



Impact of individual molecular components in determining primary sensitization to latex

M. De Amici^a, F. Barocci^{b,*}, C.E. Barzaghi^c, L. Sacchi^d, A. De Silvestri^e, A. Licari^{f,g},
S. Caimmi^g, A. Marseglia^g, G. Testa^g, C. Torre^h, G.L. Marseglia^{f,g}

^a Immuno-Allergy Laboratory of Clinical Chemistry and Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

^b Medicine Unit Laboratory, ASST Rhodense Garbagnate Milanese, Rho, Italy

^c Allergy Clinic, ASST Rhodense Garbagnate Milanese, Rho, Italy

^d Laboratory for Biomedical Informatics "Mario Stefanelli", Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Italy

^e Servizio di Epidemiologia Clinica e Biometria Direzione Scientifica - Fondazione IRCCS Policlinico san Matteo, Pavia, Italy

^f Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Italy

^g Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

^h Laboratory of Clinical Chemistry, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

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ABSTRACT

Allergy to natural rubber latex emerged as one of the main allergies at the beginning among some professional groups and the general population. Sensitization and development of latex allergy have been attributed to exposure to products containing residual latex proteins. The prevailing cross-reactivity of latex proteins with other food allergens is of great concern. Numerous purified allergens are currently available, which greatly help in patient management, thus determining their specific profile. We conducted a multicenter study to investigate changes, from the ROC analysis, in the characteristics of patients with latex allergy by measuring its major protein components. Sensitization to latex proteins is crucial because it highlights the cross reactivity to inhalants (pollen) and food (fruit). It is very essential in an accurate and specific clinical setting.

1. Introduction

Natural rubber latex (NRL) (Alenius et al., 2002) is an intracellular cytosol secreted from a rubber tree, *Hevea brasiliensis* (Hev b), which functions as a protective sealant and, for its excellent elastic properties, is widely used in the manufacture of medical devices and a variety of everyday articles such as catheters, gloves, condoms and balloons. Undiagnosed latex allergy is potentially severe for patients and is increasingly recognized as a significant contributing factor to morbidity and mortality during medical and surgical procedures.

The most frequent clinical manifestations of NRL are related to type I hypersensitivity mediated by immunoglobulin E (IgE), including the "latex fruit syndrome."

This study is to highlight epidemiological, clinical, and diagnostic aspects of natural rubber latex allergy (NRLA) (Alenius et al., 2002). The diagnosis of NRLA is based on heterogeneous clinical signs. Still, the definitive diagnosis cannot be made on the single positivity of cutaneous test using latex extract because it may represent a false positivity.

Therefore, it is preferred the use the major molecular components of latex in vitro allergy diagnosis. As described in our previous study (De Amici et al., 2021), determining recombinants allergens is essential to obtain a precise assessment of patients and reduce the medical risk and healthcare costs. The correct diagnosis is critical to prevent possible threatening allergic reactions.

The prevalence of latex allergy in the general population varies between 0 % and 2.3 % (Turjanmaa et al., 1996); therefore, our study aimed to assess the prevalence of latex sensitization in a large group of patients referred to our laboratory by the Pavia pediatric allergy services or by the attending allergologist of Milan.

Natural Rubber Latex Allergy (NRLA) is often IgE-mediated but can also be cell-mediated.

IgE-mediated clinical manifestations are variable in severity and site: they can affect the skin, respiratory system, and oral and conjunctival mucous membranes up to causing true anaphylaxis involving multiple systems.

In the mid-'90s, a latex allergy epidemic was observed among health

* Corresponding author at: Formerly Medicine Unit Laboratory, ASST Rhodense - Garbagnate Milanese, Rho (Milan), Italy.

E-mail address: fiorellabarocci@yahoo.it (F. Barocci).

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workers. It was later shown that it was caused by the inhalation of latex particles carried by the powder used for lubrication of surgical gloves. The elimination of powder substantially reduced the prevalence of this condition.

About 40 % of NRLA patients react to fruit (banana, kiwifruit, avocado, chestnut) with symptoms of oral allergic syndrome usually without severe systemic symptoms and constitute Latex-Fruit Syndrome. The time course of clinical manifestations may vary from person to person: the allergy to some fruits, such as bananas, may be the first or, in other cases, it may follow the allergy to the natural latex.

Latex allergy is still highly prevalent in risk subjects such as patients with spina bifida (at high risk because of repeated surgery and the daily use of catheters) or among certain workers. In contrast, the relevant allergen varies according to the length and the modes/routes of contact with the natural latex.

Clinical history (previous spina bifida surgery, work environment, type of manifestations) is essential for diagnosis, as well as research of specific IgEs and recombinant antigens. The latter makes it possible to assess in the individual patients' profile depending on their unique molecular allergy and the risk of developing anaphylaxis when exposed to latex in a hospital/surgical setting. Thus, molecular diagnosis may reduce healthcare costs by limiting the number of NRLA patients who need to be admitted to a latex-free environment.

The recombinant allergens allow us to define the level of risk and the cross-reactive allergens (Hev b 2, 6, 7, 8...) that cause latex-fruit syndrome.

The present work aimed to evaluate the prevalence of allergic sensitization to latex (k82) and to the proteins (Raulf-Heimsoth et al., 2007): k215 - rHev b 1, k218 - rHev b 5, k219 - rHev b6.01, k220 - rHev b 6.02, k221 - rHev b 8, k222 - rHev b 9, k224 - rHev b 11, in relation to different geographical areas, according to the patient's age and in correlation with clinical history.

2. Methods

The study was conducted on patients referred to the pediatric allergy unit of the Foundation IRCCS Policlinico San Matteo of Pavia (Italy), from Milan and surrounding areas, in recent years. Immuno-fluorometric assay (FEIA, ThermoFisher Sc) was used to evaluate specific IgE levels for Latex (k82) and the following protein components (Nowakowska-Świrta et al., 2019): rHev b1, rHev b5, rHev b6.01 (currently no more available for purchase), rHev b6.02, rHev b8, rHev b9, rHev b11 (Garnier et al., 2012; Kurup et al., 2005). Results are expressed in KU/L, international IgE reference which is calibrated in units that are traceable to mass measures (2.4 ng/mL IgE Δ 1 IU/mL = kU/L) available for use in total serum IgE assays (Kleine-Tebbe et al., 2016); and considered as positive for values higher than 0.35 KU/L.

Latex (k82) is included in the standard panel of allergens that are tested on all sera from subjects with pollinosis or unidentified allergies. In some cases, the treating physicians had directly requested the molecular investigation.

The age of the patients ranged from 70 to 8 years, in Pavia, whose average age was 8 (sd 4), of which males were 46 %, and in Milan, whose average age was 37 (sd 21) was 46 %. The difference in median age is because the patients in Pavia come from a pediatric population, whereas patients in Milan come from an adult population.

The number of patients being analyzed for k82 (Latex) are 1990 tests for Pavia, and 325 tests for the Milan area. Cut-off values for k82 (Latex) levels and recombinant positivity are determined with ROC curves.

ROC analysis quantifies the accuracy of diagnostic tests or other evaluation modalities used to discriminate between two states or conditions. The analysis uses the ROC curve, a graph of the sensitivity versus 1-specificity of the diagnostic test.

The global performance of a diagnostic test is commonly summarized by the area under the ROC curve (AUC). This area can be interpreted as the probability that the result of a diagnostic test of a randomly selected

abnormal subject will be greater than the result of the same diagnostic test from a randomly selected normal subject. The greater the AUC, the better the global performance of the diagnostic test.

The patients in the Pavia area were part of a first-level in vitro allergology screening, and no specific data on clinical manifestations of adverse reactions are available. Only in a small sample group of patients selected in Rho (MI) was correlation between clinical data and recombinant dosage.

Statistical analyses were conducted with Matlab®. Normality distribution of data was evaluated through the Kolmogorov–Smirnov test. Patients were divided by the provenience. Chi-squared test was performed to verify a statistical difference between sensitization to geographic provenience and the possible influence of age.

3. Results and discussion

For 2315 patients analyzed the percentage of positive k82 (Latex) is 72 %. However, of this value, 95 % corresponds to the Pavia area and the remaining 5 % to the Milan and surrounding areas (ManabuKawai and Nakajima, 2020). Also, the level of specific IgE for the single proteins does not correlate with age, except for rHev b 6.01 ($p = 0.03$). This correlation is due to the presence of some outliers in the levels of rHev b 6.01 for 4 patients. The significance of the provenience's groups was found to be $p < 0,0001$. For a group of 15 patients from the Milan area, it was possible to retrieve data on adverse reactions to latex. Considering this sample of 15 patients who were checked for clinical manifestations of adverse reactions to latex, 5 patients showed such manifestations (Table A.1). Within these 5 patients, no patient was found with positive rHev b 1. This confirmed the specificity of this recombinant to individuals with congenital pathology (spina bifida) (Tosi et al., 1993) that were not included in our dataset. The results show positivity to rHev b 5, rHev b 6.01, rHev b 6.02 in atopic patients with pollinosis and clinical symptoms. In particular, 2, 1, and 3 subjects were positive for the Skin Prick test (SPT). It is also evident among the subjects screened 10 out of 100 were positive for rHev b 8 Profilin, but only 3 were positive for the SPT.

From the ROC analysis (Table A.2), it results that rHev b 8 Profilin has the highest positive predictive value (PPV) to predict sensitization to latex allergy considering the pooled centers (PPV = 0.67). Noticeably, rHev b8 Profilin is not associated with a primary latex allergy, but with cross-reactivity to food or pollen (Radauer et al., 2011). In this case, it is not necessary to provide a latex-free environment as in the processing procedure, the rHev b 8 Profilin component is deteriorated.

The results on rHev b 8 Profilin are confirmed when considering the two centers separately (Table A.3 for Pavia and Table A.4 for Milan).

rHev b 6.02 shows higher accuracy, negative predictive value (NPV) and area under the curve (AUC) in the Pavia population. Figs. 1 and 2 depict the results of Table A.3 and Table A.4 for specific IgE rHev b 6.02. In particular, the Figures show average values of specific IgE k82 (LATEX) in patients who resulted positive and negative for IgE specific rHev b 6.02, and the ROC curves built by considering rHev b 6.02 values.

Table A.1

Clinical manifestations of adverse reaction to latex in a sample of 15 patients. Patients selected in the ambulatory clinic of hospital Rho (ASST Rhodense).

	Overall (N = 15)	Adverse reaction to Latex (N = 5)	NO adverse Reaction (N = 10)
rHev b 1	7♂ 8♀ 1 positive	1♂ 4♀ 0 positive	6♂ 4♀ 1 positive
rHev b 5	2 positive	2 positive	0 positive
rHev b 6.01	2 positive	1 positive	1 positive
rHev b 6.02	4 positive	3 positive	1 positive
rHev b 8 Profilin	10 positive	3 positive	7 positive
rHev b 11	1 positive	0 positive	1 positive

Table A.2

ROC analysis to determine k82 (Latex) cutoffs considering data from all the centers (Pavia and Milan). TP: true positives, TN: true negatives, FP: false positives, FN: false negatives, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve.

ROC PV + MI	cutoff	Sensitivity	Specificity	TP	TN	FP	FN	Accuracy	PPV	NPV	AUC
rHev b 1	7.7	0.50	0.86	2	65	11	2	0.8375	0.15	0.97	0.68
rHev b 5	5.80	0.57	0.83	4	58	12	3	0.805195	0.25	0.95	0.70
rHev b 6.01	1.41	0.70	0.68	7	36	17	3	0.68254	0.30	0.92	0.69
rHev b 6.02	8.31	0.42	0.92	5	33	3	7	0.791667	0.63	0.83	0.67
rHev b 8 Profilin	0.78	0.61	0.68	20	21	10	13	0.640625	0.67	0.62	0.64
rHev b 11	11.33	0.50	0.87	1	27	4	1	0.848485	0.20	0.96	0.69
At least one	1.19	0.55	0.75	28	33	11	23	0.642105	0.72	0.59	0.65

Table A.3

ROC analysis to determine k82 (Latex) cutoffs considering data from the Pavia area only. TP: true positives, TN: true negatives, FP: false positives, FN: false negatives, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve.

ROC Pavia	cutoff	Sensitivity	Specificity	TP	TN	FP	FN	Accuracy	PPV	NPV	AUC
rHev b 1	13.15	0.50	0.90	1	26	3	1	0.870968	0.25	0.96	0.70
rHev b 5	7.72	0.67	0.84	2	21	4	1	0.821429	0.33	0.95	0.75
rHev b 6.01	7.72	0.75	0.85	3	22	4	1	0.833333	0.43	0.96	0.80
rHev b 6.02	7.72	0.67	0.88	4	21	3	2	0.833333	0.57	0.91	0.77
rHev b 8 Profilin	1.24	0.91	0.67	10	10	5	1	0.769231	0.67	0.91	0.79
rHev b 11	11.33	1.00	0.81	1	17	4	0	0.818182	0.20	1	0.90
At least one	1.46	0.82	0.81	13	14	3	3	0.818182	0.82	0.81	0.82

Table A.4

ROC analysis to determine k82 (Latex) cutoffs considering data from the Milan area only. rHev b 11 not used in Milan. TP: true positives, TN: true negatives, FP: false positives, FN: false negatives, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve.

ROC MI	cutoff	Sensitivity	Specificity	TP	TN	FP	FN	Accuracy	PPV	NPV	AUC
rHev b 1	7.7	0.50	0.87	1	41	6	1	0.857143	0.14	0.98	0.69
rHev b 5	0.60	1.00	0.44	4	20	25	0	0.489796	0.14	1.00	0.72
rHev b 6.01	1.41	0.50	0.81	3	22	5	3	0.757576	0.37	0.88	0.66
rHev b 6.02	1.15	0.50	0.58	3	7	5	3	0.555556	0.38	0.70	0.54
rHev b 8 Profilin	0.78	0.45	0.69	10	11	5	12	0.552632	0.67	0.48	0.57
At least one	1.03	0.44	0.68	19	15	9	19	0.548387	0.63	0.50	0.56

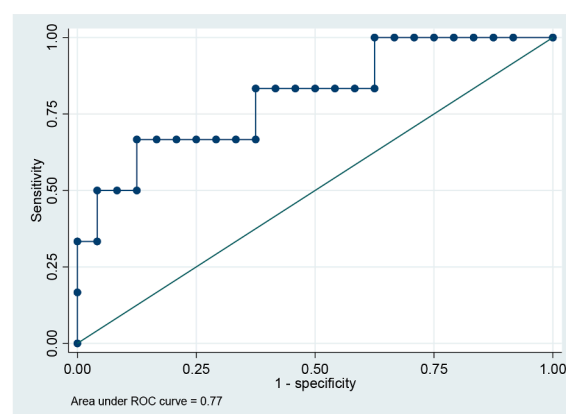
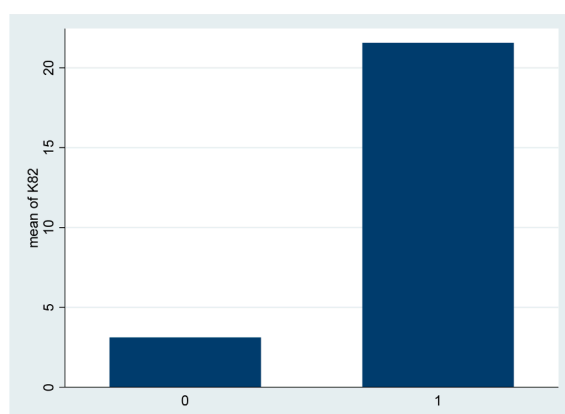


Fig. 1. Average specific IgE for rHev b 6.02 (0) = 3.11 KUA/l and specific IgE for rHev b 6.02 (1) = 21.46 KUA/l. Significant difference $p = 0.0016$ was found for Pavia population. ROC analysis shows sensitivity and specificity of specific IgE Test for rHev b 6.02 and identifies the correct in vitro diagnosis of Pavia population AUC = 0.77.

We also run a ROC analysis considering a case as positive if at least one of the single proteins was positive. In this case, we obtained a PPV of 0.72.

4. Conclusion

Therefore, we can say that the pool of patients tested for the major latex recombinants are allergic subjects with pollinosis and Oral Allergic Syndrome (OAS) after fruit consumption, oculorhinitis, contact

urticaria, edema of the glottis after use of latex gloves (Parisi et al., 2021; Ansotegui et al., 2020).

It is essential to evaluate latex-specific IgE and IgEs for its main proteins. The evaluation of single molecular components is needed for appropriate diagnosis (Nucera et al., 2020).

Our results show that the use of the model of sensitization to latex proteins plays an essential role in highlighting the cross-reaction towards food. The best PPV is obtained for rHev b 6.02 (k220), rHev b 8 profilin, which are the components associated to sensitizations in

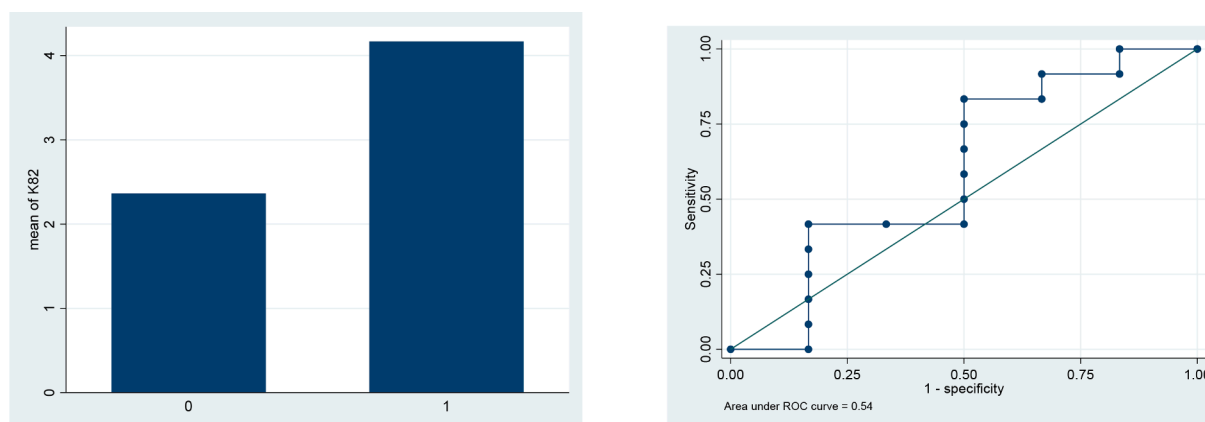


Fig. 2. Average specific IgE for rHev b 6.02 (0) = 2,36 KUA/l and specific IgE for rHev b 6.02 (1) = 4,17 KUA/l. No Significant difference $p = 0.49$ was found for Milan population. ROC analysis shows sensitivity and specificity of specific IgE Test for rHev b 6.02 and identifies the correct in vitro diagnosis of Milan population $AUC = 0.54$.

patients who frequently present a cross-reaction to inhalants and food. Therefore, an accurate diagnosis requires the performance of specific in vitro tests as well (Wu et al., 2016).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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