Short Communication

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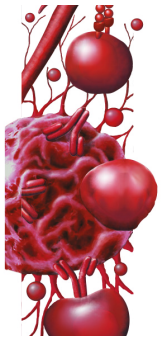
Immunotherapy



Safety of uSCIT-MPL-4: prevalence and risk factors of systemic reactions in real life

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Aim: We assessed the safety of allergoid adjuvanted by monophosphoryl lipid A (uSCIT-MPL-4) in a real-life setting. **Materials & methods:** Patients treated with uSCIT-MPL-4 were followed-up for 1 year. Systemic reactions (SRs) were registered and the association with potential risk factors was evaluated. **Results:** 2929 patients were included. Grade 0, 1, 2, 3 and 4 SR reactions were observed respectively in 3.3, 1.5, 0.31, 0.07 and 0.07% of patients. A significant association was detected between Grade ≥1 SRs and: female gender, number of administrations, previous local reactions. **Conclusion:** uSCIT-MPL-4 is safe. Local reactions should be accurately assessed as they may represent a risk factor for Grade ≥1 SRs, together with gender and number of doses/year.

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Keywords: adjuvants • allergic asthma • allergic rhinitis • immunotherapy • local reactions • risk factors • safety • subcutaneous immunotherapy • systemic reaction grading system • systemic reactions

Specific allergen immunotherapy (SIT) is currently the only immune-modifying treatment for allergic asthma and rhinitis. In addition to its proven clinical efficacy, it can potentially alter the natural history of allergic disease and produce sustained clinical remission after discontinuation [1–6]. The safety of subcutaneous allergen immunotherapy (SCIT) is well documented and fatal reactions related to SCIT are rare: 1 event in 2.5 million injections has been reported in the USA [7] and none in Europe [8]. The potential risk of injection-related allergic systemic reactions (SRs), being a fundamental parameter in the overall assessment of the treatment, has been estimated over the years in several studies [7–9]. However, the risk of SRs remains a major concern: in fact, though the overall safety of SCIT is well accepted, it should be demonstrated for the single extracts of each brand through a uniform SRs classification and grading system. Studies providing evidence for SIT are only available for some marketed products [8], however, because of a lack of differentiation between products, this evidence often is taken 'granted' for all SIT products. These generalizations are not consistent and should therefore be avoided [10]. Furthermore a uniform classification system for grading SCIT-associated SRs should be used in all the studies on that topic and on this regard, the World Allergy Organization has provided a subcutaneous immunotherapy systemic reaction grading system (WAO SCIT SRs Grading System) [11] which allows an easy and standardized data comparison.

In order to improve SCIT effectiveness, different optimized and enhanced formulations have been produced, being less allergenic and maintaining the potential for developing immune tolerance. An optimized SCIT formulation, which consists in an allergoid-adjuvant by monophosphoryl lipid A (uSCIT-MPL-4), has been developed. It maintains characteristics comparable to traditional SCIT in terms of immunogenicity, safety and tolerability. The lipopolysaccharide component of Salmonella Minnesota R 595 represents the source of monophosphoryl lipid A (MPL®). In order to preserve its potent adjuvant activity, primarily and mainly sustained by its interaction with

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Toll-like receptor 4, and to avoid at the same time unacceptable toxicity, a phosphate and fatty acid group have been removed from the lipid A portion of the endotoxin. MPL® is the result of the above described manipulation. The adjuvant activity of MPL® promotes primarily a T helper type 1 (Th1) response [14–16]. MPL® has been shown to be well tolerated and to enhance both humoral and cellular immune responses. Its long-term efficacy has been demonstrated by a recently published study [17] investigating the effect of uSCIT-MPL-4 up to 6 years after the treatment cessation. According to the authors' findings, when analysing symptoms control immediately after the treatment stop and 3–6 years after, no significant changes occurred. Furthermore IgG antibodies, although decreasing after the treatment cessation, appeared to be significantly higher in comparison with nontreated subpopulation, at every time-point. An ultra-short pre-seasonal schedule (Rush Immunotherapy, RIT) is recommended, with only one injection every 4 weeks for 4 months, and therefore it is expected to be convenient in terms of adherence and cost-benefit sustainability [18]. To the best of our knowledge few data on a small population are currently available about uSCIT-MPL-4 safety in real life [19]. In this study, we aimed at assessing safety and tolerability of uSCIT-MPL-4, as well as their determinants, in a large real-life setting.

Materials & methods

We conducted a spontaneous prospective observational survey involving 13 Allergy Units in Italy. The study included consenting patients with type I allergy caused by pollen allergens and affected by rhino-conjunctivitis and/or asthma, consecutively addressed to the first cycle of uSCIT-MPL-4. The severity of asthma and rhinitis was graded according to GINA and ARIA guidelines [20,21] respectively. Patients were collected between October 2012 and February 2013 and followed-up for 1 year. Each participating centre was asked to record data from patients, including age, gender, rhinitis ARIA classification, asthma GINA classification, sensitization profile, SIT allergen extract and treatment schedule. During regular visits data concerning SIT tolerability and safety were recorded and SRs were encoded according to the World Allergy Organization subcutaneous immunotherapy SR grading system [11]. An extra grade, labelled Grade 0, was included to define non-specific symptoms not likely to be associated with a SCIT injection, such as headaches or arthralgia. Participating physicians were also asked to notify the use of epinephrine if needed. The Review Boards approved the procedure and written informed consent was given by all participants.

Statistical analysis

The relevance of patient-related variables, including sex, age, disease type and severity, previous local reactions (LRs) and treatment-related factors, including allergen and schedule, as risk factors for severe SRs were investigated through multivariate logistic regression. In order to adapt statistical analysis to the sample size the Fisher's Exact Test was applied and Pearson $\chi 2$ test (Chi-square) was used for comparing nominal data. The Student's "t" Test for independent samples was applied for data expressed as continuous variables. A p-value equal to or less than 0.05 ($p \leq 0.05$) was considered the threshold of statistical significance. Statistical analyses were done using IBM SPSS® 23.0 [22].

Results

Overall 2929 patients (female: 61.5%; male: 38.5%; mean age: 32.8 ± 14.4) were included. Table 1 summarizes the patients' characteristics and a study findings overview. Most of subjects (83.4%) were affected by allergic rhinitis (AR), with or without allergic asthma (AA). AR and AA alone were observed in 36.1 and 16.6% of patients, respectively; in 47.3% both the diseases were present (Figure 1). The distribution of SIT allergen extracts was as follows: grass (most prescribed, 45.5%), parietaria (19.0%), ragweed (15.4%), trees (8.5%), allergen mix (5%), olive (4.10%), birch (2.4%) and mugwort (0.03%) (Figure 2). Poly-sensitization was detected in 46.7% of patients. Grade 0, 1, 2, 3 and 4 SR reactions were observed respectively in 3.3, 1.5, 0.31, 0.07 and 0.07% of patients (Figure 3). Epinephrine was used in five cases (0.17%) and no fatal events have been recorded. Table 2 (Appendix) provides an overview of patients' characteristics and SRs details. Female gender and severity of AR (ARIA classification) were significantly associated with Grade 0 SRs (OR = 2.67; 95% CI = 1.65–4.28; p = 0.0001). A significant clinical association with female gender was detected for Grade $\geq$1 SRs as well (OR = 2.56; 95% CI = 1.27–5.16; p = 0.009). Furthermore, a correlation between the treatment schedule (number of doses) and Grade $\geq$1 SRs was identified (OR = 1.32; 95% CI = 1.06–1.63; p = 0.012). Similarly, previous LRs showed a statistically significant association with Grade $\geq$1 SRs (OR = 5.80; 95% CI = 2.53–13.30; p = 0.0001). No relevant associations with other patient/SCIT related-factors were highlighted.

Table 1. Study population.

	Number	Gender (female/male)	Mean age, years (range)	GINA classification				ARIA classification				N grade 1 SRs	N grade 2 SRs	N grade 3 SRs	N grade 4 SRs	N grade 5 SRs
				1	2	3	4	1	2	3	4					
Center 1	199	105/94	34 (7–67)	42	67	24	1	38	80	35	44	7	1	0	0	0
Center 2	150	100/50	32 (9–60)	23	12	20	2	36	17	64	33	0	0	0	0	0
Center 3	121	70/51	37 (12–68)	33	13	23	0	16	28	34	39	5	1	0	0	0
Center 4	187	119/68	31 (8–63)	35	29	21	1	29	42	57	59	0	0	0	0	0
Center 5	189	106/83	34 (6–66)	55	38	29	5	64	45	42	38	2	0	0	0	0
Center 6	191	127/64	33 (7–72)	60	19	17	1	51	40	47	50	6	0	0	0	0
Center 7	184	117/67	24 (4–71)	33	19	5	1	33	50	54	47	20	3	1	0	0
Center 8	188	113/75	37 (12–68)	38	46	38	9	53	31	74	30	3	3	0	0	0
Center 9	196	117/79	34 (9–69)	36	40	45	4	80	36	22	58	0	1	0	0	0
Center 10	274	176/98	30 (4–68)	83	59	24	2	71	40	63	100	0	0	0	0	0
Center 11	117	59/58	32 (8–63)	29	15	2	0	20	2	20	75	1	0	0	0	0
Center 12	401	252/149	32 (4–67)	87	113	29	9	49	96	102	154	0	0	0	0	0
Center 13	532	340/192	35 (6–70)	82	89	75	9	125	95	153	159	0	0	1	2	0
Total	2929	1801/1128	32.8 (4–72)	636	559	352	44	665	602	767	886	44	9	2	2	0

GINA classification: 1, mild intermittent asthma; 2, mild persistent asthma; 3, moderate persistent asthma.
ARIA classification: 1, mild intermittent rhinitis; 2, mild persistent rhinitis; 3, moderate-severe intermittent rhinitis; 4, moderate-severe persistent rhinitis.
SR: Systemic reactions.

Table 2. Overview of patients' characteristics and systemic reactions' details.

Gender	Age (years)	GINA classification	ARIA classification	Allergen immunotherapy extract	Injections (n)	N grade 1 SRs	Description	N grade 2 SRs	Description	N grade 3 SRs	Description	N grade 4 SRs	Description	Epinephrine	AIT interruption	Previous local reaction
Female	27	2	2	Parietaria	4	0		1	Asthma	0		0		No	Yes	Yes
Male	52	2	3	Grass + Parietaria	4	1	Rhinitis	0		0		0		No	No	No
Male	25	No asthma	4	Grass + Mugwort	4	2	Rhinitis and conjunctivitis	0		0		0		No	No	No
Male	24	3	4	Grass pollen	4	1	Rhinitis	0		0		0		No	No	No
Male	20	3	4	Grass + House Dust Mites	4	1	Rhinitis	0		0		0		No	No	No
Female	54	3	4	Grass + Parietaria	4	1	Conjunctivitis	0		0		0		No	No	No
Female	23	2	4	Parietaria + House Dust Mites	4	1	Rhinitis and cough	0		0		0		No	No	No
Female	36	2	4	Olive + Mugwort	4	1	Rhinitis	0		0		0		No	No	No
Female	28	2	4	Grass + Cat	5	1	Rhinitis	1	Urticaria and rhinitis	0		0		Yes	No	No
Male	31	3	4	Grass + Parietaria	5	1	Rhinitis	0		0		0		No	No	No
Female	29	2	3	Parietaria	5	1	Rhinitis	0		0		0		No	No	No
Female	57	3	4	Parietaria	5	1	Rhinitis	0		0		0		No	No	No
Female	39	1	4	Grass + House Dust Mites	5	1	Rhinitis	0		0		0		No	No	No
Female	32	3	4	Parietaria + Birch	7	1	Rhinitis	0		0		0		No	No	No
Female	45	3	4	Grass + Parietaria	7	1	Conjunctivitis	0		0		0		No	No	No
Female	49	2	3	Birch pollen	4	1	Rhinitis	0		0		0		No	No	No
Female	36	No asthma	3	Grass pollen	14	1	Urticaria	0		0		0		No	No	No
Male	40	No asthma	1	Grass pollen	5	1	Rhinitis	0		0		0		No	No	No
Female	36	1	2	Parietaria	7	1	Urticaria	0		0		0		No	No	No
Female	27	1	1	Parietaria	7	1	Urticaria	0		0		0		No	No	No
Female	43	1	1	Parietaria	7	1	Rhinitis	0		0		0		No	No	No
Male	29	No asthma	2	Parietaria	14	0		0		1	Asthma	0		Yes	No	Yes

GINA classification: 1, mild intermittent asthma; 2, mild persistent asthma; 3, moderate persistent asthma.
ARIA classification: 1, mild intermittent rhinitis; 2, mild persistent rhinitis; 3, moderate-severe intermittent rhinitis; 4, moderate-severe persistent rhinitis.
AIT: Allergen immunotherapy; SR: Systemic reactions.

Table 2. Overview of patients' characteristics and systemic reactions' details (cont.).

Gender	Age (years)	GINA classification	ARIA classification	Allergen immunotherapy extract	Injections (n)	N grade 1 SRs	Description	N grade 2 SRs	Description	N grade 3 SRs	Description	N grade 4 SRs	Description	Epinephrine	AIT interruption	Previous local reaction
Female	41	No asthma	2	Grass pollen	6	1	Rhinitis	0		0		0		No	No	No
Female	13	1	2	Grass pollen	14	0		1	Urticaria and rhinitis	0		0		No	No	No
Male	22	2	2	Grass pollen	6	1	Rhinitis	0		0		0		No	No	No
Male	7	No asthma	3	Grass pollen	7	0		1	Urticaria and rhinitis	0		0		No	No	No
Male	4	No asthma	3	Grass pollen	7	1	Conjunctivitis	0		0		0		No	No	No
Male	13	No asthma	2	Grass pollen	7	1	Rhinitis	0		0		0		No	No	No
Male	9	No asthma	4	Grass pollen	7	1	Rhinitis	0		0		0		No	No	No
Male	9	No asthma	1	Grass pollen	7	0		1	Urticaria and rhinitis	0		0		No	No	No
Female	5	No asthma	4	Grass pollen	7	1	Nausea	0		0		0		No	No	No
Male	19	No asthma	3	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Male	4	No asthma	2	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Female	14	No asthma	2	Grass pollen	7	1	Rhinitis	0		0		0		No	No	No
Female	3	No asthma	4	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Female	7	No asthma	1	Grass pollen	7	1	Nausea	0		0		0		No	No	No
Male	4	No asthma	3	Parietaria	7	1	Rhinitis and cough	0		0		0		No	No	No
Female	71	No asthma	1	Grass pollen	7	1	Nausea	0		0		0		No	No	No
Male	4	No asthma	1	Grass pollen	7	1	Conjunctivitis	0		0		0		No	No	No
Female	9	No asthma	3	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Female	11	No asthma	3	Grass pollen	7	1	Rhinitis	0		0		0		No	No	No
Female	8	No asthma	2	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Male	8	No asthma	4	Grass pollen	7	1	Nausea	0		0		0		No	No	No
Female	15	No asthma	4	Grass pollen	7	1	Rhinitis and cough	0		0		0		No	No	No
Male	41	3	3	Grass pollen	4	1	Rhinitis	0		0		0		No	No	No
Male	30	No asthma	4	Grass pollen	4	1	Rhinitis	0		0		0		No	No	No
Female	47	1	3	Trees pollen	4	0		1	Urticaria and rhinitis	0		0		No	No	Yes
Female	44	2	3	Mugwort	4	0		1	Urticaria and rhinitis	0		0		No	No	Yes
Female	44	2	3	Mugwort	4	0		1	Urticaria and rhinitis	0		0		No	No	Yes

GINA classification: 1, mild intermittent asthma; 2, mild persistent asthma; 3, moderate persistent asthma.
ARIA classification: 1, mild intermittent rhinitis; 2, mild persistent rhinitis; 3, moderate-severe intermittent rhinitis; 4, moderate-severe persistent rhinitis.
AIT: Allergen immunotherapy; SR: Systemic reactions.

Table 2. Overview of patients' characteristics and systemic reactions' details (cont.).

Gender	Age (years)	GINA classification	ARIA classification	Allergen immunotherapy extract	Injections (n)	N grade 1 SRs	Description	N grade 2 SRs	Description	N grade 3 SRs	Description	N grade 4 SRs	Description	Epinephrine	AIT interruption	Previous local reaction
Male	28	4	1	Grass pollen	7	1	Urticaria	0		0		0		No	No	Yes
Female	26	2	2	Parietaria	4	1	Urticaria	0		0		0		No	No	Yes
Female	52	1	2	Mugwort	2	0		1	Urticaria and rhinitis	0		0		No	No	No
Female	21	No asthma	1	Parietaria	1	0		0		0		1	Hypotension	Yes	Yes	Yes
Male	50	2	2	Grass pollen	4	0		0		1	Asthma	0		Yes	No	No
Male	21	No asthma	3	Grass pollen	5	0		0		0		1	Hypotension	Yes	Yes	Yes

GINA classification: 1, mild intermittent asthma; 2, mild persistent asthma; 3, moderate persistent asthma.
ARIA classification: 1, mild intermittent rhinitis; 2, mild persistent rhinitis; 3, moderate-severe intermittent rhinitis; 4, moderate-severe persistent rhinitis.
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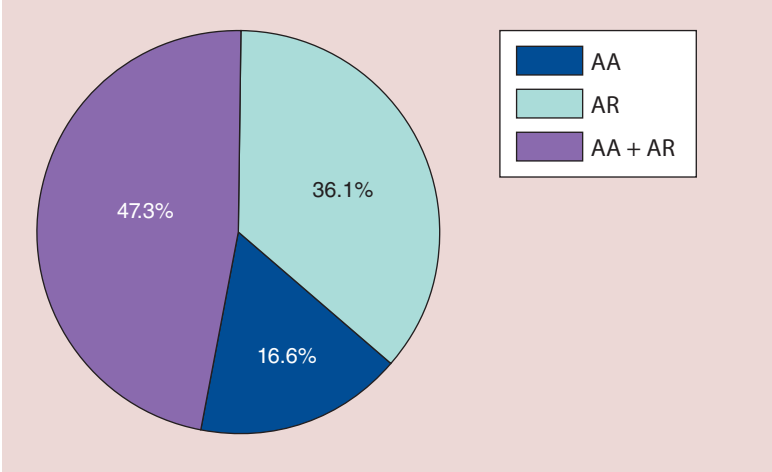

Figure 1. Prevalence of respiratory allergic diseases in the study population at baseline assessment. AA: Allergic asthma; AR: Allergic rhinitis.

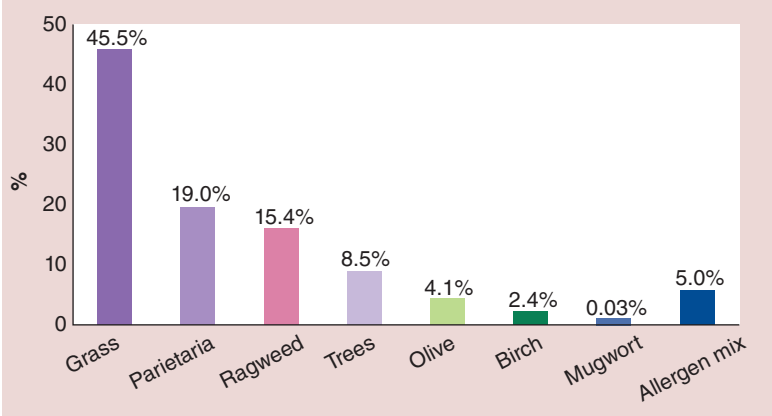

Figure 2. Proportion of different allergen extracts contained in the prescribed immunotherapy.

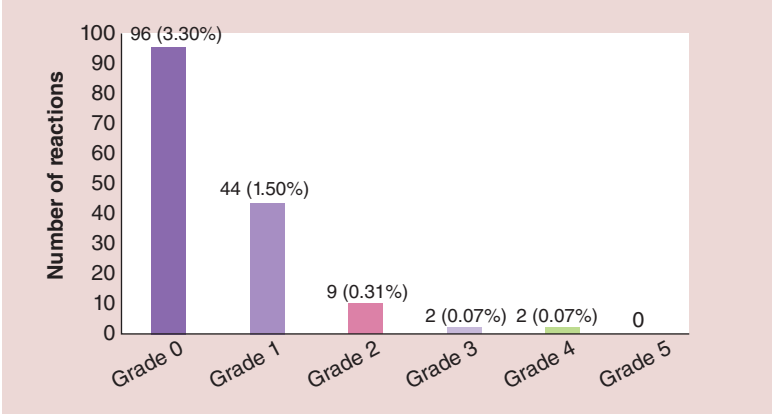

Figure 3. Frequency and severity of systemic reactions that occurred during the study time frame (1 year).

Discussion

The aim of this study was to evaluate the prevalence of adverse reactions and their determinants of safety in a real-life population undertaking uSCIT MPL4 for pollen allergy. As a main result, we described a high safety profile and the relevance of treatment schedule and previous LR and as potential risk factors for grade ≥1 SRs. The low rate of SRs, mostly grade ≤2 reactions, support the good risk/benefit ratio of allergoid uSCIT and the adjuvant monophosphoryl lipid A.

According to AAAAI/ACAAI annual prospective study from 2008 to 2012 and earlier retrospective studies, the number of fatal reactions to SCIT appears to be declining [23–27]. A large Danish survey [9], involving 1038 patients treated with SCIT for inhalant allergens and Hymenoptera Venom for a follow-up period of 3 years,

evaluated that SRs were corresponding to 33% of patients of which grade 3 and 4 SRs accounted respectively for 20 and 1% of the overall SRs. Although unproven, this recent decline in reported fatal reactions could be attributable to heightened awareness among prescribing allergists of contributing clinical factors to such events and implementation of practice measures to mitigate risk [27]. A careful risk assessment of patients, including the potential contraindications, and optimal administration procedures may significantly decrease the risk of adverse events (AEs). The European Academy of Allergy and Clinical Immunology (EAACI) Task Force on 'Contraindications to Allergen Immunotherapy' (AIT) [10] has clearly defined as absolute contraindications uncontrolled asthma, active malignant neoplasias, AIDS, age <2 years and pregnancy (for initiation of AIT) while relative contraindications are partially controlled asthma, use of β-blockers and ACE inhibitors, cardiovascular diseases, HIV infection, immunodeficiency, psychiatric and mental disorders and the use of immunosuppressive drugs.

However the risk of SRs remains a major concern: in fact, though the overall safety of SCIT is well accepted, it should be demonstrated for the single extracts of each brand through a uniform SRs classification and grading system.

Safety of uSCIT MPL4 have been evaluated by few studies up to now. A randomized, double-blind, placebo-controlled Phase IIb study assessed efficacy and safety of ragweed extract on 228 patients in with allergic rhino-conjunctivitis [28]. A standardized grading system was not applied, but the authors reported that headache, rhinorrhea and urticaria were the only drug-related non-local adverse reactions experienced by more than 2% of subjects in the active groups. No severe SRs, or deaths occurred and no epinephrine use was recorded. A 3-year post-marketing surveillance study evaluated safety data on a cohort of 3114 patients affected by allergic rhinitis, conjunctivitis and/or asthma [29]. In three patients SRs occurred. Rhinitis was reported in one case and two patients reported other SRs (excluding conjunctivitis, breathing problems, generalized urticaria and anaphylactic shock). No anaphylactic reactions or serious AEs were reported. Crivellaro *et al.* [19] assessed the safety of u-SCIT MPL4 in a 3-year multicentre real life trial conducted in Italy and involving 510 patients. Authors reported a similar SRs rate: overall 7 SRs were observed, corresponding to 1.37% of patients and 2.11/1000 injections; all SRs were classified as Grade 1 or 2 and epinephrine was not required for any of the reactions. Although conducted on a much larger population, our study reported similar results: Grade 0, 1, 2, 3 and 4 SR reactions were observed respectively in 3.3, 1.5, 0.31, 0.07 and 0.07% of patients. Epinephrine was used in five cases (0.17%) and no fatal events have been recorded. Basing on the available data, uSCIT MPL 4 seems to be at least as safe as the traditional SCIT, though our results have to be confirmed in larger studies.

A similar product, a fast up-dosed immunologically enhanced SCIT formulation with an optimized allergen to adjuvant aluminium hydroxide ratio, has been investigated by Hauswald *et al.* [30] in an open-label, uncontrolled, noninterventional study. Even in this case the most frequent AEs (24.5% of all patients) were mild to moderate LRs while SRs were recorded in 7.9% of all patients and the most were rated as mild (5.1%) or moderate (3.0%) while in 1.4% of patients the reaction was severe. Similar results are reported by Pfaar *et al.* [31].

In regard to the role of potential risk factors affecting the safety profile, we found out that previous LRs could be considered strictly related to the risk of SRs, as a strong association has been detected between grade ≥1 SRs and previous LRs (OR = 5.80; 95% CI = 2.53–13.30; p = 0.0001). Just few studies have specifically investigated this correlation [32–35] with controversial results. LRs are more common during the build-up phase than during maintenance, but do not predict subsequent occurrence of SRs [36]; furthermore some authors have demonstrated the lack of accuracy of LRs in predicting SRs at the next injections and suggest that LRs should be ignored [4,35,36]. On the other hand, recently published studies seem to controvert those results [37]. Although LRs cannot be considered strict predictors of SRs at the next injections, Calabria *et al.* [38] described a higher frequency of SRs in a subgroup of patients experiencing LRs during their immunotherapy course. According to Roy and co-workers [39], the rate of SRs was almost four-time higher among patients who reported LRs compared with those who never experienced any LRs. Kartal *et al.* [40] published in 2015 the results of a 30-year experience about the safety profile of a single SCIT brand. The authors identified LRs, when large (≥5 cm in diameter) and recurrent (≥2 times) as risk factors for SRs. Also, they investigated the potential risk factors for LRs, which according to their findings include: female gender, depot extracts and calcium phosphate-adsorbed extract. However, the role and the importance of LRs as well as their management are still matter of debate [33]. For this reason the clinical relevance of LRs and the role of dose adjustment protocols should be further explored and better evaluated for every marketed product, given the different characteristics of the extracts from different manufacturers; moreover, their importance as predictors of SRs and consistent dose adjustment protocols should be reconsidered as part of a more complete risk assessment [8]. Another determinant related to the risk of SRs seems to be the treatment schedule. According to our data an

association between the incidence of SRs and the number of administered doses can be identified. In other words the risk of SR seems to increase according to the number of injections performed, despite the allergen quantity. This finding should be confirmed by a direct comparison between different treatment schedule, but a similar trend is highlighted also by Crivellaro *et al.* [19]. According to these observations the safety profile of uSCIT MPL4 seems to be increased if the schedule suggested by the manufacturer is applied.

In the past years the use of fast regimens, though characterized by a reduced number of injections, has been associated with a higher number of SRs but more recent studies have shown that their safety profile is similar to conventional schedule [41]. In this respect, the good tolerability of cluster schedules in adult patients has been examined in a large retrospective observational multicentre study by Serrano *et Al.* [42] The study involved 1147 allergic patients and SRs were recorded in 0.6% (n = 42) of all injections and in 3.4% (n = 39) of all patients. The use of modified allergens and the consequent enhanced safety profile of the extracts may account for it. A faster schedule may also result in a better compliance to SIT, which is essential for successful treatment however non-compliance rates are known to be high [43].

Conclusion

The findings of the present study support the uSCIT MPL4 high safety profile and the good risk/benefit ratio of an extract including an allergoid and an adjuvant. Some limitations, which could weaken our findings, have to be taken into consideration; particularly the spontaneous observational real-life study design did not allow to specifically powering the study itself from a statistical point of view. Nevertheless, to the best of our knowledge the present survey provides the first large Italian real-life observatory of SRs relating to a single brand SCIT product, and one of the largest survey on uSCIT MPL4 safety. Our findings support the safety of uSCIT-MPL-4 for all the available allergens and suggest to accurately assessing LRs, as they may represent a risk factor for Grade $\geq$1 SRs, together with the number of doses/year. Specifically designed studies are needed in order to confirm the relevance of SRs risk factors.

Future perspective

It is well known that allergen immunotherapy is the unique disease-modifying treatment for allergic respiratory diseases [41]. Adjuvant molecules have been more recently investigated as a strategy to increase allergy immunotherapy efficacy and effectiveness. Adjuvants are able to modulate immunotherapy mechanisms at different steps, from delivery to interaction with the patient's immune system. In vitro studies have demonstrated that allergen extracts conjugated with adjuvants, when compared with traditional formulations, sustain a faster and broader immune response in treated subjects in the early phases [12–18]; furthermore, under a long-term perspective, a stronger long lasting immune response has been observed [17,44]. As a result of the enhanced immunotherapy effect, a reduction of allergen dose and administration frequency and a shorter immunotherapy course represent expected clinical outcomes.

The currently available adjuvants basically include delivery systems (such as Aluminium hydroxide, calcium phosphate, microcrystalline tyrosine) and immune-modulatory agents [44]. uSCIT MPL4 belongs to the last group, which primarily and mainly interact with Toll-like receptor (TLR) 4, one of the gates between innate immunity and the nonself environment. It has been demonstrated that atopy might be associated with impaired TLRs function, so that modulating that target apparently means addressing the deep background of the allergic response [45]. Also, according to the so-called 'hygiene Hypothesis' concept, that paved the way to several and successful investigations in the field, interacting with innate immunity seems to represent a 'physiological' way to shift the immune system reactivity from hypersensitivity to normal response.

Under this perspective some authors have speculated about the possibility of curing respiratory allergies without allergen extract but with adjuvants only. Few studies have been conducted on TLRs agonists; the results are quite controversial, although the theoretical rationale is strong enough [46].

However, although extremely fascinating, the clinical use of immunotherapy extracts conjugated with adjuvants, and even more the potential use in the future of adjuvants alone, deserves some critical considerations. In terms of efficacy, the superiority of the new formulations when compared with the traditional ones has been demonstrated by experimental and in vitro studies. That kind of evidence is needed and represents a strong background but cannot replace head to head studies aiming to directly compare the same formulation, meaning the same allergen and the same schedule, with and without adjuvants. In fact that is the only way to definitely prove the added value

of adjuvants in clinical practice. To the best of our knowledge up to now no head to head studies are available in the literature.

The second critical aspect is safety. From a speculative point of view, the conjugation with an adjuvant allows to decrease the dose of allergen in the therapeutic extract, and to modulate the immune response in a very 'physiological way' through the interaction with TLRs. Both these aspects sustain an optimal safety background, together with the evidence coming from several trials and real-life studies, which report brilliant data on safety and tolerability. On the other hand, manipulating innate immunity deserves long-term safety surveillance, especially in the real-life setting where treated patients may present comorbidities and multiple drug regimens. Especially in the case of concomitant immunologic diseases targeting innate immunity should be carefully evaluated and monitored.

Head to head comparative studies and long term follow up data should be part of the scientific agenda, in order to further reinforce the daily use of a more than promising therapeutic option for curing allergic respiratory diseases.

Summary points

- The safety of subcutaneous allergen immunotherapy (SCIT) is overall well documented and fatal reactions related to SCIT are rare.
- Given the different characteristics of the extracts from different manufacturers, the safety profile of every brand should be specifically investigated.
- According to the present study, the prevalence of Grade 1, 2, 3 and 4 systemic reactions (SRs) to uSCIT-MPL-4, over a 1-year observation, was respectively: 1.5, 0.31, 0.07 and 0.07%. No Grade 5 SRs occurred.
- A statistically significant association of treatment schedule (number of doses) and previous local reactions (LRs) with Grade $\geq$1 SRs was observed. No relevant associations with other patient/SCIT related-factors were highlighted.
- In the case of uSCIT-MPL-4, the number of injections performed, more than the allergen quantity, seems to be related to a higher risk of SRs.
- Assessment of local reactions should be regularly performed before SCIT start, as they may represent a major risk factor for Grade $\geq$1 SRs.

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Ethical conduct of research

All human studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The Review Boards approved the procedure and written informed consent was given by all participants.

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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