

## Diurnal Variation of Nasal Nitric Oxide Levels in Healthy Subjects

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Palabras clave: Variación diurna. Óxido nítrico nasal.

Nasal nitric oxide (nNO) has been suggested as a valuable marker of upper airway inflammatory diseases, especially allergic rhinitis [1-3]. In order to interpret nNO values derived from single measurements, knowledge about a potential diurnal variation is mandatory. The existing data, however, do not yield a consistent picture [4-6]. The aim of this study was to assess the presence of diurnal variations of nNO levels in healthy subjects by closely spaced measurements.

Measurements were performed in 19 healthy nonsmokers (12 female and 7 male). nNO determinations were done individually at fixed time points within 6 consecutive 2-hour periods between 7 AM and 7 PM. nNO was assessed with a chemiluminescence analyser (NOA 280, Sievers, Boulder, Colorado, USA) using a nasal olive in 1 nostril, with the other nostril open and a suction flow of 240 mL/min. Velum closure was confirmed by oral exhalation against a resistance, and nNO was assessed when a plateau was reached. Measurements were made in triplicate for each nostril. In addition to paired *t* tests, repeated-measures analysis of variance (ANOVA) with Newman-Keuls post hoc comparisons was used for statistical analysis.

There were no differences between the values obtained for each nostril; thus, the mean of these values was used. Comparison of the data assessed during each time period in each subject revealed statistically significant changes over the course of the day (ANOVA,  $P < .0001$ ). The mean (SEM) values for the 6 measurements repeated at 2-hour intervals between 7 AM and 7 PM were 1505 (113), 1650 (105), 1736 (109), 1730 (105), 1740 (95), and 1670 (103) parts per billion (Figure). Post hoc comparisons

showed that the measurements made between 7 AM and 9 AM were significantly different from those of all other time periods ( $P < .01$ ), while according to pairwise *t* tests, measurements between 3 PM and 5 PM also differed significantly from those between 5 PM and 7 PM ( $P = .019$ ).

These data indicate that nNO levels change over the day in healthy subjects. We did not have the opportunity to measure nNO during the night, but a lower nNO output during this period was recently described [7]. An early study found no difference in nNO over the course of the day [4]. However, consistent with our results, a steady increase has been described when comparing measurements performed from 8 AM - 10 AM, 11:30 AM - 1:30 PM, and 3 PM - 5 PM [5], while another study found higher nNO between 1 PM and 3 PM compared to 7 AM - 9 AM [6]. Performing a larger number of measurements, we observed an increase of nNO, starting with low morning values, reaching a plateau during the day, and then decreasing between 5 PM and 7 PM.

Considering these findings alongside existing data [5-7], a diurnal cycle of nNO with a minimum during the night and a maximum during the day could be postulated. This underlines the need for standardization of measurement time in clinical studies. The neglect of diurnal variation might be one reason for the inconsistency of data in allergic rhinitis compared to healthy controls, as some studies showed elevated nNO in allergic rhinitis but others similar levels [1]. Further studies should assess diurnal variation in subjects with diseases

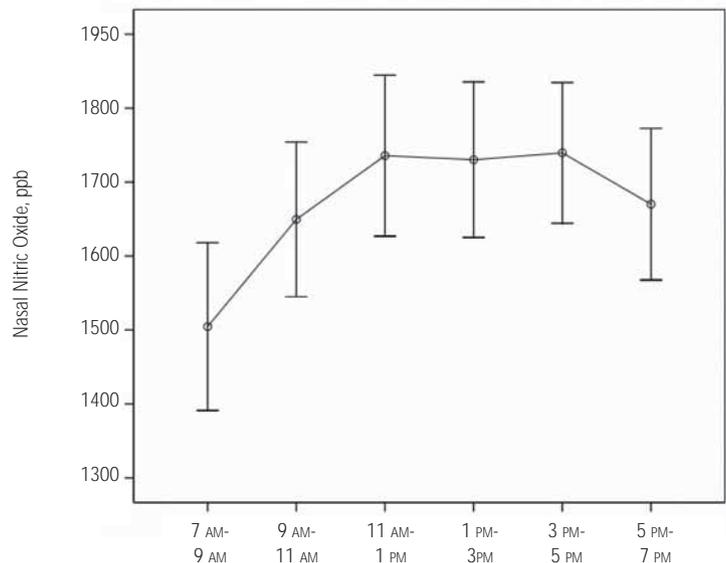


Figure. Diurnal variation of nasal nitric oxide in 19 healthy nonsmokers.  $P < .001$  by analysis of variance. Data are shown as means with SEM indicated by whiskers. ppb indicates parts per billion.

such as allergic rhinitis or with other possible interfering characteristics. Whether these subjects show diurnal variation or not, the presence of such changes in healthy subjects makes it recommendable to consider the time of measurement when making comparisons.

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## Late Patch Test Reaction to Dichlorophene

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Key words: Allergic contact dermatitis. Late reactions. Patch test. Dichlorophene.

Palabras clave: Dermatitis alérgica de contacto. Reacciones retardadas. Pruebas epicutáneas. Diclorofeno.

A 29-year-old white woman consulted for a 10-year history of intensely itching lesions on her face, hands, and feet. She also reported an erythematous reaction to adhesive plaster and itching after contact with cheap jewelry.

Examination revealed facial eczema, eczematous lesions with some fissures on the back of hands and in the interdigital spaces, and vesicular-bullous lesions on the dorsal aspect of the feet, in areas that usually come into contact with shoes.

We performed patch tests with leather-related materials and the standard hapten series recommended by SIDAPA (Italian Society of Allergologic, Professional, and Environmental Dermatology) using haptens from FIRMA (Florence, Italy) and Hayes Test Chambers (Hayes Service BV, Alphen, The Netherlands). At 48 hours, reactions to colophony (+++), nickel sulphate (++) and *p*-tert-butylphenol (++) were observed. At 72 hours, 2 more sensitizations—to Kathon CG (++) and pine oil (++)—were observed. All reactions persisted at 96 hours.

At day 10 after application, the patient complained of a new reaction to the patch test. The late-reacting substance was identified as dichlorophene G4 1%; retesting with this substance 2 months later reproduced the intensity (++) and late reaction of the first test.

Further investigation revealed that dichlorophene was used in the manufacturing cycle of the leather shoes worn by our patient. Use of differently manufactured leather shoes was recommended, and, after healing, no relapses were observed during a 6-month follow-up.

Dichlorophene is a halogenated phenol with bactericidal and fungicidal properties. It is used mainly as a component (and hapten) of cosmetics, although it is also used in the manufacturing process of several products, including leather. To the best of our knowledge, this is the first report of a late reaction to dichlorophene.

Late patch test reactions have been reported for several haptens and are not always easy to explain [1]. In the case of allergy to corticosteroids (the most frequent in clinical practice), it has been suggested that the pharmacologic properties of the molecules tested could delay the onset of the reaction in some subjects [2]. For other haptens, several studies have explained late patch test reactions as a result of active sensitization due to the test itself [3-4]. In our patient, this possibility was ruled out, because retesting also led to late reaction. In the case of active sensitization, the second test should react within the usual time frame of 48-96 hours, as suggested elsewhere [1].

The possible (and not mutually exclusive) causes of late patch test reaction are as follows: i) physical and chemical characteristics of some haptens that delay their absorption and/or binding to carrier proteins; ii) cutaneous features (pH, transepidermal water loss, sebaceous secretion) that affect the penetration of haptens; and iii) individual variability in the activity of enzymes involved in hapten metabolism and/or binding of haptens to carrier proteins.

Late patch test reactions are probably underestimated because physicians and patients are rarely aware that they occur. Further readings after 96 hours would be ideal to reveal such reactions; however, since this is not always possible, patients should be at least instructed to monitor new lesions in the area where patch tests have been applied and return for a follow-up visit should any appear.

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## Tolerability of Lumiracoxib in Patients With Analgesic Intolerance

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Key words: Lumiracoxib. Analgesic intolerance. COX-2 inhibitors. Oral challenges.

Palabras clave: Lumiracoxib. Intolerancia a analgésicos. Inhibidores de COX-2. Pruebas de provocación oral.

Analgesic intolerance is a clinical entity characterized by rhinitis, conjunctival irritation, bronchospasm, urticaria, and anaphylaxis. It usually occurs within 3 hours after the ingestion of acetylsalicylic acid and most nonsteroidal anti-inflammatory drugs (NSAIDs) [1].

The incidence of analgesic intolerance in the general population is nearly 1%, while it is 14% to 35% in patients with nasal polyps and chronic sinusitis, and 20% to 40% in those with chronic urticaria [2,3]. Although the exact mechanism has yet to be defined, inhibition of the cyclooxygenase (COX)-1

enzyme, which results in blocking of prostaglandin E<sub>2</sub>, seems to be responsible for these reactions [4].

Drugs such as celecoxib and rofecoxib, which selectively inhibit COX-2, have been shown to be safe in analgesic-intolerant patients [5,6].

We present the results of an oral challenge in 4 analgesic-intolerant patients using a novel selective COX-2 inhibitor, lumiracoxib (Prexige), which was marketed in June 2007 in Turkey, but withdrawn in August due to serious liver toxicity.

The study was prospective and started in June 2007, but enrollment was halted in August 2007 when the drug was withdrawn. A confirmed clinical history of 2 or more intolerance events with the same analgesic was required for diagnosis. If only 1 event had occurred, then single-blind and placebo-controlled oral challenges were performed to verify the diagnosis and to find safe alternative analgesics. All the oral challenges were started with lactose as the placebo. The drugs were applied at 30-min intervals at the following doses: 50 mg, 100 mg, 250 mg, and 500 mg for aspirin; 3 mg, 5 mg, 10 mg, and 20 mg for codeine; and a quarter, a half, three-quarters and all of the 7.5-mg, 50-mg, 100-mg, 100-mg, and 500-mg tablets of meloxicam, benzydamine, nimesulide, lumiracoxib, and paracetamol, respectively. Testing was stopped when a reaction developed, as described elsewhere [7], or when the highest test dose was reached. Patient characteristics and test results are summarized in the table.

Table. Patient Characteristics and Test Results

| Case                                   | 1  | 2                                   | 3                                      | 4                                       |
|--|--|-------------------------------------|--|---|
| Age/Gender                             | 49/Female  | 57/Female                           | 47/Female                              | 51/Female                               |
| Diagnosis                              | Chronic rhinosinusitis, recurrent angioedema, AI | Urticaria, recurrent angioedema, AI | Urticaria, recurrent angioedema, AI    | Asthma, chronic rhinosinusitis, AI      |
| Reactions according to history         | Aspirin<br>Naproxen<br>Flurbiprofen              | Flurbiprofen                        | Paracetamol<br>Ibuprofen<br>Diflunisal | Flurbiprofen<br>Metamizole              |
| Safe analgesics according to history   | Paracetamol                                      | Not known                           | Not known                              | Paracetamol                             |
| Positive reactions in oral challenges  | Aspirin  | Flurbiprofen                        | Meloxicam<br>Paracetamol<br>Nimesulide | Flurbiprofen<br>Nimesulide<br>Meloxicam |
| Negative reactions in oral challenges  | Nimesulide<br>Meloxicam                          | Paracetamol<br>Meloxicam            | Codeine                                | Benzydamine                             |
| Oral provocation test with lumiracoxib | Negative   | Negative                            | Negative                               | Negative                                |

Abbreviation: AI, analgesic intolerance.

NSAIDs manifest their effects through inhibition of COX-1 and COX-2 enzymes. Side effects of NSAIDs such as gastric damage and analgesic intolerance are related to the inhibition of COX-1 while anti-inflammatory, analgesic, and antipyretic actions are due to inhibition of COX-2 [1]. To minimize side effects, a new class of drugs that selectively inhibit COX-2 (including celecoxib, rofecoxib, and valdecoxib) was marketed.

Several authors have shown that selective COX-2 inhibitors can protect the gastric mucosa and are tolerated well by analgesic-intolerant patients [8]. Unfortunately, these drugs had to be withdrawn from the market due to an increased risk of coronary ischemia. However, their widespread use and success during their time on the market has led the pharmaceutical industry to develop newer and safer selective COX-2 inhibitors.

Towards the end of 2006, a new COX-2 inhibitor, lumiracoxib, was marketed for the first time in the United Kingdom and eventually in Turkey in June 2007. However, like some of its predecessors, it was withdrawn in August 2007 due to serious hepatic side effects, including 2 deaths from liver injury. Most patients were taking the 200-mg dose.

We were only able to perform 4 oral challenges with lumiracoxib before the drug was withdrawn, and we observed no reactions in any of the patients, although 2 had previously developed reactions with meloxicam and nimesulide, which are generally found to be safe in analgesic-intolerant patients.

This is the first report on the tolerability of lumiracoxib in analgesic-intolerant patients. If the drug is remarketed after withdrawal, as was the case of nimesulide, this data would be helpful in the management of patients with analgesic intolerance. It would be also be a useful addition to more comprehensive studies on this compound.

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## Allergen-Specific IgE to Inhalant and Food Allergens and Total IgE Values in China: Comparison of 2 Commercial Immunoassays

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Key words: Allergy. Immunoglobulin E. Pollen. House dust mites. Food allergy.

Palabras clave: Alergia. Inmunoglobulina E. Polen. Ácaros de polvo. Alergia alimentaria.

A characteristic feature of type I allergies is the involvement of allergen-specific immunoglobulin E (sIgE); thus, sIgE detection is an important tool in modern allergy diagnostics [1]. Historically, sIgE to various allergens was analyzed by radioallergosorbent test (RAST) using allergen-coupled cellulose paper discs [2,3]. Later on, the enzyme allersorbent test (EAST) and more recently the reverse enzyme allersorbent test (REAST) were used for sIgE detection [2,3]. The vast majority of today's test systems use allergens immobilized on a solid support such as cellulose discs or membranes, or so-called carrier polymer (CAP). The ALLERG-O-LIQ System (Dr. Fooke Laboratorien GmbH, Neuss, Germany) follows the REAST protocol using anti-IgE-coated microtiter plates and biotinylated allergens combined with streptavidin-horseradish peroxidase conjugate. State of the art allergy diagnosis includes detailed patient history, physical examination, skin prick testing (SPT), and in vitro tests for sIgE detection based on EAST or REAST protocols [2,3].

To compare the effectiveness of 2 commercially available immunoassays, serum samples were collected at Guangzhou Institute of Respiratory Disease based on the results of sIgE measurement by ImmunoCAP (Phadia, Upsalla, Sweden). Where possible, an equal number of positive and negative samples was included for each allergen. Samples were tested

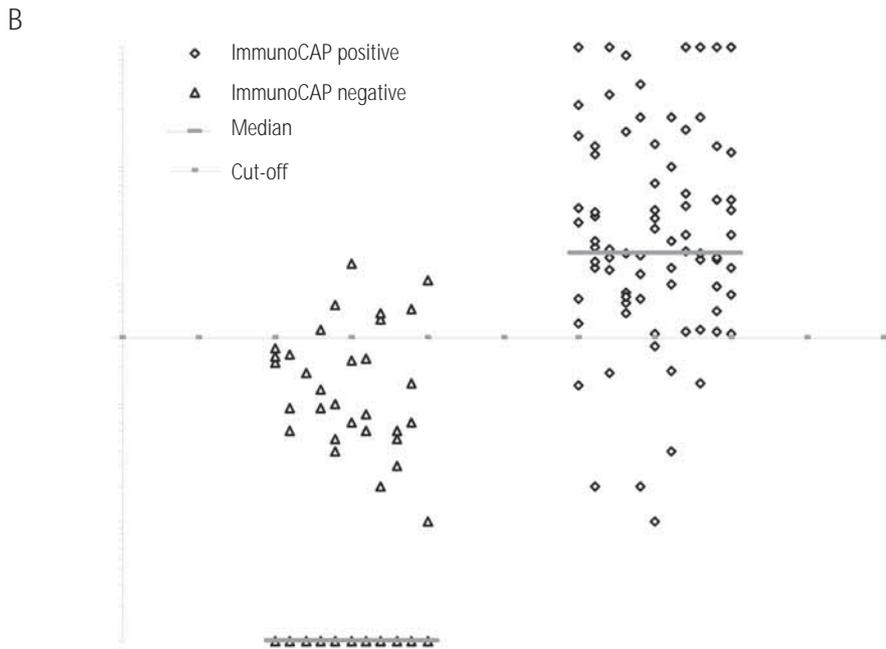
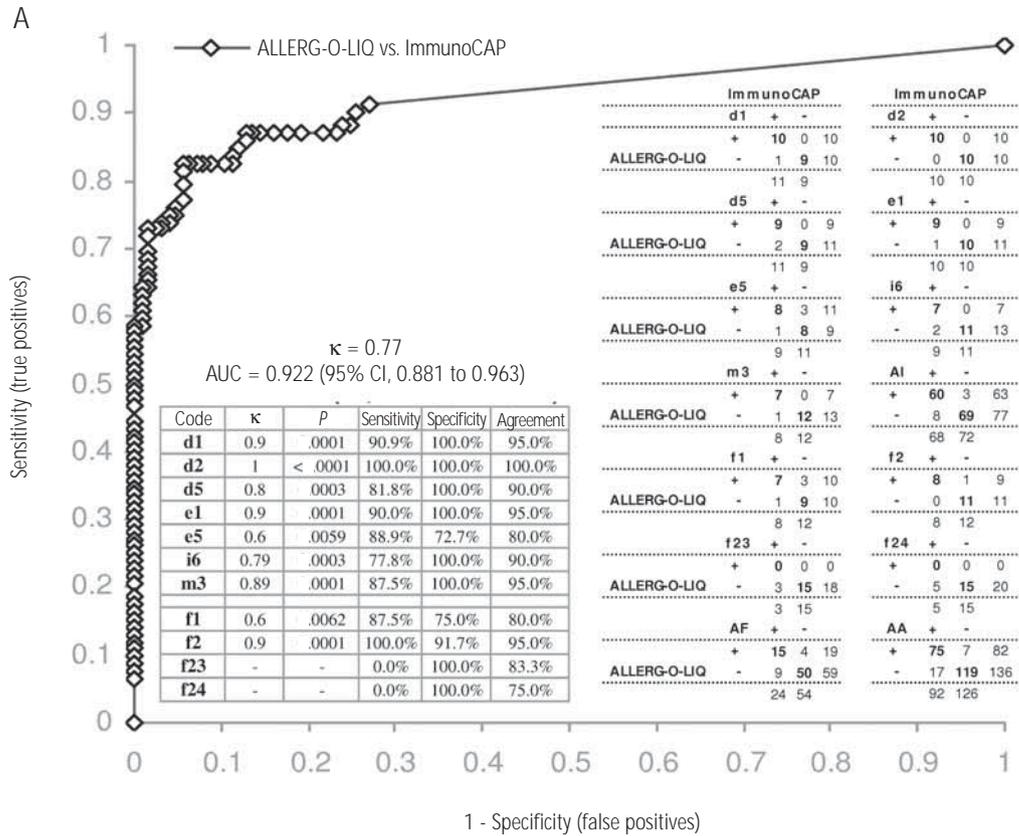


Figure. Receiver operating characteristic (ROC) analysis and comparative descriptive analysis of the specific immunoglobulin E (sIgE) results. ROC analysis (A) and comparative descriptive analysis (B) show good differentiation between ImmunoCAP-positive and negative samples using the sIgE test of ALLERG-O-LIQ as expressed by the area under the curve (AUC) of 0.922 (95% confidence interval, 0.881 - 0.963) and a qualitative kappa agreement value of 0.77, respectively. Qualitative comparisons are given for each allergen and in groups. In the comparative descriptive analysis (B), values below 0.001  $k_AU/L$  are shown as 0.001  $k_AU/L$  and values above 100  $k_AU/L$  as 100  $k_AU/L$ . Median values are indicated by horizontal lines. AI indicates all inhalant allergens; AF, all foods; AA, all allergens; CI, confidence interval.

for sIgE to 7 inhalant and 4 food allergens and total IgE by ALLERG-O-LIQ and ImmunoCAP. Results were statistically evaluated (Fisher exact test,  $\chi^2$  test, and kappa agreement) using the Microsoft Excel plug-in Analyse-it (Version 1.62).

The prevalence of positive test results ranged from 0/20 (f24) to 11/20 (e5) for ALLERG-O-LIQ and from 3/18 (f23) to 11/20 (d1/d5) for ImmunoCAP. The qualitative agreement between the 2 methods was between 75% (f24) and 100% (d2), depending on the allergen. The overall qualitative agreement between results for inhalant (n = 140), food (n = 78), and a combination of all allergens tested (n = 218) was 92.1% ( $\kappa = 0.84$ ), 83.3% ( $\kappa = 0.58$ ), and 89.0% ( $\kappa = 0.77$ ), respectively. Receiver operating characteristic and comparative descriptive analysis showed good discrimination (area under the curve, 0.922) between ImmunoCAP-positive and negative samples when using the results of sIgE testing with ALLERG-O-LIQ. The results including kappa agreement, *P* values, sensitivity, specificity, and agreement (%) are summarized in the Figure. The agreement between the total IgE results (n = 79) was found at *r* = 0.87 (*P* < .001, Pearson correlation coefficient). Mean and median values were 329.7 kU<sub>A</sub>/L/121.2 kU<sub>A</sub>/L and 570.8 kU<sub>A</sub>/L/137.0 kU<sub>A</sub>/L for ALLERG-O-LIQ and ImmunoCAP, respectively. Although no single method has been officially designated as the gold standard for IgE detection, the Pharmacia CAP System is in worldwide use and is a de facto standard against which other methods are compared [2,3]. Therefore, most studies that were designed to evaluate the accuracy of sIgE assays used the ImmunoCAP System as the reference method [4]. The ALLERG-O-LIQ and the ImmunoCAP System were first compared in 2004, and as in our study, the agreement between the methods was good to excellent, with higher concordance in the inhalant allergen than in the food allergen group [5]. Despite the fact that they are often promoted as tests for allergy diagnosis, sIgE immunoassays are best regarded as tests for the presence or absence of detectable sIgE. IgE is normally present in the serum, and sIgE can be found in patients with allergic diseases as well as in about 15% of asymptomatic healthy individuals [6-9]. Although the clinical background of sample donors in the present study was not available, based on the good to excellent agreement between ALLERG-O-LIQ and ImmunoCAP for IgE detection we conclude that the ALLERG-O-LIQ System represents a reliable test for quantitative IgE determination.

*The results of this study were presented as a poster at the World Allergy Congress; 2007 Dec 2-6; Bangkok, Thailand. M. Mahler acknowledges his work at Dr. Fooke Laboratorien GmbH, the company that developed the ALLERG-O-LIQ System, as a potential conflict of interest.*

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## Wasp Venom-Specific IgE: Towards a New Decision Threshold?

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Key words: CAP. Diagnosis. Immunoglobulin E. Wasp.

Palabras clave: Alergia. CAP. Diagnóstico. Inmunoglobulina E. Avispa.

Immunoglobulin (Ig) E-mediated hypersensitivity to Hymenoptera venom constitutes a potentially life-threatening condition. Venom-specific immunotherapy is highly effective

and can be life saving, but requires correct identification of the culprit venom [1]. Generally, physicians rely upon quantification of specific IgE (sIgE) antibodies and skin tests to confirm their clinical suspicion. Unfortunately, these tests lack absolute sensitivity and specificity, making the diagnosis of Hymenoptera venom allergy not always straightforward [2-4]. Historically, the decision threshold for sIgE has been arbitrarily set at 0.35 kU<sub>A</sub>/L, irrespective of the allergen. The objective of this study was to assess whether diagnosis of wasp (*Vespa vulgaris*) venom allergy could benefit from a low-level sIgE assay with a detection limit of 0.10 kU<sub>A</sub>/L.

Sera from 10 patients with a compelling history of wasp venom hypersensitivity and sIgE to wasp and honeybee (*Apis mellifera*) < 0.35 kU<sub>A</sub>/L by traditional fluorescent enzyme immunoassay (ImmunoCAP FEIA, Phadia AB, Brussels, Belgium) were re-examined using a low-level ImmunoCAP FEIA system with a detection limit of 0.10 kU<sub>A</sub>/L (ImmunoCAP FEIA, Phadia AB, Brussels, Belgium). Diagnosis of wasp venom allergy was confirmed with a positive basophil activation test (BAT) in all patients. Four patients had positive skin tests. Ten individuals who had received stings and were tolerant of wasp and honeybee venom and with negative BAT served as stung controls. A second control group (atopic controls) included 15 atopic patients tolerant of wasp and honeybee venom and with a total IgE (tIgE) > 500 kU<sub>A</sub>/L (range, 540-1185 kU<sub>A</sub>/L). In 12 atopic controls, sensitization to cross-reactive carbohydrate determinants (CCD) was investigated by means of sIgE to bromelain and MUXF3. Repeatability (within-run precision) was evaluated by 10 consecutive measurements of a pooled serum sample within a single run. Wasp venom sIgE results for patients and stung control individuals were compared by Kruskal-Wallis  $\chi^2$  test.

Within-run coefficients of variation were 4.70% for wasp (mean, 0.17 kU<sub>A</sub>/L) and 15.06% for honeybee (mean, 0.02 kU<sub>A</sub>/L) venom sIgE in the low-level range < 0.35 kU<sub>A</sub>/L. Individual results are shown in the Figure. Seven out of 10 patients had wasp venom sIgE between 0.10 and 0.35 kU<sub>A</sub>/L (median, 0.18 kU<sub>A</sub>/L). In contrast, all stung control individuals had wasp venom sIgE < 0.10 kU<sub>A</sub>/L ( $P = .002$ ). All patients and stung control individuals had honeybee venom sIgE < 0.10 kU<sub>A</sub>/L. In the atopic controls, sIgE ranged between < 0.10 and 4.45 kU<sub>A</sub>/L (median, 0.39 kU<sub>A</sub>/L) for wasp venom and between < 0.10 and 2.25 kU<sub>A</sub>/L (median, 0.11 kU<sub>A</sub>/L) for honeybee venom. In 4 and 2 out of 12 atopic controls sensitization to CCDs was confirmed by the presence of sIgE to bromelain and MUXF3, respectively.

This low-level sIgE technique with a detection limit of 0.10 kU<sub>A</sub>/L appeared useful to support the diagnosis of wasp venom allergy in a small group of otherwise seronegative (sIgE < 0.35 kU<sub>A</sub>/L) patients with an obvious history of wasp venom allergy. This increase in sensitivity did not encompass a change in specificity, given the sustained seronegativity (sIgE < 0.10 kU<sub>A</sub>/L) for honeybee venom in the patients group and sustained seronegativity for both wasp and honeybee venom in the stung control group. However, atopic individuals with a tIgE > 500 kU<sub>A</sub>/L exhibited elevated sIgE antibodies for both wasp and honeybee venom. This may partly reflect sensitization to CCDs rather than true sensitization to Hymenoptera venom [5] and has to be considered in the interpretation of sIgE results [6].

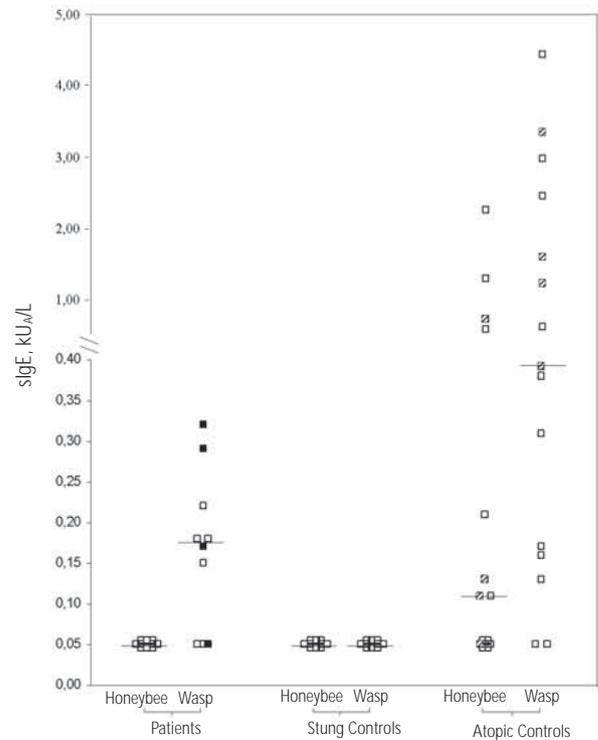


Figure. Specific immunoglobulin E (sIgE) results for wasp and honeybee venom in 10 patients with confirmed wasp venom allergy (patients), 10 wasp venom-tolerant individuals (stung controls), and 15 atopic individuals with total IgE levels > 500 kU<sub>A</sub>/L (atopic controls). Filled boxes indicate a positive skin test. Hatched boxes indicate presence of specific IgE to bromelain. Horizontal lines indicate median values.

These data support the suggestion that in vitro diagnosis of IgE-mediated wasp venom allergy could benefit from the recently developed low-level sIgE assays. However, additional studies with larger numbers of patients and control individuals are necessary to assess whether we can abandon the historical decision to use a threshold of 0.35 kU<sub>A</sub>/L and to evaluate whether these low-level techniques can be adopted in mainstream diagnostic use.

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