$), patients (256 ± 48 kU/l vs 188 ± 66 kU/l; $P = 0.011$). In the latter group, total IgE was significantly higher than in immunoblot-negative, challenge-negative patients ($n = 48$; 39 ± 7 kU/l; $P = 0.0006$).

A positive CAP test for IgE against latex was significantly more frequent among immunoblot-positive, challenge-positive than immunoblot-positive, challenge-negative patients (96.9% vs 61.5%; $P < 0.0001$), and more frequent among the latter group than in immunoblot-negative, challenge-negative patients (4.2%; $P < 0.0001$).

The proportion of patients sensitized to latex was about double in individuals sensitized to other food or inhalant allergens. The proportions of the latex-sensitized among atopic (SX1- or fx5-positive) and nonatopic patients (SX1- and fx5-negative) were, according to AlaSTAT, 45% and 23% ($P = 0.14$), to CAP 67% and 33% ($P < 0.001$), and to immunoblot 79% and 39% ($P < 0.001$), respectively. However, the pattern of frequent immunoblot bands was similar among atopic and nonatopic individuals (Table 3).

To determine whether a set of bands would be more helpful than a simple "yes-or-no" approach (any band stained or not) to separate challenge-positive from

Table 2. Prevalence of immunoblot bands in sera of spina bifida patients asymptomatic ($n = 74$) or symptomatic ($n = 34$) upon latex challenge. All results significant: $P < 0.001$

Distance from dye front (mm)	Estimated MW (kDa)	Asymptomatic (%)	Symptomatic (%)
11.5	15.3	18.9	82.4
15.0	19.5	5.4	52.9
18.0	24.0	14.9	55.9
19.5	26.6	9.5	67.6
21.0	29.5	4.1	50.0
22.5	32.7	6.8	52.9
24.0	36.3	10.8	50.0
25.5	40.3	8.1	50.0
27.0	44.7	10.3	70.6
28.5	49.5	6.8	67.6
30.5	56.9	6.8	50.0
31.5	61.0	5.4	50.0
35.0	77.6	2.7	50.0
36.0	83.2	1.4	50.0

-negative patients, we chose those 14 bands found in $\geq 50\%$ of the challenge-positive patients. As shown in Table 4, it was possible to increase the rather low specificity of the immunoblots to over 90% by increasing the number of bands set as threshold for a positive test result. However, as the specificity went up, the sensitivity decreased.

This relationship was also true for other quantitative assays, as exemplified in a receiver operator characteristic curve (Fig. 2). If the shortest distance possible is measured from the top left-hand corner of the graph (sensitivity and specificity 100%) to the curves or symbols representing the different diagnostic tools, it becomes evident that the CAP method with a cutoff between classes 2 and 3 would be closest to the ideal.

We also investigated whether a combination of two test results (immunoassay, immunoblotting, and SPT) could improve the diagnosis of latex allergy. Patients were regarded as allergic if both test results were positive (Table 1). The highest sensitivity was achieved by a combination of CAP immunoassay and SPT

Table 3. Prevalence of immunoblot bands in sera of nonatopic ($n = 25$) and atopic ($n = 33$) spina bifida patients

Distance from dye front (mm)	Estimated MW (kDa)	Nonatopic (%)	Atopic (%)	P
11.5	15.3	84.0	63.6	0.086
15.0	19.5	24.0	48.5	0.057
18.0	24.0	48.0	54.5	0.621
19.5	26.6	40.0	60.6	0.120
21.0	29.5	32.0	36.4	0.729
22.5	32.7	32.0	45.5	0.299
24.0	36.3	36.0	48.5	0.342
25.5	40.3	40.0	39.4	0.963
27.0	44.7	64.0	69.7	0.647
28.5	49.5	40.0	54.5	0.272
30.5	56.9	36.0	39.4	0.792
31.5	61.0	32.0	39.4	0.562
35.0	77.6	28.0	36.4	0.502
36.0	83.2	28.0	33.3	0.664

Table 4. Diagnostic values in relation to number of stained immunoblot bands among 14 bands frequently found in latex-challenge-positive spina bifida patients (34 patients challenge-positive, 74 patients challenge-negative)

	Immunoblot bands positive			
	≥1	≥4	≥7	≥10
Sensitivity	94.1	67.6	50.0	47.1
Specificity	68.9	89.2	94.6	97.3
PPV	58.2	74.2	81.0	88.9
NPV	96.2	85.7	80.5	80.0
Efficiency	76.9	82.4	80.6	81.5

(94.1%), but the sensitivity of SPT alone (97.1%) was not exceeded. Specificity was improved and highest in a combination of SPT and AlaSTAT immunoassay. The highest proportion of correctly diagnosed patients (efficiency) was found in a combination of SPT with either immunoassay (CAP or AlaSTAT) or immunoblotting (86.1%).

The concordance between SPT and patient history was 63.0% (history-negative and SPT-positive, 33.3%; history-positive and SPT-negative, 3.7%). The concordance between SPT and CAP results was 90.8% (SPT-positive and CAP-negative, 4.6%; SPT-negative and CAP-positive, 4.6%). The concordance between SPT and AlaSTAT results was 79.6% (SPT-positive and AlaSTAT-negative, 17.6%; SPT-negative and AlaSTAT-positive, 2.8%). The concordance between patient history and CAP results was 66.6% (history-positive and CAP-negative, 1.9%; history-negative and CAP-positive, 31.5%). The concordance between patient history and AlaSTAT results was 75.9% (history-positive and AlaSTAT-negative, 4.6%; history-negative and AlaSTAT-positive, 19.4%).

Discussion

This study shows that in the diagnosis of latex allergy the efficiency of immunoblotting alone as an alternative diagnostic tool is not superior to SPT or established specific IgE assays. However, when used as an additional tool, it does increase specificity and positive predictive value slightly (Table 1). Earlier reports that latex-allergic patients exhibit an IgE immune response to a multitude of latex antigens are confirmed (16–19). This is the first study to investigate the spectrum of IgE-binding immunoblot bands against latex in latex-challenged patients. There is no single band that marks all patients reacting allergically in a latex-exposure test. However, some bands were more prevalent among the challenge-positive spina bifida patients (Table 2), and limiting the criteria for a positive test to these bands further increases the specificity.

There is an inverse relationship between the measures of specificity and sensitivity. Raising the threshold to increase specificity will decrease sensitivity, and vice versa. The diagnostic aim guides the definition of the

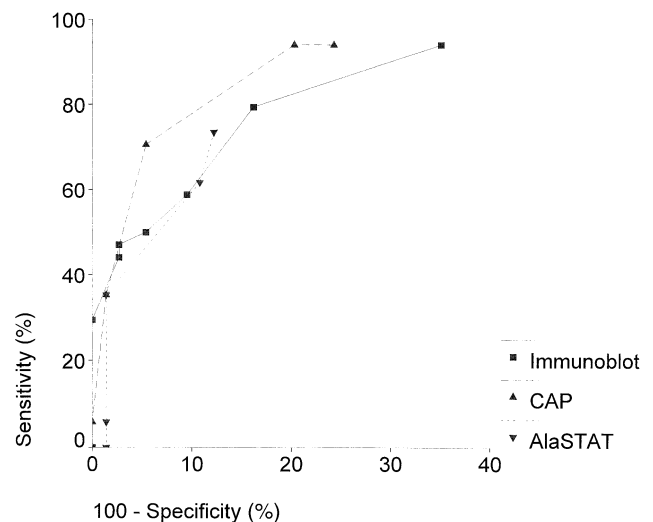


Figure 2. Receiver operator characteristic curve for *in vitro* diagnostic tools. Symbols on curves represent different cut-offs for a positive test, beginning on the right hand side: RAST classes 1-6 (AlaSTAT and CAP system); 1, 4, 7, or 10 stained bands (AlaBLOT immunoblots).

threshold level. Presurgical screening to assess the risk of perioperative anaphylaxis requires tests with a high sensitivity. CAP immunoassay, SPT, and immunoblot offer a sensitivity of >90%. Similar sensitivity rates have been reported previously for SPT and CAP immunoassay in spina bifida patients (7, 10, 20) and other patients (21–23). The assumption that the CAP system gives an unduly conservative measurement compared to the AlaSTAT system, based on the results of blood-donor sera found to be positive in the AlaSTAT system and retested in the CAP system, could not be confirmed (13). CAP results correlated well with AlaSTAT results. The low sensitivity rate found in one study in which health-care workers were classified as allergic on the basis of clinical history could not be replicated (24). It is noteworthy that these studies included other risk groups because study results may reflect different reactivity to latex proteins among spina bifida patients and other populations. The sensitivity of SPT is obviously highly dependent on the allergen extract employed (7, 24). In our study, the high sensitivity of the SPT using a natural latex extract reflects rates found before in a study with spina bifida patients (7). However, the sensitivity was not increased by a second diagnostic test.

High specificity may be desirable in the context of adequate distribution of preventive and therapeutic resources. Clinical history alone cannot be regarded as an efficient diagnostic tool in children with spina bifida because of the extremely low sensitivity found in this study and two other studies (7, 25). The low concordance of clinical history with the *in vitro* assay and SPT results in this study underlines the limited value of clinical history in this risk population. In spina

bifida patients, the number of surgical procedures has been identified as a major risk factor for latex allergy (4, 20, 26), and challenge-positive patients without a history of latex allergy may represent a subpopulation with a high risk of future clinical manifestations of latex allergy. History may be more reliable in chronically latex exposed health-care workers, and it may facilitate a more timely diagnosis of latex allergy in this risk group. However, only a fraction of sensitized health-care workers are type I allergic by history (27).

In our study, the AlaSTAT immunoassay offered the best specificity (nearly 90%). High specificity for the AlaSTAT system has been found in another history-based study (28), which also reports high specificity for the CAP system at the cost of sensitivity, but considerably lower sensitivity for another FDA-cleared assay (Hycor HYTEC). It remains to be elucidated whether a different study population contributed to this difference.

A high specificity of SPT was reported previously for spina bifida patients (20) and patients without spina bifida defined as latex allergic on the basis of clinical history (21, 22). Elevation of test criteria such as cutoffs in immunoblots or quantitative assays can further increase specificity. In this study, the combination with an IgE assay or immunoblotting increased the specificity and efficiency of SPT while maintaining a high sensitivity.

Latex proteins reported to be particularly prevalent in spina bifida patients include Hev b 3 (23–27 kD) [29–31] and Hev b 1 (rubber elongation factor, 14.6 kDa) (32–34), which shares 47% of its sequence with Hev b 3. Different routes and sources of sensitization have been proposed as possible explanations for the differences observed between spina bifida patients and other populations. In addition to proteins in the molecular mass areas of Hev b 1 and 3, a number of other proteins have been found frequently in this study among challenge-positive patients. Many of them are in the weight region of previously reported latex proteins, but some are not (Table 2 and Fig. 1). It is possible that some of these proteins are breakdown products of higher molecular mass proteins. The relevance of these bands to clinical latex allergy remains unclear.

Previous studies demonstrated a positive correlation of specific IgE titers and more severe symptoms on latex challenge (26). In this study, we found a different pattern of stained immunoblot bands in challenge-positive and -negative patients and a positive correlation of the number of stained bands and the observed severity of clinical reaction to latex challenge. A band of 40 kDa was recognized by all patients with a more severe reaction on latex challenge. This suggests that, in addition to differences in the quantity of specific IgE titers, qualitative differences in sensitization to latex allergens may be of importance for clinically relevant

latex allergy. Another study found sera of health-care workers with a history of urticaria on latex exposure to be invariably immunoblot-negative, and patients with positive immunoblots to have multiple organ disorder. A band of 40 kDa was recognized by all immunoblot-positive sera. However, the two patients with a history of anaphylaxis were immunoblot-negative (35).

We studied only spina bifida patients. Because of the proposed route of sensitization and the incriminated latex products for this risk group, caution should prevail if the study results are extended to other risk groups, who may be preferably sensitized via inhalation of dust containing latex particles or skin contact with medical equipment. There is only a partial overlap of IgE-binding immunoblot bands against latex allergen between spina bifida patients and health-care workers (18). The same allergen extracts are used for AlaSTAT and AlaBLOT allergens. However, it cannot be ruled out that the protein configuration was altered during manufacture or that heterogeneous allergen preparations used for these tests and the CAP system or the SPT influenced the results of the study.

In addition, some *in vitro* results may be coinfluenced by IgE against unrelated allergens binding to latex proteins. Total IgE was higher in false-positive (immunoblot-positive, challenge-negative) than in true-negative (immunoblot-negative, challenge-negative) serum samples. However, total IgE was even higher in true positives (immunoblot-positive, challenge-positive). The specific IgE against latex followed the same pattern. These findings suggest that the dose-dependent possibility to react positively to latex provocation with higher specific latex IgE titers is a consequence of more frequent exposure to latex during surgical intervention (4, 36). In addition, the similar pattern of frequent immunoblot bands in atopic (screening test for unrelated specific-IgE-positive) and nonatopic individuals as an indicator for patients who are more prone to be also sensitized to allergens cross-reactive with latex is not suggestive of a major bias (Table 3). However, some of the bands identified as frequent among latex-challenge-positive spina bifida patients are in the molecular mass region of proteins that cross-react with Bet v 2 (15 kDa) or class I chitinases (32 kDa) (37, 38).

In conclusion, immunoblotting was not superior to more global specific IgE tests such as *in vitro* assays or SPT in the diagnosis of latex allergy among spina bifida patients. As an additional tool, immunoblotting does slightly increase the specificity of the diagnostic process. Furthermore, immunoblotting may have a valuable role in research settings outside the scope of this study such as monitoring intraindividual courses in the natural course of the disease or under therapy. Additional studies are needed to confirm the results found for spina bifida patients and possibly to extend some of these findings to other risk groups.

On the basis of this study, a step-wise approach to diagnosis of latex allergy in spina bifida patients is proposed: because of its superior sensitivity, SPT should be performed in all patients evaluated for latex allergy. For confirmation, sera of patients showing a positive SPT reaction should be analyzed in a specific IgE assay. These assays are highly automated. If there is still doubt, latex challenge may confirm clinical manifestation of latex allergy. For a routine diagnostic test in a clinical laboratory, immunoblotting offers no striking advantage over other assays since it is time-consuming and the molecular mass of bands seems to be

of limited use for the diagnostic outcome. Ideally, regardless of the test results, spina bifida patients should be treated latex-free because of their high risk of latex sensitization and subsequent clinical latex allergy.

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References

1. SLATER JE, MOSTELLO LA, SHAER C. Rubber-specific IgE in children with spina bifida. *J Urol* 1991;**146**:578–579.
2. BEAUDOUIN E, PRESTAT F, SCHMITT M, KANNY G, LAXENAIRE MC, MONERET-VAUTRIN DA. High risk of sensitization to latex in children with spina bifida. *Eur J Pediatr Surg* 1994;**4**:90–93.
3. KELLY KJ, KURUP V, ZACHARISEN M, RESNICK A, FINK JN. Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993;**91**:1140–1145.
4. MICHAEL T, NIGGEMANN B, MOERS A, SEIDEL U, SCHEFFNER D, WAHN U. Risk factors for latex allergy in patients with spina bifida. *Clin Exp Allergy* 1996;**26**:934–939.
5. TOSI LL, SLATER JE, SHAER C, MOSTELLO LA. Latex allergy in spina bifida patients: prevalence and surgical implications. *J Pediatr Orthop* 1993;**13**:709–712.
6. YASSIN MS, SANYURAH S, LIERL MB, et al. Evaluation of latex allergy in patients with myelomeningocele. *Ann Allergy* 1992;**69**:207–211.
7. NIGGEMANN B, BUCK D, MICHAEL T, WAHN U. Latex provocation tests in patients with spina bifida: who is at risk of becoming symptomatic? *J Allergy Clin Immunol* 1998;**102**:665–670.
8. LIEBKE C, NIGGEMANN B, WAHN U. Sensitivity and allergy to latex in atopic and non-atopic children. *Pediatr Allergy Immunol* 1996;**7**:103–107.
9. SPANER D, DOLOVICH J, TARLO S. Hypersensitivity to natural latex. *J Allergy Clin Immunol* 1989;**83**:1135–1137.
10. NIGGEMANN B, MICHAEL T, VON MOERS A, et al. Comparison of three immunoassays for diagnosing sensitization to latex in children with spina bifida. *Pediatr Allergy Immunol* 1996;**7**:164–166.
11. KLEINE-TEBBE J, EICKHOLT M, GÄTJEN M, BRUNNÉ T, O'CONNOR A, KUNKEL G. Comparison between Magic Lite- and CAP-system: two automated specific IgE antibody assays. *Clin Exp Allergy* 1992;**22**:475–484.
12. WILLIAMS PB, DOLEN WK, KOEPKE JW, SELNER JC. Comparison of skin testing and three *in vitro* assays for specific IgE in the clinical evaluation of immediate hypersensitivity. *Ann Allergy* 1992;**68**:35–45.
13. OWNBY DR, OWNBY HE, MCCULLOUGH J, SHAER AW. The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors. *J Allergy Clin Immunol* 1996;**97**:1188–1192.
14. ERIKSSON NE. Allergy screening with Phadiatop® and CAP Phadiatop® in combination with a questionnaire in adults with asthma and rhinitis. *Allergy* 1990;**45**:285–292.
15. BAUR X, CHEN Z, RAULF-HEIMSOOTH M, DEGENS P. Protein and allergen content of various natural latex articles. *Allergy* 1997;**52**:661–664.
16. CHAMBEYRON C, DRY J, LEYNADIER F, PECQUET C, TRAN XUAN THAO. Study of the allergenic fractions of latex. *Allergy* 1992;**47**:92–97.
17. BREITENEDER H, SCHEINER O. Molecular and immunological characteristics of latex allergens. *Int Arch Allergy Immunol* 1998;**116**:83–92.
18. KURUP VP, ALENUS H, KELLY KJ, CASTILLO L, FINK JN. A two-dimensional electrophoretic analysis of latex peptides reacting with IgE and IgG antibodies from patients with latex allergy. *Int Arch Allergy Immunol* 1996;**109**:58–67.
19. CHARDIN H, MAYER C, DESVAUX FX, SÉNÉCHAL H, PELTRE G. Latex allergy: characterization of major allergens and isotypic expression. *Allergy* 1999;**55**:872–877.
20. DE SWERT LF, VAN LAER KM, VERPOORTEN CM, VAN HOEYVELD EM, CADOT P, STEVENS EA. Determination of independent risk factors and comparative analysis of diagnostic methods for immediate type latex allergy in spina bifida patients. *Clin Exp Allergy* 1997;**27**:1067–1076.
21. BLANCO C, CARRILLO T, ORTEGA N, ALVAREZ M, DOMINGUEZ C, CASTILLO R. Comparison of skin-prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy* 1998;**28**:971–976.
22. EBO DG, STEVENS WJ, BRIDTS CH, DE CLERCK LS. Latex-specific IgE, skin testing, and lymphocyte transformation to latex in latex allergy. *J Allergy Clin Immunol* 1997;**100**:618–623.
23. KIM KT, SAFADI GS. Relation of latex-specific IgE titer and symptoms in patients allergic to latex. *J Allergy Clin Immunol* 1999;**103**:671–677.
24. KIM KT, SAFADI GS, SHEIKH KM. Diagnostic evaluation of type I latex allergy. *Ann Allergy Asthma Immunol* 1998;**80**:66–70.
25. LEBENBORN-MANSOUR MH, OESTERLE JR, OWNBY DR, JENNET MK, POST SK, ZAGLANICZY K. The incidence of latex sensitivity in ambulatory surgical patients: a correlation of historical factors with positive serum immunoglobulin E levels. *Anesth Analg* 1997;**85**:44–49.
26. MAZON A, NIETO A, ESTORNELL F, NIETO A, REIG C, GARCIA-IBARRA F. Factors that influence the presence of symptoms caused by latex allergy in children with spina bifida. *J Allergy Clin Immunol* 1997;**99**:600–604.
27. BROWN RH, SCHAUBLE JF, HAMILTON RG. Prevalence of latex allergy among anesthesiologists. Identification of sensitized but asymptomatic individuals. *Anesthesiology* 1998;**89**:292–299.

28. HAMILTON RG, BIAGINI RE, KRIEG EF, and the Multi-Center Latex Skin Testing Study Task Force. Diagnostic performance of Food and Drug Administration-cleared serologic assays for natural rubber latex-specific IgE antibody. *J Allergy Clin Immunol* 1999;**103**:925–930.
29. ALENUS H, PALOSUO T, KELLY K, et al. IgE reactivity to 14 kD and 27 kD natural rubber proteins in latex-allergic children with spina bifida and other congenital anomalies. *Int Arch Allergy Immunol* 1993;**102**:61–66.
30. LU LJ, KURUP VP, HOFEMAN DR, KELLY KJ, MURALI PS, FINK JN. Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J Immunol* 1995;**155**:2721–2728.
31. YEANG HY, CHEONG KF, SUNDERASAN E, et al. The 14.6 kd rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 1996;**98**:628–639.
32. ALENUS H, KALKKINEN N, YIP E, et al. Significance of rubber elongation factor as a latex allergen. *Int Arch Allergy Immunol* 1996;**109**:362–368.
33. CHEN Z, CREMER R, POSCH A, RAULF-HEIMSOTH M, RIHS HP, BAUR X. On the allergenicity of Hev b 1 among health care workers and patients with spina bifida allergic to natural rubber latex. *J Allergy Clin Immunol* 1997;**100**:684–693.
34. YLITALO L, ALENUS H, TURJANMAA K, PALOSUO T, REUNALA T. IgE antibodies to prohevein, hevein, and rubber elongation factor in children with latex allergy. *J Allergy Clin Immunol* 1998;**102**:659–664.
35. MARAIS GI, FLETCHER JM, POTTER PC. *In vivo* and *in vitro* diagnosis of latex allergy at Groote Schuur Hospital. *S Afr Med J* 1997;**87**:1004–1008.
36. BERNARDINI R, NOVEMBRE E, LOMBARDI E, et al. Risk factors for latex allergy in patients with spina bifida and latex sensitization. *Clin Exp Allergy* 1999;**29**:681–686.
37. DIEZ-GOMEZ ML, QUIRCE S, CUEVAS M, et al. Fruit-pollen-latex cross-reactivity: implication of profilin. *Allergy* 1999;**55**:951–961.
38. BLANCO C, DIAZ-PERALES A, COLLADA C, et al. Class I chitinases as potential panallergens involved in the latex-fruit syndrome. *J Allergy Clin Immunol* 1999;**103**:507–513.