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ORIGINAL PAPER

# Percutaneous reactivity to natural rubber latex proteins persists in health-care workers following avoidance of natural rubber latex

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**Summary**

*Background* Long-term avoidance of natural rubber latex [*Hevea brasiliensis* (Hev b)] is currently recommended for health-care workers (HCWs) with established natural rubber latex (NRL) allergy. Percutaneous sensitivity to eight Hev b NRL allergens was evaluated in HCWs in 2000. To date, no studies have evaluated the longitudinal effects of NRL avoidance on percutaneous sensitivity to NRL allergens.

*Objective* The aims of this study were to evaluate changes in percutaneous reactivity to non-ammoniated latex (NAL) and NRL allergens in HCWs 5 years after a recommendation to avoid NRL and to evaluate factors that predict the persistence of *in vivo* sensitivity to NAL and NRL allergens.

*Methods* Skin prick testing was performed with NAL, seven NRL allergens (Hev b 1, 2, 3, 4, 6.01, 7.01, and 13), and recombinant Hev b 5 (rHev b 5) in 34 HCWs who were initially evaluated in 2000 for occupationally related NRL allergy. Serial 10-fold dilutions of NAL and NRL allergens were employed in skin testing. Sera from the HCWs were assayed for latex and enhanced latex (rHev b 5-enriched allergosorbent)-specific IgE antibodies using the ImmunoCAP® assay.

*Results* The prevalence of work-related symptoms significantly decreased between 2000 and 2005 with avoidance of NRL ($P < 0.05$). A ⩾100-fold reduction in percutaneous sensitivity to Hev b 2 and Hev b 7 was less likely in those with prior history of systemic reactions to NRL ($P = 0.0053$), reported history of reaction to cross-reactive foods ($P = 0.014$), continued local reactions to NRL gloves ($P < 0.0001$), or high NRL glove exposure since the initial study ($P = 0.0075$). The diagnostic sensitivity and specificity of the latex-specific IgE serology was 54% and 87.5%, respectively, in comparison with NAL skin tests. The addition of rHev b 5 to the ImmunoCAP® (enhanced latex) allergosorbent altered the diagnostic sensitivity and specificity of the ImmunoCAP® to 77% and 75%, respectively.

*Conclusion* While symptoms may resolve quickly with NRL avoidance therapy, detectable IgE indicating continued sensitization remains beyond 5 years, and thus continued avoidance of NRL should be recommended.

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## Introduction

One of the earliest reports of IgE-mediated allergy to natural rubber latex (NRL) was published in 1979 [1]. By 1997, the US Food and Drug Administration (US FDA) had received more than 1700 reports of severe allergic reactions associated with NRL in medical use [2]. NRL gloves have been the most common source of occupational NRL allergen exposure for health-care workers (HCWs) and patients [3–5]. Clinical manifestations of NRL allergy reported in HCWs include rhinoconjunctivitis, bronchospasm, contact urticaria, and anaphylaxis [1, 6–10].

By the 1990s, the prevalence of NRL allergy in HCWs had been estimated to be 5–40% [4, 11–21]. More recently, the apparent prevalence of NRL allergy has decreased, with one recent study finding a 0.56% prevalence among

HCWs in Wales [22]; however, no firm statistics are available in the United States. The decrease in new cases of NRL allergy has been attributed to an overall reduction in NRL exposure by the introduction of low-allergen (often powder-free) NRL gloves or allergen-free non-NRL non-sterile examination and sterile surgeon gloves in health-care facilities [4, 23–26].

Avoidance of NRL-containing products is currently recommended as the primary intervention for individuals with an established NRL allergy [27]. Long-term avoidance of NRL has been associated with reduction or disappearance of symptoms in a majority of NRL-allergic HCWs [28]. Allmers et al. [29] reported a significant reduction in the incidence of contact urticaria in HCWs over 4 years following the introduction of non-NRL gloves. A 5-year prospective study evaluating 1040 HCWs demonstrated a decreased prevalence in percutaneous reactivity to commercial NRL extract (Lofarma Allergeni, Milan, Italy) among NRL-allergic workers after conversion to non-NRL gloves [28]. While symptoms are reduced or absent in a majority of occupationally exposed individuals with avoidance of NRL, more than 20% of HCWs have continued to be symptomatic with exposure to NRL gloves [28, 30]. Of the symptomatic HCWs, only 6% have continued to exhibit percutaneous sensitivity to NRL extract [28]. Risk factors for the persistence of positive skin prick test (SPT) to NRL have not been well evaluated [28].

Thirteen allergenic proteins that bind human IgE antibodies have been purified from crude NRL and designated as *Hevea brasiliensis* (Hev b) NRL allergens [31, 32]. In 2000, we performed a prospective skin testing study in 62 HCWs with NRL allergy and confirmed that Hev b 2, 6.01, 13, and recombinant Hev b 5 (rHev b 5) are the principal allergens involved in NRL sensitivity as defined by those eliciting percutaneous reactivity in more than 50% of NRL-sensitized workers [33]. To date, no studies have evaluated the longitudinal effects on percutaneous sensitivity to NRL allergens. The aims of this study were twofold. The first was to evaluate changes in percutaneous reactivity to non-ammoniated latex (NAL) and NRL allergens in NRL-allergic HCWs 5 years after a recommendation to avoid NRL in 2000. The second was to investigate factors that predict the persistence of *in vivo* reactivity to NRL and NRL allergens.

## Materials and methods

### *Study population*

Numerous attempts were made to contact all 62 HCWs originally evaluated in 2000 [33]. Attempts were made to locate individuals who had moved through online resources using last known phone number, address, and social security number. Thirty-four of the original 62 HCWs were successfully recruited and re-tested. No subject recruited for the study declined participation. The inclusion criteria for the present study was that a subject was enrolled as part of the original 2000 study. Owing to concerns over systemic allergic reactions triggered by NRL skin testing, participants were excluded if: (1) forced expiratory volume in 1 s ($FEV_1$) was $<70\%$ predicted; (2) asthma symptoms occurred $>3$ times/week within 2 weeks before skin testing; (3) noctural asthma episodes occurred within the previous 2 weeks; and (4) a rescue inhaled β-agonist was being used. Criteria for exclusion were if subjects: (1) were pregnant women, nursing, or not using acceptable methods of birth control; (2) were unable to discontinue use of drugs known to inhibit histamine; (3) were identified with a skin condition (e.g. dermatographism) that interfered with accurate interpretation of skin tests; (4) reported having anaphylaxis or acute asthma after prior testing with NRL; or (5) were receiving β-blockers. All 34 of the original 62 participants met the inclusion criteria and had no exclusions. The study was approved by the CDC/NIOSH Human Subjects Review Board.

### *Study design*

This was a historical prospective cohort study. The study was conducted during two consecutive clinic visits separated by 1–2-week intervals. On the initial visit, complete history and physical, spirometry (if asthmatic), and sera samples (stored at $-60\,^{\circ}C$) were collected. A detailed questionnaire regarding clinical symptoms, NRL exposure, and occupational changes since 2000 was administered to all 34 HCWs. On the second visit, skin prick titration testing was performed as originally described with the test allergens utilized in the previous 2000 study, which included NAL, seven native, purified NRL allergens (Hev b 1, 2, 3, 4, 6.01, 7.01, 13), and rHev b 5 [33].

### *Latex-specific immunoglobulin E*

Latex-specific IgE was measured in both 2000 and 2005 using an ImmunoCAP® (Phadia, Uppsala, Sweden) system, using reagents and standards acquired from the manufacturer [33]. Allergosorbents used were latex (k82) and enhanced latex (Rk82, fortified with rHev b 5 allergen), both obtained from Phadia. The latex reagent (k82) is US Food and Drug Administration (US FDA) 510 (k) cleared as an *in vitro* diagnostic reagent, while the enhanced latex (Rk82) is considered an analyte-specific reagent by the US FDA.

### *Skin testing reagents*

NAL was kindly provided by Dr Robert Hamilton (Johns Hopkins University) that was obtained from the Malaysian *H. brasiliensis* (clone RRIM 600) latex preparation as previously described [34]. The seven NRL allergens were

 

purified from fresh NAL obtained from *H. brasiliensis* trees [33]. Hev b 1 and Hev b 3 proteins were isolated from the rubber particle fraction, and Hev b 2, Hev b 4, Hev b 6.01, and Hev b 13 proteins were purified from lutoid B-serum [35]. Hev b 7.01 was prepared from latex C serum by fractionation [33]. The method reported by Slater et al. [36] was used to prepare rHev b 5. After SDS-PAGE, the identity and specificity of the proteins were characterized by Western blot analysis.

### *Skin test procedures*

Allergen skin prick testing was performed with a bifurcated needle skin prick test device (Accu-Set$^{TM}$, Alk-Abelló, Round Rock, TX, USA). For NAL, serial 10-fold dilutions in PBS were prepared from a starting concentration of 1000 μg/mL. For the NRL allergens, stock solutions at 50 μg/mL were prepared on the day of testing by using PBS-containing 2.0% NaCl, pH 7.4 [33]. Tenfold dilutions of the NRL allergens were prepared from 50 μg/mL and tests were performed over a range of $5 \times 10^{-5}$ to 50 μg/mL of protein. For NAL, SPTs were performed over a range of $1 \times 10^{-6}$ to −1 mg/mL. Respective concentrations were increased by 10-fold until a positive test was observed, which was defined as a weal of ⩾3 mm compared with the diluent control. The concentration eliciting a positive SPT was defined as the end-point percutaneous threshold concentration (EPC). Histamine phosphate (5 mg/mL) and diluents were used as positive and negative reference controls, respectively. To assure safety and minimize risk in patients with current asthma, peak expiratory flow rates and $FEV_1$ were performed before skin testing. If $FEV_1$ declined by 15% from the screening visit (visit 1), skin testing was postponed until lung function recovered to within 5% of the initial value. If systemic allergic reactions occurred during treatment, no further skin testing was performed. Atopy was previously evaluated using a panel of 12 common aeroallergens [33].

### *Case definitions*

All subjects had a positive SPT to NAL in 2000 and were classified, based on symptoms related to NRL glove exposure, in the original study and grouped according to the case definitions in Table 1. For each subject, exposure to NRL products was categorized as: (1) *high exposure* defined as *direct personal contact or indirect contact via fellow employees* with powdered NRL gloves and/or direct skin contact with non-powdered NRL gloves; or (2) *no exposure* defined as no contact with NRL products. Reduced percutaneous sensitivity was defined as: (1) at least a 100-fold ($2 \log_{10}$) increase in the EPC of NAL or NRL allergens compared with baseline EPC in 2000; or (2) complete loss of SPT reactivity to any antigen during current testing.

### *Data analysis*

Data were analysed using SAS Software 9.1 (SAS Institute Inc., Cary, NC, USA). In order to ascertain if there were any differences in the 34 of 62 subjects retested in 2005, vs. the 28 who could not be contacted, EPCs for NAL and each Hev b, prevalence of occupational asthma, occupational rhinitis, atopy, and levels of latex-specific IgE (measured by both k82 and Rk82) dichotomized on tested/not tested were evaluated using Mann–Whitney rank sum tests. Wilcoxon sign rank tests were used to perform analysis of differences between NAL EPC and ImmunoCAP® levels between 2000 and 2005. The proportions sensitized in 2000 and 2005 were compared using McNemars's test. Diagnostic performance of the *in vitro* assays was computed using the following definitions: TP, true-positive diagnostic test result; TN, true-negative diagnostic test result; FN, false-negative diagnostic test result; and FP, false-positive diagnostic test result. Sensitivity ($TP/[TP+FN] \times 100$) was computed as the percentage of positive test responses in subjects with a positive SPT response. Specificity ($TN/[FP+TN] \times 100$) was computed as the percentage of negative tests in subjects with a negative PST response. Linear regression was used to evaluate the correlation between ImmunoCAP® levels and EPC for NAL. Specific factors associated with a clinically significant decrease in sensitization were evaluated using the $\chi^2$ test with Fisher's exact to calculate an odds ratio (OR) and 95% confidence intervals (CIs). A zero cell adjustment of 0.5 was used when a cell with a frequency of zero was present [37]. Given the non-normal distribution of the log differences in sensitization to the latex allergens, non-parametric analysis was performed to evaluate smaller changes in sensitization. Log differences in SPT positivity

**Table 1.** Case definitions

| NRL associated symptom | Case definition |
|---|---|
| Work-related contact urticaria | Hives with itching occurring during the skin contact with NRL gloves |
| Work-related rhinitis | Sneezing, nasal congestion, and eye itching associated with exposure to powdered NRL gloves |
| Work-related asthma | Immediate onset wheezing, shortness of breath, and chest tightness associated with exposure to powdered NRL gloves |
| Work-related anaphylaxis | Immediate onset dyspnea, diffuse urticaria, or hypotension requiring emergency treatment with epinephrine |

NRL, natural rubber latex.

 

and specific factors associated with any change in sensitization were evaluated using the Wilcoxon rank sum test. A *P*-value of 0.05 or less was considered statistically significant.

## Results

### *Study participants*

Of the 62 NRL-sensitized HCWs from the initial study, 34 (55%) were successfully recruited. There were no significant differences ($P = 0.139–0.997$) in NAL or Hev b SPT sensitivity, the prevalence of occupational asthma, occupational rhinitis, atopy, or serum latex-specific IgE concentrations in the 34 recruited vs. the 28 subjects who could not be contacted from the 2000 study. Thirty-two females accounted for the majority of participants (94%). The mean age was 45 years (range 26–73 years). A period of $56.2 \pm 2.2$ months (mean $\pm$ SD) elapsed between the two evaluations. While 10 (29%) of 34 subjects reported that they were no longer HCWs, continued work-related exposure to NRL was reported in 14 (41%) of 34 subjects. Thirty (88%) of 34 subjects were atopic. All participants completed the study protocol, and no systemic allergic reactions occurred during skin testing with NAL or NRL allergens.

At the initial evaluation in 2000, participants were classified by case definitions (Table 1). The HCWs were reclassified according to case definitions for this study and evaluated for change over time (Fig. 1). Work-related urticaria was present in all 34 subjects in 2000 and significantly decreased to seven of 34 (20.6%, $P < 0.0001$) in 2005. Work-related rhinitis was identified in 27 of 34 (79.4%) in 2000 and significantly decreased to 10 of 34 (29.4%, $P < 0.0001$) in 2005. Reported frequency of work-related asthma, which was found in 18 of 34 (52.9%) in 2000, was identified in only two of 34 (5.9%, $P < 0.0001$) in 2005. Work-related anaphylaxis was rare, present in two of 34 (5.9%) in 2000 but was not reported during the intervening period from 2000 to 2005.

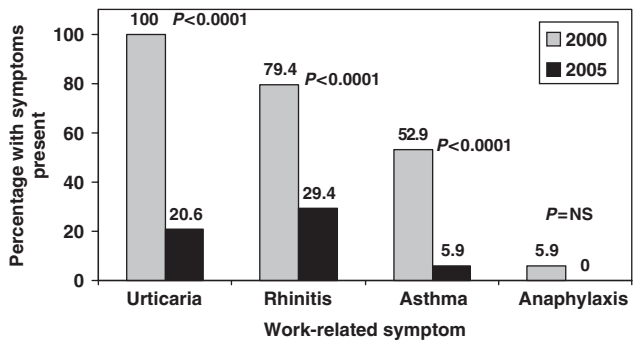


Fig. 1. Change in the prevalence of work-related symptoms among health-care workers studied between 2000 and 2005. NS, non-significant.

### *Skin test results*

In 2000, all subjects were SPT positive to NAL [33]. In 2005, 24 of 34 (70.6%) were still SPT positive to NAL, indicating no SPT evidence of continued sensitization in the remaining 10 HCWs (29.4%) (Fig. 2). The median EPC to NAL significantly increased over time from 0.0001 mg/mL in 2000 to 0.01 mg/mL in 2005 ($P < 0.03$), indicating an overall decrease in *in vivo* sensitivity. The prevalence of SPT reactivity for four NRL allergens decreased significantly over time: Hev b 2 (41% decrease), rHev b 5 (44% decrease), Hev b 6.01 (37% decrease), and Hev b 7.01 (63% decrease) (Fig. 2).

### *Performance of ImmunoCAP® compared with skin prick testing*

The mean level of latex-specific IgE for all HCWs as measured in the ImmunoCAP® using the standard latex allergosorbent was 5.25 $KU_A$/L in 2000. It was significantly reduced to 2.2 $KU_A$/L in 2005 ($P < 0.01$). Use of the rHev b 5-enhanced allergosorbent also identified a decrease in mean IgE anti-latex levels from 8 $KU_A$/L in 2000 to 3 $KU_A$/L in 2005 ($P < 0.01$).

The ImmunoCAP® assay using the standard latex allergosorbent was positive in 18 of 34 NAL SPT positive subjects in 2000 (diagnostic sensitivity: 53%) [33] and 14 of 26 with percutaneous sensitization to NAL in 2005 (diagnostic sensitivity: 54%). Use of the rHev b 5-enhanced NRL allergosorbent increased the diagnostic sensitivity to 62% (21 positive of 34 SPT-positive subjects) in 2000 [33] and 20 of 26 in 2005 (diagnostic sensitivity: 77%). Of those with a negative SPT to NAL in 2005, one of eight (test specificity: 87.5%) had a positive standard latex ImmunoCAP® result (diagnostic specificity: 87.5%) and two of eight had a positive IgE anti-latex serology with the Hev b 5-enhanced allergosorbent (diagnostic specificity: 75%).

No significant correlation was observed between the change in NAL-specific IgE levels between 2000 and 2005 and the change in NAL EPC between 2000 and 2005 using either the standard or rHev b 5-enhanced allergosorbent.

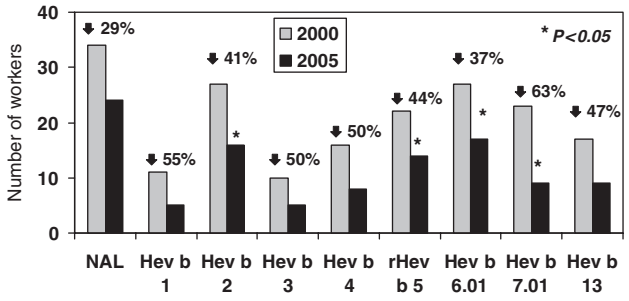


Fig. 2. Number of health-care workers with positive skin prick test results to non-ammoniated latex (NAL) and the individual natural rubber latex allergens as studied from 2000 to 2005. Hev b, *Hevea brasiliensis*, rHev b 5, recombinant Hev b 5.

 

A weak correlation was found between specific IgE level and NAL EPC determined in 2005 (adjusted $r^2 = 0.17$, $P = 0.008$, standard latex allergosorbent), which improved by using the Hev b 5-enhanced NRL allergosorbent (adjusted $r^2 = 0.24$, $P = 0.0018$).

### *Predictors of a 100-fold decrease in sensitization to natural rubber latex allergens*

The prevalence of subjects with sensitization to NAL and all NRL allergens decreased between 2000 and 2005 (Fig. 2). Several clinical measures were identified, which were associated with at least a 100-fold decrease in EPC for Hev b 2 or Hev b 7.01 (Fig. 3). Systemic reactions related to NRL exposure that were reported before 2000 were associated with a reduced likelihood of an interim (2000–2005) decrease in percutaneous sensitivity to Hev b 2 (OR 0.044, 95% CI 0.0009–0.60, $P = 0.0053$). Reported reactions to cross-reactive foods before 2000 were also associated with lower likelihood of an interim decrease in percutaneous sensitivity to Hev b 2 (OR 0.067, 95% CI 0.0014–0.76, $P = 0.014$). Reactions to cross-reactive foods between 2000 and 2005 were associated with a decreased likelihood of reduced sensitivity to Hev b 2 (OR 0.15, 95% CI 0.013–1.20, $P = 0.051$). For Hev b 7.01, HCWs with high exposure to NRL gloves occurring between 2000 and 2005 were less likely to have an interim decrease in sensitivity to Hev b 7.01 (zero cell corrected OR 0.037, 95% CI 0.0017–0.79, $P = 0.0075$). Local reactions to NRL gloves between 2000 and 2005 were associated with decreased odds of reduced percutaneous sensitivity to Hev b 7.01 (zero cell corrected OR 0.0095, 95% CI 0.0003–0.28, $P < 0.0001$).

### *Predictors of any change in percutaneous sensitivity to natural rubber latex allergens*

The mean EPC for NAL and NRL allergens demonstrated significant decreases between 2000 and 2005 (Table 2).

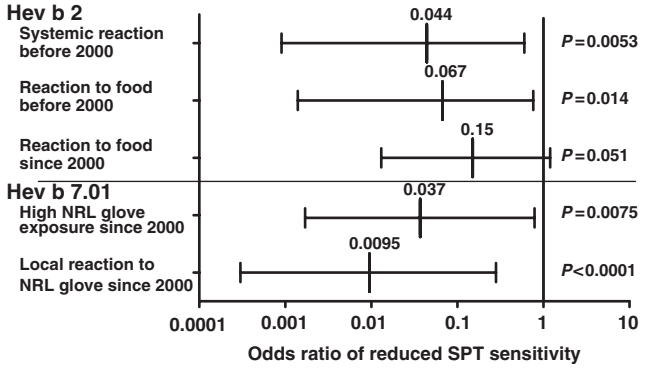


Fig. 3. Clinical and demographic characteristics associated with a 100-fold reduced skin prick test (SPT) sensitivity to natural rubber latex (NRL) allergens. Hev b, *Hevea brasiliensis*.

Table 2. Changes in the mean SPT reactivity for subjects followed in 2000 and 2005

| NRL protein | Mean log initial EPC (2000) | Mean logs difference (2000–2005) | *P* of signed rank |
|---|---|---|---|
| NAL | 4.15 | 2.71 | <0.0001 |
| Hev b 1 | 1.82 | 0.53 | 0.014 |
| Hev b 2 | 2.27 | 1.29 | <0.0001 |
| Hev b 3 | 2 | 0.71 | 0.016 |
| Hev b 4 | 2.13 | 0.94 | 0.0023 |
| rHev b 5 | 2.39 | 1.38 | <0.0001 |
| Hev b 6.01 | 2.27 | 1.15 | 0.0002 |
| Hev b 7.01 | 2.52 | 1.82 | <0.0001 |
| Hev b 13 | 2.28 | 1.09 | 0.0044 |

SPT, skin prick test; NRL, natural rubber latex; EPC, end-point percutaneous threshold concentration; NAL, non-ammoniated latex; Hev b, *Hevea brasiliensis*; rHev b 5, recombinant Hev b 5.

Given the non-normally distributed log differences in EPC to NRL allergens, non-parametric analysis was performed to evaluate <100-fold changes in percutaneous sensitivity. NAL, Hev b 2, rHev b 5, and Hev b 7.01 all showed significant decreases in percutaneous sensitivity in relation to several factors (Table 3). Low NRL glove exposure between 2000 and 2005 was associated with decreased percutaneous sensitivity to NAL ($P = 0.051$), rHev b 5 ($P = 0.017$), and Hev b 7.01 ($P = 0.046$). The absence of a systemic reaction associated with NRL exposure before 2000 was associated with a longitudinal reduction in percutaneous sensitivity to Hev b 2 ($P = 0.015$) and rHev b 5 ($P = 0.021$). The absence of a local reaction between 2000 and 2005 was associated with decreased sensitivity to Hev b 2 ($P = 0.031$), rHev b 5 ($P = 0.020$), and Hev b 7.01 ($P = 0.018$).

## Discussion

The results of this study demonstrate that avoidance of NRL exposure by NRL-allergic HCWs was associated with reduced clinical symptoms and percutaneous reactivity to NAL and Hev b antigens. In 2000, avoidance of NRL was recommended to all 62 NRL-allergic HCWs participating in our initial study [33]. In this longitudinal follow-up of a subset of the same HCWs, the prevalence of NRL-associated urticaria, rhinitis, and asthma significantly decreased. This is consistent with previous reports of the effectiveness of NRL avoidance [4, 23, 24, 26, 28, 38, 39] in reducing clinical manifestations of NRL allergy.

This is the first study to evaluate the relationship between NRL avoidance and its impact on longitudinal changes in percutaneous reactivity to NRL allergens. It was noteworthy that persistence of percutaneous reactivity to Hev b 2 was associated with prior systemic reactions after donning NRL gloves (reported in 2000) as well as

**Table 3.** Clinical and demographic characteristics associated with any change in SPT sensitivity to NAL or NRL allergens

| | NAL | | Hev b 2 | | rHev b 5 | | Hev b 7.01 | |
|---|---|---|---|---|---|---|---|---|
| | Mean rank score* | Exact *P* | Mean rank score | Exact *P* | Mean rank score | Exact *P* | Mean rank score | Exact *P* |
| NRL glove exposure 2000–2005 | | | | | | | | |
| No | 19.3 | 0.051 | | | 19.8 | 0.017 | 19.4 | 0.046 |
| Yes | 12.9 | | | | 12.3 | | 12.8 | |
| Systemic reaction before 2000 | | | | | | | | |
| No | | | 20.4 | 0.015 | 20.2 | 0.021 | | |
| Yes | | | 12.2 | | 12.5 | | | |
| Local reaction 2000–2005 | | | | | | | | |
| No | | | 17.5 | 0.031 | 17.6 | 0.020 | 17.8 | 0.018 |
| Yes | | | 10.1 | | 9.7 | | 9.3 | |

*The analysis was performed using the Wilcoxon rank sum test. The higher the mean rank score is the greater the reduction in sensitization.
SPT, skin prick test; NAL, non-ammoniated latex; NRL, natural rubber latex; Hev b, *Hevea brasiliensis*; rHev b 5, recombinant Hev b 5.

prior and interim-reported allergic reactions to foods known to cross-react with NRL. Persistent percutaneous reactivity to Hev b 7.01 was associated with interim high exposure to NRL (2000–2005) and interim-reported local reactions associated with NRL exposure. It is unclear why food reactions were associated with persistent sensitivity to Hev b 2 vs. Hev b 6.01 (prohevein), which is known to cross-react with avocado and banana [40]. It is also difficult to explain why persistent reactivity to Hev b 7.01, a patatin-like protein, was associated with persistent exposure and local contact reactions, because in the original 2000 study, we found this protein to be a minor *in vivo* Hev b allergen in comparison with Hev b 2, rHev b 5, Hev b 6.01, and Hev b 13. Hev b 7.01 exhibited SPT reactions in 45% of 62 workers [33, 41–43].

Avoidance and less-severe reactions were associated with reduced percutaneous reactivity to Hev b 2, rHev b 5, and Hev b 7.01. Significant changes in percutaneous reactivity with Hev b 2 and rHev b 5 could be attributed to these being major allergens involving a majority of HCWs, thereby providing a larger sub-sample to observe longitudinal changes in EPC responses. Although Hev b 7.01 is not a major *in vivo* NRL allergen, it appears to be sensitive to a reduction or increase in reported NRL exposure status.

We performed our study in 2005 with the same lots of NRL allergens that were used in 2000. It could be construed that the observed reduction in percutaneous reactivity to NRL allergens could be due to loss of allergenic potency of these allergens after 5 years of storage at −80 °C. However, 6–12% of subjects tested both in 2000 and 2005 to identical allergens had essentially no change in EPC. Thus, given the findings in this subgroup, it is highly unlikely that there was an interval loss of allergenic potency of the NRL test reagents used in this study.

Another potential approach to investigate longitudinal changes in NRL allergen sensitivity, which has been used, is serial determinations of *in vitro*-specific IgE [44, 45]. Although we would have liked to compare our skin test results with *in vitro* specific IgE data, this was not possible due to inadequate amounts of NRL native proteins originally prepared from crude NAL before the initial study.

Skin prick testing with NAL is a highly sensitive method for evaluating NRL sensitization [18, 46, 47]. Test sensitivity of NAL SPT reagent has been reported as high as 100% in a European study using a crude NRL commercial extract [47]. However, an FDA-approved NRL skin test reagent is not commercially available in the United States [27, 48]. As a consequence, most allergists (64%) utilize NAL-specific IgE serology as the initial test in the evaluation of NRL allergy [49]. US FDA-cleared serological assays for NRL-specific IgE are readily available. However, in comparison with the SPT, estimates of the diagnostic sensitivity and specificity are less than stellar and they vary as a function of the allergosorbent used. For the ImmunoCAP®, diagnostic sensitivities of 50–90% and specificities of 80–96% have been reported [50–53]. Our results confirm these past performance characteristics and show a statistically significant but weak correlation between changes in IgE anti-latex as measured in the ImmunoCAP® and EPC for NAL SPTs. Enriching the ImmunoCAP®'s allergosorbent with rHev b 5 improved this correlation ($r^2 = 0.24$); however, the association remained weak. The diagnostic sensitivity of the ImmunoCAP® can be considered marginal (53.8%), and it was improved with rHev b 5 enrichment (76.9%). With the improved sensitivity, however, came a minimal loss in diagnostic specificity (87.5–75%). These data support the elimination of the standard latex allergosorbent in favour of the exclusive use of the rHev b 5-enriched allergosorbent.

Despite improvement, the rHev b 5 enriched NRL-specific IgE ImmunoCAP® assay still exhibits an inferior diagnostic sensitivity compared with SPT. It has been speculated that this is less a result of the limited sensitivity of the serological assay and possibly more a result of the fact that immunoreactive NRL-specific IgE remains

 

resident on cellular FcεR1 receptors long after it decreases below detectable levels in the blood. The IgE anti-NRL serology alone exhibits an insufficient diagnostic sensitivity to permit accurate assessment of NRL sensitivity. There is a continued urgent need for an US FDA-approved latex skin testing material.

The results of this longitudinal study confirm that avoidance of NRL products among NRL-allergic HCWs appears to be an effective method for reducing symptoms and to some extent percutaneous sensitivity to NAL over time. Interestingly, despite avoidance and reduced clinical symptoms, most HCWs continue to retain percutaneous reactivity to NAL, even with a higher EPC (decreased sensitivity). Our results confirm that avoidance of NRL decreases but does not generally eliminate *in vivo* sensitization to NAL [30, 54]. While symptoms may resolve quickly with avoidance of NRL, based on our results, sensitization remains even after 5 years, and continued avoidance of NRL should be recommended. Future longitudinal studies of sensitized HCWs need to investigate the long-term natural history of NRL allergy following complete cessation of exposure.

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