

Expert Opinion

1. Introduction
2. Data from the literature
3. Results of the systematic review
4. Conclusion
5. Expert opinion

Safety of hymenoptera venom immunotherapy: a systematic review

Cristoforo Incorvaia[†], Franco Frati, Ilaria Dell'Albani, Anna Robino, Eleonora Cattaneo, Marina Mauro, Marie David, Rosanna Qualizza & Elide Pastorello

[†]Allergy/Pulmonary Rehabilitation, Istituti Clinici di Perfezionamento, Milan, Italy

Introduction: The efficacy of venom immunotherapy (VIT) in patients with insect sting allergy is not questioned. However, its safety, especially when honeybee is used, is a matter of concern.

Areas covered: A systematic review of the literature on VIT was done, with both aqueous and depot extracts, to compare the frequency of systemic reactions to honeybee and vespid venoms. A Medline search was performed using the keywords 'venom immunotherapy', 'safety' and 'tolerability'. The articles obtained were analyzed regarding the total number of patients treated with either honeybee or vespid VIT, the number and severity of systemic reactions during therapy, the type of extract used (aqueous or depot) and the administration regimen.

Expert opinion: The incidence of systemic reactions to VIT was 25.1% for honeybee venom and 5.8% for vespid venom ($p < 0.0001$), while it was similar with aqueous and depot extracts in the whole population of patients. This confirms that during VIT systemic reactions are significantly more frequent with honeybee venom compared with vespid venom, while there are no significant overall differences in systemic reactions between aqueous and depot extracts.

Keywords: aqueous, depot, honeybee, hymenoptera venom, systemic reactions, venom immunotherapy, vespids

Expert Opin. Pharmacother. (2011) 12(16):2527-2532

1. Introduction

Hymenoptera venom allergy affects ~ 2 – 3% of the general population and up to 40% of at-risk populations such as beekeepers [1]. Although fatalities are rare, allergy to insect stings has a significant impact on patients' quality of life. Venom immunotherapy (VIT) is accepted as a very effective treatment that offers full protection for 83 – 95% of subjects with a history of systemic allergic reactions to an insect sting [2,3] and remains effective once the therapy is stopped [4,5]. Since the introduction of venom extracts in the 1970s, VIT has become increasingly popular, and several different administration regimens have been developed to shorten the time required to reach the maintenance period and to minimize side effects. To achieve the maintenance dose considered to be protective (100 µg), the slow conventional regimen requires several weeks or months, the cluster regimen a few weeks, the rush regimen a few days and the ultrarush regimen a few hours [6]. Adverse reactions to VIT are relatively common during the build-up phase and systemic reactions occur in up to one-third of patients [6], but the numbers vary depending on the VIT regimen used. Most studies have demonstrated that patients treated with honey bee venom are at higher risk of systemic reactions to VIT than patients treated with vespid venom [6]. Several different approaches have been developed to reduce the

informa
healthcare

Article highlights.

- Venom immunotherapy (VIT) is on the whole a safe treatment, and so far no fatal reactions – in contrast to immunotherapy with inhalant allergens – have been reported.
- The rate of systemic reactions to VIT reported in single studies is generally higher in patients treated with honeybee venom than in patients treated with vespid venom, and some studies have reported better safety for depot venom compared with aqueous venom.
- This systematic review of the literature, analyzing an overall number of 3615 patients treated with VIT, confirms that systemic reactions to VIT are significantly more frequent using honeybee venom than vespid venom (25.1 vs 5.8%; $p < 0.0001$).
- The incidence of systemic reactions was similar with aqueous and depot extracts in the overall population of patients and in those treated with vespid venom. It was lower with the depot compared with the aqueous extract for honeybee venom; but the data were obtained from studies using different regimens.
- The analysis is limited by the quality of the studies evaluated (mostly retrospective), by the various definitions of systemic reaction used in the different studies, and by the lack of description of possible confounders of systemic reactions.

This box summarizes key points contained in the article.

incidence of adverse reactions and to improve the safety of VIT. Premedication with antihistamines significantly reduces the number and severity of large local reactions and mild systemic reactions such as urticaria [7,8], and one study has also reported a reduced incidence of severe systemic reactions [8]. To establish a safer therapy, various modifications of the venom used for VIT have been proposed, including chemical modification with monomethoxy polyethylene glycol [9], use of tolerogenic peptides of major allergens [10] and adsorption of allergens to insoluble substances such as aluminium hydroxide [11]. However, a number of variables may influence the safety of VIT and these must also be taken into account when comparing clinical studies. To obtain reliable data on the incidence of adverse reactions to VIT, the reactions of a large population of patients must be considered.

The aim of this study was to review the literature on VIT with both aqueous and depot extracts and to estimate the frequency of systemic adverse reactions to such therapy.

2. Data from the literature

A search of the literature in the Medline database for the period 1980 – 2011 was done using the following keywords: 'venom immunotherapy', 'safety' and 'tolerability'. The search was augmented by scanning the references cited in the articles identified. Only full-length articles in English published in referenced journals were considered. Full copies of the articles were retrieved for analysis, and the relevant data

were extracted and entered into a database management program. Only articles with the following characteristics were considered in the systematic review: both build-up phase and maintenance phase of VIT were evaluated; a maintenance dose of 100 µg was used; the number of treated patients and the number of adverse reactions to VIT were reported; and a distinction was made between the type of venom (honeybee and vespid venom) and the type of extract used (aqueous extracts and depot extracts).

The incidence of systemic reactions to VIT was compared in the different treatment groups with a chi-square test: i) honeybee venom versus vespid venoms; ii) aqueous extract versus depot extract; iii) aqueous extract versus depot extract taking into account the type of venom used. A level of probability $> 5\%$ ($p < 0.05$) was regarded as significant.

Using the Medline search as well as manual methods, 80 articles were identified, 22 of which met the criteria for inclusion in the systematic review. Sixteen studies used an aqueous extract, two used a depot extract, and four studies compared aqueous and depot extracts. In these studies, almost all the patients treated with an aqueous extract underwent rush immunotherapy, while those treated with a depot extract underwent a conventional slow protocol or cluster immunotherapy.

3. Results of the systematic review

Details of the number of patients treated and the frequency of systemic reactions during immunotherapy with honeybee or vespid venoms are reported in Table 1 (aqueous extract) and Table 2 (depot extract). In total, 3511 patients received an aqueous extract, 1026 with honeybee venom and 2485 with vespid venom; 154 patients received a depot extract, 90 with honeybee venom and 64 with vespid venom. The global incidence of systemic reactions to VIT was 11.8%. A significantly higher incidence of systemic reactions was found in patients treated with honeybee venom (25.1%) than in those treated with vespid venom (5.8%, $p < 0.0001$; Table 3). For all the patients, the incidence of systemic reactions was comparable to the aqueous (12.2%) and depot extracts (9.7%). There was also no difference with aqueous and depot extracts of vespid venom (5.8 vs 7.8%), but the incidence of SR was significantly lower in patients treated with the depot extract of honeybee venom (11.1%) than in those treated with the aqueous extract (26.3%, $p = 0.013$; Table 3).

4. Conclusion

The present systematic review analyzed the published data on the safety of VIT from studies reporting both build-up and maintenance phase, confirming the well-known finding of a higher safety profile for vespid than for honeybee venom immunotherapy. In terms of the safety of aqueous and depot extracts, no significant differences in systemic reactions were found in the overall population of patients receiving the different preparations as well as in those treated with vespids

Table 1. Studies with aqueous extracts.

Ref.	No. of patients	No. of SR (%)	Honeybee venom		Vespid venoms	
			No. of patients	No. of SR (%)	No. of patients	No. of SR (%)
Lockey <i>et al.</i> , 1990 [13]	1410	168 (11.9)	224	90 (40.2)	1236	78 (6.3)
Müller <i>et al.</i> , 1992 [14]	205	33 (16.1)	148	31 (20.9)	57	3 (5.3)
Birnbaum <i>et al.</i> , 1993 [15]	284	34 (12)	91	24 (26.4)	193	10 (5.2)
Bernstein <i>et al.</i> , 1994 [16]	77	4 (5.2)	10	3 (30)	67	1 (1.5)
Youlten <i>et al.</i> , 1995 [17]	109	24 (22)	26	12 (46.1)	83	12 (14.4)
Laurent <i>et al.</i> , 1997 [18]	97	7 (7.2)	24	4 (16.7)	73	5 (6.8)
Quercia <i>et al.</i> , 2001 [19]	40	7 (17.5)	40	7 (17.5)	-	-
Sturm <i>et al.</i> , 2002 [20]	101	8 (7.9)	52	7 (13.5)	49	1 (2)
Birnbaum <i>et al.</i> , 2003 [21]	325	36 (11.1)	90	27 (30)	235	9 (3.8)
Wenzel <i>et al.</i> , 2003 [22]	178	23 (12.9)	26	8 (30.8%)	152	7 (4.6)
Rüeff <i>et al.</i> , 2004 [23]	42	16 (38.1)	42	16 (38.1)	-	-
Schiavino <i>et al.</i> , 2004 [24]	57	4 (7)	9	1 (11.1)	48	3 (6.2)
Steiss <i>et al.</i> , 2006 [25]	43	0	16	0	27	0
Pasaoglu <i>et al.</i> , 2006 [26]	14	1 (7.1)	7	1 (14.3)	7	0
Roll <i>et al.</i> , 2006 [27]	80	10 (12.5)	33	4 (12.1)	47	6 (12.8)
Quercia <i>et al.</i> , 2006 [28]	40	8 (20)	40	8 (20)	-	-
Alessandrini <i>et al.</i> , 2006 [29]	73	5 (6.8)	-	-	73	5 (6.8)
Gorska <i>et al.</i> , 2008 [30]	118	14 (11.9)	74	11 (14.9)	44	1 (2.2)
Kalogeromitros <i>et al.</i> , 2010 [31]	65	9 (13.8)	29	7 (24)	36	2 (5.5)
Spoerl <i>et al.</i> , 2011 [32]	103	10 (10.3)	45	9 (20)	58	1 (1.7)
Total	3511	414 (11.8)	1026	270 (26.3)	2485	144 (5.8)

SR: Systemic reactions.

Table 2. Studies with depot extracts.

Ref.	No. of patients	No. of SR (%)	Honeybee venom		Vespid venoms	
			No. of patients	No. of SR (%)	No. of patients	No. of SR (%)
Mosbech <i>et al.</i> , 1986 [11]	12	3 (25)	-	-	12	3 (25)
Wyss <i>et al.</i> , 1993 [33]	40	0	22	0	18	0
Quercia <i>et al.</i> , 2001 [19]	15	0	15	0	-	-
Rüeff <i>et al.</i> , 2004 [23]	23	8 (34.8)	23	8 (34.8)	-	-
Quercia <i>et al.</i> , 2006 [28]	30	2 (6.7)	30	2 (6.7)	-	-
Alessandrini <i>et al.</i> , 2006 [29]	34	2 (5.9)	-	-	34	2 (5.9)
Total	154	15 (9.7)	90	10 (11.1)	64	5 (7.8)

SR: Systemic reactions.

venom, while a significantly lower frequency of reactions with the depot extract was detected in patients treated with honeybee venom. Given that different administration regimens were used – rush with the aqueous extract and cluster with the depot extracts – additional studies comparing the two extracts and using a larger number of patients and the same administration regimen are needed to confirm our observation.

5. Expert opinion

Specific immunotherapy with aqueous allergen extracts was introduced at the beginning of the 20th century for the treatment of respiratory allergies. In the 1920s, this therapy

was applied to patients allergic to Hymenoptera venom using a whole-body extract. The whole-body extract was replaced in the late 1970s by venom extracts after these were demonstrated to have significant efficacy compared with the mere placebo effect associated with the whole-body extract [2,3]. In the 1940s, depot extracts were developed using inhalant allergens adsorbed to insoluble substances and slowly released from the injection site. These preparations were associated with fewer adverse reactions and, over time, depot extracts almost completely replaced aqueous extracts (especially in Europe) in immunotherapy with inhalant allergens, owing to their superior safety profile. One study in a large population of patients treated with aeroallergen extracts reported that an

Table 3. Comparison of subgroups based on type of venom and type of extracts.

Comparison	No. of systemic reactions per patient (%)	Significance
Honey bee vs vespids	280/1116 (25.1)	p < 0.0001
Aqueous vs depot	vs. 149/2549 (5.8)	
	421/3511 (12)	NS
	vs. 15/154 (9.7)	
Aqueous vs depot honeybee	270/1026 (26.3)	p = 0.013
	vs. 10/90 (11.1)	
Aqueous vs depot vespids	144/2485 (5.8)	NS
	vs. 5/64 (7.8)	

8-week induction regimen using alum depot extracts showed a significantly lower frequency and severity of systemic reactions compared with aqueous extracts delivered via the same regimen [12].

However, so far, VIT has mainly been performed using aqueous extracts; these have an acceptable risk profile and allow for the more rapid induction regimens that are often required in a potentially life-threatening condition such as Hymenoptera venom hypersensitivity. A number of studies have been published on the efficacy and safety of aqueous and depot extracts for VIT [13-33]. In any case, the comparison of the safety of the different extracts is limited by the scarcity of data, as the majority of such studies have involved only one or a few research centers and limited populations of patients, and very few studies have compared the different types of extracts.

The present systematic review aimed to collect the published data on the safety of VIT, focusing on studies that i) reported the number of patients treated and the number of adverse reactions to VIT, ii) specified the type of venom (honeybee vs vespids) and the type of extract (aqueous vs depot) and iii) used the same maintenance dose of 100 µg. A total of 22 studies were analysed, 16 using aqueous extract, 2 using depot extract and 4 comparing aqueous and depot extracts. The global incidence of systemic reactions to VIT was 11.8%, with a highly significant greater incidence of systemic reactions in patients treated with honeybee venom (25.1%) than in those treated with vespids venom (5.8%). This result confirms the common observation from a number of studies (such as those reported in Tables 1 and 2), that honeybee venom causes many more adverse reactions than vespids venom; the reasons for this difference remain unclear. By contrast, the incidence of systemic reactions was similar with aqueous and depot extracts among the overall patient population as well as

among those treated with vespids venom, but it was significantly lower ($p = 0.013$) in patients treated with depot than in those treated with aqueous extracts of honeybee venom. In 2000, a multicenter European study compared 656 patients treated with aqueous and 122 patients treated with depot extracts and found no difference in the rate of systemic reactions, but no separate data on the use of honeybee or vespids venom were reported [34]. Subsequent studies evaluating depot venom extracts included only small groups of patients and the total number of patients available for the systematic review was just 154 patients (90 treated with honeybee and 64 treated with vespids venoms). Of these studies, those directly comparing aqueous and depot extracts reported a better tolerability of depot VIT in terms of local reactions [19,23,28,29], but only one reported a significantly lower frequency of systemic reactions to honeybee venom with the depot extract than with the aqueous extract [28]. It is important to note that these studies used a cluster or conventional regimen ('slow' schedules) for depot immunotherapy and a rush or ultrarush regimen ('rapid' schedule) for aqueous immunotherapy, making the comparison flawed, as it has been reported that rapid regimens are a risk factor for developing systemic adverse reactions to VIT [34]. In fact, when both aqueous and depot extracts were administered using a cluster schedule and honeybee venom, the rates of systemic reactions reported were 5 and 3.4%, respectively, compared with a rate of 36.8% for an aqueous extract administered via a rush regimen [28].

The limits of our analysis are: i) the quality of the evaluated studies, which were mostly retrospective, only a few prospective studies being available; ii) the various definitions of systemic reactions used in the different studies; and iii) the lack of description of possible confounders of systemic reactions such as comorbidities and use of drugs associated with an increased risk of systemic reactions to VIT, for example ACE inhibitors [35]. Moreover, other factors of emerging importance related to safety were not considered in the studies included in the systematic review. These are the measurement of baseline serum tryptase, a high concentration of which was recently reported as a risk factor for systemic reactions to vespids venom in the build-up phase [36], and the use of purified venom preparations, which are supposedly better tolerated than nonpurified venoms [37].

Declaration of interest

C Incorvaia is a scientific consultant for Stallergenes Italy. A Robino has received a grant from Stallergenes Italy for this study. All other authors have no conflict of interest.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

1. Antonicelli L, Bilo MB, Bonifazi F. Epidemiology of Hymenoptera allergy. *Curr Opin Allergy Clin Immunol* 2002;2:341-6
2. Hunt KJ, Valentine MD, Sobotka AK, et al. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978;299:157-61
- **Introduced venom immunotherapy.**
3. Ross RN, Nelson HS, Finegold I. Effectiveness of specific immunotherapy in the treatment of hymenoptera venom hypersensitivity: a meta-analysis. *Clin Ther* 2000;22:351-8
4. Lerch E, Muller UR. Long-term protection after stopping venom immunotherapy: results of re-sting in 200 patients. *J Allergy Clin Immunol* 1998;101:606-12
5. Golden DB, Kagey-Sobotka A, Lichtenstein LM. Survey of patients after discontinuing venom immunotherapy. *J Allergy Clin Immunol* 2000;105:385-90
6. Bonifazi F, Jutel M, Bilo MB, et al. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;60:1459-70
- **The European guidelines on venom immunotherapy.**
7. Reimers A, Hari Y, Muller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine: a double-blind, placebo-controlled trial. *Allergy* 2000;55:484-8
8. Brockow K, Kiehn M, Riethmuller C, et al. Efficacy of antihistamine pretreatment in the prevention of adverse reactions to Hymenoptera immunotherapy: a prospective, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 1997;100:458-63
9. Muller U, Lanner A, Schmid P, et al. A double blind study on immunotherapy with chemically modified honey bee venom: monomethoxy polyethylene glycol-coupled versus crude honey bee venom. *Int Arch Allergy Appl Immunol* 1985;77:201-3
10. Muller U, Akdis CA, Fricker M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;101:747-54
11. Mosbech H, Malling HJ, Biering I, et al. Immunotherapy with yellow jacket venom. A comparative study including three different extracts, one adsorbed to aluminium hydroxide and two unmodified. *Allergy* 1986;41:95-103
12. Møllerup MT, Hahn GW, Poulsen LK, Malling H. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and systemic side effects during induction treatment. *Clin Exp Allergy* 2000;30:1423-9
13. Lockey RF, Turkeltaub PC, Olive ES, et al. The Hymenoptera venom study. III: Safety of venom immunotherapy. *J Allergy Clin Immunol* 1990;86:775-80
- **The first evaluation of safety of venom immunotherapy.**
14. Muller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J Allergy Clin Immunol* 1992;89:529-35
15. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy: comparative safety of three protocols. *Clin Exp Allergy* 1993;23:226-30
16. Bernstein JA, Kagen SL, Bernstein DI, Bernstein IL. Rapid venom immunotherapy is safe for routine use in the treatment of patients with Hymenoptera anaphylaxis. *Ann Allergy* 1994;73:423-8
17. Youten LJF, Atkinson BA, Lee TH. The incidence and nature of adverse reactions to injection immunotherapy in bee and wasp venom allergy. *Clin Exp Allergy* 1995;25:159-65
18. Laurent J, Smiejan JM, Bloch-Morot E, et al. Safety of Hymenoptera venom rush immunotherapy. *Allergy* 1997;52:94-6
19. Quercia O, Rafanelli S, Puccinelli P, Stefanini GF. The safety of cluster immunotherapy with aluminium hydroxide-adsorbed honey bee venom extract. *J Investig Allergol Clin Immunol* 2001;11:27-33
20. Sturm G, Kranke B, Rudolph C, Aberer W. Rush Hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. *J Allergy Clin Immunol* 2002;110:928-33
21. Birnbaum J, Ramadour M, Magnan A, Vervloet D. Hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. *Clin Exp Allergy* 2003;33:58-64
22. Wenzel J, Meissner-Kraemer M, Bauer R, et al. Safety of rush insect venom immunotherapy. The results of a retrospective study in 178 patients. *Allergy* 2003;58:1176-9
23. Rueff F, Wolf H, Schnitker J, et al. Specific immunotherapy in honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. *Allergy* 2004;59:589-95
24. Schiavino D, Nucera E, Pollastrini E, et al. Specific ultrarush desensitization in Hymenoptera venom-allergic patients. *Ann Allergy Asthma Immunol* 2004;92:409-13
25. Steiss JO, Jodicke B, Lindemann H. A modified ultrarush insect venom immunotherapy protocol for children. *Allergy Asthma Proc* 2006;27:148-50
26. Pasaoglu G, Sin BA, Misirligil Z. Rush hymenoptera venom immunotherapy is efficacious and safe. *J Investig Allergol Clin Immunol* 2006;16:232-8
27. Roll A, Hofbauer G, Ballmer-Weber BK, Schmid-Grendelmeier P. Safety of specific immunotherapy using a four-hour ultra-rush induction scheme in bee and wasp allergy. *J Investig Allergol Clin Immunol* 2006;16:79-85
28. Quercia O, Emiliani F, Pecora S, et al. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: comparison of standardized quality depot versus aqueous extract. *Allergy Asthma Proc* 2006;27:151-8
29. Alessandrini AE, Berra D, Lodi Rizzini F, et al. Flexible approaches in the design of subcutaneous immunotherapy protocols for Hymenoptera venom allergy. *Ann Allergy Asthma Immunol* 2006;97:92-7
30. Gorska L, Chelminska M, Kuziemiński K, et al. Analysis of safety, risk factors and pretreatment methods during rush

- hymenoptera venom immunotherapy. *Int Arch Allergy Immunol* 2008;147:241-5
31. Kalogeromitros D, Makris M, Koti I, et al. A simple 3-day rush venom immunotherapy protocol: documentation of safety. *Allergol Immunopathol (Madr)* 2010;38:69-73
32. Spoerl D, Bircher AJ, Scherer K. Safety profile of hymenoptera venom immunotherapy (VIT) in monosensitized patients: lack of new sensitization to nontreated insect venom. *J Investig Allergol Clin Immunol* 2011;21:22-7
33. Wyss M, Cheitlin T, Stadler BM, et al. Immunotherapy with aluminum hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. *Allergy* 1993;48:81-6
34. Mosbech H, Muller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. *Allergy* 2000;55:1005-10
- **The largest multicenter prospective study on safety of venom immunotherapy.**
35. Stumpf JL, Shehab N, Patel AC. Safety of angiotensin-converting enzyme inhibitors in patients with insect venom allergies. *Ann Pharmacoter* 2006;40:699-703
36. Rueff F, Przybilla B, Bilo MB, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol* 2010;126:105-11
37. Bilo MB, Antonicelli L, Bonifazi F. Purified vs. nonpurified venom immunotherapy. *Curr Opin Allergy Clin Immunol* 2010;10:330-6

Affiliation

Cristoforo Incorvaia^{†1}, Franco Frati², Ilaria Dell'Albani², Anna Robino³, Eleonora Cattaneo², Marina Mauro⁴, Marie David⁵, Rosanna Qualizza⁶ & Elide Pastorello³

[†]Author for correspondence

¹Allergy/Pulmonary Rehabilitation, Istituti Clinici di Perfezionamento, Via Bignami 1, 20126 Milan, Italy
Tel: +39 02 5799 3289; Fax: +39 02 5799 3262;
E-mail: cristoforo.incorvaia@gmail.com

²Scientific Department, Stallergenes, viale Certosa2, 20155 Milan, Italy

³Niguarda Ca' Granda Hospital, Unit of Allergology and Immunology, piazza Ospedale Maggiore 3, 20162 Milan, Italy

⁴Sant'Anna Hospital,

Allergy Service,
via Napoleona 60,
22100 Como, Italy

⁵Scientific Department,
Stallergenes, rue Alexis de Tocqueville 92183
Antony,
France

⁶Allergy Service,
Istituti Clinici di Perfezionamento,
via Farini 9, 20154 Milan,
Italy