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THE ROLE OF ALLERGEN COMPONENTS FOR THE DIAGNOSIS OF LATEX-INDUCED OCCUPATIONAL ASTHMA

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Running title: Specific latex IgE and occupational asthma

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Author Contributions

OV had full access to all the data and assumes responsibility for the integrity and accuracy of the data analysis. OV, AF, CP and MR designed the study, supervised experiments, contributed to data interpretation and analysis, as well as writing and reviewing of the manuscript. AF, UM, HPR, CR and SS performed the in vitro experiments, contributed to data collection, interpretation and analysis, and reviewed the manuscript. JJ contributed to data analysis and interpretation, and reviewed the manuscript.

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List of abbreviations

NPV: Negative predictive value
NRL: Natural rubber latex
OA: Occupational asthma
PPV: Positive predictive value
SIC: Specific inhalation challenge
rHev b: Recombinant *Hevea brasiliensis* allergen

ABSTRACT

Background: Recombinant *Hevea brasiliensis* (rHev b) natural rubber latex (NRL) allergen components have been developed to assess the patients' allergen sensitization profile and to improve the diagnosis of NRL allergy.

Objective: To examine whether the determination of specific IgE (sIgE) reactivity to a panel of recombinant allergen components would be helpful for diagnosing NRL-induced occupational asthma (OA) in predicting the outcome of a specific inhalation test.

Methods: sIgE levels to NRL extract and 12 recombinant NRL allergen components were assessed in 82 subjects with OA ascertained by a positive specific inhalation challenge (SIC) with NRL gloves and in 25 symptomatic subjects with a negative challenge.

Results: The sensitivity, specificity, positive predictive value and negative predictive value of a NRL-sIgE level ≥ 0.35 kU_A/L as compared to the result of SICs were 94%, 48%, 86 %, and 71 %, respectively. The positive predictive value increased above 95% when increasing the cut-off value to 5.41 kU_A/L. Subjects with a positive SIC showed a significantly higher rate of sIgE reactivity to rHev b 5, 6.01, 6.02 and 11 than those with a negative SIC. A sIgE sum score against rHev b 5 *plus* 6.01/6.02 ≥ 1.46 kU_A/L provided a positive predictive value >95% with a higher sensitivity (79%) and diagnostic efficiency (Youden index: 0.67) as compared with a NRL-sIgE ≥ 5.41 kU_A/L (49% and 0.41, respectively).

Conclusion: In suspected OA, high levels of sIgE against rHev b 5 combined with rHev b 6.01 or 6.02 are the most efficient predictors of a bronchial response to NRL.

Word count: 250 words

Key words: Bronchial provocation test; component-resolved diagnosis; latex; occupational asthma; recombinant allergens.

INTRODUCTION

Natural rubber latex (NRL) was recognized as a major cause of IgE-mediated occupational asthma (OA) in the early nineties, especially in health care facilities (1-3). Because of successful prevention strategies aimed at reducing exposure to NRL protein allergens in

gloves, the incidence of NRL-induced allergy and OA has markedly decreased (4, 5), even though this condition may still remain a cause of concern in workers with frequent NRL exposure, especially in fast developing countries (6).

An accurate diagnosis of OA caused by NRL is a crucial step in implementing appropriate interventions aimed at minimizing the adverse health and socioeconomic impacts of the disease (2, 7-9). Because facilities for performing safe and accurate specific inhalation challenges (SIC) with NRL are not widely available (10, 11), physicians generally rely on the clinical history, the presence of nonspecific bronchial hyperresponsiveness, and the documentation of specific IgE (sIgE) antibodies directed against NRL through in vitro assays and/or skin-prick testing for diagnosing NRL-induced OA. However, these tests have a high sensitivity, but a low specificity for diagnosing NRL-induced OA as compared to the results of SICs with NRL gloves (12, 13). Additionally, in several European countries, NRL extracts for skin-prick testing are no longer commercially available leading to a deficit in diagnostic tools.

Fifteen NRL allergens have been currently characterized and most of them are available as recombinant allergens (14-16) (WHO/IUIS Committee List [<http://www.allergen.org>]). The application of these recombinant *Hevea brasiliensis* (rHev b) allergens in the development of sIgE in vitro assays could provide new opportunities for improving the diagnosis of NRL allergy. Although a number of studies have evaluated sIgE antibodies against recombinant or purified allergen components to characterize the sensitization profile of patients with NRL allergy (16-20), this technique has not been specifically applied to the diagnosis of NRL-induced OA.

Therefore, the aim of this study was to investigate whether the determination of sIgE against NRL and its recombinant allergen components could be helpful in identifying NRL-induced OA as compared with SIC to NRL

METHODS

Study Design

In this retrospective study, the records of all subjects investigated through an SIC with NRL gloves for possible OA from January 1993 to December 2010 at a tertiary centre were reviewed. All patients completed a standardized diagnostic assessment that included a detailed medical and occupational questionnaire, skin-prick testing with a commercial extract of non-ammoniated NRL and 20 common inhalant allergens (Stallergènes, Waterloo, Belgium), assessment of nonspecific bronchial hyperresponsiveness to histamine, and SIC. The results of skin-prick tests with NRL were not analyzed in this study because these tests were performed with various batches of the commercial NRL extracts over time. A blood sample was collected to determine the level of NRL-sIgE antibodies at the time of the diagnostic assessment and an aliquot of serum was stored at -20°C. The protocol of the study was approved by the *Comité d'éthique médicale* of the *Centre Hospitalier Universitaire de Mont-Godinne* (approval number 82/2013 and BE039201421354). A statement of informed consent was not required because the data were analysed retrospectively in an anonymous way.

Specific Inhalation Challenges

SICs with powdered NRL gloves were completed according to a previously described protocol (12) and international recommendations (11, 21). Asthma medications were withdrawn according to their duration of action (21) and inhaled corticosteroids were halted 72 hours prior to the tests. During the SIC, spirometry (22, 23) was obtained at baseline, immediately after each challenge exposure, then every 15 minutes for the first hour, every 30 minutes for the second hour, and hourly thereafter for a total of at least six hours after the end of exposure. A SIC was considered positive when a sustained $\geq 20\%$ fall in FEV₁ was recorded on two consecutive assessments. The baseline level of nonspecific bronchial hyperresponsiveness to histamine (24) was assessed at the end of the control day and re-

assessed 6-8 hours and/or 24 hours after the active challenges. The degree of nonspecific bronchial hyperresponsiveness was determined using the tidal breathing method and expressed as the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) (25).

On a control day, the subjects were asked to handle vinyl gloves for 30 minutes to ensure that FEV₁ fluctuations were $\leq 12\%$ of the baseline value. On the following day(s), the subjects were challenged with NRL using a realistic approach. The subjects were asked to shake NRL gloves in a 5-m³ challenge room, one pair of gloves every five minutes. The first 38 SICs were performed using the same lot of sterile NRL gloves (Triflex powdered, Baxter Healthcare Corporation, Lessines, Belgium) while the following procedures were completed with powdered nonsterile NRL gloves (Glads, Mönlycke, Göteborg, Sweden). The subjects wore protective overalls, caps, overshoes, and vinyl gloves to avoid, as much as possible, direct skin contact with NRL. The duration of exposure was gradually increased (i.e. 1 min, 4 min, 10 min, 15 min, 30 min, and 60 min) until a $\geq 20\%$ fall in FEV₁ occurred or a cumulative exposure of two hours was completed. When exposure to NRL gloves failed to induce a 20% fall in FEV₁ on the first challenge day, the procedure was repeated for 2 hours on the next day. In addition, further challenges were proposed when there was a significant (>3 -fold) decrease in the post-challenge PC₂₀ value as compared to the control day values (24).

Immunoglobulin E Antibody Assessment

The determination of sIgE antibodies was performed using the ImmunoCAP system (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). All sera were analysed for NRL- sIgE using the ImmunoCAP k82 (supplemented for rHev b 5) according to the instructions of the manufacturer. sIgE levels against the recombinant NRL component allergens rHev b 1, 3, 5, 6.01, 6.02, 8, 9, and 11 were assessed in all subjects using the ImmunoCAP method. In addition, rHev b 7, 10, 12 and 15 were produced in *E. coli* in fusion with maltose-binding protein (16, 19) and coupled to streptavidin ImmunoCAP as previously described (26).

ImmunoCAP containing horse radish peroxidase (HRP) (Ro 400) was used to detect sIgE binding to cross-reactive carbohydrate determinants. sIgE values of 0.35 kU_A/L or greater were considered positive.

Analysis of Results

Quantitative data are presented as median and 25th-75th percentiles. Patients with positive and negative SICs with NRL gloves were compared using the Wilcoxon rank sum test, chi-square or Fisher's exact test as appropriate, and correlations between variables were assessed through Spearman rank correlations using the IBM SPSS Statistics V.19.0 software (SPSS Inc, Chicago, Illinois, USA). Receiver-operating characteristic (ROC) analyses were conducted using the MedCalc 14.8.1 software (Mariakerke, Belgium) to determine the diagnostic performance of sIgE levels against: 1) the whole NRL extract; 2) the individual recombinant NRL allergen components that were the most discriminant between positive and negative SIC (i.e. rHev b 5, 6.01, 6.02, and 11); and 3) combined sIgE scores derived by summing the individual sIgE titers against these recombinant allergens. Sensitivity, specificity, positive predictive values (PPV), negative predictive value (NPV), likelihood ratio, areas under the curve (AUC) values, and Youden index (i.e. sensitivity + specificity – 1) (27) with their 95% CI values were calculated for the following sIgE cut-off values: 1) the usual sIgE cut-off of 0.35 kU_A/L for the NRL extract and the individual recombinant allergens; 2) the sIgE threshold value that provided a PPV >95% for the NRL extract and the individual recombinant allergens; and 3) the "optimal" cut-off value identified as the sIgE level that yielded the highest Youden index for the NRL extract, individual recombinant allergens, and combined sIgE sum scores. All statistical tests were two-tailed. A P value <0.05 was considered significant

RESULTS

Characteristics of the Subjects

During the period from 1993 to 2010, a total of 129 subjects exposed to NRL gloves who reported work-related asthma symptoms completed a SIC procedure with NRL gloves. Serum samples were available for 107 subjects, of whom 82 (77%) showed a positive SIC response in terms of the changes in FEV₁. The demographic and clinical characteristics of the subjects with positive and negative SICs are summarized in Table 1. The majority of these subjects (87%) worked in healthcare environments. When compared to the subjects who developed an asthmatic response during SIC, those with a negative SIC were slightly older, were more often smokers or ex-smokers, and reported less frequently contact urticaria and work-related rhinitis. The pattern of the asthmatic reactions induced by challenge exposure to NRL gloves was immediate in 67%, dual in 29%, isolated late in 2% and atypical progressive in 1%. The baseline histamine PC₂₀ value was >8 mg/ml in six (24%) subjects with a negative SIC, but also in 16 (20%) of those with a positive SIC. Notably, only one of 11 subjects with a positive SIC showed a post-challenge PC₂₀ value >8 mg/ml, while the post-challenge PC₂₀ remained in the non-asthmatic range in the six subjects with a negative SIC who demonstrated a baseline PC₂₀ value >8 mg/ml.

Specific IgE against NRL Extract

A level of sIgE against the NRL extract k82 equal to or above the usual cut-off value of 0.35 kU_A/L was detected in 77 of 82 (94%) subjects with a positive SIC and in 13 of 25 (52%) subjects with a negative SIC (P <0.001). The PPV of a NRL-sIgE level ≥0.35 kU_A/L for a positive SIC result was 86% while the NPV was 71%. The AUC of the ROC curve was 0.84 (95% CI: 0.76-0.90; p<0.001) (Figure 1). The optimal NRL-sIgE cut-off level based on the highest Youden index (0.61, 95%CI: 0.42-0.81) was ≥1.12 kU_A/L, providing a sensitivity of 85% and a specificity of 76%. The minimal cut-off value that provided a PPV >95% was 5.41 kU_A/L which produced a sensitivity of 49% and a low diagnostic efficiency (Youden index of 0.41 [95%CI: 0.24-0.58]).

The characteristics of the five subjects with a positive SIC who failed to show detectable NRL-sIgE antibodies are detailed in Table 2. All of them were non-smoking female healthcare workers. Their demographic and clinical characteristics did not differ from those of the subjects with a significant NRL-specific IgE level (data not detailed), with the exception that they required a longer duration of exposure to NRL gloves to elicit an asthmatic reaction during the SIC (median [25th-75th percentile], 105 min [58-262] vs. 45 min [10-120], $p=0.068$). Notably, the post-challenge histamine PC₂₀ value was significantly decreased (>3 fold) as compared to the baseline value in the three subjects for whom this assessment was performed 7 hours ($n=2$) or 24 hours ($n=1$) after the end of the challenge exposure to NRL gloves. The median (25th-75th percentile) total IgE level was significantly lower in these five subjects (26 k_AU/L [7.5-110]) as compared to those with positive NRL-sIgE antibodies (96 k_AU/L [42-226]; $p=0.024$).

Among the 13 subjects with a negative SIC but a positive NRL-sIgE result, 12 (92%) experienced contact urticaria and rhinoconjunctivitis at work and three (23%) reported symptoms suggestive of a fruit allergy. Among the 12 subjects with a negative SIC and NRL-sIgE reactivity, contact urticaria was reported by seven subjects (58%), rhinoconjunctivitis at work by eight (68%), and fruit allergy by seven (58%) subjects; $p=0.073$, 0.160, and 0.111, respectively, when compared to the former group of 13 subjects with a positive NRL-sIgE.

Specific IgE against NRL Component Allergens

Table 3 presents the prevalence rates of specific IgE reactivity to the individual recombinant NRL allergen components. The median (25th-75th percentile) level of sIgE against NRL and the allergen components Hev b 1, 5, 6.01, 6.02, and 11 was significantly higher in subjects with a positive SIC as compared to those with a negative SIC. Among subjects with a positive NRL-sIgE result (i.e. 77 subjects with a positive SIC and 13 with a negative SIC), the recombinant allergens that were predominantly recognized by the subjects' IgE antibodies

were rHev b 6.01 (72/90, 80%), rHev b 6.02 (70/90, 78%), rHev b 5 (55/90, 61%), rHev b 11 (21/90, 23%), and rHev b 7 (18/90, 20%). Importantly, none of the subjects who showed a positive SIC and a negative NRL-sIgE result demonstrated a sIgE reactivity higher than $>0.35 \text{ kU}_A/\text{L}$ to any of the tested recombinant NRL allergen components.

The individual patterns of sIgE reactivity to the NRL recombinant allergen components and HRP are detailed in Table 4. There was a strong and significant correlation between sIgE levels against rHev b 6.01 and 6.02 ($r_s=0.96$; $p<0.001$), while there were only modest, though significant, correlations between the other allergen components (rHev b 5, 6.01, 6.02, and 11) with r_s values ranging from 0.48 to 0.63 (all p values <0.001). Only four subjects showed discordant results for rHev b 6.01 and 6.02, of whom two subjects showed isolated sIgE reactivity to rHev b 6.01 and one to rHev b 6.02. Among the 77 subjects with a positive SIC and positive NRL-sIgE, 11 demonstrated sIgE reactivity neither to Hev b 6.01 nor to Hev b 6.02, of whom 8 subjects showed sIgE binding to Hev b 5. Of the three subjects in whom sIgE was not detected neither to Hev b 5 nor to Hev b 6.01/6.02, two subjects displayed isolated sIgE reactivity to Hev b 8 and one subject showed IgE reactivity to none of the tested recombinant allergens. The latter subject showed low levels of sIgE against the NRL extract ($0.49 \text{ kU}_A/\text{L}$), rHev b 6.01 ($0.24 \text{ kU}_A/\text{L}$) and rHev b 6.02 ($0.16 \text{ kU}_A/\text{L}$) while sIgE levels against the other allergen components were $<0.004 \text{ kU}_A/\text{L}$. In the whole study population, four subjects demonstrated sIgE binding to horse radish peroxidase (HRP) with relatively low HRP-sIgE values ($0.4\text{-}1.15 \text{ kU}_A/\text{L}$), including three subjects with a positive SIC and sIgE against Hev b 5 ($n=3$), Hev b 6.01 ($n=3$), Hev b 6.02 ($n=3$), Hev b 7 ($n=1$), Hev b 10 ($n=1$), Hev b 11 ($n=3$), and Hev b 12 ($n=1$). One subject with a negative SIC showed sIgE antibodies only against HRP in the same low range ($0.47 \text{ kU}_A/\text{L}$) as her NRL-sIgE level ($0.55 \text{ kU}_A/\text{L}$). Clinical fruit allergy was not associated with a specific pattern of sensitization to the NRL allergen components (data not detailed). Among subject with a positive SIC, the magnitude of the asthmatic reaction during the SIC, expressed as the maximum fall in FEV_1 compared to pre-challenge value, showed a slight correlation with the sum of sIgE levels

against the 12 recombinant allergens ($r_s=0.27$; $p=0.14$), but not with the number of allergen components recognized by the subjects.

Subjects with a positive SIC showed significantly more often sIgE reactivity to more than one recombinant allergen component (52/82, 63%) than those with a negative SIC (7/25, 28%, $p=0.003$). When compared to the subjects with a negative SIC (Table 3), those with a positive SIC showed a significantly higher rate of positive sIgE result against rHev b 5 (63% vs. 12%, $p < 0.001$), rHev b 6.01 (78% vs. 32%, $p < 0.001$), rHev b 6.02 (78% vs. 24%, $p < 0.001$), and rHev b 11 (24% vs. 4%, $p=0.023$). ROC analysis of the individual sIgE levels against these component allergens showed AUC values ranging from 0.72 to 0.82 ($p < 0.001$) (Table 4). Using the usual 0.35 kU_A/L cut-off, sIgE measurements for these recombinant allergens provided specificity rates (68% to 96%) and PPVs (89% to 95%) that were somewhat higher than those provided by NRL-sIgE assessment (48% and 86%, respectively), but yielded lower sensitivity rates (24% to 78%) and NPVs (28% to 51%) compared to NRL-sIgE (94% and 71%, respectively). A similar trend was observed when using the optimal cut-off values based on the highest Youden indexes for the individual allergen components (Table 5).

Based on the optimal cut-off values derived from the highest Youden indexes, the combined sIgE scores derived by summing the individual sIgE titers directed against the recombinant allergens rHev b 5 and 6.01 or 6.02 yielded a sensitivity of 79% (or 78%, respectively), a specificity of 88%, a PPV of 96%, and a NPV of 56% (or 55%) (Table 5). When using a target PPV >95%, the diagnostic efficiency of the combined sIgE score against rHev b 5 *plus* rHev b 6.01 (cut-off ≥ 1.46 kU_A/L; sensitivity [95% CI]: 79% [69-87]; Youden index [95% CI]: 0.67 [0.50-0.84]) was higher than that provided by a sIgE level against the whole NRL extract ≥ 5.41 kU_A/L (sensitivity: 49% [38-60]; Youden index: 0.41 [0.24-0.58]) as well as against the individual recombinant allergens rHev b 5 (Youden index: 0.54 [0.38-0.71]), rHev b 6.01

(Youden index: 0.56 [0.39-0.74]), and rHev b 6.02 (Youden index: 0.55 [0.35-0.75]). The sIgE sum score against rHev b 5 *plus* rHev b 6.02 yielded a similar diagnostic performance (i.e. sensitivity: 78% [68-86]; Youden index: 0.66 [0.49-0.83]). Adding sIgE against rHev b 11 to these recombinant sIgE scores failed to provide additional diagnostic benefit (Table 5). Combining positive sIgE results for the NRL extract and the recombinant allergen components rHev b 5, 6.01, 6.02, and 11 provided similar AUC (0.84-0.85) and Youden index (0.61-0.65) values (data not detailed), but did not improve the diagnostic performance.

DISCUSSION

This study shows that, in subjects exposed to NRL gloves who experience work-related asthma symptoms, a high serum level of sIgE against NRL or against its recombinant allergen components rHev b 5 (i.e. acidic protein) combined with rHev b 6.01 (i.e. prohevein) or rHev b 6.02 (i.e. hevein) has a high predictive value for a positive SIC with NRL gloves and strongly supports a diagnosis of NRL-induced OA. Although SICs with the suspected occupational agent is still acknowledged as the “reference method” for diagnosing sensitizer-induced OA (7-9, 28), this procedure is very infrequently completed due to the lack of availability of appropriate facilities, the time and expertise required to perform the test, the cost of the procedure, and concerns about the potential risk of inducing severe asthmatic reactions (10, 11, 29). Our data indicate that the measurements of NRL-sIgE and, more efficiently, the level of sIgE antibodies against the NRL recombinant allergens rHev b 5 *plus* Hev b 6.01 or 6.02 are useful in selecting the patients for whom an SIC would be required in order to achieve the highest level of confidence in establishing or excluding a diagnosis of NRL-induced OA. Namely, a diagnosis of NRL-induced OA could have been made without performing an SIC with NRL gloves in subjects with a high level of sIgE against NRL (i.e. ≥ 5.41 kU_A/L) or more efficiently in those with a high sIgE sum score against rHev b 5 *plus* b 6.01/6.02 (i.e. ≥ 1.46 kU_A/L). In our population referred to a tertiary center for possible OA

caused by NRL, this approach would have eliminated the need for performing a SIC in 39% or 64% of the subjects when using either the sIgE level against NRL extract or the sum score of sIgE against Hev b 5 *plus* Hev b 6.01/6.02, respectively.

There is scarce information on the relationship between NRL-sIgE concentrations and the outcome of the SIC with NRL gloves or NRL extracts. The findings of this study confirmed that the determination of sIgE level against a whole NRL extract has a high sensitivity (94%), but a low specificity (48%) for predicting the result of the SIC. The NPVs of the NRL-sIgE assessment was low (70%), indicating that a sIgE concentration below the minimal cut-off values does not allow for excluding a diagnosis of NRL-induced OA without performing additional investigations. On the other hand, the PPV of NRL-sIgE could be increased above 95% by increasing the cut-off value to 5.41 kU_A/L or greater, but this decreased the sensitivity of the test to 49%. These results are quite similar to those reported by Quirce et al. who found that the sensitivity and specificity of the CAP assay for NRL were 95% and 40%, respectively, using a sIgE cut-off value of 0.35 kU_A/L in 30 subjects who completed a quantified environmental challenge with powdered NRL gloves (13).

The role of recombinant allergens in the diagnosis of NRL allergy has been investigated in recent years. Component-resolved diagnosis studies based on the traditional singleplexed sIgE assays and multiplex microarray techniques have demonstrated that panels of NRL allergens that include Hev b 1, 3, 5, and 6.01/6.02 identify almost all NRL-allergic patients (17-20). Studies have suggested that IgE-mediated sensitization to certain NRL allergens are associated with specific clinical phenotypes of NRL allergy as in healthcare workers (Hev b 5 and Hev b 6.01/6.02) and in those suffering from spina bifida (Hev b 1 and b 3) (30). In addition, the component-resolved approach allows discrimination between genuine NRL allergy and IgE cross-reactivity due to the profilin component of NRL (Hev b 8) (20) or to carbohydrate epitopes (31). In our study that included mostly healthcare workers, sIgE binding was predominantly detected for rHev b 6.01 (80%), rHev b 6.02 (78%), rHev b 5 (61%), rHev b 11 (23%), and rHev b 7 (20%) among subjects with a NRL-sIgE titer ≥ 0.35

kU_A/L. Interestingly, the sum of sIgE concentrations against rHev b 5 and rHev b 60.1/6.02 yielded a high predictive value for a positive SIC, but showed a higher sensitivity (79%) and diagnostic efficiency (0.67) as compared with a high level of NRL-sIgE (49% and 0.41, respectively).

Nevertheless, none of the subjects with a positive SIC and a negative NRL-sIgE result showed IgE binding to any of the tested recombinant NRL allergen components. Accordingly, the determination of sIgE to recombinant allergens failed to improve the NPV of the NRL-sIgE assay. Although purely speculative, a potential explanation for this finding would be that these subjects were sensitized to NRL allergens that were not included in our test panel or to currently unidentified allergens. Indeed, no reliable recombinant proteins are currently available for Hev b 2, 4, 13 and 14. In addition, Hev b 2 and 13 are glycosylated molecules and the role of glycosylation in allergenicity remains uncertain for these allergens (32-34), although native proteins may elicit reactions in skin-prick testing (17). Moreover, the total IgE concentrations of the subjects with undetectable NRL-sIgE were very low compared to those with positive NRL-sIgE. This may suggest that the level of circulating IgE in these patients is low, but a specific IgE-mediated mechanism could be involved at the level of target organs. These patients might be candidates for further investigation using basophil-activation tests (35). Another potential explanation would be that the changes in FEV₁ observed during SICs might have resulted from a non-allergic mechanism: This hypothesis seems unlikely since the asthmatic reactions were associated with a post-challenge increase in nonspecific bronchial hyperresponsiveness to histamine as demonstrated in three of the five subjects for whom the post-challenge histamine PC₂₀ value was determined. Such changes in the level of nonspecific bronchial hyperresponsiveness are in line with asthmatic reactions induced by common inhalant allergens (36). It is also unlikely that a long interval of time between the last NRL exposure at work and the diagnostic assessment could account for the failure to detect sIgE reactivity to NRL and recombinant NRL allergens in 6% of the subjects with a positive SIC since the median (25th-75th percentile) time elapsed between the last exposure and the

diagnostic evaluation did not differ between subjects with positive NRL-sIgE (0.1 month [0.1-4.2]) and those with undetectable NRL-sIgE (0.2 month [0.1-11]). Unfortunately, the results of sIgE determinations could not be compared to those of skin-prick tests with NRL extracts in our retrospective study because these skin testing was performed with various batches of commercial NRL extracts over time. In a recent European multicentre study (37), skin-prick testing with five commercial NRL showed sensitivity rates ranging from 67% to 89% and specificity rates of 92-96% as compared to the ImmunoCAP determination of sIgE against NRL extract. These findings emphasize the need to further compare the diagnostic performance of skin-prick tests and sIgE assessments compared to NRL challenge tests in prospective studies.

In conclusion, the level of sIgE against against rHev b 5 *plus* rHev b 6.01/6.02 in the sera of workers experiencing work-related asthma symptoms is an efficient predictor for a positive SIC to NRL. These data have clinical implications since they may help clinicians to reduce the number of SICs without impairing the diagnostic accuracy. The sum of sIgE levels against the recombinant Hev b 5 and Hev b 6.01/6.02 has a higher sensitivity than the level of NRL-sIgE antibodies in identifying the subjects who will develop a positive SIC response and is, thus, more efficient in selecting the subjects for whom a SIC procedure could be avoided. However, these data also confirm that levels of sIgE against NRL and currently available related components below the usual cut-off level of 0.35 kU_A/L do not allow excluding the diagnosis of NRL-induced OA

LEGEND TO FIGURES

Figure 1

Receiver operating characteristic (ROC) plots for the level of specific IgE antibodies (sIgE) against natural rubber extract (NRL-sIgE; continuous line) and the sum of the concentrations of sIgE against rHev b 5 and rHev b 6.01 (Hev b 5 sIgE + Hev b 6.01 sIgE; dotted line) for

predicting the development of an asthmatic reaction during specific inhalation challenges with NRL gloves.

Table 1. Clinical characteristics of the subjects

	Negative SIC (n=25)	Positive SIC (n=82)	p-value
Gender (male), n (%)	1 (4)	7 (8.5)	0.678
Age, yr *	40 (32-45)	34 (30-39)	0.012
Healthcare workers, n (%)	19 (76)	71 (87)	0.205
Current/ex-smokers, n (%)	5/7 (20/28)	7/8 (8/10)	0.008
Atopy†, n (%)	16 (64)	58 (71)	0.523
Total serum IgE level, kU/L*	71 (28-276)	89 (39-218)	0.839
Work-related urticaria, n (%)	19 (76)	75 (91)	0.038
Work-related rhinitis, n (%)	20 (80)	77 (94)	0.037
Fruit allergy, n (%)	8/16 (50)	35/55 (64)	0.326
Asthma preexisting to NRL exposure, n (%)	3 (12)	23 (28)	0.118
Duration of NRL exposure before asthma onset, mo*	67 (36-160)	72 (36-120)	0.868
Duration of asthma before SIC, mo *	28 (10-57)	32 (14-70)	0.605
Delay since last work exposure, mo *	1 (0.1-20)	0.2 (0.1-11)	0.370
Medication, n (%):			
Short-acting beta ₂ -agonist	14 (58)	51 (65)	0.580
Inhaled corticosteroid	9 (38)	33 (40)	0.809
Long-acting beta ₂ -agonist	4 (17)	14 (17)	1.000
Oral H ₁ -antihistamine	14 (58)	39 (48)	0.353
Baseline FEV ₁ , % pred *	99 (91-108)	96 (86-102)	0.226
Baseline FEV ₁ /FVC, % *	80 (76-83)	77 (74-81)	0.077
Baseline FEV ₁ <80% pred and FEV ₁ /FVC <70%	0 (0)	4 (5)	0.571
Pre-challenge histamine PC ₂₀ , mg/mL *	3.74 (0.86-8.33)	1.40 (0.40-5.55)	0.085
Pre-challenge histamine PC ₂₀ >8 mg/mL, n (%)	6 (24)	16 (20)	0.147
Post-challenge histamine PC ₂₀ >8 mg/mL, n/n tested	6/6	1/11	<0.001

Legend: Data are presented as n and % of available data unless otherwise specified.

FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; NRL: natural rubber latex; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁; SIC: specific inhalation challenge with latex gloves.

* Median value with 25th-75th percentiles in parentheses

† Atopy was defined by a positive skin reaction to one or more common inhalant allergens.

Table 2. Clinical and functional characteristics of the subjects with a positive SIC and undetectable NRL-specific IgE

Subject	Age, yrs	Atopy	Total IgE, kU/L	Associated symptoms	Duration of challenge exposure, min	Baseline FEV ₁ , % pred	Maximum fall in FEV ₁ , % from baseline	Pattern of asthmatic reaction	Histamine PC ₂₀ , mg/ml	
									Pre-SIC	Post-SIC†
Fp 1	34	-	39	R, U	105	110	23	I	1.0	NA
Qm 2	40	+	181	R	300	101	40	L	16.0	0.80 (24h)
Di 3	39	-	26	R, U	225	93	25	I	7.15	1.33 (7h)
Bv 4	34	+	12	R, U, FA	90	80	32	I	1.0	0.21 (7h)
Tp 5	39	+	3	R, U	25	85	26	D	0.1	NA

Legend: SIC: Specific inhalation challenge; NRL: natural rubber latex; R: Rhinoconjunctivitis; U: Urticaria; FA: fruit allergy; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; I: Immediate reaction; L: Late reaction; D: Dual reaction; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁; NA: not available.

† Post-challenge histamine PC₂₀ values measured 7 hours post-exposure in two subjects and 24 hours post-exposure in one subject (with an isolated late reaction).

Table 3. Specific IgE reactivity against recombinant NRL component allergens

Allergen	Negative SIC (n=25)	Positive SIC (n=82)	p-value
NRL extract (k82) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25 th -75 th percentile) sIgE level, kU _A /L	13 (52) 0.36 (0.06-0.36)	77 (94) 4.9 (0.05-16)	<0.001 <0.001
rHev b 1 (rubber elongation factor) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	1 (4) 0.01 (0-0.04)	5 (6) 0.02 (0.01-0.073)	1.000 0.027
rHev b 3 (small rubber particle-associated protein) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	0 (0) 0.004 (0-0.025)	2 (2) 0.007 (0-0.03)	1.000 1.000
rHev b 5 (acidic protein) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	3 (12) 0.01 (0-0.065)	52 (63) 1.81 (0-6.64)	<0.001 <0.001
rHev b 6.01 (prohevein) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	8 (32) 0.11 (0.035-0.56)	64 (78) 1.88 (0.47-4.1)	<0.001 <0.001
rHev b 6.02 (hevein) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	6 (24) 0.04 (0.007-0.41)	64 (78) 1.50 (0.40-3.75)	<0.001 <0.001
rHev b 7 (patatin-like protein) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L Median (25th-75th percentile) sIgE level, kU _A /L	3 (12) 0.04 (0.02-0.013)	15 (18) 0.02 (0-0.21)	0.556 0.22
rHev b 8 (profilin) No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	1 (4)	4 (5)	1.000

Median (25th-75th percentile) sIgE level, kU _A /L	0.01 (0.002-0.055)	0.03 (0.01-0.07)	0.192
rHev b 9 (enolase)			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	0	0	1.000
Median (25th-75th percentile) sIgE level, kU _A /L	0.01 (0.005-0.04)	0.02 (0.01-0.033)	0.314
rHev b 10 (manganese superoxide dismutase)			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	0	2 (2)	1.000
Median (25th-75th percentile) sIgE level, kU _A /L	0.03 (0.005-0.045)	0.03 (0.02-0.05)	0.493
rHev b 11 (class I chitinase)			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	1 (4)	20 (24)	0.023
Median (25th-75th percentile) sIgE level, kU _A /L	0.02 (0.004-0.045)	0.055 (0.02-0.36)	0.001
rHev b 12 (non-specific LTP 1)			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	1 (4)	3 (4)	1.000
Median (25th-75th percentile) sIgE level, kU _A /L	0.05 (0.03-0.12)	0.06 (0.04-0.07)	1.000
rHev b 15 (serine protease inhibitor)			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	0	3 (4)	1.000
Median (25th-75th percentile) sIgE level, kU _A /L	0.02 (0.01-0.045)	0.01 (0.01-0.03)	0.149
Horse radish peroxidase			
No. (%) of subjects with sIgE level ≥ 0.35 kU _A /L	1 (4)	3/81 (4)	1.000
Median (25th-75th percentile) sIgE level, kU _A /L	0.04 (0.02-0.06)	0.03 (0.02-0.05)	0.620

Legend: LTP: lipid transfer protein; rHev b: recombinant *Hevea brasiliensis* allergen; NRL: natural rubber latex; SIC: specific inhalation challenge with NRL gloves.

Table 4. Patterns of sIgE reactivity to NRL allergen components

Positive sIgE level (>0.35 kU _A /L)	Positive SIC (n=82)	Negative SIC (n=25)
NRL extract (k82)	77	13
No recombinant allergen	n=6	n=14
One recombinant allergen:	n=12	n=4
rHev b 5	7	0
rHev b 6.01	1	2
rHev b 6.02	2	0
rHev b 7	0	1
rHev b 8	2	0
Horse radish peroxidase (HRP)	0	1
Two recombinant allergens:	n=13	n=2
rHev b 6.01, 6.02	12	2
rHev b 5, 7	1	0
Three recombinant allergens:	n=25	n=3
rHev b 6.01, 6.02, 5	21	2
rHev b 6.01, 6.02, 11	2	0
rHev b 6.01, 6.02, 7	1	1
rHev b 6.01, 6.02, 8	1	0
Four recombinant allergens:	n=13	n=2
rHev b 6.01, 6.02, 5, 11	6	0
rHev b 6.01, 6.02, 5, 7	4	0
rHev b 6.01, 6.02, 5, 1	1	1

rHev b 6.01, 6.02, 7, 15	1	0
rHev b 6.01, 5, 1, 3	1	0
rHev b 11, 7, 8, 12	0	1
Five recombinant allergens:	n=8	n=0
rHev b 6.01, 6.02, 5, 11, 7	3	0
rHev b 6.01, 6.02, 5, 11, 15	1	0
rHev b 6.01, 6.02, 5, 11, HRP	1	0
rHev b 6.01, 6.02, 5, 7, 1	1	0
rHev b 6.01, 6.02, 11, 7, 1	1	0
rHev b 6.01, 6.02, 11, 12, 8	1	0
Six recombinant allergens:	n=3	n=0
rHev b 6.01, 6.02, 5, 11, 7, 15	1	0
rHev b 6.01, 6.02, 5, 11, 7, HRP	1	0
rHev b 6.01, 6.02, 5, 11, 10, 12	1	0
Seven recombinant allergens:	n=2	n=0
rHev b 6.01, 6.02, 5, 11, 7, 1, 3	1	0
rHev b 6.01, 6.02, 5, 11, 10, 12, HRP	1	0

Legend: rHev b: recombinant *Hevea brasiliensis* allergen; HRP: horse radish peroxidase; NRL: natural rubber latex; SIC: specific inhalation challenge with NRL gloves.

Table 5. ROC analysis of specific IgE levels against NRL extract and NRL recombinant allergens.

	slgE cut-off value (kU _A /L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Likelihood ratio	Youden Index	Area under the curve¥
NRL extract (K82)	≥0.35	94 (86-98)	48 (28-72)	86 (77-92)	71 (44-90)	1.8 (1.2-2.6)	0.42 (0.22-0.62)	0.84 (0.76-0.90)
	≥1.12*	85 (76-92)	76 (55-91)	92 (84-97)	61 (42-78)	3.6 (1.8-7.2)	0.61 (0.42-0.81)	
	≥5.41 [†]	49 (38-60)	92 (74-99)	95 (84-99)	35 (24-48)	6.1 (1.6-23.5)	0.41 (0.24-0.58)	
rHev b 5	≥0.35	63 (52-74)	88 (69-98)	94 (85-99)	42 (29-57)	5.3 (1.8-15.5)	0.51 (0.35-0.68)	0.79 (0.70-0.86)
	≥0.51*	62 (51-73)	92 (74-99)	96 (87-100)	43 (29-57)	7.8 (2.0-29.7)	0.54 (0.38-0.71)	
rHev b 6.01	≥0.35	78 (68-86)	68 (46-85)	89 (79-95)	49 (31-66)	2.4 (1.4-4.4)	0.46 (0.26-0.66)	0.81 (0.72-0.88)
	≥0.86*	68 (57-78)	88 (69-98)	95 (86-99)	46 (31-61)	5.7 (1.9-16.6)	0.56 (0.39-0.74)	
rHev b 6.02	≥0.35	78 (67-86)	76 (55-91)	91 (82-97)	51 (34-68)	3.2 (1.6-6.6)	0.54 (0.35-0.73)	0.82 (0.73-0.89)
	≥0.31*	79 (69-87)	76 (55-91)	92 (82-97)	53 (36-70)	3.3 (1.6-6.7)	0.55 (0.35-0.75)	
rHev b 11	≥0.35	24 (16-35)	96 (80-100)	95 (76-100)	28 (19-39)	6.1 (0.9-43.2)	0.20 (0.08-0.32)	0.72 (0.62-0.80)
	≥0.08*	43 (32-54)	92 (74-99)	95 (82-99)	33 (22-45)	5.3(1.4-20.6)	0.35 (0.18-0.51)	
rHev b 5, 6.01 [‡]	≥1.46*	79 (69-87)	88 (69-98)	96 (88-99)	56 (40-72)	6.6 (2.3-19.2)	0.67 (0.50-0.84)	0.86 (0.78-0.92)
rHev b 5, 6.02 [‡]	≥1.47*	78 (68-86)	88 (69-98)	96 (88-99)	55 (38-71)	6.5 (2.2-18.9)	0.66 (0.49-0.83)	0.86 (0.79-0.92)
rHev b 5, 6.01, 11 [‡]	≥1.49*	79 (69-87)	88 (69-98)	96 (88-99)	56 (40-72)	6.6 (2.3-19.2)	0.67 (0.50-0.84)	0.85 (0.77-0.91)
rHev b 5, 6.02, 11 [‡]	≥1.50*	79 (69-87)	88 (69-98)	96 (88-99)	56 (40-72)	6.6 (2.3-19.2)	0.67 (0.50-0.84)	0.86 (0.79-0.92)

Legend: 95% confidence intervals are presented between parentheses; ROC: receiver-operating characteristics.

* Optimal cut-off value based on the highest Youden Index (i.e. sensitivity + specificity – 1).

[†] Minimal cut-off value providing a positive predictive value >95%.

* All P-values <0.001.

[‡] slgE sum score defined as the sum of slgE titers against individual recombinant allergens.

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