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*Review*

Treating moderate-to-severe allergic asthma with anti-IgE monoclonal antibody (omalizumab). An Update
G. D'Amato, M. Perticone, E. Bucchioni, A. Salzillo, M. D'Amato, G. Liccardi 135

*Original articles*

Cross reactivity between European Hornet and Yellow Jacket venoms
M.G. Severino, B. Caruso, P. Bonadonna, D. Labardi, D. Macchia, P. Campi, G. Passalacqua 141

GAPP Italy: "A survey on asthma on Italian physicians and patients"
F. Fumagalli, R. Baena-Cagnani, E. Compalati, F. Braido, G.W. Canonica, C.E. Baena-Cagnani 146

Anaphylaxis to apple: is fasting a risk factor for LTP-allergic patients?
A. Arena 155

*Case report*

Co-sensitisation (but co-recognition also) to novel banana and tomato allergen
R. Asero, G. Mistrello, S. Amato 159

*News* 163



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# Treating moderate-to-severe allergic asthma with anti-IgE monoclonal antibody (omalizumab). An Update

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**Key words**

*Allergic asthma, allergic respiratory diseases, anti-IgE therapy, Monoclonal anti-IgE antibody, Omalizumab, Therapy of asthma, airways hyperresponsiveness*

**Summary**

*Increased asthma severity is not only associated with enhanced recurrent hospitalisation and mortality but also with higher social costs. Most cases of asthma are atopic in nature, with the trigger for acute asthma attacks and chronic worsening of inflammation being allergens inducing an immune response through immunoglobulins of IgE class. Currently antiinflammatory treatments are effective for most of asthma patients, but there are subjects whose disease is incompletely controlled by inhaled or systemic corticosteroids and these patients account for about 50% of the healthcare costs of asthma. Omalizumab is a humanized recombinant monoclonal anti-IgE antibody developed for the treatment of allergic diseases and with clear efficacy in adolescent and adult patients with moderate-to-severe allergic asthma.. The anti-IgE antibody inhibits IgE functions blocking free serum IgE and inhibiting their binding to cellular receptors. By reducing serum IgE levels and IgE receptor expression on inflammatory cells in the context of allergic cascade, omalizumab represents a really new approach to the treatment of atopic asthma . Omalizumab improves quality of life of patients with severe persistent allergic asthma that is inadequately controlled by currently available asthma medications. This therapy is well tolerated and significantly improves symptoms, disease control, reducing asthma exacerbations and the need to use high dosage of inhaled corticosteroids. In other words, omalizumab may fulfil an important need in patients with moderate-to-severe asthma.*

## Introduction

Even though the pathogenesis of bronchial asthma is not completely understood, it is evident that this clinical condition has a multifactorial etiology and a body of evidence suggests that bronchial asthma has become more common worldwide in recent years and is recognized as a higly prevalent health problem in the developed and developing world (1-4). It is estimated that about two-thirds of asthma has an allergic background and about 50% of patients with severe asthma have allergic-atopic asthma (5), although many previously published data demonstrated that the disease is less frequent in atopic adult-onset asthma (6-8).
Allergic bronchial asthma is a T-helper 2-lymphocites (Th2) mediated chronic inflammatory disease of the airways and immunoglobulin E (IgE) antibodies, Th2 derived cytokines and eosinophils play a major role in the development of chronic airway inflammation, which is

observed even in subjects with very mild disease (9-11). Airway inflammation plays a central role in the pathogenesis of bronchial asthma and is associated with an increase in airway responsiveness to a several trigger factors such as aeroallergens which induce bronchoconstriction in atopic asthma patients.

The development of inflammation in asthma involves a complex array of several inflammatory mediators that promote the recruitment and activation of various different immune cells and regulate inflammatory cell trafficking into the lungs .

Activation of chemokine receptors triggers multiple cascades of intracellular signaling events that lead to recruitment and activation of immune effector cells. The inhibition of specific chemokines and receptors could prevent the excessive recruitment of leukocytes to sites of inflammation.

A number of selective chemokine receptor antagonists are currently at various stages of development for clinical use.

Elevated serum levels of specific IgE towards common environmental allergens are a key component in the pathogenesis of allergic asthma . IgE antibodies cause chronic airway inflammation through effector cells such as mastcells, basophils etc, activated via high-affinity (FcεRI) or low-affinity (FcεRII) IgE receptors.

There is also high association between serum IgE levels and FcεRI receptors on precursor dendritic cells, suggesting that IgE participates in the differentiation and activation of allergen-specific Th2 lymphocytes. The expression of these receptors on antigen presenting cells such, as dendritic cells, is increased in asthmatic patients (12).

Since the discovery of IgE antibody our knowledge of the mechanisms of allergy has improved to such an extent that now it is possible to modulate the IgE-mediated allergic response.

IgE antibodies have been viewed as a target for novel immunological drug development in asthma, and a number of strategies aimed at inhibiting its proinflammatory action despite an increase in recent years in the availability of drugs used for asthma therapy have been developed.

Current treatment for asthma suggested by Global Initiative for Asthma (GINA) guidelines includes several reliever and controller drugs, in particular corticosteroids which reduce recruitment and activation of inflammatory cells in the airways (13). The available anti-asthma treatments are effective for most of these patients. However, there are asthmatic subjects who continue to experience severe debilitating disease, since their broncoobstruction is incompletely controlled by inhaled or systemic corticosteroids associated with other drugs such as beta2bronchodilators (short and long-acting), antileucotrienes etc.

Several studies have indicated that increased asthma severity is not only associated with enhanced recurrent hospitalisation and mortality within 1 year of initial hospitalisation, but also with higher costs (14-16)

Therapeutic anti-IgE antibodies, omalizumab, able to reduce free IgE levels avoiding the binding of IgE to FcεRI without the following development of allergic reaction (crosslinking IgE and triggering degranulation and syntesis of new-generated chemical mediators of IgE-sensitized cells) have been developed (17-27). This non-anaphylactogenic anti-IgE monoclonal antibody (omalizumab) binds IgE at the same site of Fc fragment defined Cε3 domain as these antibodies bind FcεRI and FcεRII. Consequently, IgE effector functions are inhibited, because the IgE binding to high-affinity receptors on IgE effector cells is blocked, as well as the following activation of mast cells and basophils (28-35) (Table 1). In other words, in allergic subjects omalizumab prevents the acti-

***Table 1*** - Biological characteristics of omalizumab

- Omalizumab expresses a high degree of isotype specificity and can neutralize serum free IgE without affecting other antibody classes
- Omalizumab binds to serum free IgE and reduces IgE serum concentration, while do not binds to high- or low-affinity IgE receptors on inflammatory cells. However, it blocks IgE binding to these receptors and the IgE effector cells of inflammation are "disarmed".
- Long-term treatment with Omalizumab down-regulates the high-affinity receptors on basophils and dendritic cells.
- Omalizumab do not induces extensive immune complex formation.
- Omalizumab acitivity does not depend from the allergic sensitisation to various type of aeroallergens (seasonal, perennial) and is active in case of sensitisation to one or more allergens.

vation of cellular response and the occurrence of asthma symptoms.

Studies in patients with atopic asthma showed that anti-IgE antibodies decrease serum IgE levels in a dose-dependent manner and allergen-induced bronchoconstriction during both the early and late-phase responses to inhaled allergen (20, 21).

Serum free IgE are rapidly reduced after omalizumab administration and the expression of high-affinity receptors is significantly reduced after three months treatment (36). Also skin test reactivity is reduced by omalizumab (37). Nevertheless, when omalizumab was withdrawn after few months of therapy, the serum IgE levels returned to pretreatment values as well as the number of IgE receptors on the basophils surface (38). This structural "involution" reflects the trend of the symptoms related to the ashmatic disease, leading patients to increase the dosage of standard therapy. Nopp et al. investigated the long-term efficacy of 6-year-therapy with omalizumab in 18 patients with moderate-severe IgE-mediated asthma 1 year (39) and 3 years (40) after the withdrawal of omalizumab. In both cases the Authors documented the stabilization of the asthma-related symptoms, similar to that observed during the treatment period with omalizumab, as well as the downregulation of basophil allergen sensitivity.

In patients who experience asthma associated with allergic rhinitis there is an improvement also in nasal symptoms (41-45). The treatment with omalizumab should be potentiated by specific immunotherapy which is active by using other mechanisms (24).

In several clinical controlled trials omalizumab resulted to be able to reduce asthma-related symptoms, to decrease corticosteroid use and to improve quality of life of asthmatic patients (28-34). Recent studies show the benefits of anti-IgE as add-on therapy in patients with moderate and severe persistent asthma who are inadequately controlled by antiasthma pharmacological therapy. The anti-IgE approach to asthma treatment has several advantages, including concomitant treatment of other IgE-mediated diseases (allergic conjunctivitis and rhinitis, atopic dermatitis and food allergy) and a favorable side-effect profile regardless of the type of allergic sensitisation (seasonal or perennial) (28-34, 41-45). No anti-omalizumab antibody response has been observed in patients treated subcutaneously. Omalizumab was shown not only to inhibit mast cell and basophil responses but also to have inhibiting effect on the inflammatory cells, such as eosinophils, T lymphocytes and B lymphocytes which are fundamental to the chronic inflammatory response in allergic diseases such as asthma. This increased understanding places anti-IgE therapy firmly in the domain of an anti-inflammatory treatment for chronic allergic disease, with effect on multiple cell types. (Tab. 2).

Severe or refractory asthma remains a frustrating disease for both patients and the clinicians treating them (46, 47). Severe asthma has been defined as persisting symptoms due to asthma despite high-dose inhaled steroids (1000 mcg beclometasone dipropionate or equivalent) plus long-acting beta2agonist, with the requirement for either maintenance systemic steroids or at least two rescue courses of steroids over 12 months and despite trials of add-ons such as leukotriene-receptor antagonist or theophylline.

The Global Initiative for Asthma (GINA) guidelines for patients with severe persistent asthma (step 5 therapy) recommend the use of high-dose inhaled corticosteroids

***Table 1*** - Omalizumab in clinical studies in allergic asthma patients showed to be able

- To decrease IgE-induced bronchoconstriction during both the early and late-phase responses to inhaled allergen during the bronchial provocation tests.
- To reduce skin prick test response to allergenic extracts.
- To reduce asthma exacerbations regardless of the type of seasonal or perennial allergic sensitisation.
- To have a corticosteroid sparing effect.
- To reduce the use of bronchodilators.
- To improve also the nasal symptoms in subjects whit allergic rhinitis associated with asthma .
- To improve quality of life in patients with asthma, also in those with severe persistent allergic asthma that is inadequately controlled by currently available asthma medication.
- To have a reassuring safety profile similar to that of placebo. No anaphylactic reactions, nor any immune complex disease has been observed.

plus a long-acting beta2agonist (LABA), and, if required, one more additional controller. Currenlty several studies showed benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy.

The INNOVATE (INvestigatioN of Omalizumab in seVere Asthma TrEatment) study was specifically designed to evaluate the efficacy and safety of add-on therapy with omalizumab in this difficult asthma population (48).

In the INNOVATE trial were enrolled patients aged 12-75 years with severe persistent allergic asthma (GINA step 3 or 4 clinical features despite step 4 therapy).

The primary efficacy variable was the rate of clinically significant asthma exacerbations (defined as a worsening of asthma symptoms requiring treatment with systemic corticosteroids). A total of 419 patients were included in the efficacy analyses (omalizumab, n=209;placebo,n=210). The rate of clinically significant asthma exacerbations, after adjusting for an observed imbalance in asthma exacerbation history prior to randomization, was significantly reduced by 26.2% with omalizumab versus placebo (0.68 and 0.91, respectively; p=0.042).

Compared with placebo, treatment with omalizumab significantly reduced the rate of severe asthma exacerbations (0.24 vs 0.48, p=0.002) and the rate of total emergency visits for asthma (0.24 vs 0.43, p=0.038). Significantly greater improvements were achieved with omalizumab compared with placebo in AQLQ scores (overall and individual domains), with a significantly greater proportion of patients receiving omalizumab achieving a clinically meaningful (>0.5-point) improvement from baseline compared with placebo recipients (61% and 48%, respectively; p=0.008).

Recently several "real life" studies confirmed the efficacy and tolerability of omalizumab in severe persistent allergic asthma patients.

The first was an observational study performed in French (49). The second one was a prospective post-marketing surveillance trial that evaluated the efficacy and tolerability of omalizumab in real-life in Germany (50), and the third study was a prospective multicenter real-life study conducted in Belgium, the PERSIST study (51), the fourth a small questionnaire-based observational study in 65 patients in the UK, who had continued with omalizumab therapy beyond 16 weeks, conducted by Niven et al. (52).

In the first study, the authors evaluated 154 patients. The analysis performed during the treatment period and compared to the previous year, showed that patients with a follow-up of at least 5 months experienced 62% fewer exacerbations requiring oral corticosteroids, 65% fewer emergency department visits and 29% fewer hospitalisations per year. Korn and co-workers reported the results of the observation of 280 patients followed-up for 6 months. After 6 months of specific anti-IgE therapy, omalizumab was demonstrated to reduce the daily (–76%) and nocturnal symptoms (–84%), exacerbations (–82%), unscheduled medical assistance (–81%), hospitalizations (–78%) and increase quality of life (Mini-AQLQ: score increase from 2.9 to 4.5). Overall, efficacy of omalizumab was rated as excellent or good by the majority of physicians (82%) and patients (86%).

In the PERSIST Study Brusselle and co-workers evaluated the 15- and 52-week effectiveness of add-on omalizumab treatment in 158 enrolled subjects. After 16 weeks of therapy, a good/excellent GETE was achieved by > 82% (p<0.001), the total AQLQ scores improved in > 82% by > 0.5 points (p<0.001) and > 91% of the subjects were exacerbation -free. At 52 weeks, the same results were achieved by > 72% (p<0.001), > 84% (p<0.001) and > 65% (p<0.001), respectively. In addition, a significant reduction in healthcare utilization compared the year prior to treatment was observed.

Niven and coll. found that out of 33 patients taking oral corticosteroid at baseline, 18 (54.5%) had reduced their oral corticosteroid and 8 (24.2%) had stopped oral corticosteroid altogether. The mean relative reduction in oral corticosteroid dose from baseline was 49% (22.6–11.6 mg, prednisolone equivalent).

All these studies show that anti-IgE treatment has a reassuring safety profile. It is very well tolerated, and its overall adverse event profile is similar to that of placebo .

In a recent review Corren and co-workers evaluated the safety of omalizumab in a pooled analysis of data from 15 randomized multicentric studies involving more than 7500 patients (adults, adolescents and children). All patients suffered from severe persistent allergic asthma and the majority of them received omalizumab for almost 24 weeks at the dose of 150-300 mg every four weeks or 225, 300 or 375 mg every two weeks. In all studies the number of adverse events (AEs) was similar between groups and the majority of AEs were mild or moderate. The most frequent AE observed in both groups was nasopharyngitis; no difference indicative of omalizumab specific tossicity was detected between groups. The only AE with >2% difference between groups was sinusitis, observed in 10.1% of patients treated with omalizumab and in 12.2%

in the placebo groups. The assessment of laboratory parameters did not showed any significant effect of omalizumab on blood cells counts, renal and liver function; however, no previous data exist about patients with previous renal or hepatic impairment treated with omalizumab, thus caution should be used in administering the drug in these sets of patients. This review confirmed that add-on omalizumab is an effective and well tolerated treatment in patients with moderate-to-severe IgE-mediated asthma (53), and its cost-effectiveness is similar to other chronic disease biologics (54). Furthermore, the same Authors highlight that, despite its cost, omalizumab used as an add-on therapy in this setting of patients improves quality-adjusted survival (QALYs) at an increase in direct medical costs, and that this value is directly related to the duration of the therapy.

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# Cross reactivity between European Hornet and Yellow Jacket venoms

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**Key words**

*Hymenoptera venom allergy, Vespa crabro, Vespula germanica, Cross-reactivity, CAP-inhibition, immunoblotting*

**Summary**

***Background:*** *Cross-reactions between venoms may be responsible for multiple diagnostic positivities in hymenoptera allergy. There is limited data on the cross-reactivity between Vespula spp and Vespa crabro, which is an important cause of severe reactions in some parts of Europe. We studied by CAP-inhibition assays and immunoblotting the cross-reactivity between the two venoms.* ***Methods:*** *Sera from patients with non discriminative skin/CAP positivity to both Vespula and Vespa crabro were collected for the analyses. Inhibition assays were carried out with a CAP method, incubating the sera separately with both venoms and subsequently measuring the specific IgE to venoms themselves. Immunoblotting was performed on sera with ambiguous results at the CAP-inhibition.* ***Results****: Seventeen patients had a severe reaction after Vespa crabro sting and proved skin and CAP positive also to vespula. In 11/17 patients, Vespula venom completely inhibited IgE binding to VC venom, whereas VC venom inhibited binding to Vespula venom only partially (<75%). In 6 subjects the CAP-inhibition provided inconclusive results and their sera were analysed by immunoblotting. The SDS-PAGE identified hyaluronidase, phospholipase A1 and antigen 5 as the main proteins of the venoms. In 5 sera the levels of IgE against antigen 5 of Vespa crabro were higher than IgE against Vespula germanica, thus indicating a true sensitisation to crabro.* ***Conclusion:*** *In the case of multiple positivities to Vespa crabro and Vespula spp the CAP inhibition is helpful in detecting the cross-reactivities.*

## Introduction

The choice of the vaccine for immunotherapy (IT) is crucial in hymenoptera venom allergy (HVA), since specific desensitization may confer an almost complete protection and avoid severe reactions (1). Therefore, it is important to know if the skin and CAP positivities to multiple venoms are due to independent sensitisations or, rather, if is due to cross-reacting epitopes. In this latter case, the vaccination with the primary sensitising venom is sufficient. Cross reactivities among venoms of different stinging insects, including *Polistinae* and *Vespinae* (2, 3) or bees and wasps (4), have been previously described, and in the case of Vespidae, the cross reactivity seems to be remarkably frequent (3). The CAP-inhibition technique maybe a helpful method to approach the problem.

European Hornet (*Vespa crabro*) is largely present in many European countries and is now recognized as an important cause of severe reactions in patients with HVA (5). There are, so far, few data available on the possible cross-reactivity between the venoms of *Vespa crabro* (VC) and *Vespula* spp (6-8).

We evaluated the presence and extent of cross-reactivity between the venoms of *VC* and yellow jacket in patients with severe reactions to VC stings. The cross reactivity was evaluated with CAP-inhibition techniques. In addition an immunoblotting was carried out on selected sera, for which the CAP-inhibition provided inconclusive results.

## Methods

Sera from patients with severe reactions (grade III and IV according to Mueller), and who unequivocally recognized *VC* as the stinging insects responsible for the reaction were collected for the CAP-inhibition experiments. All patients underwent the standard diagnostic work-up (9), including clinical history, skin prick test, intradermal tests and specific IgE measurement by the commercial CAP-RAST (UniCap, Phadia, Uppsala, Sweden) assay. Prick tests were performed with standardized extracts at increasing concentrations from 0.01 to 100 μg/ml, whereas intradermal tests involved the injection of 0.02 mL extract at 0.001 to 1 μg/ml concentration. The tests were carried out with *Apis mellifera*, *Vespula* spp (Stallergènes, Milan, Italy), *Polistes dominulus* and *Vespa crabro* (Anallergo, Florence, Italy).

The inhibition assays were performed following a slightly modified Straumann's procedure (4), thus a specific IgE level greater than 1 kU/L was required. Briefly, 200 μL of serum were incubated for 12 hours at 4°C with 100 μL of venom at increasing concentrations (0; 0.3; 3.0; 30, 300 μg/ml). Inhibitor venoms were the same used for IT and skin testing and the commercial reagent, containing American and European Vespula venoms (including germanica) was the substrate in the CAP inhibition. Subsequently, specific IgE against each of the venoms were determined in the samples prepared as above. The CAP inhibition test was carried out with a specific program in UniCap 250 (Phadia, Uppsala, Sweden). The extent of homologous (blockage of venom-specific IgE by the same venom) and heterologous (blockage of the venom-specific IgE by the other venom) inhibition at the maximum venom concentration was computed with the following formula: %inhibition= 100-[IgE inhibited

***Table 1*** - Characteristics of the patients and results of CAP-RAST and intradermal test. VC = *Vespa crabro*; Vspp = V*espula* Species

| | | | Allergen-specific IgE (CAP) | | | | Intradermal test wheal (concentration in μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| N Pat | Age/ Sex | Total IgE kU/L | bee kU/L | Polistes dominulus kU/L | VSpp kU/L | VC KU/L | bee | Polistes dominulus | VSpp | VC |
| 1 | 59/m | 19 | <0.35 | 1.00 | 2.30 | 1.79 | 8 mm (1) | 6 mm (1) | 8 mm (0.1) | 9 mm (0.1) |
| 2 | 51/m | 118 | <0.35 | <0.35 | 2.08 | 1.11 | 7 mm (1) | NEG | 9 mm (0.1) | 10 mm (0.1) |
| 3 | 63/f | 91 | <0.35 | 1.70 | 2.36 | 3.30 | NEG | 7 mm (1) | 11 mm (0.1) | 12 mm (0.1) |
| 4 | 43/m | 127 | 0.85 | <0.35 | 4.95 | 1.36 | 8 mm (1) | 8 mm (0.1) | 10 mm (0.01) | 9 mm (0.1) |
| 5 | 50/f | 80 | <0.35 | 1.0 | 10.5 | 5.25 | NEG | 8 mm (0.01) | 8 mm (0.001) | 12 mm (0.001) |
| 6 | 39/f | 346 | 0.88 | <0.35 | 11.0 | 2.28 | NEG | NEG | 11mm (0.01) | 10 mm (0.1) |
| 7 | 51/m | 260 | 0.70 | 0.60 | 4.50 | 3.50 | 6 mm (1) | 6 mm (1) | 9 mm (0.1) | 10 mm (0.1) |
| 8 | 47/f | 173 | 0.35 | 0.49 | 4.29 | 2.81 | NEG | 8 mm (0.01) | 9 mm (0.01) | 8 mm (0.1) |
| 9 | 17/m | 85 | 0.75 | 0.90 | 2.90 | 1.60 | 7 mm (1) | 8 mm (1) | 9mm (0.1) | 10mm (0.1) |
| 10 | 52/m | 209 | 0.92 | 0.94 | 84.9 | 5.50 | NEG | 8 mm (0.01) | 9 mm (0.0001) | 11mm (0.0001) |
| 11 | 30/m | 69 | 0.35 | 1.40 | 1.60 | 3.49 | 8 mm (1) | 9 mm (0.1) | 10 mm (0.1) | 11 mm (0.1) |
| 12 | 46/m | 191 | 0.80 | 0.35 | 4.27 | 5.26 | NEG | 11 mm (0.01) | 10 mm (0.01) | 12 mm (0.01) |
| 13 | 33/m | 168 | 7.90 | 2.40 | 6.50 | 10.8 | 9 mm (1) | NEG | 10 mm (0.1) | 9mm (0.1) |
| 14 | 75/f | 280 | 4.12 | 12.0 | 15.2 | 6.57 | 10 mm (1) | 11.5 mm (1) | 11 mm (0.1) | 13 mm (1) |
| 15 | 70/m | 175 | 9.27 | 0.68 | 6.78 | 7.37 | 10 mm (1) | 11 mm (1) | 13 mm (0.1) | 12 mm (0.1) |
| 16 | 60/m | 116 | 0.77 | 1.72 | 6.06 | 2.92 | NEG | 11 mm (1) | 12 mm (0.1) | 11.5 mm (0.1) |
| 17 | 30/m | 151 | 3.00 | 1.93 | 2.66 | 2.75 | 6.5 mm (1) | 10 mm (0.1) | 10.5 mm (0.1) | 10 mm (0.1) |

sample (kU/L)X100/IgE antivenom (kU/L) at zero concentration]. An inhibition ≥75% was considered indicative of full cross-reactivity.

For immunoblotting, the proteins of venoms were separated through an SDS-PAGE (Bio-Rad, gel Criterion XT 12% and Bio-Rad, XT Reducing Agent 20x) in MES buffer (Bio-Rad, MES Running buffer) under reducing and denaturing conditions. Eight mcg of venom, 5 mcg of molecular weight standard and 8 mcg of Parietaria extract (as control) were run for 1 hour at 200V. Parietaria was chosen since it is an uncommon allergen in North-east Italy, where all patients proved skin negative for it. Protein bands were revealed by Coomassie blue staining and quantified by densitometry. In parallel, another gel was run for immunoblotting onto nitrocellulose membranes. Membranes were incubated with patients' sera, then with peroxydase-conjugate anti-IgE (Sigma, St.Louis, Anti-Human IgE peroxidase Conjugate). Bound IgE were detected by a chemiluminescent reaction (GE Healthcare, ECL Plus, catalog RPN2132). Final results were expressed as the ratio between the staining intensity obtained in the immunoblotting and that of the Coomassie blue, in order to avoid the bias due to the different content of proteins in the separation bands.

## Results

**Patients.** Seventeen patients (12 male, mean age 45.3 years) had a severe reaction (10 grade IV and 7 grade III) unequivocally provoked by VC. Thirteen of them had previous stings by yellow jacket, two could not recognize the insect at previous stings and two (patients 1 and 2) reported one VC sting in the past. In all cases, the previous stings had provoked only local reactions. All subjects had skin and CAP-RAST positivity to both VC and Vespula spp. with specific IgE of 3.98 ± 2.55 and 10.2 ± 19.6, respectively (p= NS). Some patients had also a positive skin test and/or CAP-RAST for honeybee and four for Polistes dominulus, but they have had never been stung by these insects. The results of the diagnostic workup are summarized in Table 1.

***Table 2*** - Results of the CAP-inhibition assays. The inconclusive results are highlighted in light grey. VC= *Vespa crabro*; VSp= *Vespula* Species

| | Heterologous | | Homologous | |
|---|---|---|---|---|
| | % inhibition of VC-specific IgE by VSp venom | % inhibition of VSp-specific IgE by VC venom | % inhibition of VSp-specific IgE by VSp venom | % inhibition of VC-specific IgE by by VC venom |
| 1 | 75 | 48 | 85 | 80 |
| 2 | 90 | 39 | 89 | 70 |
| 3 | 67 | 73 | 94 | 79 |
| 4 | 82 | 62 | 94 | 83 |
| 5 | 96 | 67 | 99 | 98 |
| 6 | 87 | 42 | 95 | 70 |
| 7 | 95 | 36 | 92 | 90 |
| 8 | 75 | 88 | 100 | 95 |
| 9 | 75 | 52 | 98 | 75 |
| 10 | 90 | 87 | 98 | 93 |
| 11 | 83 | 52 | 85 | 79 |
| 12 | 98 | 32 | 94 | 99 |
| 13 | 77 | 61 | 89 | 77 |
| 14 | 95 | 68 | 94 | 98 |
| 15 | 97 | 83 | 90 | 98 |
| 16 | 92 | 92 | 93 | 95 |
| 17 | 86 | 78 | 94 | 83 |

**CAP inhibition.** At the CAP-inhibition assays, pre-incubation with each venom efficiently blocked the specific IgE for that venom (homologous inhibition >90%) as expected. Concerning the heterologous inhibition, in 11/17 patients, Vespula venom completely inhibited IgE binding to VC venom, whereas VC venom inhibited binding to *Vespula* venom only partially (<75%) (Table 2). This means that pre-incubation with VC venom did not bind the Vespula-specific IgE. In 6 subjects (n. 3, 8, 10, 15-17) the CAP-inhibition test provided inconclusive results, therefore the sera of these patients were analysed by immunoblotting. Examples of different inhibition curves (patients 14 and 16) are shown in Figure 1.

**SDS-PAGE.** The SDS-PAGE procedure separated three major bands, corresponding to hyaluronidase (45 kD), phospholipase A1 (35 kD) and antigen 5 (23 kD) (11) (Fig. 2). The 45 kD band had a too low intensity and was not analysed. At the immunoblotting, the serum from patient 15 proved positive also for the negative control (Parietaria) and was not included in the evaluation. In the 5 sera evaluated, the levels of IgE (optical density) against phospholipase of VC and V germanica were similar. On the other hand, IgE against Vespula antigen 5 were significantly lower than IgE against VC antigen 5 (Fig. 3), thus indicating at least a greater affinity of the IgE for the VC antigen 5 epitopes.

## Discussion

The cross-reactivity among different allergens is quite common and occurs, in fact, with vegetables, pollens (10) and drugs. In the case of HVA, cross-reactions among venoms may produce multiple diagnostic positivities, with the consequent prescription of multiple vaccines, also when one single IT would be sufficient. This frequently occurs with Vespidae, whose venoms are quite similar in the allergenic composition. In our experience, the positivity to both yellow jacket and VC (European hornet), often makes difficult the choice of the vaccine, although it has been previously suggested that one wasp venom can protect also against VC (8). Thus, we attempted to define if a patient truly had IgE against unique epitopes in both venoms or if the reactivity with one of the venoms was entirely due to cross-reactivity. The CAP-inhibition assay, indeed evidenced that the two venoms extensively cross-react in 67% of patients, and that vespula venom efficiently binds the VC-specific IgE. In those patients, yellow jacket vaccination is reasonably expected to be adequate. Similar findings were reported some years ago in a case series of 24 patients

***Figure 1*** - Examples of two inhibition experiments. On X axis the concentration of the inhibitor, and on Y axis the % of inhibition. Homologous and heterologous inhibitions with VC and *Vespula* spp venoms are shown.

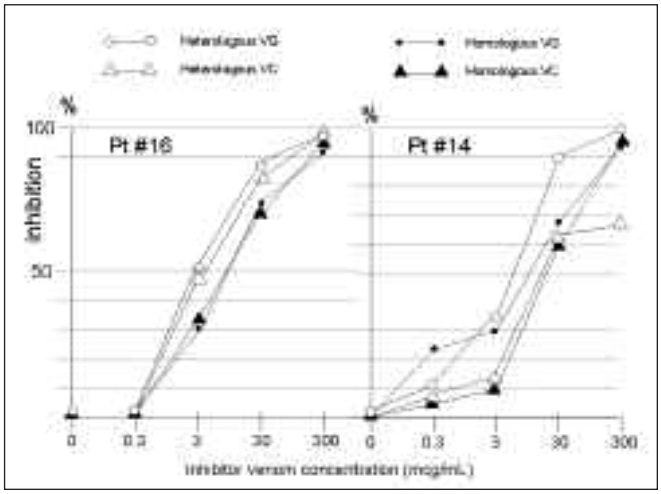



***Figure 2*** - Immunoblotting assays of the 6 sera with inconclusive results at the CAP inhibition assay. From left to right lanes: molecular standard, *Vespula*, VC, parietaria. The serum #15 (right) proved positive also for the negative control and was excluded

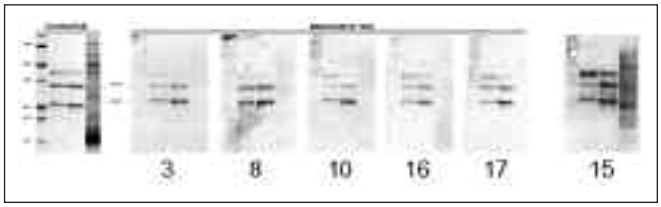
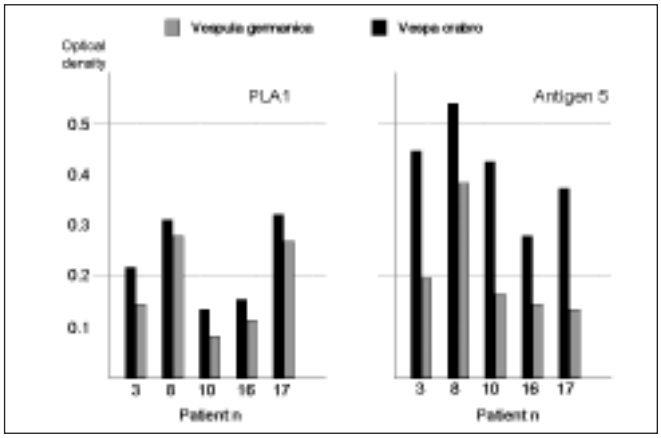



***Figure 3*** - Specific IgE (optical density) against phospholipase (left) and antigen 5 (right) of VC and Vespula in the five sera shown in figure 1



(8). In such cases, it can be hypothesized that patients are primarily sensitised to yellow-jacket, and the cross-reactivity of venoms is responsible for the severe reactions to European hornet. Our results partly differ from those reported in a Spanish study (7), but this may be attributed, at least in part, to the different presence and distribution of the insect in different geographical regions. As a partial limitation, in this study we could not identify the exact nature of the cross-reactive epitope, although it is conceivable that part of the cross-reactivity is due to carbohydrate determinants, as previously described for honeybee and yellow jacket (12, 13). Another possible limitation is that the extract used for skin tests and as inhibitor is a mix of different Vespula species, including *Vespula germanica*. This is due to the fact that a purified Vespula germanica venom for in vitro and in vivo diagnosis is not available.

The CAP-inhibition assay, which is a sensitive technique, largely used in allergy since decades, is helpful in identifying those patients. Where the CAP-inhibition provided ambiguous results, the immunoblotting assay clearly showed that the patients had higher levels of IgE against one allergen of VC, thus they should be vaccinated with a VC extract, which is of note available only in few European countries. Certainly, the clinical evaluation remains the basic criteria, but the CAP-inhibition, which is relatively simple, can be regarded as an useful tool to better detail the diagnosis and the consequent therapeutic approach. In this regard, the identification of the correct venom to use for vaccination may counterbalance the cost of the technique itself.

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# GAPP Italy: "A survey on asthma on Italian physicians and patients"

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**Key words**

*Asthma control, GAPP survey, physician-patient communication, treatment of asthma*

**Summary**

*Guidelines recognize the importance of achieving and maintaining asthma control: the treatment strategies now available allow the control of the great majority of patients with asthma but despite many efforts only 5% of patients achieve guideline-defined asthma control. The GAPP (The Global Asthma Physician and Patient survey) is a global quantitative survey with the aim of identifying barriers to optimal management of asthma. Physicians and adult patients with persistent asthma have been interviewed with closed-ended questions questionnaire. This study has been conducted in 16 countries. In Italy the survey has revealed that physicians prescribe a combination of ICS and LABA more often in the other countries. They consider ICS the first-line treatment for mild persistent asthma. They are not completely satisfied with ICS because of local and systemic side effects. At the same time, the reason why patients change asthma medication is the potential for side effects. The two group responses were found to differ about the time spent discussing how to improve the management of asthma. A better communication between physician and patient and a new treatment option with lower side effect profile could be the key point to achieve asthma control in a larger number of patients.*

## Introduction

During the last 25 years a large number of studies on asthma have been made with the aim to improve the knowledge of this worldwide disease. Nowadays we have a clear global and regional view of asthma deriving from the two main important epidemiological studies, the ISAAC (1) and the ECRHS (2).

Relevant advances in the pathogenesis of asthma have been achieved: starting from the knowledge of inflammatory mechanisms (3), through the smooth muscle pathophysiology (4, 5), to the new insight into remodeling (6).

At present asthma diagnosis is mainly based on clinical features and on spirometric lung function evaluation, but other new instruments seem to be useful, such as induced-sputum, eNO, pulmonary function in infants, HRCT, etc.

The treatment strategies now available allow the control of a great majority of patients affected by asthma; new drugs such as anti-IgE, are important in severe asthma therapy. Moreover, the Global Initiative for Asthma guidelines (GINA), its successive updating (7) and National Institute of Health guidelines (NIH) (8) have been published with the aim to divulge a clear trace in the management and treatment of this world spread disease. Unfortunately, despite these tools, only 5% of patients achieve guideline-defined asthma control (9).

Four important studies have been conducted to evaluate how asthmatic patients are assessed and treated in real life, covering almost every continent of the world.

The AIRE study (Asthma Insight and Reality in Europe) revealed that asthma has an important impact on different daily life aspects (9, 10).
A large survey has been conducted in the United States to explore asthma prevalence, the frequency and severity of symptoms, the use of emergency care, quality of life, and quality of care issues. The study revealed that the asthma management has not reached the goals established by the National Heart, Lung, and Blood Institute (NHLBI) (11). The same findings have been shown in the south of America by the AIRLA survey (12).
Despite the strong efforts, there is evidence that asthma is far from a good control in the great majority of cases and it seems that part of the energy invested in its management is wasted in some elements of the system. For these reasons and because of the unanswered questions from AIRE and the other studies it has been necessary to develop another study, the Global Asthma Physician and Patient (GAPP) survey, that was designed to uncover asthma attitudes and treatment practices among physicians and patients, with the goal of identifying barriers to optimal management (Fig. 1).
The GAPP is a global quantitative survey with the aim to evaluate the same themes both in patients and physicians by asking similar questions in the two groups. It has been conducted during 2005 in 16 countries (Australia, Belgium, Brazil, Canada, France, Germany, Ireland, Italy, Japan, the Netherlands, Poland, South Africa, Spain, Switzerland, the UK and the US).
This survey was announced and supported by the World Allergy Organization (WAO) and the American College of Allergy, Asthma and Immunology (ACAAI) scientists.
The main objectives were:

- Enhance understanding and awareness of likely contributors to suboptimal asthma management.
- Explore the content and dynamics of physician-patient communications.
- Enhance treatment compliance and outcomes.

Physicians (including primary care physicians/family practitioners, pulmonologists and allergists) and adults affected by persistent asthma have been interviewed with a 20-minute questionnaire with closed-ended questions.
The key global findings regard the compliance of patients that can be enhanced by improving the communication between physician and patients and through their education on asthma. Moreover, this important goal can be achieved by administering treatment options with lower side effect profiles.

***Figure 1*** – Global key findings of GAPP Survey.


## The GAPP survey in Italy

*Patients and methods*

5,480 physicians and patients worldwide have been evaluated.
In Italy a group of 105 adults affected by asthma has been recruited and screened from Harris Interactive online panel; the patients were at least 18 years aged.
101 physicians were recruited and screened from existing national databases. All of them used to treat adults and 51 were generalists (including general practitioners and internal medicine practitioners) and 50 were specialists (allergists and pulmonologists). To be included in this study they were required to be practicing medicine at least for three up to thirty years, see at least three adult asthmatic patients per week and write at least one asthma treatment prescription per week.
The number of interviews was determined to guarantee statistical significance when the data were measured globally and in each country. The interviews were performed by experienced interviewers in their native language. Before being used, the questionnaire was tested on 10 people from each country to ensure that the questions had been understood.
The patients were asked screening questions to ensure that they were affected from asthma. Chronic obstructive pulmonary diseases patients were not excluded.
Table 1 and table 2 contain patients' and physicians' questionnaire. Patients and physicians were not requested to sign a specific informed consent, however they agreed their responses could be used in public in an aggregate, anonymous and confidential way.

*Data management and analysis*

In Italy, as well as in the other countries except for USA, the data were not weighed. These samples are not probability samples: since simple size is 100 the margin of error was ±10.

## Results

Mild, moderate and severe asthma show a homogeneous distribution globally in the study, both in Europe and in Italy when the patients' self-reported perception of asthma severity is considered. The 50% of patients describe their

***Table 1*** - Survey questions for patients

| | |
|---|---|
| Q1a | Which type of doctor or healthcare professional do you usually see to treat your asthma? |
| Q2a | Overall, based on your symptoms, how would you describe your asthma? |
| | a) Mild b) Moderate c) Severe |
| Q3a | Overall, how much has your asthma limited your ability to do your daily activities? |
| | a) A great deal b) Somewhat c) Not much d) Not at all |
| Q4a | During the past 12 months, have you [insert options] for your asthma? |
| | a) Made unscheduled calls to your doctor because of your asthma |
| | b) Made unscheduled visits to your doctor because of your asthma |
| | c) Gone to a hospital emergency room because of your asthma |
| | d) Been admitted to hospital because of your asthma |
| Q5a | Which of the following medications are you currently taking to treat your asthma?* |
| Q6a | Overall, how satisfied or dissatisfied are you with the following features of you current asthma medication(s)? |
| | a) Ease of use d) How many times per day you take it |
| | b) Effectiveness e) Potential for side effects |
| | c) Fast acting f) Safety |
| Q7a | Since being diagnosed with asthma, have you ever switched from one medication to another or discontinued an asthma medication because…? |
| | a) Your asthma symptoms lessened or went away |
| | b) You experienced side effects |
| | c) You were concerned about the potential for side effects |
| | d) The asthma medication was too expensive |
| | e) The asthma medication was difficult or inconvenient to use |
| Q8a | After you doctor told you your asthma can be triggered by allergies, has he or she…? |
| | a) Told you how to avoid allergic triggers |
| | b) Explained that you should be on continuous preventer medication |
| | c) Referred you to an allergist or other specialist |
| Q9a | Who at your doctor's office typically explains your treatment options and techniques for successful management of your asthma? |
| | a) Treating physician |
| | b) Nurse |
| | c) No one |
| Q10a | During a typical visit with your doctor or health care professional, what percentage of the time do you or did you spend discussing how to improve techniques for successful management of your asthma? |

Q11a Does your doctor or other healthcare professional in his or her office discuss any of the following with you?

a) A plan for treating asthma

b) Correct inhaler technique

c) Keeping daily symptom/ medication diaries

d) Monitoring peak expiratory flow

e) Contacting patient support organizations

Q12a Is the following statement true or false or are you not sure? Asthma attacks can be fatal in patients with mild asthma.

Q13a When you discuss or discussed side effects of asthma medications with your doctor or other health care professional, who typically brings up the topic, you or your doctor or health care provider?

Q14a How often do you or did you discuss short-term side effects or your asthma medications related to your mouth or throat – such as fungal infection, sore throat or hoarseness – with your doctor or other health care professional?

a) Never

b) Rarely

c) Sometimes

d) Always

Q15a How often do you or did you discuss long-term side effects of your asthma medications – such as weight gain, weakening of the bones or changing bone density, cataracts or glaucoma – with your doctor or other health care professional?

a) Never

b) Rarely

c) Sometimes

d) Always

Q16a The following is a list of potential side effects of inhaled corticosteroids. ON a scale of 1-10 where "1" mean "not at all concerned" and "10" means "extremely concerned", how concerned have you been with the following potential side effects, or were you not previously aware of these as potential side effects?

Q17a The following is a list of potential side effects of inhaled corticosteroids. ON a scale of 1-10 where "1" mean "not at all concerned" and "10" means "extremely concerned", how concerned have you been with the following potential side effects, or were you not previously aware of these as potential side effects?

Q18a While taking asthma medications, have you experienced….?

a) Decreased cortisol production

b) Long-term side effects

c) Short-term side effects

Q19a Have any of the asthma medication side effects you experienced since being diagnosed caused you to….?

a) Consider switching medications

b) Switch medications

c) Consider skipping doses

d) Skip doses

e) Consider stopping medications

f) Stop taking medications

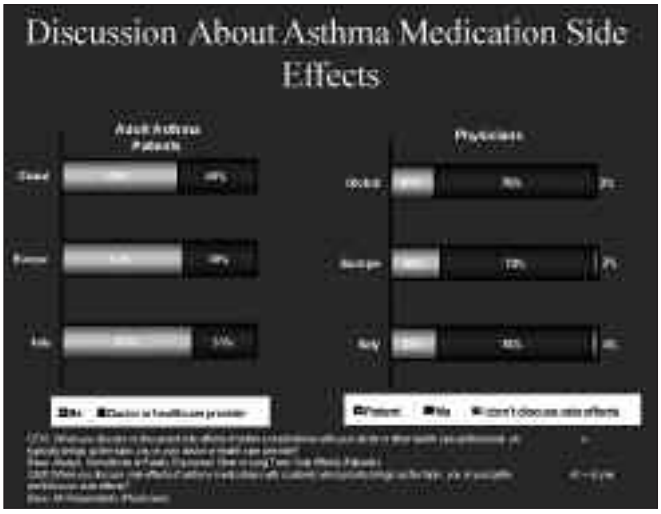
g) Change dosage

| | |
|---|---|
| Q20a | What percentage of the time do or did you take your asthma medication according to your doctor or other health care professional's instructions? |
| | a) Never |
| | b) 1-25% |
| | c) 26-50% |
| | d) 51-75% |
| | e) 76-99% |
| | f) All |
| Q21a | On a scale of 1- 10 where "1" means "not at all important" and "10" means "extremely important", how important are the following reasons you don't or didn't always take your asthma medication as instructed? |
| Q22a | Have you ever experienced the following if you don't or didn't take your asthma medication as instructed? (Asked to patients who took asthma medication less than 100% of the time) |
| | a) Increased symptoms |
| | b) Limited physical activity |
| | c) Increased use of bronchodilator |
| | d) Nighttime awakenings |
| | e) More frequent asthma attacks or exacerbations |
| | f) More severe asthma attacks |
| | g) More physician visits |
| | h) More hospitalizations or ER visits |
| | i) Absences from work |
| | j) Life-threatening asthma attacks |
| | k) Less interaction with friends and family |
| Q23a | Do you think there is a need for new medication options for people with asthma? |
| Q24a | If a new inhaled corticosteroid asthma medication were to become available, using a scale of 1 -10 where "1" means "not at all important" and "10" means "extremely important", please rate how important each of the following would be to you. |
| | a) Lower potential for unwanted long-term side effects |
| | b) Works at least as well as other ICS |
| | c) Fewer side effects in the mouth and throat |
| | d) A drug that is activated in the lung |
| | e) Once-daily dosing |
| | f) No mouth rinse issues |
| | g) A dose counter |
| | h) Quickly eliminated from the body |
| | i) Can use without a spacer |
| | j) High lung deposition |

asthma as mild, while around the 40% say their asthma is moderate and the 11% of patients consider it as severe.
In Italy, more than in the other countries of the GAPP study, patients' daily activities are limited since up to one third of them state that their abilities are somewhat reduced. Moreover, around the 24% of the asthmatic patients paid unscheduled visits, the 8% applied to the emergency department and the 5% has been admitted to the hospital due to asthma.
In Italy the 54% of asthmatic patients are treated by the specialist: this percentage is higher than the global (31%) and the European (36%) one; unfortunately, on the other

hand, we have one fifth of subjects who don't see any doctor, PCP either or the specialist.
In Italy the 86% of patients have taken asthma medication at any time; in the 65% of cases they have been treated with the combination of inhaled corticosteroids (ICS) and long acting beta2-agonist: this percentage is much higher than in the other countries of the survey. Inhaled corticosteroids are considered the "gold standard" treatment for asthma, as a matter of fact they are used most frequently as a first-line treatment for all patients; there is a wide agreement in the treatment of the inflammation with the aim to reduce the risk of broncho-constriction. At the same time physicians are not completely satisfied with local and systemic side effects.
On this topic the patients' responses are similar: they are satisfied with medication efficacy, but they are not with potential side effects.
What's interesting is that in Italy, more than in other countries, physicians prefer to prescribe corticosteroids as first-line treatment for mild intermittent asthma both in combination and singularly. The 72% suggests the beta2-short acting; moreover, Italy has the highest percentage of doctors who give leukotriene receptor antagonist for this kind of asthma.
The 74% of Italian patients with mild persistent asthma have been given the combination, but the difference with those who received only inhaled corticosteroids was small. In the same view, physicians administer leukotriene antagonist much more often than in other countries (53% versus 36% in Europe and 42% considering all countries).
The average physicians' satisfaction with corticosteroids administered as monotherapy in Italy is the lowest compared to the other countries; accordingly physicians' satisfaction with ICSs is the lowest on both systemic and local side effect issues. In parallel, patients' satisfaction with current asthma treatment is much lower on "potential for side effects" compared to other countries of the survey (51% versus 72%) and the reasons why patients change asthma medications concern potential side effects.
Both patient and healthcare provider recognize the importance of taking care of the patient's education and agree that the physician is the most responsable healthcare provider for this task.
Physicians appear available and helpful on discussing about the asthma management plan, the inhaler use technique and about the usefulness and side effects of steroids and bronchodilators.
In Italy the 43% of physicians says that they spend the whole time of the visit discussing how to improve techniques for the successful management of patients' asthma; on the other side, only the 32% of patients say that the whole visit has been spent for this topic. Anyway, a very low number of subjects followed for asthma say that the percentage of time used for this issue was less than at least the 50%.
Even if these results are encouraging, a lot of work has to be done yet.
Indeed, asthma education is not ideal, since three-quarters of the patients in Italy don't recognize that asthma attacks in mild patients can be fatal; moreover, the physician's perspectives on side effects differ deeply from patient's point of view: patients are more concerned about long-term side effects than physicians and their opinions differ about who must start the discussion on asthma medication side effects. In Italy as well as in the other countries of the survey, more than the 60% of patients (67% in Italy) state that they bring up the topic "drug side effects", while, at least the 73% of physicians say that they do (Fig. 2).
Both patients and physicians are most concerned about the long-term side effects of ICS, compared to the short-term ones. More often in Italy, this leads patients to consider switching medication, skipping or changing doses or stopping the therapy; according to physicians this seems to be due to fear of steroids and the concern for side effects (Figs. 3, 4)
Compared with the rest of the world in Italy adult patients with asthma and physicians are, on average, discussing asthma management less frequently and a greater proportion of adult asthmatic patients don't know that

***Figure 2*** - Different opinion about who must initiate the discussion on asthma medication side effects.



asthma attacks can be fatal in patients with mild asthma. In addition, patients are equally aware of side effects and they are concerned about the decreased production of cortisol and short term side effects but are less likely to be concerned about long term side effects.
Italian physicians need new asthma medication more than physicians of other countries, while their patients believe that there's a need for new asthma drugs only in the 47% of cases, a percentage very low compared to other countries where it is estimate around 70%.
When doctors and patients were asked which specific attributes were of primary importance if a new inhaled corticosteroid medication were to become available, the physicians' replay was the once-daily dosing, together with at least the same efficacy, fewer side effects in the mouth and throat and a lower potential for unwanted long-term side effects; this attitude confirms their awareness of the necessity to apply strategies to enhance the subjects' compliance (Tab. 3).
In confirmation of this aspect, a study evaluated the adherence to twice-daily inhaled corticosteroid therapy in 50 adults with moderate-to-severe asthma monitoring electronically for 42 days. Average adherence was the 63%; the 54% of patients recorded at least the 70% of the prescribed number of inhaled steroid actuations; the compliance to the therapy decreased progressively over the weeks of the study. Factors associated with poor adherence included: less than 12 years of formal education, poor patient-clinician communication, household income lower than $ 20,000, non–English-speaking patient and minority status (12).
Ciclesonide is a new inhaled steroid that has to be taken once-daily; a study showed that at 160 or 40 mcg/day it had no detectable effects on growth velocity, as assessed by stadiometer height, in children with mild persistent asthma (13-15). CIC demonstrated no effect on the growth in children from different regions, while differences in growth rates between children with asthma from North and South America may reflect genetic and socioeconomic differences. This drug could improve patients' adherence to therapy since it can be taken only once daily.

***Figure 3*** - Actions taken by patients due to medication side effects.

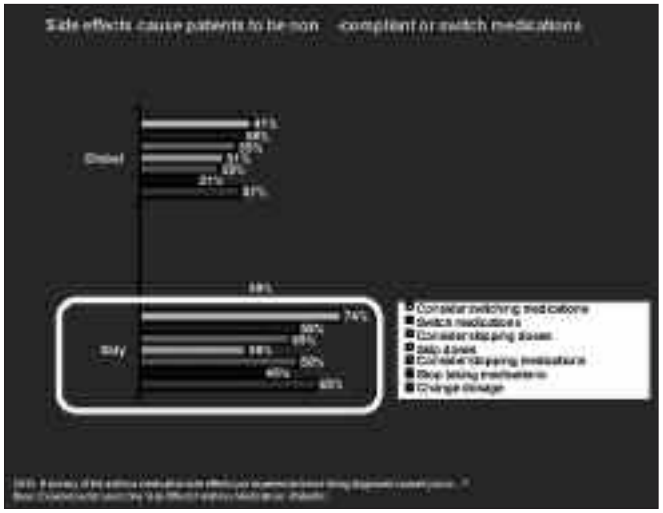
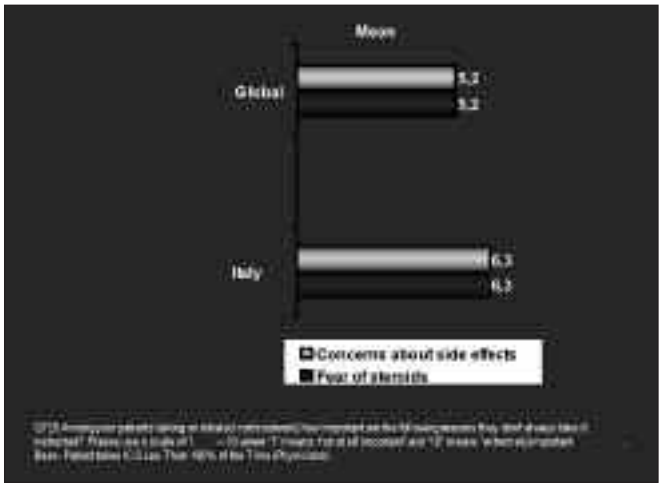



***Figure 4*** - Physicians' Perceptions of Why Patients Do Not Always Take ICS as Instructed.




## Discussion

The GAPP survey has studied the current management and treatment of asthma by evaluating patients' and physicians' point of view.
Both groups were questioned on asthma diagnosis and symptoms, communications with their respective patients or physician, the quality of life, their experience with asthma medications, side effects from asthma drugs, the concern and awareness of side effects and interest in new drugs for asthma.
The GAPP has the aim to identify barriers to optimal management of asthma through enhancing the understanding and the awareness of likely contributors to suboptimal asthma management, exploring content and dynamics of physician-patient communications, finally enhancing treatment compliance and outcomes.

***Table 3*** - Main results in Italy, Europe and globally.

| | Italy | Europe | Global |
|---|---|---|---|
| Patients activities are limited | 36% | 32% | 31% |
| Unscheduled visits to doctor | 24% | 28% | 27% |
| Patients admitted to hospital due to asthma | 5% | 6% | 6% |
| Patients treated by specialist | 54% | 31% | 36% |
| Patients not treated by doctors | 20% | 11% | 11% |
| Patients treated with combination ICS+LABA | 65% | 29% | 27% |
| ICS considered the gold standard treatment for asthma by physicians | 80% | 95% | 95% |
| Leukotriene antagonist in mild persistent asthma | 53% | 36% | 42% |
| Patients assert to start discussion on asthma medication side effects | 67% | 62% | 60% |
| Physicians assert to start discussion on asthma medication side effects | 74% | 73% | 76% |
| Patients believe there is a need for a new asthma medication | 47% | 73% | 76% |
| Physicians believe there is a need for new asthma medication | 98% | 80% | 81% |

From the evaluation of the patients' self-reported perception of asthma severity, unscheduled resources use and limitation to daily activities it is evident that asthma is far from a satisfactory control. This could be due to the patients' lack of knowledge or awareness about their disease or to the physicians' inability to face key issues. Moreover, in Italy the 20% of patients is not treated by any doctor.

Both patients and healthcare providers recognize the importance of the provider in patient education. However, there is disagreement about the time devoted to asthma education and discussing how to improve techniques for the successful management of asthma.

The most frequent asthma treatment taken is the combination of ICS and LABA. Italian physicians agree that it is important to treat bronchial inflammation and that ICS are the gold-standard therapy for asthma: in this view these are the first-line treatment for mild persistent asthma; at the same time, they are not fully satisfied with their potential local and systemic side effects. In Italy, more frequently than in the other countries, patients are treated with leukotriene antagonist.

The GAPP survey also revealed that potential side effects are for the cause of patients' concern and this is considered one of the key points that can lead to switch medication, to skip doses, or to stop taking medication. Another important point is the time devoted to patients' information by the health provider. Patients' information should improve awareness of the disease and educate on the necessity to take medication in order to cure and prevent symptoms. The barriers to adherence can be identified in the relationship between the patient, the provider and the health care system. As a matter of fact, poor provider-patient communication can lead to a poor understanding of the disease and of the benefits and risks of treatment by the patients. Moreover, the patient has a poor understanding of the medication if the physician prescribes a complex course of treatment or switches to different formulary.

Despite our knowledge and our efforts on asthma treatment, some unmet needs still exist. As a matter of fact, even if GINA guide lines greatly improved asthma therapy approach over the time, asthma control is still too low, Quality of Life has a significant impact on patients' lives, side effects are relevant and compliance is not ideal. Moreover, the 70% of asthma death could be prevented. Asthma patient education seems to be a crucial point to exploit and must be improved through discussions with physicians during office visits.

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A. Arena

# Anaphylaxis to apple: is fasting a risk factor for LTP-allergic patients?

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**Key words**

*Food allergy, Lipid transfer protein, Apple allergy, Allergens*

**Summary**

***Background:*** *Primary apple allergy is frequent in Mediterranean countries where hypersensitivity to lipid transfer protein (LTP) is common. Due to its stability upon pepsin digestion, LTP may cause systemic allergic reactions. This study investigated the potential risk associated with an isolated intake of apple while fasting in LTP-hypersensitive patients with clinical allergy to peach but not to apple.* ***Patients and methods:*** *Based on the observation of 6 patients who experienced 7 apple-induced anaphylactic reactions that in 6 cases followed the ingestion of the fruit after fasting, open food challenges were carried out in 12 patients LTP-hypersensitive patients with peach allergy but tolerant to apple.* ***Results:*** *Four out of the 12 patients (33%) reacted to apple upon oral challenge.* ***Conclusion:*** *Fasting seems to play a relevant role in the clinical expression of allergy to LTP. It is possible that in an empty gastrointestinal tract the allergen is absorbed more rapidly. Alternatively, pepsin might digest the food matrix more efficiently, thus increasing the concentration of the purified allergen that comes in contact with the gut mucosa.*

## Introduction

Primary allergy to Rosaceae fruits is frequent in the Mediterrean area (1-3). In central and northern Europe Rosaceae allergy is associated with birch pollinosis and is clinically mild and restricted to the oropharyngeal mucosa (4, 5), whereas in Southern Europe primary sensitization to Lipid transfer protein (LTP) is frequent (6, 7). LTPs are heat- and pepsin-stable, and can cause systemic reactions (6, 8). It is generally accepted that Pru p 3, the peach LTP, represents the primary sensitizer to this allergen (7). In LTP-hypersensitive patients allergic to peach, apple allergy may occur due to the high homology between Mal d3 , the apple LTP and Pru p3 (7, 9).
In LTP-hypersensitive subjects the clinical presentation of apple allergy can be severe and not always preceded by other symptoms, as the OAS (1, 10, 11).

The observation of a group of six LTP-allergic patients, who experienced seven anaphylactic episodes induced by apples with peel, that in 6 cases were ingested at least two hours after a meal and without eating anything else, prompted us to investigate the potential risk associated with the isolated intake of apple in patients with peach-allergy.

## Patients and methods

### *Patients*

29 LTP-hypersensitive patients with a history of peach allergy but clinically apple-tolerant, negative on SPT with birch pollen extract, seen at the Allergy center of Azienda Sanitaria of Messina (Italy) from 2007 to 2009 were asked to participate to the study.

All the patients scored positive on SPTs with Golden Delicious (GD) fresh apple (peels and pulp separately), according to prick-by-prick method (12), and with a commercial peach extract containing uniquely lipid transfer protein (Alk Abellò ; LTP 30 μg/ml). SPTs were carried out and read following the EAACI recommendations (13) using histamine hydrochloride (10 mg/mL) and saline as positive and negative controls, respectively.

*Specific IgE*

Specific IgE against apple and Pru p 3 were measured by CAP-System (Phadia©, Uppsala, Sweden), according to the instructions of the manufacturer.

*Challenge tests*

Twelve out of 29 subjects accepted to undergo the apple challenge. An informed consent was obtained from each patient before the challenge.
GD apples, bought at the local market, were used in the challenges. Open food challenges (OFC) were performed by administering slices of fresh apple with peel on patients fasting for at least two hours . One slice of apple (approximately 10 g) was administered at the beginning and the dose was then doubled every 60 min. The test continued until the patient had convincing symptoms, or a total of approximately 70 g of apple had been ingested (3 h). Before all challenges and SPTs, medication was discontinued according to the guidelines on skin testing of the European Academy of Allergology and Clinical Immunology (EAACI) (14).

**Results**

Results are shown in Table 1. Four out of 12 (33%) subjects scored positive upon apple challenge. All 4 experienced itch, urticaria, abdominal pain and nausea.
No significant differences were found in Pru p 3 and apple IgE between subjects who responded or tolerated apple on oral challenge (Table 2).

***Table 1*** - Patients submitted to oral challenge with fresh, unpeeled apple

| N. | Age | Sex | Peach* | Cap Pru p3 | Cap Apple | Other food allergies** | Clinical symptoms during OFC*** * | Dose challenge |
|---|---|---|---|---|---|---|---|---|
| 1 | 44 | F | U, AP | 22,7 | 69,3 | Ha (U-A) | I,N,AP | 10 |
| 2 | 18 | F | OAS, P | 1,85 | 7,11 | Al (U) | T | 70 |
| 3 | 33 | F | OAS | 6,31 | 19,8 | Ha (OAS) | T | 70 |
| 4 | 38 | F | OAS | 4,75 | 63,8 | Pn (OAS) | T | 70 |
| 5 | 20 | F | CU | 1,35 | 0,8 | W (U) | T | 70 |
| 6 | 19 | F | OAS | 0,95 | 0,61 | ------- | T | 70 |
| 7 | 46 | F | OAS | 1,5 | 0,5 | Pn, Al (SOA) | AP, I | 30 |
| 8 | 23 | M | OAS | 2,34 | 2 | ------- | T | 70 |
| 9 | 20 | M | OAS | 9,57 | 1,8 | Pn(SOA) | T | 70 |
| 10 | 32 | F | OAS | 3,8 | 0,61 | ------- | T | 70 |
| 11 | 29 | M | D,CU | 12,3 | 13,4 | Pn (U) | U,N | 30 |
| 12 | 28 | M | CU | 3,96 | 1,2 | ------- | N,I | 30 |

* A, angioedema; AP, abdominal pain; D, dyspnoea; N, nausea; I, itch; U, urticaria; UC, contact urticaria; OAS, oral allergy syndrome
** Apr, apricot; Al, almond; Ha, hazelnut; Pn, peanut; W noce;
*** T, tolerated

***Table 2*** - Specific IgE

| | | Positive on apple oral challenge (n= 4) | Negative on apple oral challenge (n= 8) |
|---|---|---|---|
| Pru P3 | kU/l (mean[range]) | 10,1 (1,5-22,7) | 3,8 (0,9-6) |
| Apple | kU/l (mean[range]) | 21,1 (0,5-69,3) | 12,06 (0,6-63,8) |

## Discussion

The observation that in 6/7 (85%) cases of apple-induced anaphylaxis the fruit had been eaten while fasting prompted us to carry out the present study. In a group of LTP-hypersensitive subjects with a history of peach allergy but clinically tolerant to apple (albeit sensitized to apple on SPT and in-vitro assays) submitted to open food challenge with increasing doses of unpeeled apple, one third experienced a systemic reaction following apple ingestion while fasting.

Recent guidelines recognize that there is no absolute correlation between pepsin digestion and allergenicity but suggest that rapid and extensive degradation may be helpful in increasing allergen availability (15). The proteolysis of food allergens is strongly dependent on the pepsin to allergen ratio (16). Pepsin secretion by human stomach is influenced by quantity and type of food ingested (17). Digestibility and allergenicity of some proteins, such as peanut and β-lactoglobulin, is the of interactions between allergens and other food ingredient (18-20).

It is possible that in an empty gastrointestinal tract the LTP is absorbed more rapidly. Alternatively, pepsin might digest the food matrix more efficiently, thus increasing the concentration of the purified allergen that comes in contact with the gut mucosa.

There are several different facilitating factors in food allergy: exercise (21-23), various drugs (24) or both (25). Fasting has never been described as a risk factor for systemic reaction to foods.

These observations allow to hypothesize that the absence of food in the stomach may influence allergen presentation to the immune system, thus representing an eliciting factor for clinical allergy in apple-allergic subjects.

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# Co-sensitisation (but co-recognition also) to novel banana and tomato allergens

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**Key words**
*Food allergy, banana, tomato, cross-reactivity, allergens*

**Summary**
*An unusual case of both banana and tomato allergy is reported. In vitro tests showed that both co-sensitization to and co-recognition of allergen in the two fruits were present. Interestingly, the patients showed IgE reactivity to hitherto not described, high molecular weight allergens.*

## Introduction

Tomato and banana allergy are not uncommon. Most cases are found in patients with seasonal airborne allergy due to the cross-reactivity between pollen profilin and the homologous food protein. Further, banana allergy has been frequently described following primary natural rubber latex allergy due to cross-reactivity between latex and banana allergens. In contrast, primary sensitisation to these two foods is rather rare. This study reports an unusual case of co-sensitisation (but of co-recognition as well) to novel tomato and banana allergens.

## Patients and methods

*Case report*

A 17-year-old boy was recently seen at the allergy outpatient clinic of this clinic with a history of slight rhinoconjunctivitis in springtime during the last 2 years, and two distinct episodes of angioedema of the face, hypotension, and diarrhoea during the last 2 months, both occurring about 30 min after the ingestion of banana and lasting for about 1 hour. Further, the patient reported a typical oral allergy syndrome (immediate itching of oral mucosa) following the ingestion of raw tomato. Both banana- and tomato-induced symptoms were not related to the onset of the seasonal rhinoconjunctivitis, and the patient reported good tolerance of all other foods. SPT with commercial extracts (Allergopharma, Reinbeck, Germany) of the main airborne allergens present in this area including pollens (grass, mugwort, ragweed, pellitory, plantain, birch, cypress, and olive), house dust mites, molds (Alternaria, Aspergillus, Cladosporium, Candida), and danders (cat and dog) showed moderate skin reactivity to grass and ragweed pollen. SPT with a series of commercial extracts (ALK-Abello, Madrid, Spain) of food allergens including egg white and yolk, cow's milk, shrimp, pork, cod, wheat, maize, soybean, peanut, walnut, hazelnut, tomato, sunflower, carrot, orange, celery, banana, kiwi, and sesame (all 1:20 w/v), and peach LTP (30 μg/ml) were performed as well.

*In-vitro assays*

Tomato and banana extracts were prepared as previously described. Briefly, 100 g of fresh tomato including both pulp and peel and 100 g of banana were homogenized. Both homogenates were mixed with 300 ml of pre-cooled acetone and equilibrated at -20°C overnight. The precipitates were washed twice with acetone and once with acetone/ether (1:1, v/v) and dried. The resulting powders were extracted (1); protein concentrations of the extracts were 3 mg/ml and 0.8 mg/ml for banana and tomato, respectively (2) (Bio-Rad). In direct ELISA assays 1 μg of tomato or banana extracts both diluted in 100 μl of coating buffer (15 mM $Na_2CO_3$, and 35 mM $NaHCO_3$)/well, were used for coating 96-microtitre plates (Maxisorp, Nunc) (3). After washings with 0.1 M PBS, pH 7.4, and 0.05% Tween 20 (Sigma), wells were saturated with 2% BSA in PBS for 2h at RT. After further washing, 100 μl of undiluted serum were added per well and incubated for 2 h at RT. After washing bound specific IgE was detected by adding a peroxidase-conjugated anti-human IgE from goat (Biospacific, USA; 1:1500). The enzyme reaction, induced using tetramethyl-benzidine/$H_2O_2$ as substrate, was stopped after 20 minutes by 1 mol/L HCl. Absorbance was read at 450 nm and expressed as optical density (OD). In order to assess a possible cross-reactivity between tomato and banana allergen, ELISA cross-inhibition experiments were carried out pre-absorbing patient's serum with 10 μg of either tomato or banana protein; in case of significant inhibition, a curve was built by measuring IgE reactivity after pre-adsorption of serum with 1 μg and 0.1 μg of extract as well. Patient's IgE reactivity was further investigated by immunoblot under reducing conditions against tomato and banana extracts. Electrophoresis of extracts (15 μg/lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel with MES buffer according to manufacturer's instructions (Invitrogen) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane (4). The membrane was saturated with 0.1 mol/L Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera. After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1000 in saturation buffer; Biospacific) using an ECL western blotting kit (Amersham) as substrate.

## Results

*Skin tests*

SPT showed strong skin reactivity to commercial extracts of tomato (mean wheal diameter 8 mm), banana (6 mm) and hazelnut (8 mm). SPT with fresh tomato both raw and boiled at 100°C for 5 min scored intensely positive with no difference between the raw and the heat-processed food (mean wheal diameter 6 mm in both cases). In contrast, no skin reactivity to natural rubber latex extract (500 μg protein/ml) and to purified date palm profilin [(Pho d 2; 50 μg protein/ml (5)] (both by ALK-Abello) was recorded.

*In-vitro assays*

Direct ELISA showed significant IgE reactivity to both banana (854 OD) and tomato (3285 OD). Cross-inhibition experiments showed that pre adsorption of serum with banana extract caused a dose-dependent reduction of IgE reactivity to tomato, whereas pre adsorption with 10 μg of tomato extract caused very little inhibition of IgE reactivity to banana (Tab. 1), thus showing partial cross-reactivity between banana and tomato allergens and sug-

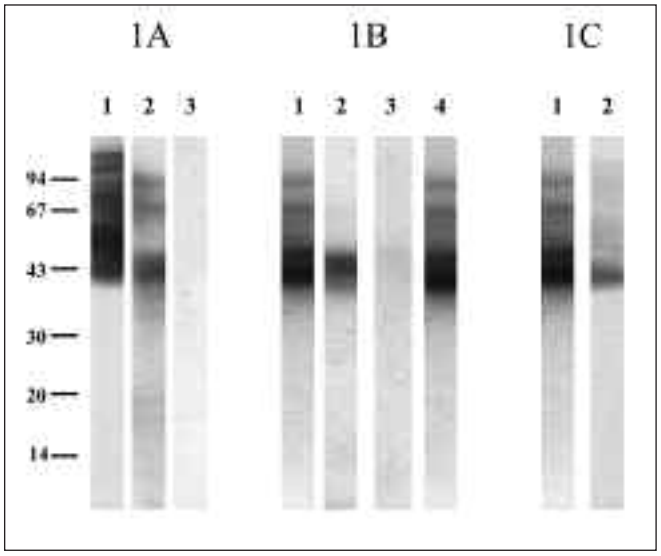
***Table 1*** - ELISA and cross-inhibition studies results.

| | OD | % inhibition | |
|---|---|---|---|
| IgE reactivity to tomato extract | Uninhibited serum | 3284 | |
| | Serum pre adsorbed with banana extract (10 μg) | 1203 | 63 |
| | Serum pre adsorbed with banana extract (1 μg) | 1999 | 39 |
| | Serum pre adsorbed with banana extract (0.1 μg) | 3079 | 6 |
| IgE reactivity to banana extract | Uninhibited serum | 854 | |
| | Serum pre adsorbed with tomato extract (10 μg) | 563 | 34 |
| | Serum inhibited with house dust mite extract | 889 | 0 |

*IgE reactivity is expressed as optical density (OD); based on the mean values found in normal sera levels < 150 OD were considered negative*

gesting banana as the possible primary sensitizer. On immunoblot analysis IgE reactivity against proteins from 43 to 90 kDa in banana extract and against 43, 67, and 94 kDa proteins in tomato extract was found (Fig. 1A). Pre-absorption of patient's serum with banana abolished IgE reactivity to 67 and 94 kDa tomato allergens, whereas IgE reactivity against the 43-kDa-zone remained unchanged (Fig. 1B). In view of the reported presence of cross-reactive carbohydrate determinants (CCD) in tomato extracts (6) we investigated whether patient's IgE-reactivity to tomato and banana extract was at least in part directed to CCD. To this end we treated tomato extract-blotted nitrocellulose strip with sodium periodate in order to oxidise possible glycoprotein oligosaccharides (7). The IgE-binding pattern was then compared with that from the untreated strip. Periodate treatment induced the loss of IgE-binding to 67 and 94 kDa tomato components while IgE-reactivity against 43 kDa zone allergen was only partially reduced (Fig. 1C), suggesting that the 2 higher m.w. components were expressed as glycoproteins and that the IgE reactivity to 67 and 94 kDa was possibly due to CCD in both tomato and banana.

***Figure 1*** - A) Immunoblot analysis of patient's serum IgE reactivity to banana and tomato. Lane 1: IgE reactivity to banana extract; lane 2: IgE reactivity to tomato extract; lane 3 : IgE reactivity to banana or tomato extract of a normal control serum. B) Lane 1: IgE reactivity to tomato extract; lanes 2-4: IgE reactivity to tomato extract after pre-absorption of serum with banana extract, tomato extract, and house dust mite extract, respectively. C) IgE reactivity to tomato extracts before (lane 1) and after (lane 2) treatment of extract with sodium periodate.




Unfortunately the lack of patient's serum did not allow us to perform same experiments with banana extract to reinforce our hypothesis. The persistence of IgE reactivity to tomato 43 kDa allergen following pre-absorption of serum with banana extract suggests a co-sensitization to both foods.

## Discussion

Several tomato allergens have been described to date, including Lyc e 1 [profilin, m.w. 14 kDa (8)], Lyc e 2 (fructofuranosidase; 50 kDa), Lyc e 3 (lipid transfer protein, 6 kDa), Lyc e chitinase (31 kDa), Lyc e glucanase (55 kDa), and Lyc e peroxidase (44 kDa). The clinical relevance of each of these allergens is ill defined, with the exception of profilin which may cause oral allergy syndrome (9). The IgE profile of our patients does not correspond to any of these allergens with the possible exception of peroxidase. If so, this would be the proof that tomato peroxidase sensitisation can be clinically relevant. Patient's hypersensitivity to banana is very interesting as well. Banana allergy has been mostly described following primary natural rubber latex allergy due to hypersensitivity to 1,3-beta-glucanase (Hev b 2) and class-1 chitinase (Hev b 6/Mus a 2), or in patients with pollen allergy due to cross-reactivity with profilin (Mus a 1), but in this case reactivity to both natural rubber latex and profilin was ruled out. This patient seemingly reacted to hitherto not yet described tomato and banana allergens.

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# News

**Record Pollen Season Brings Misery across Country Allergists Offer Survival Tips**

ARLINGTON HEIGHTS, Ill. – Record snow, heavy early spring rains, followed by a rapid warm up have created the perfect storm for allergy season. But allergists from the American College of Allergy, Asthma and Immunology can offer ways to help people find relief.

"It's one of the worst seasons we have seen for tree pollens, but there's no reason to suffer, you can get relief," said allergist Dr. Sami Bahna, president of the American College of Allergy, Asthma and Immunology (ACAAI). "In addition to over the counter medications, relief options include immunotherapy, allergy testing and vaccine and prescription medications."

Eight in 10 patients in an ACAAI consumer survey said taking matters into their own hands with self-medication falls short of being "very effective." The survey found that those who had seen an allergist were nearly three times more likely to say their treatment was effective than those who took over-the-counter medicine.

Allergists recommend allergy sufferers:

Know your triggers. You may think you know that pollen is causing your suffering, but other substances may be involved as well. More than two-thirds of spring allergy sufferers actually have year-round symptoms. An allergist can help you find the source of your suffering and stop it, not just treat the symptoms.

Work with your allergist to devise strategies to avoid your triggers, such as:

- Monitor pollen and mold counts — most media report this information during allergy seasons.
- Keep windows and doors shut at home, and in your car during allergy season.
- Stay inside during mid-day and afternoon hours when pollen counts are highest.
- Take a shower, wash hair and change clothing after being outdoors working or playing.
- Wear a mask when doing outdoor chores like mowing the lawn. An allergist can help you find the type of mask that works best.

One of the most effective ways to treat pollen allergies is with immunotherapy. These injections slowly introduce a little bit of what causes your allergy, so your body learns to tolerate it rather than react with sneezing, a stuffy nose or itchy, watery eyes.

Visit www.AllergyAndAsthmaRelief.org to take a simple online test to gauge allergy symptoms, obtain a personalized plan on how to get relief or to find an allergist.

**About ACAAI**

The ACAAI is a professional medical organization headquartered in Arlington Heights, Ill., that promotes excellence in the practice of the subspecialty of allergy and immunology. The College, comprising more than 5,000 allergists-immunologists and related health care professionals, fosters a culture of collaboration and congeniality in which its members work together and with others toward the common goals of patient care, education, advocacy and research.

Editor's Note: Allergists across the country are available to talk about the allergy season. Please contact Sara Brazeal at 312-558-1770 to arrange an interview.

**Herbal Remedies Linked to Poor Asthma Control**

ARLINGTON HEIGHTS, Ill. – Use of herbal remedies results in poorer quality of life and increased frequency of symptoms in asthma patients, according to a study published this month in Annals of Allergy, Asthma & Immunology, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

"Results indicate patients using herbal remedies are less likely to take their prescribed medications," said Angkana Roy, M.D., lead author, Department of Pediatrics, Mount Sinai School of Medicine, New York. "These patients report worse asthma control and poorer quality of life than patients who follow medica-

tion plans. Underuse of prescribed medication is one of the main factors contributing to poor outcomes in asthma patients."
The study tracked 326 asthma patients over a 33-month period. Of those, 25 percent reported herbal remedy use and lower adherence to use of prescribed inhaled corticosteroids (ICS). Patients using herbal remedies were younger, more likely to have been hospitalized or intubated for asthma, have concerns about possible adverse effects of ICS and have difficulty following a medication schedule.
"Patients interested in herbal remedies need to use them to complement treatment and not as an alternative, or they will not maximize their health and may actually hinder it as this study shows," said Leonard Bielory, M.D., ACAAI Integrative Medicine Committee chair. "Remember, asthma is a serious disease and needs to be treated that way. Always ask your allergist about medication concerns and discuss use of herbal remedies."
Consumers and patients can take a simple online test to gauge their asthma symptoms and obtain a personalized plan on how to get relief at www.AllergyAndAsthmaRelief.org.
"Anyone with asthma should be able to feel good, be active all day and sleep well at night," said Dr. Bielory. "No one should accept anything less."

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To learn more about allergies and asthma and to find an allergist, visit www.AllergyAndAsthmaRelief.org