

CHALLENGE TESTS IN THE DIAGNOSIS OF LATEX ALLERGY

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The aim of this study is to evaluate the sensitivity, specificity and safety of challenge tests and their usefulness in the diagnosis of latex allergy. Forty adult subjects (F/M = 34/6, aged 18-66 yrs) with a history of adverse reactions after latex exposure and positive prick test and/or specific IgE to latex were enrolled. They were compared with 20 control subjects. They underwent provocative (cutaneous, mucous-oral, sublingual, conjunctival, nasal, bronchial, vaginal) tests. Symptoms and drug scores were recorded for each patient during challenges. All patients reacted to at least one of the following: cutaneous, nasal and conjunctival tests. No systemic reactions requiring epinephrine occurred. Of the challenges, the vaginal test resulted as the safest, but it had low sensitivity and many limits related to the procedure. According to our data, bronchial and nasal tests had the highest sensitivity (76% and 82% respectively), and were more precise than other tests in determining latex exposure and symptoms, but the bronchial test also presented the highest rate of risk. Mucous and cutaneous tests resulted as the most reliable. For all the tests, specificity and positive predictive value were 100%. All control subjects resulted negative to all challenges. There were no statistically significant changes in skin and serologic tests between the first and second visits. Correlations between MIS and skin tests and between MIS and serum tests were not found. Challenges can be considered safe diagnostic procedures. Tests that most faithfully reproduce natural exposure, on the basis of a patient's history, are preferable.

Allergic hypersensitivity to natural rubber latex (NRL) has become an important problem of public health in recent years. Symptoms of NRL allergy (ranging from contact urticaria and asthma, to systemic anaphylaxis) are elicited by direct contact with NRL items (i.e. medical devices) or by inhalation of latex airborne proteins. Diagnosis is formulated on the basis of the personal history and

an accurate allergological evaluation. Nevertheless, patient personal history can not be considered a good diagnostic tool because of its extremely low sensitivity: in fact, it has been reported as failing to identify about 50% of patients found to be symptomatic in a latex provocation test (1-2). The most common diagnostic tools are skin prick and serologic tests for the identification of NRL-

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specific IgE-mediated reactions: these tests vary in sensitivity and specificity (3-8). If the clinical history and skin and blood tests are discordant, provocative procedures are particularly important to verify the latex allergic status. Furthermore, challenges may uncover non-IgE-mediated sensitization to latex.

Several methods of performing challenge tests have been reported. The glove-use test (cutaneous challenge) was performed by donning a latex glove for 15 minutes and recording the symptom score after an additional 15 minutes. Other investigators asked patients to wear a latex glove finger and then the whole glove on a wet hand for an additional 15 min (9). Palczynski et al. performed a nasal provocation test instilling an NRL solution (prepared by washing pieces of NRL gloves of different brands) into the nostrils for 5 min (10-11). The conjunctiva challenge test was performed by instilling one drop of latex solution into the inferior fornix, starting with the highest dilution and progressively increasing the concentration every 15 min, to reach the threshold dose (12). Also for the bronchial test many methods are available, the newest being the Hooded exposure chamber system procedure, which monitors respiratory parameters before and after exposure to a cloud of latex-associated cornstarch particles (13-14). Acero et al. performed a bronchial provocation with aerosolized particles of latex extract, generated by a continuously pressurized nebulizer (15). Other reports are available on provocation by inhalation, such as the procedure of Vandenplas et al. (16). In order to improve its sensitivity, Hamilton et al. employed a 2-stage glove provocation test: patients had to blow up a powdered latex glove and gradually expel the air into their face while inhaling (17). The aim of this study is to evaluate the sensitivity, specificity and safety of challenge tests, and their usefulness in the diagnosis of latex allergy.

MATERIALS AND METHODS

Forty adult subjects (female-male ratio = 34/6), aged 18-66 yrs (mean $\pm$ SD = 35.6 $\pm$ 10.5 yrs), were enrolled in the study. They all had a personal history of reactions (52 episodes reported) consistent with latex allergy and positive prick test and/or specific IgE to latex. Thirty-eight patients (95%) had muco-cutaneous symptoms

(generalized urticaria-angioedema or itching erythema in 16 patients); 22 patients (55%) had respiratory symptoms (bronchial asthma in 15 patients). No episodes of anaphylactic shock were reported (details in Table I).

Of 25 patients occupationally exposed to latex, 20 reported the first allergic reaction in their work environment (13 of them were healthcare workers): 18 manifested cutaneous symptoms (only local symptoms after glove use in 14 patients), 14 had respiratory symptoms (latex-related asthma in 10 patients). Fourteen patients manifested latex adverse reactions during surgical procedures (3 episodes), gynecological and dental examinations (6 and 9 episodes respectively). Ten atopic and ten non-atopic control subjects with no clinical history of latex allergy and negative latex skin and serologic tests were also studied. All patients underwent a second allergological evaluation 1 month after the last challenge to verify whether challenges may increase patients' reactivity to latex.

Skin tests

Skin prick and patch tests were carried out with a standard latex extract (500 μ g/ml; ALK Abellò, Madrid, Spain) and a 1-cm² piece of surgical glove latex material (prick-by-prick method: a puncture device is pushed into the skin through a piece of surgical latex glove manufactured by Triflex Allegiance Health Care Co., McGaw Park, IL). Prick tests were performed with a Morrow-Brown needle (ALK, Abellò) on the volar surface of the forearm. Negative (glycerine solution) and positive (10 mg/ml of histamine) controls were also performed. A positive response was defined as one producing a wheal greater than 3 mm in diameter 20 minutes after application, without a reaction to the negative control.

Patch tests were performed to exclude a type IV cell-mediated delayed hypersensitivity to latex: their results were checked 72 h after the patch had been placed, assessing positivity according to the North American Contact Dermatitis Group criteria: negative reaction (0); macular erythema (?); erythema, infiltration, and possibly papules (1+); erythematous papules and/or vesicles (2+); and spreading blisters and/or crust with ulceration (3+) (18).

Serologic tests

Serum levels of total and specific anti-NRL immunoglobulin IgE were measured by means of fluorescent enzyme immunoassay (UniCAP System; Pharmacia, Uppsala, Sweden). Samples with specific IgE values > 0.35 kU/L were regarded as positive. *In vitro* detection of serum specific anti-NRL IgG4 antibodies was obtained with a latex-specific fluorescent enzyme immunoassay (CAP-FEIA; Pharmacia). Serologic tests

were performed during the first and last visits.

Exclusion criteria

Exclusion criteria were pregnancy or lactation, regular use of asthma or allergy medications (antihistamines, steroids), autoimmune diseases, asthma or urticaria-angioedema exacerbation 24 h before the tests, the use of β -blocking drugs, any severe renal and cardiovascular diseases.

Challenge tests

Provocative procedures were performed under a day-hospital regimen in accordance with good clinical practice after being approved by our hospital's Ethics Review Board. Patients gave their fully-informed written consent. Resuscitative equipment and personnel were available. Antihistamines were withheld for 10 days before each challenge. Expiratory peak flow, pulse rate and blood pressure monitoring were carried out during each challenge. Patients remained under medical control for 2 hours after each challenge. Cutaneous, mucous, sublingual, conjunctiva and vaginal tests were considered positive if one or more symptoms occurred (local or generalized itching erythema, urticaria, angioedema, conjunctivitis, rhinitis, cough, dyspnea, asthma, etc). If patients experienced respiratory symptoms they were treated with an inhaled bronchodilator (salbutamol) and corticoid (beclometasone). If mucocutaneous symptoms occurred patients were administered antihistamines (ocular ketotifene, oral cetirizine or loratadine; im/iv clorfenamine), corticoids (ocular dexamethasone; nasal budesonide; oral/im/iv methylprednisolone) and im epinephrine, according to symptom severity. If symptoms were mild, no medication was administered.

For sublingual, conjunctival, nasal and bronchial tests, latex solutions at increasing concentrations were prepared with latex extract (500 $\mu\text{g/ml}$; Alk Abellò) diluted in saline.

Cutaneous test

Before the challenge, patients had to wash and perfectly dry their hands. Patients had to wear a latex glove (Allegiance Triflex) until symptoms appeared. The maximum exposure time was 1 h. As a control they had to wear a synthetic (vinyl) glove on the opposite hand. Then the gloves were taken off and their hands were washed again. A physician assessed possible clinical reactions. The test was considered positive if symptoms occurred without reactions to the control glove. Patients with active hand dermatitis at the time of the challenge were excluded. Patches were applied to cover any cutaneous lesion to avoid an abnormal entrance of latex proteins through the skin.

Mucous (oral) test

The mucous oral test was carried out by asking the patients to hold a latex-gloved test tube in their mouths for 1 h or until the occurrence of symptoms. A preliminary control test with a vinyl glove was performed. Patients with oral lesions at the time of the challenge and with a positive control test were excluded.

Sublingual test

One drop of latex solution (from the smallest concentration of $500 \times 10^{-8} \mu\text{g/ml}$ to the largest of 500 $\mu\text{g/ml}$) was put under the tongue every 20 minutes. The threshold dose was defined as the smallest allergen concentration able to induce the appearance of symptoms. A preliminary control test with a placebo (saline) was performed. Patients with oral lesions at the time of the challenge and with a positive control test were excluded.

Conjunctival test

One drop of latex solution was instilled every 20 minutes into the inferior fornix, starting with the smallest concentration of $500 \times 10^{-8} \mu\text{g/ml}$ to the largest of 50 $\mu\text{g/ml}$, until the occurrence of symptoms. A control test was performed with saline in the opposite eye. Patients with active conjunctivitis at the time of the challenge and with a positive control test were excluded.

Nasal test

The percent decrease of nasal airflow was measured (by anterior rhino-manometry, Rhinospir 164, Sibelman) 20 min after the inhalation of increasing doses of latex solution (from $500 \times 10^{-4} \mu\text{g/ml}$ to 500 $\mu\text{g/ml}$). The intranasal insufflation of latex solution was performed using a dosimeter (Mefar), calibrated ($1.5 \text{ Kg/cm}^2 \cdot 0.4 \text{ s}$) and connected to an ampulla with olive-shaped nasal adapters. If the airflow decreased by 40% the test was assessed as positive. If the response was negative, the larger concentration was administered. We excluded patients whose baseline nasal airflow was less than 150 cm^3/s . The nostril with the higher baseline airflow was challenged. Before the challenge patients underwent a control test with physiologic saline. Patients with a positive control test were excluded.

Bronchial test

The variation of respiratory parameters (FEV_1 , PEF) was measured after the inhalation of latex solution, administered with Mefar dosimeter. Patients inhaled 10 μg of solution in each breath, starting with the smallest concentration of $500 \times 10^{-8} \mu\text{g/ml}$, and progressively decreasing the dilution, every 20 minutes if the test was negative, until the largest concentration of $500 \times 10^{-1} \mu\text{g/ml}$ was reached. The test was considered positive if

the respiratory parameters decreased by 20%. When the test was assessed as positive, the patient was administered an inhaled bronchodilator (salbutamol 200 µg) and re-evaluated after 20 minutes. We excluded patients whose baseline FEV1 was less than 80% of the predictive value. Patients were instructed to avoid inhaled bronchodilators beginning 12 hours before challenge. Patients underwent a preliminary control test by inhaling physiologic saline: those with a positive test were excluded. Patients were monitored for any late asthmatic response: auscultation and measurement of expiratory peak flow were recorded every hour for 6 hours.

Vaginal test

It was performed inserting a latex-covered hygienic tampon into the vagina until symptoms appeared (the maximum exposure time was 1 h). A gynaecological examination was performed before the challenge and when the tampon was taken out. A preliminary control test with a neoprene-gloved hygienic tampon was performed. Patients with active vaginitis at the moment of the challenge, with a positive control test or those who were pregnant were excluded.

Reactivity scoring during provocation tests

Symptom scores

Ocular (tearing, itching, edema, conjunctival edema), respiratory (rhinorrhea, nasal itching, nasal blockage, sneezing, wheezing, cough, dyspnea, laryngospasm) and muco-cutaneous symptoms (generalized pruritus, erythematous-papular rash, urticaria-angioedema, oral and pharyngeal itching) were recorded during challenges and scored on a 4-point scale: 0 (absent), 1 (mild), 2 (moderate), 3 (severe).

Drug scores

On the basis of drug consumption during challenges and within 2 hours after they had finished, for each patient, a score was recorded on a 5-point scale: 0 (no drugs), 1 (nasal/ocular steroid or antihistamine), 2 (inhaled bronchodilator and corticoid, oral antihistamine), 3 (intramuscular antihistamines, intramuscular and/or intravenous corticoids), 4 (subcutaneous epinephrine).

Mean individual score (MIS)

This is the mean value of the total score (symptom + drug score), recorded for each patient. In order to assess patient's reactivity, MIS was graded as following: 0-1: mild; 1-4: moderate; >4 severe.

Statistical analysis

Analyses were performed using the SPSS software package (release 13.0 for Windows, Chicago, USA).

Correlation of MIS with latex-specific IgE and the skin test result was calculated by a simple linear regression. Comparison of latex-specific IgE and the skin test result in patients with mild and moderate reactivity was evaluated by the Mann-Whitney test. A $p < 0.05$ was considered as significant.

RESULTS

Forty patients were diagnosed as suffering from IgE-mediated latex allergy (positive latex skin and/or serologic tests). Demographic data are reported in Table I. Skin and serologic test results were negative in all the control subjects. All patients had negative patch tests.

All patients reacted at least to one challenge and particularly to one of the following: cutaneous, nasal and conjunctival tests. Thirty-three patients (82%) reacted to at least three challenges (one patient reacted to all seven challenges). The cutaneous challenge was performed on all patients and was positive in 28 patients (70%), the most frequent symptom being local urticaria (79%). Four patients had respiratory symptoms, but only one patient presented a 20% decrease of PEF. Four patients (14%) manifested reactions requiring therapy: ocular antihistamine (2 patients), oral antihistamine (2 patients); inhaled bronchodilator and corticoid (1 patient). Two patients (7%) had an individual score > 4. No severe reactions occurred. No patients developed reactions on the opposite control hand. All the control subjects were negative.

The mucous (oral) challenge was carried out on 39 patients (1 patient was excluded because of active stomatitis) and was positive in 18 of them (46%). Seventeen patients (94%) presented muco-cutaneous symptoms (labial/oral edema, generalized itching erythema), one patient manifested respiratory symptoms with a decrease of respiratory parameters. Seven patients (39%) manifested reactions requiring therapy: oral antihistamine (6 patients); inhaled bronchodilator and corticoid (2 patients), intramuscular steroid (2 patients). Three patients (19%) had an individual score >4. No severe reactions occurred. All the control subjects were negative. No subjects developed reactions during the control test.

The sublingual challenge test was carried out on 40 patients and resulted positive in 17 patients

Table 1. *Patients' data.*

Patients (N°)	40
Sex (M/F)	34/6
Age (ys) (Mean $\pm$ SD)	18-66 (35.6 $\pm$ 10.5)
N° of patients (%) with personal history of - Atopic diseases - Asthma - Multiple operations - Professional exposure - Latex-fruit syndrome	15 10 2 25 23
Setting of allergic reactions (N° patients) - Work environment - Surgical/medical procedures - Home (latex gloves) - Condom use	20 14 4 2
Clinical features of latex allergy (N° patients) - Mucous-skin reactions - Local erythema-urticaria - Urticaria-angioedema - Eczema - Itching erythema - Respiratory symptoms - Asthma - Rhinoconjunctivitis - Anaphylactic shock	20 11 6 5 15 15 0
Patients with latex specific IgE > 0.35 kU/L	31
Mean (kU/L)	10.9
Patients with latex prick test > 3 mm	37
Mean wheal diameter (prick test, mm)	8

(42%). The most frequent symptom was oral itching with sublingual erythema (47%). Four patients had respiratory symptoms, with a 20% decrease of PEF in 2 cases. Five patients (29%) manifested reactions requiring therapy: oral antihistamine (3 patients); inhaled bronchodilator and corticoid (2 patients). Three patients (18%) had an individual score >4. No severe reactions occurred. Six patients reacted to a dose lower than $500 \times 10^{-3} \mu\text{g/mL}$ (one of them reacted after the first dose). All the control subjects were negative. No one developed reactions to the placebo.

The conjunctiva challenge test was carried out on 38 patients (1 patient excluded for active conjunctivitis, 1 patient for a positive control test with physiologic saline): it resulted positive in 28 patients (74%). The most frequent symptom was

conjunctivitis (75%). Four patients had respiratory symptoms, with a 20% decrease of PEF in 2 cases. Six patients (21%) manifested reactions requiring therapy: ocular antihistamine (4 patients), oral antihistamine (1 patient); inhaled bronchodilator and corticoid (2 patients), intramuscular steroid (1 patient). Two patients (7%) had an individual score >4. No severe reactions occurred. Six patients reacted to a dose lower than $500 \times 10^{-3} \mu\text{g/mL}$ (2 of them reacted after the first dose). All the control subjects were negative.

The nasal challenge was carried out on 33 patients (2 patients were excluded for active rhinitis, 5 patients for a positive control test with saline): 27 (82%) presented a reduction of nasal airflow higher than 40% and manifested respiratory symptoms: 3 of them presented a 20% reduction of PEF. Four patients

Table II. *Results of challenge tests.*

Patients	Sex/ Age	Grade	Cutaneous	Mucous (oral)	Sublingual	Conjunctival	Nasal	Bronchial	Vaginal	MIS
1.	F/40	3	+	-	-	+	+	+	+	1.29
2.	F/39	1	+	+	+	-	-	+	-	1.71
3.	F/46	1	+	+	+	+	+	+	-	1.43
4.	F/30	2	+	+	+	-	-	+	-	2.14
5.	F/25	1	+	-	-	-	-	+	-	0.50
6.	F/24	1	+	-	-	+	-	-	-	0.40
7.	F/28	2	+	+	-	+	+	+	+	2.57
8.	F/40	3	+	+	+	-	-	+	+	1.20
9.	M/49	3	+	-	-	+	-	+	-	1.00
10.	F/21	2	-	+	+	-	+	+	-	1.29
11.	F/42	1	-	-	+	+	-	+	+	0.83
12.	F/45	1	+	+	+	+	+	-	-	2.33
13.	F/54	2	-	-	-	+	+	-	+	0.67
14.	F/29	1	+	+	+	+	+	-	-	1.29
15.	F/48	1	+	+	+	+	-	-	-	2.20
16.	M/38	3	-	-	-	+	-	+	-	1.00
17.	M/31	2	+	-	+	+	+	-	-	1.00
18.	M/35	1	-	-	+	+	+	+	-	1.83
19.	F/30	2	+	+	-	+	+	+	+	1.86
20.	F/40	1	+	+	+	-	-	-	+	1.33
21.	F/18	3	-	+	-	+	+	+	-	1.67
22.	F/22	2	+	+	+	+	+	+	+	3.57
23.	F/54	2	+	+	-	+	+	+	+	2.71
24.	F/36	2	-	-	-	+	+	-	-	0.33
25.	F/66	3	-	+	-	+	+	-	+	1.00
26.	M/38	3	+	-	-	+	+	+	-	1.17
27.	F/36	3	+	+	-	-	+	-	-	1.00
28.	F/42	3	+	-	-	-	-	+	-	0.80
29.	F/31	3	+	-	+	+	+	+	-	1.57
30.	F/35	3	-	-	+	+	+	-	-	0.50
31.	F/40	3	+	-	-	+	+	+	-	1.86
32.	F/29	2	-	-	-	+	+	+	-	0.57
33.	F/23	1	+	-	-	+	-	+	+	0.83
34.	F/30	1	-	+	-	-	+	+	-	1.00
35.	F/39	2	+	-	+	+	+	+	+	2.43
36.	F/18	2	+	-	+	-	-	-	-	0.43
37.	M/40	1	+	-	-	-	+	+	-	0.67
38.	F/22	2	+	+	-	+	+	-	-	5.80
39.	F/28	3	+	-	-	+	+	-	+	0.86
40.	F/41	3	-	-	-	-	+	-	-	0.14

(15%) manifested reactions requiring therapy: oral antihistamine (3 patients); inhaled bronchodilator and corticoid (4 patients). Three (11%) had an individual score >4 . No severe reactions occurred. Two patients reacted to a dose lower than $500 \times 10^{-3} \mu\text{g/mL}$ (none after the first dose). All the control subjects were negative.

Bronchial test was carried out on 33 patients (2 were excluded because of regular asthma treatment, 2 because on therapy with antihistamines, 3 for a positive control test).

Twenty-five patients (76%) resulted positive:

they presented a decrease of respiratory parameters of at least 20% after the inhalation of latex. Seven patients (21%) reacted to a dose lower than $500 \times 10^{-3} \mu\text{g/mL}$ (three after the first dose). All the controls were negative. In 12 patients (36%) therapy was required: oral antihistamine (1 patient); inhaled bronchodilator and corticoid (12 patients). One patient had an individual score >4 . Late phase reactions were not reported. The test was positive in 16 patients (out of 18 tested, 89%) with professional exposure to latex.

The vaginal test was carried out on 30 patients:

Table III. Challenge tests: statistical parameters.

	Cutaneous	Mucous (oral)	Sublingual	Conjunctival	Nasal	Bronchial	Vaginal
Performed	40	39	40	38	33	33	30
Positive	28	18	17	28	27	25	13
Sensitivity (IC)	70% (56-84)	46% (31-61)	42% (27-57)	74% (60-88)	82% (69-95)	76% (62-90)	43% (25-61)
Specificity (IC)	100% (89-100)	100% (87-100)	100% (90-100)	100% (86-100)	100% (87-100)	100% (84-100)	100% (86-100)
Positive predictive value (IC)	100% (84-100)	100% (71-100)	100% (79-100)	100% (78-100)	100% (75-100)	100% (84-100)	100% (59-100)
Negative predictive Value (IC)	63% (49-77)	48% (33-63)	45% (30-60)	65% (51-79)	75% (62-88)	70% (56-84)	48% (30-66)
Patients requiring treatment	4	7	5	6	4	12	1
Patients requiring epinephrine	-	-	-	-	-	-	-
Total score	54	57	45	61	55	70	17
Rate of risk	5.4	10.22	5.65	9.66	6.68	25.44	0.57

IC: Interval of Confidence

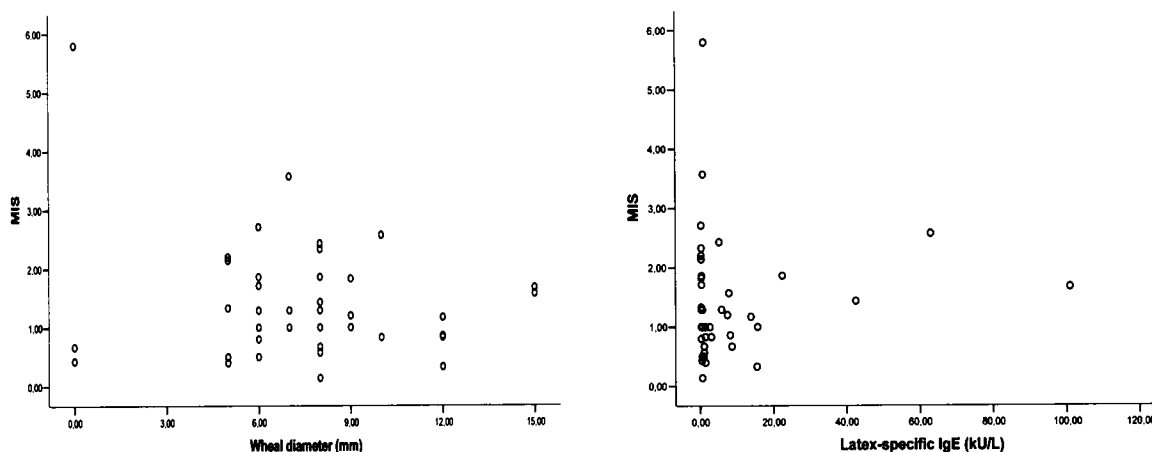
Sensitivity: proportion of true positive results among symptomatic individuals

Specificity: proportion of true negative results among non-symptomatic individuals

Positive predictive value: proportion of symptomatic individuals among those with positive test results

Negative predictive value: proportion of non-symptomatic individuals among those with negative test results

Rate of risk: total score $\times$ proportion of individuals requiring therapy

**Fig. 1.** Correlation of MIS with latex-specific IgE and with results of skin tests.

6 men (obviously not suitable for the test), 1 woman with a positive control test and 1 woman with vaginitis were excluded; 2 patients refused the test. The test resulted positive in 13 (43%); they all manifested local symptoms (erythema and/or itching). One patient (3%) had generalized urticaria which required therapy (oral antihistamine). All the control subjects were negative. It was positive in

66% patients who reported allergic reactions during gynecological examination with latex gloves.

Data on sensitivity, specificity, positive and negative predictive values, symptoms score and rate of risk are reported in Table III. No systemic reactions requiring epinephrine occurred.

The correlation of MIS with latex specific IgE ($r=0.091$, $r^2=0.008$) and with skin test results

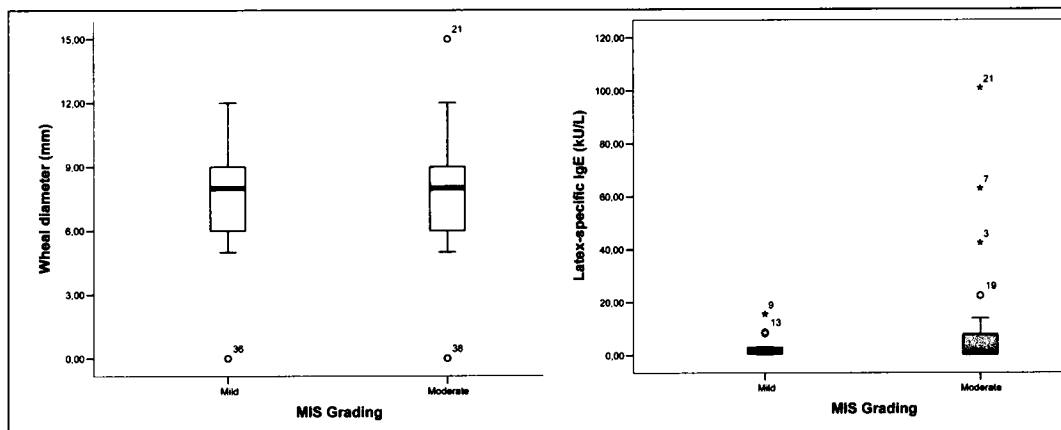


Fig. 2. Distribution according to latex-specific IgE and skin test results of patients with mild and moderate reactivity (MIS > 1 and MIS > 2 respectively).

($r=0.203$, $r^2=0.041$) was not significant (Fig. 1). There was no significant difference in terms of IgE value ($p=0.870$) and wheal diameter ($p=0.945$) between patients with mild and moderate reactivity during challenge tests. There were no statistically significant changes in latex skin and serologic tests between the first and second visits.

DISCUSSION

Challenge tests are an important tool to confirm the diagnosis and evaluate the degree of sensitization of allergic patients. Nasal, conjunctiva and bronchial challenges are commonly employed to confirm an allergy to other allergens such as pollens or house dust mites (19-21). Such procedures are likewise employed in the diagnosis of latex allergy (22).

A large number of reports are available on the usefulness of the glove-use test in the evaluation of latex-allergic patients (9, 16-17). Some authors consider it relatively safe and conclusive only in patients with cutaneous symptoms. However, some authors have shown, by a mouse model, how the cutaneous latex exposure may induce a Th2 inflammation in the lung and a systemic IgE response (23). According to our experience, also patients reporting latex-related respiratory symptoms reacted during the cutaneous challenge, presenting both respiratory and cutaneous symptoms. For these reasons, the cutaneous challenge can be considered

a valid diagnostic tool in the evaluation of latex allergy, even for patients reporting only respiratory symptoms.

The cutaneous test showed a medium rate of risk despite a low sensitivity: it resulted positive in 70% of our patients but 4 of them manifested reactions requiring therapy. These results partially confirm data from literature, which report the glove test as minimally sensitive, but not those presenting the cutaneous test as absolutely safe (1, 9). According to our data, challenges may be considered safe diagnostic procedures. In fact, all patients presented mild symptoms; severe anaphylactic reactions did not occur. Furthermore, provocative procedures did not induce sensitization in control patients, even those with atopy, and did not increase allergic patients' reactivity.

Among the challenges, the vaginal test was the safest, but it had a low sensitivity and many limits related to the procedure, which requires a gynecologist and can only be performed on adult women able to comply. Its use could be limited to specific cases (i.e. patients with a history of allergic gynecological reactions).

The mucous test had a similar low sensitivity but, even if easily feasible, it had a high rate of risk. It could be performed on patients reporting allergy after latex exposure of the oral mucosa. According to our data, bronchial and nasal tests had the highest sensitivity (76% and 82% respectively), but the

bronchial test also presented the highest rate of risk. The conjunctival test had a good sensitivity (74%).

As for reproducibility, mucous and cutaneous tests resulted as the most easily reliable, because latex gloves are easily available. Also sublingual and conjunctival tests were easily carried out but they required latex extract.

For all the tests, specificity and positive predictive value were 100%, because false positive tests did not occur in the control group. These data need further studies on a larger group of individuals to be confirmed.

The sublingual challenge seems particularly suitable as a preliminary test for patients selected for sublingual desensitization to evaluate the specific reactivity to treatment, especially when a rush induction is used. The nasal challenge is strongly recommended when a bronchial test can not be performed (for example when patients can not withhold inhaled bronchodilators and corticoids). However, in the evaluation of the performances of different challenge procedures it could be useful to establish a gold standard according to clinical history (i.e. bronchial challenge test in asthma, nasal challenge test in rhinitis, etc.) and refer sensitivity and specificity of other challenges to it. Unfortunately, because of the small number of patients, a history-based subgroup analysis was not performed for the low power of the study.

In our study, we failed to find a correlation between MIS and skin and serum tests. These data further confirm provocation tests as mandatory. In fact, a positive skin test or serum levels of specific IgE >0.35 kU/L can not be predictive of clinical reactivity to latex: sensitization, not allergy, is assessed. Diagnostic decision points were not found for identifying clinically allergic patients without provocation tests (Figs. 1, 2). In order to improve sensitivity, at least the following should be performed: cutaneous, nasal and conjunctival tests. Besides their diagnostic role, provocation tests seem particularly useful to follow the natural history of latex allergy and to assess the efficacy of specific desensitizing immunotherapy (24-28).

REFERENCES

1. 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; 102:665-70.
2. Liebke C, Niggemann B, Whan U. Sensitivity and allergy to latex in atopic and non-atopic children. *Pediatr Allergy Immunol* 1996; 7:103-7.
3. Nieto A, Mazón A, Estornell F, Reig C, García-Ibarra F. The search of latex sensitization in spina bifida: diagnostic approach. *Clin Exp Allergy* 2000; 30:264-9.
4. Grüber C, Buck D, Wahn U, Niggemann B. 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. *Allergy* 2000; 55:476-83.
5. Hamilton RG, Adkinson NF. Natural rubber latex diagnostic skin testing reagents. Comparative performance on non-ammoniated latex, ammoniated latex, and latex rubber glove extracts. *J Allergy Clin Immunol* 1996; 98:872-83.
6. Hamilton RG, Charous BL, Adkinson NF, Yunginger JW. Serological methods in the laboratory diagnosis of latex rubber allergy: study of non-ammoniated latex, ammoniated latex, and glove extracts as allergen reagent sources. *J Lab Clin Med* 1994; 123: 594-604.
7. Ownby DR, Ownby HE, McCullough J, Shafer AW. The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors. *J Allergy Clin Immunol* 1996; 97:1188-92.
8. Blanco C, Carillo T, Ortega N, Alvarez M, Dominguez C, Castillo R. Comparison of skin-prick test and specific IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy* 1998; 28:971-6.
9. Hamilton RG, Adkinson NF. Validation of the latex glove provocation procedure in latex-allergic subjects. *Ann Allergy Asthma Immunol* 1997; 79: 266-272.
10. Palczynski C, Walusiak J, Ruta U, Gorski P. Nasal provocation test in the diagnosis of natural rubber latex allergy. *Allergy* 2000; 55:34-41.
11. Bernardini R, Pucci N, Rossi ME, et al. Allergen specific nasal challenge to latex in children with latex allergy: clinical and immunological evaluation. *Int J Immunopathol Pharmacol* 2008; 21:333-41.
12. Leynadier F, Herman D, Vervloet D, Andre C.

1. Niggemann B, Buck D, Michael T, Wahn U. Latex

- Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. *J Allergy Clin Immunol* 2000; 106:585-90.
13. Kurtz KM, Hamilton RG, Schaefer JA, Adkinson NF Jr. A hooded exposure chamber method for semiquantitative latex aeroallergen challenge. *J Allergy Clin Immunol* 2001; 107:178-84.
 14. Kurtz KM, Hamilton RG, Schaefer JA, Adkinson NF Jr. Repeated latex aeroallergen challenges employing a hooded exposure chamber: safety and reproducibility. *Allergy* 2001; 56:857-61.
 15. Acero S, Alvarez MJ, Garcia BE, Echechipía S, Olaguibel JM, Tabar AI. Occupational asthma from natural rubber latex. Specific inhalation challenge test and evolution. *J Invest Allergol Clin Immunol* 2003; 13:155-61.
 16. Vandenplas O, Delwiche JP, Evrard G, Aimont P, van der Brempt X, Jamart J, Delaunois L. Prevalence of occupational asthma due to latex among hospital personnel. *Am J Respir Crit Care Med* 1995; 151:54-60.
 17. Hamilton RG, Adkinson NF Jr and the Multicentre Latex Skin Testing Study Task Force. Diagnosis of natural rubber latex allergy: multicentre latex skin testing efficacy study. *J Allergy Clin Immunol* 1998; 23:482-90.
 18. Marks JG, Belsito DV, DeLeo VA, et al. North American Contact Dermatitis Group patch results for the detection of delayed type hypersensitivity to topical allergens. *J Am Acad Dermatol* 1998; 38:911-8.
 19. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge: a clinical approach to studying allergic conjunctivitis. *Arch Ophthalmol* 1990; 108:84-8.
 20. Tan RA, Spector SL. Provocation studies in the diagnosis of occupational asthma. *Immunol Allergy Clin North Am* 2003; 23:251-67.
 21. Lopuhaa CE, de Riemer MJ, Out TA, Sjamsoedin DH, Aalberse RC, Jansen HM, van der Zee JS. Comparison of allergen-induced late inflammatory reactions in the nose and in the skin in house dust mite-allergic patients with or without asthma. *Int Arch Allergy Immunol* 2003; 130:266-74.
 22. Kurtz KM, Hamilton RG, Adkinson NF Jr. Role and application of provocation in the diagnosis of occupational latex allergy. *Ann Allergy Asthma Immunol* 1999; 83:634-9.
 23. Lehto M, Haapakoski R, Wolff H, et al. Cutaneous, but not airway, latex exposure induces allergic lung inflammation and airway hyperreactivity in mice. *J Invest Dermatol* 2005; 125:962-8.
 24. Patriarca G, Nucera E, Buonomo A, Del Ninno M, Roncallo C, Pollastrini E, De Pasquale T, Milani A, Schiavino D. Latex allergy desensitization by exposure protocol: five case reports. *Anesth Analg* 2002; 94:754-8.
 25. Nucera E, Schiavino D, Buonomo A, Roncallo C, Del Ninno M, Milani A, Pollastrini E, Patriarca G. Latex rush desensitization. *Allergy* 2001; 56:86-87.
 26. Patriarca G, Nucera E, Pollastrini E, Roncallo C, Buonomo A, Bartolozzi F, De Pasquale T, Gasbarrini G, Schiavino D. Sublingual desensitization: a new approach to latex allergy problem. *Anesth Analg* 2002; 95:956-60.
 27. Patriarca G, Nucera E, Buonomo A, Roncallo C, De Pasquale T, Pollastrini E, Schiavino D. New insights on latex allergy diagnosis and treatment. *J Invest Allergol Clin Immunol* 2002; 12:169-76.
 28. Nucera E, Schiavino D, Sabato V, Colagiovanni A, Pecora V, Rizzi A, Aruanno A, Milani M, Pollastrini E, Patriarca G. Sublingual immunotherapy for latex allergy: tolerability and safety profile of rush build-up phase. *Curr Med Res Opin* 2008; 24:1147-54.